# “Inter- and intraspecific parasitism in honeybees (*Apis mellifera* L.): the small hive beetle (*Aethina tumida* Murray) and the Cape honeybee (*A. m. capensis* Esch.)”

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# 1 Introduction

Honeybees, *Apis mellifera*, are eusocial insects with a well developed reproductive division of labour between the queen and the workers (Wilson, 1971). While, the queen usually dominates reproduction, the workers participate in all other tasks necessary to maintain the colony, e.g. brood rearing, foraging and nest defence (Ribbands, 1953). Honeybee colonies comprise of a single egg-laying queen, several thousand workers (~10,000 to 60,000) and several hundred male sexuals (= drones) depending on the season (Moritz and Southwick, 1992). Honeybees naturally nest in cavities, e.g. in hollow trees. The nest is constructed of wax, which is produced in special glands by the workers (Hepburn, 1986). It consists of a central brood nest with a surrounding pollen storage area and a honey storage area in the nest periphery (Ribbands, 1953).

The individual bees as well as the colony and its stored resources can be exploited by a wide range of parasitic organisms (Schmid-Hempel, 1998). Parasitism can be defined as the relationship between two organisms, where one organism lives at the expense of another organism, its host. Although parasites do not normally kill their hosts, many of these parasitic associations produce pathological changes in the hosts. In extreme cases, this always results in the death of the host (parasitoids, Schmid-Hempel, 1998). One can distinguish between several forms of parasitism. For example parasitism can occur within a single species (intraspecific) and between two species (interspecific; Schmid-Hempel, 1998).

Social parasitism is a common and intriguing phenomenon in social insects. Social parasitic species evolve from their social ancestors by developing mechanisms to exploit the resources of their social hosts. Social parasitism can occur both within and between species (Rinderer et al., 1985; Roubik, 1989). There are several forms of social parasitism (Wilson, 1971; Hölldobler and Wilson, 1990). In some cases workers raid the nests of their own or other species to take food resources (e.g. robbing behaviour of honeybees, Ribbands, 1953; Moritz and Southwick, 1992). Some species show only temporary social parasitism in the nest-founding phase, when mated queens usurp the nests of host species instead of establishing nests by themselves (e.g. wood ants of the genus *Formica*; Hölldobler and Wilson, 1990). Other species take slaves by stealing brood from hosts` nests (Hölldobler and Wilson, 1990). The host brood is raised in the slave maker nest and performs all tasks necessary for the maintenance of the parasite colony. An advanced form of social parasitism are inquiline species, where the worker caste is either reduced or has been lost altogether (Hölldobler and Wilson, 1990). Some of such species spend their entire life in the host nest (Hölldobler and Wilson, 1990). Social parasite species are often closely related to their hosts (= Emery's rule, Emery, 1909). This might be related to the communication between host and parasite. In order to successfully pass the host defence mechanism, social parasites must have evolved communication systems, which are very similar to their host species.

In recent decades, the frequency of biological invasions has increased to an unprecedented level (Hänfling and Kollmann, 2002). Parasites may also become invasive species, which are transferred from their endemic range into new areas and may cause substantial damage to local ecosystems and agriculture. However, the successful treatment and control of invasive parasite species requires not only comprehensive information about the biology of the parasite itself but also a good understanding of the nature of the parasites' interactions with their hosts species. In the following thesis two recent examples of invasive honeybee parasites were investigated in detail: The small hive beetle and the Cape honeybee.

**References**

Emery C (1909) Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. Biol Zentralbl 29: 352-362.

Hölldobler B, Wilson EO (1990) The Ants. Springer Verlag, Berlin, Heidelberg, New York.

Hänfling B, Kollmann J (2002) An evolutionary perspective of biological invasions, Trends Ecol. Evol. 17, 545-546.

Moritz RFA, Southwick EE (1992) Bees as superorganisms. An evolutionary reality. Springer Verlag, Berlin, Heidelberg, New York.

Ribbands CR (1953) The behaviour and social life of honeybees, Bee Research Association Limited, London, UK.

Rinderer TE, Hellmich RL, Danka RG, Collins AM (1985) Male reproductive parasitism. A factor in the Africanization of honeybee populations. Science 228, 1119-1121.

Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge, Cambridge University Press.

Wilson EO (1971) The insect societies. Cambridge, Harvard University Press.

# 2. The small hive beetle
# (*Aethina tumida* Murray, Coleoptera: Nitidulidae)

The small hive beetle, *Aethina tumida*, was first described in 1867 (Murray, 1867) and belongs to the coleopteran family Nitidulidae which contains approximately 2,800 described species in 172 genera worldwide (Habeck, 2002). This family can be distinguished from other similar beetles by their transverse procoxal cavities, grooved metacoxae, dilated tarsal segments, small fourth tarsi and three-segmented antennal club (Habeck, 2002). The Nitidulid beetles can feed on fresh, rotten and dried fruits, plant juices, carrion and crops but occasionally on flowers as well (Lin et al., 1992; Fadamiro et al., 1998; Hepburn and Radloff, 1998; Smart and Blight, 2000; Wolff et al., 2001).

Small hive beetles are honeybee parasites native to sub-Saharan Africa, where they are a minor pest only (Lundie, 1940; Schmolke, 1974; Hepburn and Radloff, 1998). In contrast, the beetles can be harmful parasites of European honeybee subspecies (Elzen et al., 1999a,b; Hood, 2000). Since 1998, the small hive beetle has raised considerable international attention because it has become an invasive species in populations of European-derived honeybees in the USA (Elzen et al., 1999a,b) and Australia (Minister for Agriculture, 2002). At present, the effects of beetle infestations seem to be different in the USA and Australia. While even strong colonies of European honeybee subspecies can be taken over and killed by small hive beetles in the USA (Elzen et al., 1999a,b), strong colonies are not affected in Australia (D Anderson, personal communication).

The natural history of *A. tumida* was described by Lundie (1940, 1951, 1952a,b) and Schmolke (1974). The adults are about 5 to 7 mm long, 3 to 4.5 mm wide and are dark brown to black in colour (Lundie, 1940; Schmolke, 1974). Adults are lighter in colour just after pupation (yellowish to red) but soon become darker (Lundie, 1940). Females (length: 5.27 +/-0.06mm; breadth 3.25 +/-0.04 mm) tend to be bigger than males (length: 5.12 +/- 0.07 mm; breadth: 3.21 +/- 0.04 mm; Lundie, 1940). Small hive beetle eggs are pearly-white, banana-shaped and about 1.4 mm long and 0.26 mm wide (~2/3 the size of a honeybee egg; Lundie, 1940; Schmolke, 1974). The larvae are whitish in colour and emerge from the egg shell through a longitudinal slit at the anterior end in 1-6 days with most hatching in 2-3 days (Lundie, 1940). The majority of the larvae grow to a length of 0.48 to 0.63 cm when four days old and up to 1.2 cm when full-grown (8 to 29 days; Lundie 1940). They have relatively large heads, spiny protuberances along the body, six fully developed legs near the head (which might facilitate their feeding within the hive; Lundie 1940) and look superficially like wax moth larvae. However, the six legs of small hive beetle larvae are larger, more pronounced and only occur near the head. This is how small hive beetle infestations differ from those of the greater wax moth, *Galleria mellonella*, because small hive beetle and wax moth infestations may simultaneously occur in one colony (Lundie, 1940).

Host finding of small hive beetles may occur by individual adults or occasionally by beetle swarms (Tribe, 2000). Then, the beetles have to bypass the host colony's guard force to successfully intrude the host colony. In African subspecies, successful beetle reproduction appears to be most successful in weak/stressed colonies or in recently abandoned nests and is far less common in strong colonies (Lundie, 1940; Schmolke, 1974; Hepburn and Radloff, 1998). In strong African colonies small hive beetles usually have to wait until absconding (= non-reproductive swarming) or seasonal migration (Hepburn and Radloff, 1998) leads to unprotected recently abandoned nests. Massive aggregations of small hive beetles and/or heavy infestations appear to induce absconding (Hepburn and Radloff, 1998; Neumann and Elzen, 2003). In contrast, successful reproduction seems to be more common in strong colonies of European subspecies in the USA but not in Australia (see above). Overwintering may also occur in European colonies (Pettis and Shimanuki, 2000). Female beetles oviposit in the host colonies (Lundie, 1940). The emerging larvae develop until the wandering stage and then leave the nest for pupation in the soil (Lundie, 1940). Newly emerged adults invade new host colonies, thereby completing the life cycle of *A. tumida*. The life cycle of *A. tumida* may also occur in alternative hosts (Armbrose et al., 2000), on alternative food sources such as fruits (Eischen et al., 1999; Ellis et al., 2002) and stored bee products (Lundie, 1940).

Mating can either occur inside or outside of the host colony (Neumann et al., 2001a,b) and multiple mating by males seems to be occur (Neumann et al., 2001a). The large number of offspring per breeding couple shows the enormous reproductive potential of this parasite (Neumann et al., 2001a; Ellis et al., 2002c), which seems to be related to the protein rich-diet (Ellis et al., 2002c). Inside of the hive, adults are able to live on pollen and honey but prefer bee brood as food even in the presence of pollen and honey (Elzen et al., 2000; Swart et al., 2001). However, in contrast to adult large hive beetles, *Hoplostoma fuligineus* which can severely damage colonies (Hepburn and Radloff, 1998; Swart et al., 2001), the adult small hive beetle itself has little impact on an African honeybee colony. It is the larvae that can cause severe damage to combs (Lundie, 1940; Schmolke, 1974; Eischen et al., 1998). Indeed, their destructive effects are comparable to those of wax moths (Hepburn and Radloff, 1998). The larvae scavenge on storage combs of weak colonies often resulting in the full structural collapse of the nest (Hepburn and Radloff, 1998). The larvae also cause fermentation of the honey (Lundie, 1940; Swart et al., 2001), resulting in the characteristic foul smell of infested colonies (Lundie, 1940). Older larvae often aggregate in the corners of frames and on the floor boards (Lundie, 1940). After 8-29 days the larvae reach the “wandering phase” (Lundie, 1940), become positively photo-tactic (Schmolke, 1974) and leave the nest to pupate. Because such larvae may be covered by a sticky film, heavily infested colonies may show a brownish coat at the outside (Lundie, 1940). The larvae usually pupate in the soil in close proximity to the nest (83% within 30 cm of the hive entrance and no beetles found at 180 cm from the hive; Pettis and Shimanuki, 2000; Hood, 2000), but can crawl considerable distances to reach a suitable pupation environment (Schmolke, 1974; >30m in a concrete building, CWW Pirk, personal communication). The pupae are whitish brown (Lundie, 1940). Pupation takes about 3-4 weeks (Lundie, 1940). It appears that the type of soil can significantly affect the ability of the larvae to pupate with light sandy soils providing a more suitable pupation medium than heavy clay soils (Pettis and Shimanuki, 2000). In sandy soils larvae, pupae and newly emerged adults were found at 1-20 cm depth with nearly 80% in the top 10 cm (Pettis and Shimanuki, 2000; Hood, 2000).

Field observations in Africa indicate that successful reproduction of the small hive beetle can be enhanced by hot and humid conditions (Swart et al., 2001). Indeed, the length of the small hive beetle life cycle can range from ~30 to more than 60 days, depending on food supply, temperature and moisture regime (Lundie, 1940; Schmolke, 1974). This is similar to other nitidulid species, where rates of development have been shown to change in a linear fashion with temperature over a range of constant temperatures (e.g. *Carpophilus* spec., James and Vogele, 2000). The adult small hive beetles can survive for at least five days without food and water in moderate temperatures (Pettis and Shimanuki, 2000; Ellis et al., 2002c). In South Africa, up to five beetle generations can be produced per year (Lundie, 1940). Small hive beetle adults are relatively long-lived. The average life span is about two to three months but may be up to six months in laboratory cultures and probably longer in host colonies (Lundie, 1940; Schmolke, 1974). This great longevity results in the overlapping of generations and in constant annoyance to the beekeeper (Lundie, 1940). The adult females reach sexual maturity from two to seven days after emergence (Lundie, 1940; Schmolke, 1974). Newly emerged adult beetles are very active, readily take flight, and orient toward the light (Lundie, 1940). After one or two days, the adults become less active and prefer less illuminated areas (Lundie, 1940).

It seems as if there is a female-biased sex ratio of offspring, with up to two females per male (laboratory rearing: Neumann et al., 2001a; wild populations: Ellis et al., 2002b; but see Schmolke, 1974). In a laboratory study (Ellis et al., 2002c) significantly more females than males were only found in the brood and pollen diets. In these diets significantly more larvae were produced than in the others (fruits, honey, etc). Larval density can act indirectly on sex ratio because of food competition and selective mortality that usually benefits female offspring (Laugé, 1985). Female insects tend to be heavier than males, which seems to be related to a general nutrient accumulation needed for their role as egg layers (Slansky and Scriber, 1985). This increase in weight might result from amplified food consumption by female larvae (Slansky and Scriber, 1985). Also small hive beetle females tend to be bigger and heavier than males (Schmolke, 1974; Ellis et al., 2002b). Thus, in cases of high larval density (e.g. in highly infested colonies or protein rich diets) female larvae may be more competitive, leading to the selective mortality of male larvae.

Many aspects of the biology of the small hive beetle are still poorly understood. However, successful and sustainable control efforts require a detailed understanding of the invasion dynamics and of the biology of an invasive species such as *A. tumida*. Therefore, both laboratory and field studies on the small hive beetle are included in this thesis, which are addressed in the following chapters.

## Research goals and conclusions:

### 2.1 Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera, Nitidulidae)

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A simple and fast method for laboratory rearing of small hive beetles is developed. The results show that small hive beetles have an enormous reproductive potential, which is probably related to the parasitic life history. A significant female-biased sex ratio was also found in the offspring which is interesting with respect to observations that males mate multiply and tend to infest host colonies before females.

### 2.2 Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets

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The longevity and reproductive success of adult small hive beetles assigned different natural diets were determined. The pupation success and sex ratio of small hive beetle offspring were also analysed. Longevity in honey-fed small hive beetle adults was significantly higher than on other diets. Small hive beetles fed empty brood comb lived significantly longer than unfed beetles. Small hive beetle offspring were produced on honey/pollen, pollen, bee brood, fresh kei apples, and rotten kei apples but not on honey alone, empty brood comb, or in control treatments. The highest reproductive success occurred in pollen fed adults. The data also show that small hive beetles can reproduce on fruits alone, indicating that they are facultative parasites. However, the reproductive success on fruits was much smaller than on pollen and brood. Larvae fed pollen, honey/pollen, or brood had significantly higher pupation success rates than on the other diets. Sex ratios of emerging adults fed diets of pollen or brood as larvae were significantly skewed towards females supporting the results from chapter 1. Because longevity and overall reproductive success was highest on foodstuffs located in honeybee colonies, it is easily seen why small hive beetles are efficient at causing economic damage to colonies of honeybees.

### 2.3 The effects of adult *Aethina tumida* (Coleoptera: Nitidulidae) on nests and foraging activity of African and European honey bees (*Apis mellifera*)

**Published in:** Ellis JD, Hepburn HR, Delaplane K, Neumann P, Elzen PJ (2003) The effects of adult *Aethina tumida* (Coleoptera: Nitidulidae) on nests and foraging activity of African and European honey bees (*Apis mellifera*). Apidologie **34**: 399-408. **Own contribution:** project idea, manuscript.

Differences in the effects of small hive beetles on flight activity and nests of European-derived honeybees in the United States and Cape honeybees in South Africa were evaluated. Treatments consisted of control colonies and experimental colonies receiving beetles. Absconding day did not differ significantly between treatment or bee race but absconding was greater between the two treatments in European colonies than in Cape ones. Cape bees used significantly more propolis than European bees. Honey stores were significantly greater in Cape honeybee colonies than in European ones. Bee weight did not differ significantly between treatments or bee race. Treatment did not significantly affect bee populations, brood area, or average flight activity in Cape colonies but it did significantly lower all of these variables in European honeybee colonies. The effects of treatment in European colonies are symptomatic of preparation for absconding. Treatment significantly lowered the amount of pollen stores in Cape colonies, but this effect was not found in European colonies. The number of beetles in control colonies was significantly higher in European colonies than Cape ones while the percentage of beetles remaining in non-absconding treated colonies was higher in Cape colonies than European ones. These data indicate that adult small hive beetles are sufficient to cause significant harmful effects on colonies of European, but not Cape, honey bees.

### 2.4 Behaviour of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*

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The defensive behaviour towards adult small hive beetles by *A. m. capensis* and North American European-derived *A. mellifer*a was quantified. The results establish that Cape honeybees exhibit significantly more investigative contact and aggression behaviour towards the adult beetles than European honeybees. The study also showed that adult beetles readily accept Cape honeybee eggs as food.

### 2.5 Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis*)

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Social encapsulation of adult small hive beetles by Cape honeybee colonies was evaluated. *A. m. capensis* worker encapsulate the small hive beetle in propolis (tree resin collected by the bees). The encapsulation process lasts one to four days and the bees have a sophisticated guarding strategy for limiting the escape of beetles during encapsulation. Some encapsulated beetles died (4.9%) and some escaped (1.6%). Encapsulation has probably evolved because the small hive beetle cannot easily be killed by the bees due to its hard exoskeleton and defensive behaviour.

### 2.6 Cape (*Apis mellifera capensis*) and European (*Apis mellifera*) honey bee guard age and duration of guarding small hive beetles (*Aethina tumida*)

**Published in:** Ellis JD, Holland AJ, Hepburn HR, Neumann P, Elzen PJ (2003) Cape (*Apis mellifera capensis*) and European (*Apis mellifera*) honey bee guard age and duration of guarding small hive beetles (*Aethina tumida*). J. Apic. Res. **42**: 32-34. **Own contribution:** project idea, manuscript.

The guard age and duration of North American European-derived *A. mellifer*a and Cape honeybees guarding small hive beetle prisons were determined using three-frame observation hives, noting the commencement and termination of prison guarding by individually labelled honeybees. European honey bees in the United States began guarding small hive beetle prisons significantly earlier, and stopped guarding prisons significantly sooner than Cape honey bees in South Africa. Although the timing of prison guarding behaviour between the two subspecies is significantly different, it does not explain the differential damage to European and Cape honey bee colonies caused by small hive beetles.

### 2.7 Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier)

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The removal of small hive beetle small hive beetle eggs and larvae was studied in field colonies of African honeybees (*A. m. scutellata*). Because female beetles can protect their eggs by oviposition in small cracks unprotected eggs and protected eggs were introduced into these colonies. Whereas all unprotected eggs were removed within 24 hours, 66±12% of the protected eggs remained, showing that small hive beetle eggs are likely to hatch in infested colonies. However, all larvae introduced into the same colonies were rejected within 24 hours. Workers responded quickly to the presence of small hive beetle offspring in the colonies because 72±27% of the unprotected eggs and 49±37% of the larvae were removed within the first hour after introduction. The removal of small hive beetle eggs and larvae was not correlated with colony phenotypes (size, amount of open and sealed brood, pollen and honey areas). Our data show that African colonies remove both unprotected eggs and larvae of *A. tumida* within short periods of time. Therefore, it is concluded that this removal behaviour plays an important role for the apparent resistance of African honeybees towards small hive beetle infestations.

### 2.8 The biology of the small hive beetle (*Aethina tumida*, Murray): Gaps in our knowledge of an invasive species

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The literature on the biology and the current distribution of the small hive beetle is reviewed. The review concentrates on examining the more proximate aspects of the biology of the beetle and the host that may contribute to the invasion process. Several potential reasons may be responsible for the difference between pest severity in Africa, in the US and in Australia: 1) Different beekeeping techniques, 2) Differences between introduced small hive beetle populations, 3) Enemy release hypothesis, 4) Climatic differences, 5) Different strains of honeybees, 6) Different densities of small hive beetle populations. It is concluded that at the current state of evidence it appears premature to decide which of these factors is important for the differences between beetle damage in the US and Australia. However, the differences between the US and Africa most likely result from behavioural differences between African and European subspecies, unless massive host shifts occur in the new range or unless important small hive beetle pests/parasites have not been identified yet. The known behaviours, which are probably involved in small hive beetle resistance of African bees, such as absconding, aggression and social encapsulation also occur in susceptible populations of European honeybees. Therefore, it is obvious that the susceptibility of European bees is not due to a lack of behavioural resistance mechanisms. Resistance of African bees is probably due to quantitative differences in a series of behaviours such as absconding, aggression, removal of beetle eggs and larvae and social encapsulation. The beetles use counter-resistance tactics such as defence posture, dropping, hiding, escape, egg laying in small gaps and trophallactic mimicry. However, many of the behavioural mechanisms have only been qualitatively described, have not been tested in comparative studies between African and European bees or may even simply be unknown. Moreover, very important basic features of the life cycle of *A. tumida* are still poorly understood. Therefore, more comparative studies between parasite and host populations in Africa, Australia and in the US are urgently required. In general, there is a fragmentary knowledge of the small hive beetle, creating demand for more research in all areas of its biology. Nevertheless, small hive beetles are obviously efficient in long-range transportation (US: 1996, Australia: 2002) and can establish populations in temperate regions (e.g. in Ohio, USA) due to their overwintering capacity in the honeybee winter clusters. Host shifts to other wild bee species such as bumblebees, may also occur. Thus, small hive beetles have the potential to become a global threat to apiculture and wild bee populations.

## References

Ambrose JT, Stanghellini MS, Hopkins DI (2000) A scientific note on the threat of small hive beetles (*Aethina tumida* Murray) to bumble bees (*Bombus* sp.) colonies in the United States, Apidologie 31, 455-456.

Eischen FA, Baxter JR, Elzen PJ, Westervelt D, Wilson W (1998) Is the small hive beetle a serious pest of U.S. honey bees? Am. Bee J. 138, 882-883.

Eischen FA, Westervelt D, Randall C (1999) Does the small hive beetle have alternate food sources? Am. Bee J. 139, 125.

Ellis JD, Delaplane KS, Hood WM (2002a) Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the south-eastern United States, Am. Bee J. 142, 520-522.

Ellis JD, Neumann P, Hepburn HR, Elzen PJ (2002b) Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets, J. Econom. Entomol. 95, 902-907.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Cutts L, Wilson W, Eischen FA, Delaplane KS, Hopkins DI (1999a) Status of the small hive beetle in the U.S., Bee Culture 127, 28-29.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L., Wilson WT (1999b) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western hemisphere, Apidologie 30, 361-366.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Wilson WT (2000c) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae) in Florida, USA, Apidologie 31, 593-594.

Fadamiro HY, Gudrups I, Hodges (1998) Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles, J. Stored Products Res. 34, 151-158.

Habeck DH (2002) Nitidulidae. In: American Beetles, Volume 2, (Arnett RH, Thomas MC, Skelley PE and Frank JH eds.), CRC Press, Boca Raton, pp. 311-315.

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin, Heidelberg, New York.

Hood, WM (2000) Overview of the small hive beetle *Aethina tumida* in North America, Bee World 81, 129-137.

James DG, Vogele B (2000) Development and survivorship of *Carpophilus hemipterus* (L.), *Carpophilus mutilatus* Erichson and *Carpophilus humeralis* (F.) (Coleoptera: Nitidulidae) over a range of constant temperatures, Australian J. Zoology 39, 180-184.

Laugé G (1985) Sex Determination: Genetic and Epigenetic Factors, In: GA Kerkut and LI Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology Volume 1. Permagon Press, Oxford, pp. 313-315.

Lin HC, Phelan PL, Bartelt RJ (1992) Synergism between synthetic food odours and the aggregation pheromone for attracting *Carpophilus lugubris* in the field, Env. Entomol. 21, 156-159.

Lundie AE (1940) The small hive beetle *Aethina tumida*, Science Bulletin 220, Dep. Agr. Forestry, Government Printer, Pretoria, South Africa.

Lundie AE (1951) The principal diseases and enemies of honey bees, S. Afr. Bee J. 26, 15-16.

Lundie AE (1952a) The principal diseases and enemies of honey bees, S. Afr. Bee J. 27, 9.

Lundie AE (1952b) The principal diseases and enemies of honey bees, S. Afr. Bee J. 27, 13-15.

Minister for Agriculture (2002) Small hive beetle confirmed in Sydney bee apiary: Amery, Agricult. media releases 31 October 2002, http://minister.agric.nsw.gov.au/reader/16058.

Murray A (1867) List of Coleoptera received from Old Calabar, Ann. Magazine Nat. Hist., London 19, 167-179.

Neumann P, Elzen PJ (2003) The biology of the small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species. Apidologie 34, in press.

Neumann P, Pirk CWW, Hepburn HR, Elzen PJ, Baxter JR (2001a) Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera: Nitidulidae), J. Apic. Res. 40, 111-112.

Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001b) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.), Naturwissenschaften 88, 214-216.

Pettis J., Shimanuki H (2000) Observations on the small hive beetle, *Aethina tumida*, Murray, in the United States, Am. Bee J. 140, 152-155.

Schmolke MD (1974) A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, pp. 178.

Slansky, F, Scriber JM (1985) Food Consumption and Utilization, In: GA Kerkut and LI Gilbert (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology Volume 4. Permagon Press, Oxford, pp. 96-110.

Smart LE, Blight MM (2000) Response of the pollen beetle, *Meligethes aneus*, to traps baited with volatiles from oilseed rape, *Brassica napus*, J. Chem. Ecol. 26, 1051-1064.

Swart JD, Johannsmeier MF, Tribe GD, Kryger P (2001) Diseases and pests of honeybees, In: Johannsmeier M.F. (ed) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp 198-222.

Tribe GD (2000) A migrating swarm of small hive beetles (*Aethina tumida* Murray), S. Afr. Bee J. 72, 121-122.

Wolff M, Uribe A, Ortiz A, Duque P (2001) A preliminary study of forensic entomology in Medellin, Colombia, Forensic Sci. Internat. 120, 53-59.

# 2.1 Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera, Nitidulidae)

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**Keywords:** *Aethina tumida*, *Apis mellifera*, honeybee, laboratory rearing, parasite, small hive beetle, sex ratio

The small hive beetle (*A. tumida*, SHB) is a common honeybee (*Apis mellifera*) parasite in Africa, which causes little damage to strong colonies (Lundie 1940). However, it is a serious threat in the Western Hemisphere where the beetle has recently been introduced (Elzen *et al.* 1999) and where host colonies lack the behavioral resistance mechanisms of African honeybees (Neumann *et al.* 2001). Captive breeding of SHB is an important research technique to produce SHB under controlled conditions for experiments. Here we report on a simple technique for rearing SHB in the laboratory.

On 12.03.2000, 30 SHB adults were randomly collected from the bottom boards of several *A. m. capensis* colonies near Port Elizabeth, South Africa. The SHB were introduced into a hard plastic container (22×33×33cm) with a mesh insert (about 1mm mesh width, 20×20cm) in the middle of the lid to provide air. The container had clips on the lid and on the sides to seal it properly to prevent beetle escape. The bottom of the container was filled with two pieces of comb (approx. 30×15cm) taken from honeybee colonies containing either honey and pollen or brood. The container was kept in a dark storeroom at room temperature (ranging from 17-24°C) without normal daylight. The container was checked once daily for 21 days. The adults moved rapidly over the combs and immediately started feeding on the honey, pollen and brood provided. Four matings were observed during the initial check on day 2. SHB larvae were observed to move on and in all combs from day 4 onwards supporting an egg stage of about two days (Schmolke 1974). As soon as the first larvae showed the "wandering phase" (Lundie 1940) from day 18 onwards they accumulated in the corner of the box facing the door of the storeroom, thus showing positive phototaxis as previously reported (Schmolke 1974).

On day 21, 474 larvae were found dead in the container. All of the remaining 3866 larvae showed the wandering phase and were transferred into a new pupation container as described above, however with c. 9000 $cm^3$ of autoclaved soil instead of frames. A piece of pine wood (19×11×2cm) with 60 round holes (1×1.8cm) was placed on top of the soil. The holes were filled with water. Then, the pupation container was placed into another storeroom at room temperature and normal daylight conditions and photoperiod. One side of the container was covered with a piece of cardboard (20×20cm). The larvae rapidly moved into the soil. While several larvae were found underneath the piece of pine wood (c. 100), no larvae were observed on top of the soil or close to the uncovered walls of the container. However, when the cardboard was removed on day 22, 20 larvae were observed close to this wall of the container. On day 23 no larvae were observed close to this wall, suggesting that larvae ready for pupation show negative phototaxis. From day 24 onwards the pupation container was checked on a weekly basis when the soil was moistened with water by filling the holes of the piece of wood. From day 57 onwards, adult beetles emerged. All emerging beetles were removed from the containers and sexed (Schmolke 1974). By day 74, a total of 1646 beetles had emerged; 650 males and 996 females (average length of developmental cycle = 49±0.11 days). A significant female biased sex ratio was observed in the emerging adults ($\chi^2 = 94.4$; $P<0.0001$). 42.6% of the introduced wandering larvae emerged as adult beetles.

Our results clearly show that SHB can easily be reared in large numbers in the laboratory without sophisticated equipment. The method is inexpensive, simple and does not require labor intensive steps in contrast to previously reported rearing techniques (Schmolke 1974). Our method is probably not restricted to periods of the year when honeybee brood and honey/pollen combs are available, because frozen pieces of comb likely can be used. Breeding of SHB is also successful on a diet of honey and pollen alone (Schmolke 1974). However, in our study larvae readily accepted bee brood as food. Thus, we recommend including bee brood in the diet whenever possible. The high mortality rate in our study may be due to the fact that many larvae tried to pupate in a relative small container (only ∅ of 2.3$cm^3$ soil for each SHB). We therefore recommend using several containers to reduce larval/pupal density in the soil. The rather long developmental cycle in our technique can probably be shortened by using incubators, because Schmolke (1974) found an average cycle of about 32 days under constant 30°.

The observed female biased sex ratio of offspring SHB supports other observations (MacKay unpublished, cited in Schmolke 1974) that beetles would be found in a ratio of two females to one male (but see Schmolke 1974). The female biased sex ratio may be related to the parasitic life history of *A. tumida*, especially to observations that multiple mating by males is common in SHB (unpublished data) and that males tend to infest colonies before females (Elzen *et al.* 2000). Our data clearly show the enormous reproductive potential of SHB probably necessary for an obligate parasite. This may explain the rapid spread of SHB in regions, where honeybee host colonies show no effective behavioral resistance mechanisms (Neumann *et al.* 2001).

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## REFERENCES

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L, Wilson WT (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking honey bees in the Western Hemisphere. Apidologie 30: 361-366.

Elzen P.J, Baxter JR, Westervelt D, Randall C, Wilson WT (2000) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. Apidologie 31: 593-594.

Lundie AE (1940) The small hive beetle, *Aethina tumida*. Scientific Bulletin 220, Union of South Africa, Department of Agriculture and Forestry, 1940, 30 pp.

Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Schmolke MD (1974) A study of *Aethina tumida*: The small Hive Beetle, Project Report, University of Rhodesia (1974) 178 pp.

# 2.2 Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets

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**Abstract -** The longevity and reproductive success of newly emerged, previously unfed adult small hive beetles (*Aethina tumida* Murray) assigned different natural diets (control = unfed; honey/pollen; honey; pollen; empty brood comb; bee brood; fresh kei apples; and rotten kei apples) were determined. The pupation success and sex ratio of SHB offspring were also analysed. Longevity in honey-fed small hive beetle adults (average maximum: 167 days) was significantly higher than on other diets. Small hive beetles fed empty brood comb lived significantly longer (average maximum: 49.8 days) than unfed beetles (average maximum: 9.6 days). Small hive beetle offspring were produced on honey/pollen, pollen, bee brood, fresh kei apples, and rotten kei apples but not on honey alone, empty brood comb, or in control treatments. The highest reproductive success occurred in pollen fed adults (1773.8 ± 294.4 larvae/3 mating pairs of adults). The data also show that small hive beetles can reproduce on fruits alone, indicating that they are facultative parasites. Larvae fed pollen, honey/pollen, or brood had significantly higher pupation success rates of 0.64, 0.73, and 0.65 respectively than on the other diets. Sex ratios of emerging adults fed diets of pollen or brood as larvae were significantly skewed towards females. Because longevity and overall reproductive success was highest on foodstuffs located in honeybee colonies, it is easily seen why small hive beetles are efficient at causing economic damage to colonies of honeybees. Practical considerations for the control of small hive beetles are discussed briefly.

**Key words:** *Aethina tumida, Apis,* facultative parasite, reproductive success, pupation, success, sex ratio

## 1. INTRODUCTION

*Aethina tumida* Murray are endemic in sub-Saharan Africa and are parasites of honey bee, *Apis mellifera* (Hepburn and Radloff 1998),colonies. They rarely inflict severe damage to strong colonies in their native range (Lundie 1940,Schmolke 1974).In contrast, *A. tumida* have recently been discovered in the United States where they cause severe damage to colonies of European derived subspecies of *A. mellifera* (Elzen et al.1999). Indeed, in 1998 Florida beekeepers experienced an estimated $3 million loss due to *A. tumida*. The economic impact of *A. tumida* appears to depend on beetle longevity and ability to mass reproduce on the foodstuffs located in honey bee colonies.

Studies on the longevity of small hive beetle adults are few and contradicting. Lundie (1940) reported that small hive beetle adults fed honey and pollen can live 180-188 d but given only water and beeswax, adults lived a maximum of 19 d (Schmolke 1974). Schmolke (1974) added that adult beetles deprived of food and water died within two days while Pettis and Shimanuki (2000) reported that adult *A. tumida* can live 5 d when entirely deprived of food and water. In another study (Flügge 2001), newly emerged adults deprived of food and water lived seven days. Thus, it is unclear how long *A. tumida* can live and how this is related to different food regimes and reproductive success, which is the major factor affecting the economic impact of *A. tumida*.

Unlike other species of Nitidulidae that mainly feed and reproduce on rotten fruit (Borror et al.1989), small hive beetle adults and larvae have been reported to feed on honey bee nest contents, including pollen, honey, (Lundie 1940;Schmolke 1974,Neumann et al. 2001b) and, preferentially, honey bee brood (Elzen et al.2000). It is when small hive beetle adults and larvae are feeding on these foodstuffs that colony health begins to decline. Eischen et al.(1999)stated that *A. tumida* can feed and reproduce on fruits, indicating that *A. tumida* may only be facultative parasites. However, the relative reproductive success of small hive beetle adults on different diets afforded by a honey bee nest or by fruits has not yet been quantitatively investigated.

Successful reproduction of the small hive beetle in its native range is often restricted to weak colonies or associated with after absconding events (Hepburn et al. 1999) because of behavioural resistance mechanisms of their honey bee hosts (Elzen et al. 2001,Neumann et al. 2001a). In these cases, a variety of food stores, brood combs and freshly emerged bees, are often left behind by the absconding swarms (Hepburn and Radloff 1998). Thus, *A. tumida* are provided a range of diets in their native habitat, the reproductive affects of which are not yet known.

Sex ratios of adult small hive beetle populations in the wild show no statistically significant sex bias although females most always outnumber males

(Schmolke 1974,Ellis et al. 2002). However, Neumann et al. (2001b) showed that sex ratios could significantly favour female offspring in vitro supporting a similar observation made by Mackay (unpublished cited in Schmolke 1974). It is possible that, in instances where populations of *A. tumida* exhibit biased sex ratios, small hive beetle larval diet affects emerging adult sex ratios. That possibility is also investigated here.

In this study, we report the longevity and reproductive success of newly emerged adult *A. tumida* assigned different diets. The pupation success of larvae reared on the same diets as their parents and sex ratios of the resulting adults were also analysed. These data will shed light on the reproductive success and life history of *A. tumida* telling us if *A. tumida* are obligate or facultative parasites of honey bee colonies; the possibility of their survival outside of honey bee colonies; and their longevity as adults, which may be crucial for small hive beetle reproduction. All of these factors contribute to *A. tumida* success in causing honey bee colony collapse.

## 2. MATERIAL AND METHODS

Experiments were conducted at Rhodes University in Grahamstown, South Africa from February to October 2001. Small hive beetles were obtained from infested colonies at Grahamstown and Port Elizabeth, South Africa and were reared in the laboratory according to standard methods, being fed a mixture of bee brood, honey and pollen combs, and water *ad lib* (Neumann et al. 2001b),and sexed according to standard protocols (Schmolke 1974).

Three recently emerged unfed adult males and females were put in pairs in plastic containers (11 by 11 by 9 cm) and provided with water *ad lib*. This was replicated five times for each of the following eight treatments: (1) control (no food), (2) empty brood comb (6 by 6 cm), (3) comb with honey (6 by 6 cm), (4) comb with pollen (6 by 6 cm), (5) comb with both honey and pollen in roughly equal volumes (6 by 6 cm), (6) brood comb containing live brood of all stages (6 by 6 cm), (7) rotten Kei apples (*Dovyalis caffra*; n = 4),and (8) fresh Kei apples (n = 4). All foodstuffs were frozen before use to kill any beetle eggs. As the supply of food in the feeding chambers was exhausted, an amount of food equivalent to the original amount was added to the containers. This was repeated as needed for the duration of the experiment. The feeding containers (with adult *A. tumida* and individual diets) were kept from light and at room temperature throughout the experiment.

To determine longevity, the number of live adults in each container was counted weekly until all adults were dead. Because we were interested in determining average maximum longevity (giving us an idea how long we can expect the longest-lived adults to survive), we used data on the last date small hive beetle adults were recorded alive in each container. When larvae feeding in the same containers as their parents reached the wandering phase before pupation (which normally occurs in the ground outside of honey bee colonies, Lundie 1940),they were transferred into containers with slightly moist soil (Neumann et al. 2001b) and were kept from light and at room temperature. Because of the high number of hatching larvae on the pollen diet, larvae reaching the wandering phase were put into several soil chambers to eliminate a possible larval density effect on pupation success (Neumann et al. 2001b). Adults emerging from the pupation chambers were sexed (Schmolke 1974). Because adult *A. tumida* often congregated under the soil surface in the pupation containers the contents of the containers were sifted to collect the mature adult *A. tumida*.

### Statistical Analysis.

The number of larval and adult small hive beetle offspring produced, ratios of emerging adults per larvae, and longevity of the parental adults in the food containers were compared between the treatments using ANOVAs and Newman-Keuls post hoc comparisons. The sex ratios of emerging adults were evaluated using chi-square tests. All calculations were performed using the software package Statistica (Statistica 2001).

## 3. RESULTS

A total of 13,926 larvae was transferred into pupation containers across all diets and 8532 male and female adult beetles emerged in the pupation containers.

### Diet Effects on Longevity

Diet affected the longevity of parental adults ($F=45.15$, $df=7$, $P<0.0001$). Adults fed brood or nothing (control) lived significantly shorter periods than adults fed all other diets ($P<0.05$)(Table 1). Adults fed empty brood comb, fresh Kei apples, and rotten Kei apples had statistically similar longevities ($P<0.05$) (Table 1). Honey fed adults lived significantly longer than adults fed all other diets ($P<0.05$) (Table 1) with the longest-lived adults surviving for 176 d. The longevity of pollen fed adults was significantly different ($P<0.05$) from those of all other diets and was second only to honey fed adults (Table 1). Finally, longevities in honey-pollen fed adults, fresh Kei apple fed adults, and rotten Kei apple fed adults were statistically similar ($P<0.05$; Table 1).

### Diet Effects on Reproductive Success

There were diet effects on the number of wandering larvae put into soil chambers ($F = 97.27$, $df = 7$, $P<0.0001$). No larvae were found in the control, empty brood comb, or honey diet containers (Table 1). The numbers of larvae produced from adults fed pollen, brood, or honey-pollen were significantly different from one another and from all other treatments ($P<0.05$ level), with the pollen diet having the highest reproductive success followed by brood and honey-pollen respectively (Table 1). Adults feeding on both fresh Kei apples and rotten Kei apples did not produce significantly more larvae per three pairs of adults than any of the adults fed diets on which no larvae were produced ($P<0.05$) (Table 1). After the 81st experimental day, an estimated number of >5,000 unidentified common pollen mites infested only the pollen diet containers. Upon mite infestation, the adult *A. tumida* feeding on the pollen diets stopped reproducing.

### Diet Effects on Pupation Success

Diet affected the number of adult *A. tumida* emerging from the pupation soil chambers ($F=93.27$,df=7, $P<0.0001$). Because no larvae were found in the control, empty brood comb, or honey containers (Table 1) no adults emerged from these diets. The numbers of emerged adults from larvae fed pollen, brood, or honey-pollen were significantly different from one another and from all other treatments ($P<0.05$ level),with the pollen diet yielding more adult *A. tumida* followed by the diets brood and honey-pollen respectively (Table 1). Adults emerging from larvae feeding on both fresh Kei apples and rotten Kei apples were not significantly more numerous than on those diets where no adults emerged ($P<0.05$; Table 1).

There were also diet effects on the average ratios of adults per larvae ($F=4.95$,df=4, $P<0.01$). Adults per larvae ratios are the number of emerged adults from the number of larvae initially placed into their respective soil containers, or the proportion of larvae that pupated successfully. The adults per larvae ratios for the pollen, honey-pollen, and brood diets did not significantly differ from one another ($P<0.05$) and they were higher for these diets than for all other diets. Additionally, the adults per larvae ratios for brood and fresh Kei apples did not significantly differ from one another ($P<0.05$); neither did the adults per larvae ratios for the fresh Kei apple and rotten Kei apple diets ($P<0.05$; Table 1).

### Diet Effects on Sex Ratio

In all diets except for rotten apples, the sex ratios of adult small hive beetle offspring were skewed toward females (Table 2). For the diets pollen ($\chi2=21.77$,df=4, $P<0.00022$) and brood ($\chi2=19.09$,df=4, $P<0.00076$), the number of emerging adult female small hive beetles was significantly higher than the expected value of 50% of emerging adults being females. The number of emerging females did not statistically deviate from the expected value of 50% in honey-pollen ($\chi2=6.89$, df=4, $P=0.14$), fresh Kei apple ($\chi2=2.91$, df=4, $P=0.57$), and rotten Kei apple ($\chi2=0.41$, df=4, $P=0.98$) diets (Table 2).

## 4. DISCUSSION

### Diet Effects on Longevity

Our data and that of Lundie (1940) show that adult *A. tumida* feeding on honey alone can live for over 5 mo. Dadd (1985) states that carbohydrate (especially sugar) utilization is very important in insect longevity, which is consistent with our findings that honey-fed adults live the longest. So, it is possible that *A. tumida* can live in honey houses for at least 5 mo and reproduce once locating acceptable food sources. Therefore, beekeepers should strive to maintain clean honey houses. Pollen fed adults were also long-lived (Table 1) indicating a need for beekeepers to properly store any frames or equipment that contain pollen.

Small hive beetle adults feeding on honey-pollen, fresh Kei apple, and rotten Kei apple diets all lived less than those on honey and pollen diets (Table 1),despite the fact that the former are also high in carbohydrates. However, we still show that adult *A. tumida* can live on diets of fruit alone for >2 mo, thus identifying a potential pathway for *A. tumida* from their native range in Africa to the United States and elsewhere via fruit transports on cargo ships.

Small hive beetles living on empty brood comb survived for an average maximum of 49.8 d (Table 1), indicating nutritional food stuffs in empty brood comb (Shimanuki et al. 1992),but not enough to support reproduction. Therefore, small hive beetle adults are able to live on old comb for over a month, further strengthening recommendations (Hood 2000) to properly store beekeeping equipment.

Unfed adult *A. tumida* in this experiment had longevities similar to those found by others (Schmolke 1974, Pettis and Shimanuki 2000, Flügge 2001). Surprisingly, longevity in adult *A. tumida* feeding on brood diets did not differ significantly from those on control diets. This is likely due to the rancid environment created in those containers. A possible improvement in design would have been to separate the parental adult beetles from the feeding larvae across all treatments.

Because of our findings, we recommend to beekeepers suffering from small hive beetle infestations of their hives to properly store all equipment (especially combs) and to be assiduous in cleaning up rotten fruit piles and piles of discarded hive equipment.

### Diet Effects on Reproductive Success

Our data and that of others (Lundie 1940,Schmolke 1974) show that *A. tumida* can successfully reproduce on diets of brood, pollen, and mixtures of honey and pollen, all of which contain the proteins and carbohydrates essential for the maturation of larvae and adult reproduction (Dadd 1985). On average, pollen contains 24% protein (Buchmann 1986) and 27% carbohydrates (Schmidt and Buchmann 1992). These factors probably contributed to the high reproductive success on pollen diets, which raises the question why the honey-pollen diets were less efficacious.

A possible explanation may be that small hive beetle faeces causes honey to ferment (Lundie 1940; Schmolke 1974) creating an unhealthy environment in the chambers. We observed that fermented honey filled the bottoms of the plastic containers, possibly jeopardizing oviposition and larval health. The presence of unidentified pollen mites in the pollen chambers after day 81 likely inhibited further oviposition by female *A. tumida*, since no more larvae appeared after the initial mite infestations. Despite this, the number of larvae maturing on pollen was still significantly higher than on all other diets. It is a common practice among beekeepers to use in-hive pollen traps to collect pollen from foraging bees. The pollen is collected in an area of the trap that is separated (therefore unprotected from beetle invasion) from the bee colony. Our data suggests that pollen traps should not be left in colonies for extended periods because of the beetles` ability to successfully reproduce in pollen, especially pollen that is unguarded.

Bee brood is another source of nutrients, and an analysis of *A.* m. scutellata sealed bee brood showed them to contain 20-35% protein, 50-62% carbohydrate and ash, and 10-18% lipid (Hepburn et al. 1979). Thus, the question emerges about the less than optimal reproductive success of *A. tumida* on bee brood. Small hive beetle adults and larvae feeding on decomposing honey bee brood caused a rancid environment, which probably led to reduced oviposition and longevity on this diet.

Schmolke (1974) showed that female *A. tumida* do not oviposit on diets of honey, an observation in accord with the fact that *A. tumida* larvae never appeared in any honey container in this study. Although beetle adults were able to survive for great lengths on empty brood comb (see diet effects on longevity), no larvae occurred in any empty brood comb containers likely indicating the lack of any volume of foodstuffs in the comb to allow for small hive beetle reproduction.

Small hive beetle adults were able to reproduce on fresh and rotten Kei apples which is consistent with other findings (Eischen et al. 1999). Because larvae were not produced to the extent found in any other diet, it is likely that Kei apples and other fruits (Eischen et al. 1999) barely meet the minimum requirements needed for reproduction and larval growth. As in the brood and honey-pollen diets, the environment in the fruit containers became quite rancid shortly after the presence of larvae. This too could have had an effect on the number of larvae produced. Although the beetles were not able to reproduce in great numbers on fruit alone, our data suggests the possibility that beetles may reproduce on fruit in the wild in instances where no bee colonies are present.

### Diet Effects on Pupation Success

Larval diet also plays a critical role in pupation success (Slansky and Scriber 1985). Because of this, adults per larvae ratio is the most critical value in determining the effects of diet on pupation success and not simply the number of adults emerging from the containers. The numbers of adults emerging from the soil chambers paralleled numbers of larvae reaching the wandering phase.

The three diets (pollen, honey-pollen, and brood) with the highest adults per larvae ratios also yielded the highest number of larvae. Because these adults per larvae ratios did not significantly differ, it is inferred that all three diets are equally efficacious for pupal fitness. The adults per larvae ratios from brood and fresh Kei apples did not differ significantly, although more larvae were found in the brood containers (Table 1). Fewer larvae were produced in the fresh Kei apple diets (Table 1) providing abundant food for the small number of larvae. The sheer abundance of larvae produced on brood (Table 1) gives a great reproductive benefit to adult *A. tumida* feeding on brood as opposed to feeding on Kei apples.

The poor adults per larvae ratios in both apple diets likely reflect a non-optimum nutrition accumulation by larvae in those diets (Slansky and Scriber 1985; Dadd 1985). Although *A. tumida* can successfully reproduce on fruits alone they are not optimal diets for *A. tumida* as shown by others (Eischen et al. 1999). Nonetheless the results show that *A. tumida* are only facultative parasites because they can reproduce on a diet of fruit alone. Because beetles can feed and reproduce on fruits, it is possible that fruit transporters in the United States could spread *A. tumida* to un-infested areas.

### Diet Effects on Sex Ratio

There were significantly more females than males in the brood and pollen diets (Table 2), the same for which there were significantly more larvae than in other diets (Table 1). Laugé (1985) states that density and crowding of larvae can act indirectly on sex ratio because of food competition and selective mortality that usually benefits female offspring. In all diets except for rotten apples, there were more female offspring than male, which is consistent with other findings (G. F. MacKay, unpublished data, cited in Schmolke 1974,Neumann et al. 2001b, Ellis et al. 2002).

Female insects tend to be heavier than males (Slansky and Scriber 1985) indicating a general nutrient accumulation needed for their role as egg layers. Small hive beetle females do indeed tend to be bigger and heavier than males (Schmolke 1974,Ellis et al. 2002). Slansky and Scriber (1985) state that this generally results from increased food consumption by female larvae. In cases of crowding, female larvae might be able to out-compete male larvae when feeding, leading to the selective mortality of male larvae. Although never shown to be significant, Ellis et al. (2002) reported small hive beetle populations with numerically higher female ratios found in natural populations of *A. tumida*.

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## REFERENCES

Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1989. An introduction to the study of insects, 6th ed. Saunders, Orlando, FL.

Buchmann, S. L. 1986. Vibratile pollination in *Solanum* and *Lycopersicon*: a look at pollen chemistry ,pp. 237-252. In W. G. D´Arcy (ed.), Solanaceae biology and systematics. Columbia University Press, New York.

Dadd, R. H. 1985. Nutrition: organisms, pp. 322-341. In G. A. Kerkut and L. I. Gilbert (eds.), Comprehensive insect physiology, biochemistry, and pharmacology vol. 4. Permagon, Oxford, UK.

Eischen, F. A., D. Westervelt, and C. Randall. 1999. Does the small hive beetle have alternative food sources? Am. Bee J. 139(2): 125.

Ellis, J. D. Jr., K. S. Delaplane, and W. M. Hood. 2002. Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the south-eastern United States. Am. Bee J. 142(7): 520-522.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, K. S. Delaplane, L. Cutts, and W. T. Wilson. 1999. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western Hemisphere. Apidologie 30: 361-366.

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, and W. T. Wilson. 2000. A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae),in Florida, USA. Apidologie 31: 593-594.

Elzen, P. J., J. R. Baxter, P. Neumann, A. Solbrig, C.W.W. Pirk, R. Hepburn, D. Westervelt, and C. Randall. 2001. Behavior of African and European subspecies of Apis mellifera toward the small hive beetle, *Aethina tumida*. J. Apic. Res. 40: 40-41.

Flügge, A. M. 2001. General physiological investigations of the small hive beetle *Aethina tumida* M., a parasite of honeybees Apis mellifera L. MSc thesis, Freie Universität, Germany.

Hepburn, H. R., R. C. Cantrill, P. R. Thompson, and E. Kennedi. 1979. Metabolism of carbohydrate, lipid and protein during development of sealed worker brood of the African honeybee. J. Apic. Res. 18(1): 30-35.

Hepburn, H. R., and S. E. Radloff. 1998. Honeybees of Africa. Springer, Berlin.

Hepburn, H. R., S. Reece, P. Neumann, R.F.A. Moritz, and S. E. Radloff. 1999. Absconding in honeybees (Apis mellifera) in relation to queenstate and mode of worker reproduction. Insectes Soc. 46: 323-326.

Hood, W. M. 2000. Overview of the small hive beetle, *Aethina tumida*, in North America. Bee World 81(3): 129-137.

Laugé, G. 1985. Sex Determination: Genetic and Epigenetic Factors, pp. 313-315. In G. A. Kerkut and L. I. Gilbert (eds.),Comprehensive insect physiology, biochemistry, and pharmacology, vol. 1. Permagon, Oxford, UK.

Lundie, A. E. 1940. The small hive beetle, *Aethina tumida*. Union S. Afr. Dep. Agric. For. Sci. Bull 220.

Neumann, P., C.W.W. Pirk, H. R. Hepburn, A. J. Solbrig, F.L.W. Ratnieks, P. J. Elzen, and J. R. Baxter. 2001a. Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Neumann, P., C.W.W. Pirk, H. R. Hepburn, P. J. Elzen, and J. R. Baxter. 2001b. Laboratory rearing of A. *tumida* (Coleoptera, Nitidulidae). J. Apic. Res. 40: 111-112.

Pettis, J. S., and H. Shimanuki. 2000. Observations on the small hive beetle, *Aethina tumida* Murray, in the United States. Am. Bee J. 140(2): 152-155.

Schmidt, J. O., and S. L. Buchmann. 1992. Other products of the hive, pp. 927-977. In Joe Graham (ed.),The hive and the honey bee. Dadant and Sons, Hamilton, IL.

Schmolke, M. D. 1974. A study of *Aethina tumida*: the small hive beetle. University of Rhodesia Certificate in Field Ecology Project Report, Salisbury.

Shimanuki, H., D. A. Knox, B. Furgala, D. M. Caron, and J. L. Williams. 1992. Diseases and pests of honey bees, p. 1126-1130. In Joe Graham (ed.),The Hive and the Honey Bee. Dadant and Sons, Hamilton, IL.

Slansky, F. Jr., and J. M. Scriber. 1985. Food consumption and utilization, pp. 96-110. In G. A. Kerkut and L. I. Gilbert (eds.),Comprehensive insect physiology, biochemistry, and pharmacology, vol. 4. Permagon, Oxford, UK.

Statistica. 2001. System reference, version 6,p. 1063. Stat-Soft, Tulsa, OK.

**Table 1.** Treatment means and mean separations for number of wandering larvae produced per three mating pairs of adult small hive beetles put into soil chambers; number of adult small hive beetles emerging from soil chambers; average ratio of emerging adults per larvae per diet; and longevity of parental adults

| Diet | No. larvae | No. adults | Adults per larvae | Longevity (days) |
|---|---|---|---|---|
| Control | 0a | 0a | 9.6±4.0a | |
| Empty brood comb | 0a | 0a | 49.8±10.2b | |
| Honeycomb | 0a | 0a | 167.2±8.7c | |
| Pollen comb | 1,773.8±294.4 (8869)b | 1,096.4±236.4 (5482)b | 0.64±0.19a | 123.4±17.5d |
| Honey-pollen comb | 337.0±134.3 (1685)c | 230.6±53.3 (1153)c | 0.73±0.19a | 81.0±15.7e |
| Brood comb | 597.4±217.5 (2987)d | 353.6±55.5 (1768)d | 0.65±0.23a,b | 9.0±0a |
| Fresh Kei apples | 50.6±55.7 (253)a | 15.2±16.5 (76)a | 0.32±0.13b,c | 63.6±30.4b,e |
| Rotten Kei apples | 26.4±23.7 (132)a | 10.6±14.0 (53)a | 0.24±0.25c | 58.6±30.0b,e |

Values are means ± standard deviations; n = 5 replicate containers for all values; numbers given in brackets are total individuals produced per diet. Column means followed by the same letter are not different at the P □ 0.05 level. Mean separations were determined by ANOVAs and Newman-Keuls post hoc comparisons.

**Table 2.** Sex data on small hive beetles emerging from pupation chambers having been reared on different diets as larvae

| Diet | Adult females | Adult males | Sex ratio female to male | P values for no. adult females |
|---|---|---|---|---|
| Pollen | 587.2±137.6 (2936) | 493.4±112.9 (2467) | 1.19±0.06 | 0.00022* |
| Honey-pollen comb | 122.0±23.6 (610) | 102.4±31.5 (512) | 1.23±0.18 | 0.14 |
| Brood comb | 188.6±34.7 (943) | 142.4±28.4 (712) | 1.34±0.17 | 0.00076* |
| Fresh apples | 9.6±10.8 (48) | 5.6±5.7 (28) | 1.62±0.40 | 0.57 |
| Rotten apples | 5±7 (25) | 5.6±7.0 (28) | 0.86±0.25 | 0.98 |

Values are mean ± standard deviation; n = 5 replicate containers for all values; numbers given in parentheses, where applicable, are total number of individuals emerging per diet. Variables are number emerging adult females per diet replication; number emerging adult males per diet replication; average sex ratio of adult females/adult males per diet; and P values for number adult females, as determined by chi-square tests. An asterisk indicates significance at the $P<0.05$ level.

# 2.3 The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and flight activity of Cape and European honey bees (*Apis mellifera*)

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**Abstract -** This study identifies differences in the effects of small hive beetles on flight activity and nests of European-derived honey bees (*Apis mellifera*) in the United States and Cape honey bees (*Apis mellifera capensis*) in South Africa. Treatments consisted of control colonies (<5 beetles/colony) and experimental colonies receiving beetles (treatment). Absconding day did not differ significantly between treatment or bee race but absconding was greater between the two treatments in European colonies than in Cape ones. Cape bees used significantly more propolis than European bees. Honey stores were significantly greater in Cape honey bee colonies than in European ones. Bee weight did not differ significantly between treatments or bee race. Treatment did not significantly affect bee populations, brood area, or average flight activity in Cape colonies but it did significantly lower all of these in European colonies. The effects of treatment in European colonies are symptomatic of absconding preparation. Treatment significantly lowered the amount of pollen stores in Cape colonies, but this effect was not found in European colonies. The number of beetles in control colonies was significantly higher in European colonies than Cape ones while the percentage of beetles remaining in non-absconding treated colonies was higher in Cape colonies than European ones. These data indicate that adult small hive beetles are sufficient to cause significant harmful effects on colonies of European, but not Cape, honey bees.

**Key words:** *Aethina tumida* / *Apis mellifera* / *Apis mellifera capensis* / flight activity / honey bee nests

## 1. INTRODUCTION

Small hive beetles (*Aethina tumida* Murray) are native to honey bee colonies (*Apis mellifera* L.) of sub-Saharan Africa where the beetle's pest status is negligible (Hepburn and Radloff, 1998). Successful reproduction of the beetle in its native range is often restricted to weak host colonies, due to behavioural resistance mechanisms of their honey bee hosts (Elzen et al., 2001; Neumann et al., 2001a), or is associated with after absconding events (Hepburn et al., 1999). Absconding is frequent in African honey bee subspecies and can be triggered by parasite infestations (Hepburn and Radloff, 1998). Indeed, severe small hive beetle infestations may cause such absconding (Hepburn and Radloff, 1998).

In sharp contrast, colonies of European-derived honey bee subspecies are highly susceptible to small hive beetle depredation (Elzen et al., 1999; Hood, 2000; Wenning, 2001). This damage stems from the feeding habits of both adult and larval beetles (Hood, 2000). It has been reported that only the larval stage presents a direct threat to colony health and European colonies can host thousands of adult hive beetles without suffering visible side effects (Wenning, 2001); however, no quantitative study has confirmed this.

Such quantitatively different responses of Cape (and presumably most other African subspecies) and European host colonies towards adult small hive beetles are very likely to be reflected in colony productivity. Since European honey bees are highly susceptible, a reduction in colony productivity is more likely to be expressed in European host colonies than in Cape ones. Although the impact of hive beetles on European host colonies is striking, this effect has not yet been measured quantitatively.

Here we report the results of an intercontinental quantitative study of the productivity of artificially infested or non-infested Cape (A.m. capensis) and European honey bee colonies. The variables measured included absconding day, total propolis, honey stores, bee weight, sealed brood, number of adult bees, pollen stores, flight activity and the number of small hive beetles remaining in treated colonies of Cape honey bees in South Africa and European honey bees of mixed origin in the United States.

## 2. MATERIAL AND METHODS

### 2.1. Cape honey bees

Experiments were conducted at Rhodes University (Grahamstown, South Africa) in late summer/early fall

(April 2001). Twenty propolis-free nucleus colonies (about 20 l in volume) of Cape honey bees (an African honey bee subspecies that is geographically distributed in the region of study) were established with 3 frames of workers, 1 frame of honey, 2 frames of brood, and a laying queen. Ten treated colonies were artificially infested with 100 adult small hive beetles on a daily basis between 17:00-21:00 h for 15 consecutive days. The small hive beetles used were reared in the laboratory according to standard procedures (Neumann et al., 2001b). By the end of the experiment, 1,500 beetles (100 beetles/colony for 15 days) had been introduced into all of the treated colonies. This level of beetle infestation is high for African honey bee colonies, but is common in infested European ones. Ten control colonies (<5 beetles/colony) were otherwise treated identically to the treated colonies. All nucs were placed in the same apiary, blocked together by treatment.

The number of returning bees was counted for all colonies twice daily, 1 minute each count, between 11:00 – 11:40 and 15:00 – 15:40 h because of data indicating peak foraging times for honey bees at 11:00 and 15:00 in southern Africa (Hepburn and Magnuson, 1988). Overall flight activity was determined by averaging the number of incoming bees per minute for both times.

Each colony was monitored three times daily (11:00, 15:00, 20:00) to identify its date of absconding, immediately after which, the colony was dismantled to determine number of adult small hive beetles present; sealed brood area (cm$^2$), honey area (cm$^2$), and pollen area (cm$^2$) (using a calibrated plastic grid); and total weight of propolis (g) in the colony.

On the evening of day 16 all remaining colonies were closed up, gassed with CO$_2$, frozen at -10ºC, and then analysed. For each colony, data were collected for the amount of sealed brood, honey, and pollen (cm2), number of adult small hive beetles, total weight of bees (g), weight of a sub-sample of bees (g) and number of bees in the sample (used to derive the number of bees in the colony), and total weight of propolis (g).

### 2.2. Honey bees of mixed European origin

A modified procedure was conducted on honey bees of mixed European origin (unknown history) in Warren County, Georgia, USA in late summer/early fall (August/September 2001). Adult beetles were reared from larvae collected in the field. The larvae were supplemented on a diet of pollen, honey, and bee brood (Neumann et al., 2001b; Ellis et al., 2002b) until they reached the wandering phase (Lundie, 1940), after which they were transferred to soil chambers for pupation and emersion as adults. Each treated European colony cumulatively received 1,400 beetles (100 beetles/day for 14 days).

European colonies which did not abscond in the experimental period were collected early morning on the 17th day of the experiment, cooled at 7ºC for 1 day, and then frozen for an analysis identical to that done on non-absconding Cape bee colonies.

### 2.3. Data Analysis

The effects of treatment [small hive beetles added or not added (control)] on absconding day, total propolis content, honey area, bee weight, number of bees, sealed brood and pollen area, and average flight activity were tested with a randomised design analysis of variance, blocked on location (United States or South Africa) and accepting differences at the $\alpha \leq 0.05$ level. When the treatment × location interaction was significant, analyses were run separately by location. For the variables absconding day, total propolis content, brood and pollen area, analyses included absconding colonies. Absconding colonies were excluded from analyses of honey area, bee weight, and number of bees because these parameters were either unavailable or confounded in empty hives.

The effects of time and increasing beetle numbers on average daily bee flight activity were tested with regression analyses testing for linear, quadratic, and cubic effects.

The ending number of small hive beetles in non-absconding control colonies and the percentage of beetles remaining in non-absconding treated colonies were analysed for location effects with ANOVA. Beetle numbers in both absconding and non-absconding treated colonies were analysed separately by location because the absconding ′ location interaction was significant. All reported data are mean ± standard error; n.

## 3. RESULTS

### 3.1. Absconding

There were no treatment effects (F = 1.6; df = 1,13; P = 0.2220), location effects (F = 2.8; df = 1,13; P = 0.1201), or location × treatment effects (F = 2.6; df = 1,13; P = 0.1308) for absconding day. Treated colonies did not abscond earlier than control colonies (Tab. I). In South Africa, 44% of control and 60% of treated colonies absconded while in the United States, 10% of control and 60% of treated colonies absconded.

Prior to absconding, treated European colonies aborted much of their brood. This was evident by the piles of mutilated brood on the ground outside of each colony. Further, worker bees were seen carrying brood out of the colony and discarding it on the ground. Upon post-absconding analysis of these colonies, no uncapped brood remained.

### 3.2. Propolis

There were no treatment effects (F = 2.2; df = 1,32; P = 0.1447) or location × treatment interactions (F = 1.4; df = 1,32; P = 0.2461) for the amount of propolis in colonies. Treated colonies did not have more propolis than control colonies (Tab. I). There were location effects for the total propolis content (F = 30.1; df = 1, 32; P < 0.0001). Cape honey bee colonies had significantly more propolis than did European honey bee colonies (Tab. I).

### 3.3. Honey area

There were treatment (F = 7.5; df = 1,18; P = 0.0136) and location (F = 100.4; df = 1,18; P < 0.0001) effects for honey area. Control colonies had significantly more stored honey than treated colonies while Cape honey bees had significantly greater stores of honey than did European honey bees (Tab. I). There were no location × treatment interactions found for honey area (F = 1.4; df = 1, 18; P = 0.2455).

### 3.4. Bee weight

There were no treatment effects (F = 1.4; df = 1,18; P = 0.2495), location effects (F = 0; df = 1,18; P = 0.9746), or location × treatment interactions (F = 1.5; df = 1,18; P = 0.2361) for bee weight. There were no differences in Cape and European colonies with respect to weight (Tab. I). Bee weight was not significantly different across all tested control and treated colonies (Tab. I).

### 3.5. Brood area

There was a significant location × treatment interaction for sealed brood area (F = 9.6; df = 1,35; P = 0.0039) so analyses were run separately by location. In Cape colonies, treatment did not significantly affect the amount of sealed brood (F = 0; df = 1, 17; P = 0.9712); yet it did in European colonies (F = 12.69; df = 1,18; P = 0.0022). In European colonies there was significantly less brood in treated colonies than controls (Tab. II).

### 3.6. Number of Bees

There was a significant location × treatment interaction for number of bees (F = 7.3; df = 1,18; P = 0.0144) so analyses were run separately by location. In Cape colonies, treatment did not affect the number of bees in colonies (F = 3.2; df = 1,7; P = 0.1174) while it did in the European colonies (F = 5.2; df = 1,11; P = 0.0432). European treated colonies had significantly fewer adult bees than control colonies (Tab. II).

### 3.7. Pollen area

There was a significant location × treatment interaction for pollen area (F = 5.3; df = 1,35; P = 0.0276) so analyses were run separately by location. Treatment affected pollen area in Cape colonies (F = 5.8; df = 1,17; P = 0.0278) whereas it did not in European bee colonies (F = 1.0; df = 1,18; P = 0.3398). Cape treated colonies had significantly less pollen than control colonies (Tab. II).

### 3.8. Flight Activity

There was a significant location effect for average flight activity (F = 13.3; df = 1,474; P = 0.0003). European colonies (13.5 ± 0.5; 262) had significantly more activity than Cape colonies (10.2 ± 0.6; 216). There was also a significant location × treatment interaction for average flight activity (F = 6.4; df = 1,474; P = 0.0120). In Cape colonies, treatment did not affect average flight activity (F = 1.40; df = 1,214; P = 0.2387). Cape honey bee treated colonies had similar flight activity as control colonies (Tab. II). In contrast, treatment significantly affected average flight activity (F = 25.8; df = 1,260; P < 0.0001) in European colonies. The number of incoming bees was significantly greater in control colonies than in treated colonies (Tab. II).

Regression analyses of flight activity trends over time reveal pronounced differences between locations (Fig. 1). In Cape colonies, average flight rates increased linearly over time in both treated and control colonies. Thus, flight activity appeared unaffected by increases in beetle numbers and the sampling period was universally and increasingly favourable for foraging. However, in European colonies there were measurable differences in trends between treated and control colonies. A cubic regression model in which rates fell, then rose, then fell again over time explained flight activity in treated colonies. A quadratic model in which rates rose then fell explained flight activity in control colonies. Rates were generally lower in treated colonies. Moreover, the increasing rates of flight by control colonies early in the sampling period contrast strongly with the decreasing rates by treated colonies at the time when conditions were apparently favourable for foraging. In spite of a mid-period surge by treated colonies, rates began decreasing more rapidly in treated colonies by the end of the sampling period when foraging conditions appeared to be deteriorating universally and when rainy weather was prevalent.

### 3.9. Beetle counts

There were significant differences between locations for the number of small hive beetles present in control colonies at the end of the experiment (F = 14.0; df = 1,12; P = 0.0028) and for the percentage of beetles remaining in non-absconding treated colonies (F = 18.0; df = 1,6; P = 0.0054). There were significantly more beetles present in European control colonies (12.9 ± 1.3; 9) than in Cape control colonies (5.6 ± 1.3; 5). A significantly higher percentage of beetles remained in Cape bee non-absconding treated colonies (87.8 ± 0.7; 4) than did in European bee non-absconding treated colonies (42.1 ± 10.7; 4). Indeed, that percentage was over twice as high for Cape bee colonies.

There were location effects (F = 13.2; df = 1,16; P = 0.0022) and location × absconding effects (F = 22.7; df = 1,16; P = 0.0002) for the number of beetles remaining in absconding and non-absconding treated colonies. Cape treated colonies (pooled absconding and non-absconding) had more beetles present (713.5 ± 165.0; 10) at colony analyses than did European treated colonies (481.3 ± 83.3; 10). Because the interaction term was significant, the number of beetles left in absconding and non-absconding treated colonies was analysed by location. For Cape honey bees, there was a significant difference between the number of beetles remaining in non-absconding and absconding treated colonies (F = 656.8; df = 1,8; P < 0.0001). Non-absconding treated colonies had significantly more beetles remaining (1316.3 ± 11.2; 4) than did absconding ones (311.7 ± 30.5; 6). For European colonies, there was no difference (F = 1.1; df = 1,8; P = 0.3174) between the number of beetles remaining in absconding treated colonies (409.3 ± 95.8; 6) and the number of beetles in non-absconding treated colonies (589.3 ± 150.3; 4).

## 4. DISCUSSION

### 4.1. Absconding

An analysis of absconding is of particular interest because most African honey bee subspecies readily abscond in response to nest predation (Hepburn and Radloff, 1998) while by contrast, temperate races of *A. mellifera* very seldom abscond (Simpson, 1959; Winston, 1992). In this study, control and treated colonies alike in both locations absconded; but there were no effects of treatment or location on the latency to abscond (Tab. I). For Cape bees, 44% of the controls absconded and 60% of treated colonies absconded. Because a large percentage of both Cape treatment and control colonies absconded, we infer other factors (colony disturbance, nectar dearth, etc.) caused them to abscond and not merely the presence of large numbers of adult small hive beetles.

Because 60% of European treated colonies absconded and only 10% of control colonies, we infer that, unlike Cape bees, European colonies absconded in response to the presence of large numbers of adult beetles in the hives. European colonies exhibited "prepared absconding" because these colonies had no uncapped young brood (based on post-abscond analyses), few workers emerged after the colony absconded, and honey stores were reduced. Other authors (Woyke, 1976; Winston et al., 1979; Koeniger & Vorwohl, 1979; Koeniger & Koeniger, 1980; Punchihewa, 1990; Nakamura, 1993; Mutsaers, 1994) record these symptoms as behavior typical of colonies preparing to abscond.

Moreover, European treated colonies (including the non-absconding colonies) uncapped and discarded all or most of their capped pre-pupae and pupae, as evidenced by the piles of mutilated pupae on the ground in front of treatment colonies. Further, bees were observed pulling pupae from the combs. By the end of the experiment, there was no open brood observed in any non-absconding European treated colony. These observations are similar to those of Woyke (1989) who showed that colonies of *A. m. adansonii* ate all of their uncapped larvae and most of their sealed brood before absconding. This suggests that the remaining 4 treated colonies were going to abscond soon and this is a likely explanation for the beetle effects seen on adult bees, brood, and flight activity in these colonies. None of this behaviour was observed in the control European colonies. Therefore, our data clearly indicate that European colonies do respond to large adult small hive beetle infestations by having high, prepared absconding rates.

### 4.2. Propolis

We found that European honey bees used almost 4 times less propolis than Cape honey bees (Tab. I) which is consistent with the findings of others (Bro. Adam, 1983; Ruttner, 1988; Dietz, 1992; Hepburn and Radloff, 1998) though this difference could be due to environmental effects. Because social encapsulation of adult small hive beetles in propolis prisons appears to be a resistance mechanism of African honey bees (Hepburn and Radloff, 1998, Neumann et al., 2001a), this could be a reason European colonies are highly susceptible towards small hive beetle infestations while Cape honey bees are more resistant (Tribe, 2000). Cape bees readily use more propolis than do European bees; therefore, more propolis is available in Cape colonies for use in beetle social encapsulation systems. Although imprisoning behaviour is also present in European honey bees (Ellis, 2002) our data suggest that it may not be as efficient as African honey bee imprisoning behaviour, possibly due to the lesser use of propolis by European bees.

### 4.3. Honey area

Treatment clearly reduced the amount of honey stores in bee colonies (Tab. I). Because flight activity was not reduced, this difference could be due to the feeding habits of adult beetles (Lundie, 1940; Schmolke, 1974; Ellis et al., 2002b), or general colony stress conditions due to beetle presence. European treated colonies had no honey stores at the end of the study, possibly reflecting preparation for absconding (Winston et al., 1979; Koeniger and Vorwohl, 1979; Koeniger and Koeniger, 1980; Punchihewa, 1990).

### 4.4. Brood

It has been reported that small hive beetles feed on honey bee eggs and brood (Lundie, 1940; Schmolke, 1974; Elzen et al., 1999; Ellis et al., 2002b) and indeed, that they do so preferentially (Elzen et al., 2000). These data support our finding of significant differences in sealed brood areas between treated and control European honey bee colonies. Despite beetles feeding on bee brood, the major factor contributing to a decline in brood area between treatment and control European honey bee colonies was most likely due to the observed absconding preparation behaviour, namely brood abortion and cannibalism. On the other hand, Cape honey bees did not experience the same decline in brood area when infested with hive beetles (Tab. II), also suggesting a superior ability to cope with beetle infestations.

### 4.5. Adult bees and bee weight

The data show that the presence of adult small hive beetles lowers the number of adult bees present in European honey bee colonies, but not in Cape honey bee colonies, although beetle infestations did not compromise bee weight. However, threshold values have not yet been determined. Contrary to what has been reported by others (Wenning, 2001), this shows that beetle larvae are not the only stage of the small hive beetle's life cycle that damages honey bee colonies. European treated colonies also had significantly less brood than control colonies and this is probably related to the differences in adult bee populations between treatments in European colonies.

### 4.6. Pollen

The only striking impact small hive beetle infestations had on infested Cape honey bee colonies was a reduction in pollen stores. It is possible that beetles in these colonies were feeding on pollen stores. Although beetles preferentially feed on bee brood (Elzen et al., 2000), it is evident that Cape bees are efficient at guarding their brood because there was no significant loss of brood area in beetle infested Cape colonies. In these circumstances the beetles would have had to feed on alternative food sources, such as pollen stores. It is well established that beetles feed on pollen (Lundie, 1940; Schmolke, 1974; Elzen et al., 2000; Hood, 2000; Neumann et al., 2001b) and that they reproduce most successfully on a diet of pollen alone (Ellis et al., 2002b). In European colonies the beetles caused a significant reduction in brood area (probably by feeding and ovipositing on it and because of prepared absconding behaviour by the bees) and there were no differences in the pollen stores between the treatments. Our data suggest that beetles are restricted to pollen in Cape bee colonies, but gain access to brood in European ones, which likely triggers explosive reproduction by beetles.

### 4.7. Flight activity

The fact that European bees had greater flight activity than Cape bees is probably due to nectar flow differences in each country for the time of year the experiments were conducted. The pertinent information lies in the interactions found between location × treatment. Our data show that treatment significantly lowered average flight activity in European bee colonies but not in Cape ones. Small hive beetle presence in European colonies was sufficient to lower flight activity. Although the causes for this are unknown, it may be that small hive beetles cause general disruption in European colonies (Hood, 2000; Wenning, 2001) and flight behaviour is thus compromised. The difference appears related to prepared absconding behaviour of the treated European colonies; a behaviour that likely limited the number of available foraging workers. Further, Ellis et al. (2002a) showed that European honey bees guarding small hive beetle prisons belong to the same age cohort as foraging bees. Therefore, an increasing population of small hive beetles could cause more foraging-age bees to begin guarding beetle prisons thus explaining the overall decrease in flight activity between European treatment and control colonies seen in this study. Increasing beetle densities affected flight activity only in treated European colonies.

### 4.8. Beetle counts

All colonies in both locations were created from colonies having small populations of hive beetles. We believe all colonies started with < 5 beetles per colony (visual estimates). Therefore, the number of beetles found in Cape control colonies could be considered background noise, being close to the original population of beetles present in the colony at the beginning of the experiment. A total of 2565 small hive beetles were unaccounted for in Cape colonies by the end of the experiment. These beetles were put into the hive, but not re-collected. At the same time our data show that these beetles were not migrating into control colonies. Even though European control colonies had significantly more beetles than did Cape control colonies, they too were not heavily infested with "stray" beetles (unaccounted beetles totalling 4487 individuals in the U.S.).

Why beetles tended to migrate from European non-absconding treated colonies and not from Cape non-absconding treated colonies is unclear. This could be indicative of a superior ability of Cape bees to imprison and guard beetles more efficiently than European bees (Hepburn and Radloff, 1998; Neumann et al., 2001a; Ellis, 2002). Regardless, over half of the beetles introduced into European colonies were not in the hives at the end of the experiment. These beetles may have been host seeking, even though they were not going to control colonies.

Small hive beetle populations in both European absconding and non-absconding treated colonies were the same. This occurred regardless of the number of beetles introduced into the colonies (which totalled 1400 beetles/colony for treated colonies that did not abscond and an average of 617 beetles/colony for treated colonies that absconded). This implies a "carrying capacity" for small hive beetles in European bee colonies. It could also imply a threshold, that when met, European colony health is compromised and, even in extreme situations, absconding preparation begins.

At the same time, the carrying capacity for beetles in Cape colonies is either much higher, or non-existent. We base this on our data showing that most of the beetles put into Cape colonies stayed in those colonies. Because this large number of beetles in Cape colonies never significantly affected measured colony parameters, with the exception of reduced pollen stores (Tab. II), Cape bees must have either superior imprisoning techniques (Hepburn and Radloff, 1998; Neumann et al., 2001a), or other behavioural mechanisms (Elzen et al., 2001) that make them better able to handle large infestations of small hive beetles.

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## References

Adam Bro. (1983) In Search of the Best Strains of Bees, Northern Bee Books, Mytholmroyd, Hebden Bridge, UK.

Dietz A. (1992) Honey bees of the world, in: Graham J.M. (Ed.), The hive and the honey bee, Dadant and Sons, Hamilton, Illinois, pp. 23-71.

Ellis J.D. Jr. (2002) Life behind bars: why honey bees feed small hive beetles, Am. Bee Jour. 142(4), 267-269.

Ellis J.D. Jr., Holland, A.J., Hepburn, R., Neumann, P., Elzen, P.J. (2002a) Cape (*Apis mellifera capensis*) and European (*Apis mellifera*) honey bee guard age and duration of small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae) prison guarding, J. Apic. Res. in review.

Ellis J.D. Jr., Neumann P., Hepburn R., Elzen P.J. (2002b) Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets, J. Econ. Ent. 95(5), 902-907.

Elzen P.J., Baxter J.R., Westervelt D., Randall C., Delaplane K.S., Cutts L., Wilson W.T. (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the Western Hemisphere, Apidologie 30, 361-366.

Elzen P.J., Baxter J.R., Westervelt D., Randall C., Wilson W.T. (2000) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA, Apidologie 31, 593-594.

Elzen P.J., Baxter J.R., Neumann P., Solbrig A., Pirk C.W.W., Hepburn R., Westervelt D., Randall C. (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*, J. Apic. Res. 40, 40-41.

Hepburn H.R., Magnuson P. (1988) Nectar storage in relation to wax secretion by honeybees, J. Apic. Res. 27(2), 90-94.

Hepburn H.R., Radloff S.E. (1998) Honeybees of Africa, Springer Verlag, Berlin.

Hepburn H.R., Reece S., Neumann P., Moritz R.F.A., Radloff S.E. (1999) Absconding in honeybees (*Apis mellifera*) in relation to queenstate and mode of worker reproduction, Insectes Soc. 46, 323-326.

Hood W.M. (2000) Overview of the small hive beetle, *Aethina tumida*, in North America, Bee World 81(3), 129-137.

Koeniger N., Koeniger G. (1980) Observations and experiments on migration and dance communication of *Apis dorsata* in Sri Lanka, J. Apic. Res., 19, 21-34.

Koeniger N., Vorwohl G. (1979) Competition for food among four sympatric species of *Apini* in Sri Lanka (*Apis dorsata*, *Apis cerana*, and *Trigona irridipennis*), J. Apic. Res. 18, 95-109.

Lundie A.E. (1940) The small hive beetle, *Aethina tumida*, Sci. Bull. 220, Union of South Africa, Dept. Agric. Forestry, 30 pp.

Mutsaers M. (1994) Absconding of honey bee (*Apis mellifera adansonii*) colonies in south-western Nigeria, related to the seasonal weight of colonies and combs, Proc. 5th Int. Conference of Apic. in Tropical Climates, IBRA, pp. 3-9.

Nakamura J. (1993) Regulatory system in a honeybee colony toward resource deterioration, PhD Thesis, Tamagawa University, Japan.

Neumann P., Pirk C.W.W., Hepburn H.R., Solbrig A.J., Ratnieks F.L.W., Elzen P.J., Baxter J.R. (2001a) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.), Naturwissenschaften 88, 214-216.

Neumann P., Pirk C.W.W., Hepburn H.R., Elzen P.J., Baxter J.R. (2001b) Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera, Nitidulidae), J. Apic. Res. 40, 111-112.

Punchihewa R.W.K., Koeniger N., Howpage D. (1990) Absconding behaviour of *Apis cerana* in Sri Lanka, In: Proc. 11th Int. Cong. IUSSI, India, pp. 106-107.

Ruttner F. (1998) Biogeography and Taxonomy of Honeybees, Springer Verlag, Berlin, Germany.

Simpson J. (1959) Variation in the incidence of swarming among colonies of *Apis mellifera* throughout summer, Insectes Soc. 6, 85-89.

Schmolke M.D. (1974) A study of *Aethina tumida*: the small hive beetle, University of Rhodesia Certificate in Field Ecology Project Report, 178 pp.

Tribe G.D. (2000) A migrating swarm of small hive beetles (*Aethina tumida* Murray), South African Bee Jour. 72(3), 121-122.

Wenning C.J. (2001) Spread and threat of the small hive beetle, Am. Bee Jour. 141(9), 640-643.

Winston M.L., Otis G.W., Taylor O.R. Jr. (1979) Absconding behavior of the Africanized honeybee in South America, J. Apic. Res. 18(2), 85-94.

Winston M.L. (1992) The honey bee colony: life history, in: Graham J.M. (Ed.), The hive and the honey bee, Dadant and Sons, Hamilton, Illinois, pp. 73-101.

Woyke J. (1976) Brood-rearing efficiency and absconding in Indian honeybees, J. Apic. Res. 15(3/4), 133-143.

Woyke J. (1989) Biology and management of African bees *Apis mellifera adansonii* in Africa, 32nd Int. Apic. Cong., Apimondia, Bucharest, Romania, pp. 44-47.

**Figure 1.** Predicted daily average number of returning workers for control and treated (beetles added) colonies of Cape and European-derived honey bees. Data were measured by averaging the number of incoming bees per minute at hours 11:00 and 15:00. Each day corresponds to an increase of 100 beetles/colony. Control colonies are represented by grey squares and dashed lines while treated colonies are black triangles with solid lines.

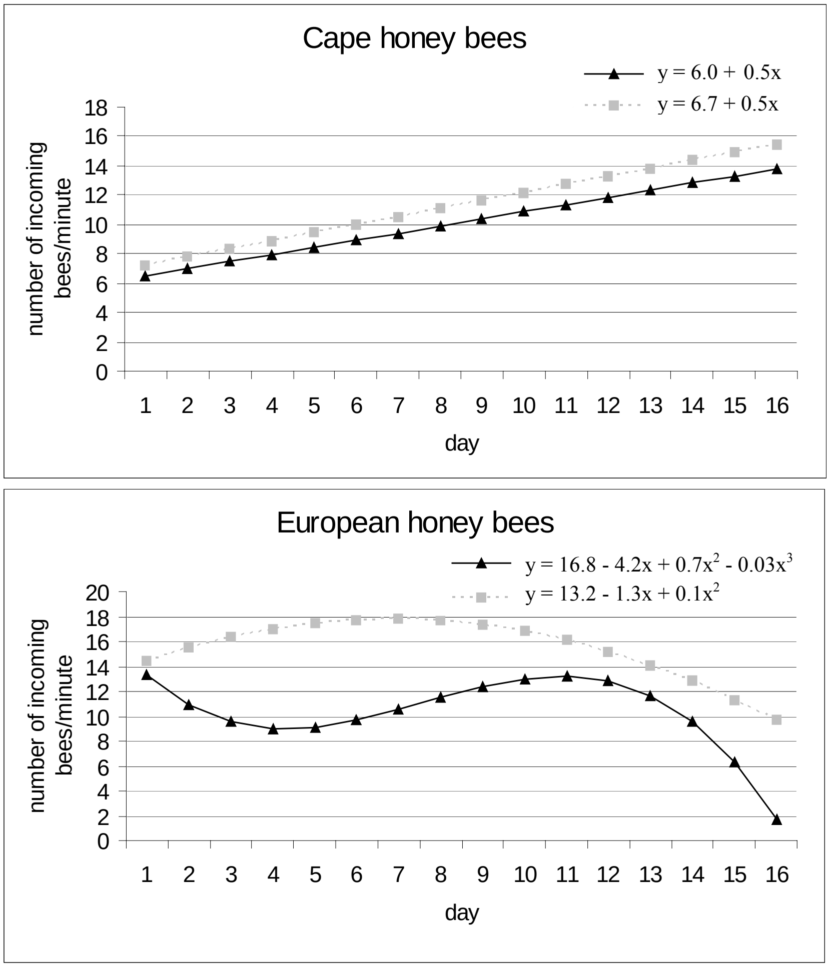

**Table I.** Analyses of absconding day, honey area ($cm^2$), total propolis (g), and bee weight (mg) for Cape and European host colonies**.** Values are mean ± standard error with sample size (*n*) in parentheses.

| | absconding day | | | | honey area | | |
|---|---|---|---|---|---|---|---|
| | treatment | control | row total | | treatment | control | row total |
| Cape | 7.3 ± 2.0 (6) | 6.3 ± 2.6 (4) | 6.9 ± 1.5 (10)a | Cape | 663.8 ± 82.7 (4) | 960.0 ± 124.9 (5) | 828.3 ± 90.5 (9)a |
| European | 7.5 ± 2.2 (6) | 17.0 ± 0 (1) | 8.9 ± 2.3 (7)a | European | 0 ± 0 (4) | 115.6 ± 38.9 (9) | 80.0 ± 30.6 (13)b |
| column total | 7.4 ± 1.4 (12)a | 8.4 ± 2.9 (5)a | | column total | 331.9 ± 131.1 (8)a | 417.1 ± 122.1 (14)b | |

| | total propolis (g) | | | | bee weight (mg) | | |
|---|---|---|---|---|---|---|---|
| | treatment | control | row total | | treatment | control | row total |
| Cape | 15.5 ± 3.1 (8) | 10.8 ± 2.3 (8) | 13.1 ± 2.0 (16)a | Cape | 91.4 ± 4.4 (4) | 91.3 ± 4.1 (5) | 91.4 ± 2.8 (9)a |
| European | 3.7 ± 0.6 (10) | 3.2 ± 0.4 (10) | 3.5 ± 0.3 (20)b | European | 87.6 ± 3.1 (4) | 95.3 ± 1.7 (9) | 92.9 ± 1.8 (13)a |
| column total | 9.0 ± 2.0 (18)a | 6.6 ± 1.4 (18)a | | column total | 89.5 ± 2.6 (8)a | 93.9 ± 1.8 (14)a | |

Row total and column total means followed by the same letter are not different at the α ≤ 0.05 level. For the variables absconding day and total propolis, analyses were run including absconding colonies. For the variables honey area and bee weight, analyses were run without including absconding colonies.

**Table II.** Location × treatment interactions for amount of sealed brood ($cm^2$), number of adult bees, stored pollen area ($cm^2$), and average flight activity (number of bees returning per minute) in Cape and European host colonies. Values are mean ± standard error with sample size (*n*) in parentheses.

| | Cape colonies | |
|---|---|---|
| | treatment | control |
| sealed brood area | 201.9 ± 78.8 (10)a | 205.6 ± 58.3 (9)a |
| number of adult bees | 6552.8 ± 675.5 (4)a | 4823.4 ± 675.4 (5)a |
| stored pollen area | 27.7 ± 11.2 (10)a | 116.9 ± 37.1 (9)b |
| average flight activity | 9.6 ± 0.7 (102)a | 10.9 ± 0.8 (114)a |
| | **European colonies** | |
| | treatment | control |
| sealed brood area | 54.1 ± 18.0 (10)a | 739.7 ± 191.6 (10)b |
| number of adult bees | 3246.8 ± 234.3 (4)a | 6321.0 ± 869.9 (9)b |
| stored pollen area | 67.5 ± 42.4 (10)a | 25.1 ± 8.6 (10)a |
| average flight activity | 10.4 ± 0.6 (103)a | 15.5 ± 0.7 (159)b |

Analyses were run separately by location for these variables. For number of adult bees, analyses were run without including absconding colonies; for sealed brood and stored pollen area analyses included absconding colonies. Row means followed by the same letter are not different at the α ≤ 0.05 level.

# 2.4 Behaviour of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*

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**Keywords:** behaviour, honey bee subspecies, small hive beetle, *Aethina tumida*, *Apis mellifera*, *Apis mellifera capensis*

The small hive beetle (SHB), *Aethina tumida*, is a newly introduced nitidulid species attacking European honeybees, *Apis mellifera*, in North America (Sanford, 1998). Adult and larval beetles feed on pollen and brood, but mostly cause damage by feeding on honey, rendering it foul and unusable. The SHB is native to sub-Saharan Africa, where it is a non-economic problem. Lundie (1940) in South Africa and later Schmolke (1974) in Zimbabwe, studied the SHB. Both report the beetle is rarely seen to inundate a hive. Lundie (1940) conducted rearing studies in an effort to identify SHB parasites and predators, but found none. A common observation of South African beekeepers is that African honeybees defend against adult and larval SHB by continuously harassing adults and removing larvae from the hive. In contrast, beekeepers in the USA with European honey bees rarely see such defence. We quantified the defensive behaviour toward the SHB by *A. m. capensis* and North American European *A. mellifera*.

Wooden hoarding cages were constructed with clear glass enclosing opposite sides of the cage. Outer dimensions of each cage were 1 3 ×1 6.5 ×9 cm. Four colonies of the Cape honey bee *(A. m. capensis*) were evaluated at Rhodes University, Grahamstown, South Africa. Two trials were conducted, each using worker bees and brood from two colonies per trial. For a trial, each colony provided bees and brood to assemble 10 cages each containing a 5 ×4 cm piece of sealed brood attached to the upper surface of each cage and between 40 and 50 adult workers from the brood nest. Food and water were provided and the cages were incubated (32°C) in darkness for 24 h. The 10 cages were randomly assigned as either a test or control cage. A single living adult SHB was released into each test cage and a black push pin similar in size to a SHB was placed into each control cage.

Adult bees were observed in each cage for 1 5 min by two observers, one on each side of the cage. Occurrences of ignoring the beetle or pin (coming within 5 mm then moving away), contacting (antennating then moving away) or defending (grasping and attempting to sting) were recorded.

The same procedures were used with European *A. mellifera* in Umatilla, Florida, USA. There, three colonies supplied brood and bees for three evaluation trials.

For each 1 5-min observation period the number of each behavioural response category was divided by the number of all responses and multiplied by 100, to provide the percentage of total responses for each response category. Resulting percentages were then arc-sine transformed (to normalize percentage data (Zar, 1974)) and appropriate means and standard errors were calculated. All test and control means for each location and between locations were compared by ANOVA and means were separated by a least significance difference test (Systat, 1997).

Table 1 shows the proportionality of the responses of European honey bees and Cape honey bees. The Cape honey bee attacked the introduced beetle significantly more than did the European honeybee (32.8 % defensive responses compared to 1 .4%). The Cape honeybee also exhibited a significantly higher proportion of contacts with the introduced SHB (and control pin) than did European honey bees.

Because many factors may explain why the SHB is not an economic problem in Africa, we tested the acceptability of Cape honey bee eggs as food for the SHB. We previously demonstrated that adult SHB will feed on European honey bee eggs in a laboratory setting, even in the presence of excess honey and pollen (Elzen *et al*., 1999). Five 0.473-litre jars were established with five adult beetles, a known number of Cape honeybee eggs in uncapped comb, and excess honey and pollen. Five control jars consisted of a known number of eggs in comb and excess honey and pollen (no beetles added). All 10 jars were held 24 h at 32°C and the number of eggs remaining were counted.

Results showed overall, within 24 h the SHB ate 94% of the Cape honeybee eggs. Our results confirm field observations both in the south-eastern USA and in South Africa: in the USA, the European honey bee exhibits only slight behavioural defensiveness against the SHB, whereas in South Africa, the Cape honey bee quite vigorously defends its colonies against this beetle. However, given the opportunity, the SHB ate eggs of the

Cape honey bee as readily as they ate European honey bee eggs in Florida (Elzen *et al*., 1999).

## REFERENCES

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L, Wilson WT(1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera: Nitidulidae), attacking European honey bees in the Western Hemisphere. Apidologie 30: 36 –366.

Lundie AE (1940) *The small hive beetle,* Aethina tumida. Scientific Bulletin 220, Union of South Africa, Dept of Agriculture and Forestry; 30 pp.

Sanford MT (1998) *Aethina tumid*a: a new beehive pest in the Western Hemisphere. *APIS* 1 6: 1 –5.

Schmolke MD (1974) *Aethina tumida:* the small hive beetle. MS thesis, University of Rhodesia, Rhodesia; 1 8 pp.

Zar JH (1974) Biostatistical analysis. Prentice-Hall Inc.; Engelwood Cliffs, NJ, USA; 620 pp.

**Table 1:** Response of European *Apis mellifera* and South African *(A. m. capensi*s) honeybees toward the small hive beetle. Location/Treatment % of Total behavioural responses observed x ± s.e.

| | **% of Total Behaviours Observed (x ±SE)** | | | **Total no.** |
|---|---|---|---|---|
| **Location/Treatment** | **Ignore** | **Contact** | **Attack** | **responses observed** |
| **Florida** | | | | |
| Bees + SHB | 86.8 (3.0) a | 11.7 (3.1) a | 1.4 (0.7) a | 775 |
| Control | 88.9 (2.6) a | 11.1 (2.6) a | 0 a | 908 |
| **South Africa** | | | | |
| Bees + SHB | 30.6 (5.3) b | 36.6 (8.2) b | 32.8 (7.3) b | 575 |
| Control | 57.8 (5.8) c | 42.3 (5.8) b | 0 a | 878 |

$^{a, b, c}$ Means within a column followed by different letters are significantly different (ANOVA, $P < 0.001$; means separated by LSD).

# 2.5 Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.)

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**Abstract -** Worker honeybees (*Apis mellifera capensis*) encapsulate the small hive beetle (*Aethina tumida*), a nest parasite, in propolis (tree resin collected by the bees). The encapsulation process lasts 1-4 days and the bees have a sophisticated guarding strategy for limiting the escape of beetles during encapsulation. Some encapsulated beetles died (4.9%) and some escaped (1.6%). Encapsulation has probably evolved because the small hive beetle cannot easily be killed by the bees due to its hard exoskeleton and defensive behaviour.

**Key words:** *Aethina tumida*, *Apis mellifera*, encapsulation, honeybee, parasite, small hive beetle

## 1. INTRODUCTION

The small hive beetle, *Aethina tumida* (SHB), is a honeybee (*Apis mellifera*) parasite endemic to Africa. It lives within honeybee nests and feeds on brood and stored food but seldom causes serious damage (Hepburn & Radloff 1998). By contrast, the SHB is proving a serious threat to European races of *A. mellifera* in the south-eastern United States since its introduction in 1998 (Elzen et al. 1999). One possible reason for this difference is that African honeybees sympatric with the SHB have evolved specific defence mechanisms.

Unlike other parasites (Moritz et al. 1991), SHB are easily detected and vigorously attacked by the workers in an African honeybee nest but it is difficult for the bees to kill or eject them (Lundie 1940; Elzen et al. 2001). Instead the bees encapsulate SHBs in propolis, tree resin which the bees collect and use for sealing cracks in the nest cavity.

## 2. MATERIAL AND METHODS

Adult male and female small hive beetles *A. tumida* (Coleoptera: Nitidulidae) infest honeybee colonies and may stay within them for a long period of time, until they can finally successfully reproduce (Lundie 1940, Schmolke 1974). SHB eggs are laid on the combs and adults as well as the emerging larvae feed on honey, pollen and brood (Lundie 1940, Schmolke 1974, Elzen et al. 1999), but seem to prefer the latter as their protein diet. After about 14 days the larvae reach the wandering phase (Schmolke 1974), leave the hives and pupate in the soil close to the hive for about 17 days (Lundie 1940, Schmolke 1974). Emerging adults leave the soil and may disperse over large distances to infest new host colonies (unpublished data). In South Africa successful reproduction of SHB is mainly restricted to small and weak colonies (Lundie 1940); but once larvae emerge, colonies quickly show "wormy" combs (Schmolke 1974, personal observations), due to the high reproductive potential of this parasite. In natural populations SHB show great variation in size (Schmolke 1974); but in general, male beetles are slightly smaller (length: 5.12±0.07 mm; breadth: 3.21±0.04 mm) than females (length: 5.27±0.06 mm; breadth: 3.25±0.04 mm; Schmolke 1974).

In contrast to African honeybees, even strong colonies of European bees are decimated by SHB (Elzen et al. 1999). This is probably caused by high infestation levels, which may exceed to ~1000 adult SHB and several hundred larvae per colony (Elzen et al. 1999). Moreover, European honeybees in the New World show significantly less aggressive and investigative contact behavior toward adult SHB than do African *A. mellifera* (Elzen et al. 2001). This clearly shows that SHB are a serious threat to the European races of *A. mellifera*.

We investigated encapsulation of SHBs by the Cape honeybee, *A. m. capensis*, in South Africa where both are endemic. Four colonies (each ~3000 bees) naturally infested with SHB were kept in observation hives and monitored daily at 09h00 and 20h00 for 21 (colonies 1 & 2) or 57 days (colonies 3 & 4). We recorded numbers of free and encapsulated SHB, the latter were checked to see if the beetles were dead or alive at the end of the observation period. The frequency of infestation and encapsulation in 40 Langstroth field colonies were determined by counting live SHB throughout the hives. All pieces of propolis were carefully examined for previously encapsulated SHB.

## 3. RESULTS

Small hive beetles were frequently found in small gaps (height: 2-4 mm) between the frame and end bars in the observation hives. As previously reported (Elzen et al. 2001) workers were usually unable to kill the well-armoured SHB; but two SHBs moved straight into a cluster of workers and were decapitated by the bees. Although an SHB in the open is vigorously attacked by workers, the beetle stays motionless and tucks its head underneath the pronotum with the legs and antennae pressed tightly to the body (much like withdrawal in a turtle). If the workers leave the SHB, the beetle scurries into hiding.

We also observed that workers added propolis at the edges of the hive around detected hidden SHBs and completely encapsulated most of them (4 managed to escape, Table 1). The four observation hives contained 15 such propolis prisons (Fig. 1) confining each 1 to ~200 SHB (12 of which died while in confinement) and 62 free SHB (Table 1). The bees had a sophisticated strategy to hinder beetle escape during encapsulation. While some workers added propolis around the SHB one or more others (mean = 2 ± 1..27) continuously guarded the SHB in both open and closed confinements day and night for up to 57 days (Fig. 1). The guard workers continuously try to attack all SHB when they move to the edges of still open confinements and thus keep them imprisoned. It took 1-4 days for the SHB to be encapsulated. Two matings in prisons and one case of cannibalism among SHB were also observed. A total of 32 free moving SHBs were found in 8 infested field hives. In two of these we also found evidence of encapsulation (Table 1). No SHB larvae were observed in any of the colonies.

**Fig. 1.** Social encapsulation of beetle parasites in a Cape honeybee colony, where two workers keep a small hive beetle within its confined area made of propolis.

## 4. DISCUSSION

Our data clearly indicate that Cape honeybee colonies use social encapsulation as an efficient defence mechanism against parasitic beetles. Although the *A. m. capensis* workers vigorously attack small hive beetles, the parasite is seldom harmed due to its hard exoskeleton and defensive behavior. Our data also support earlier observations (Schmolke 1974) that SHB typically hide in small cracks in the nest cavity. Thus, social encapsulation by the host has probably evolved in the endemic region of the small hive beetle, as an alternative mechanism to prevent or postpone successful reproduction of the parasite.

A total of only 32 free moving SHBs were found in 8 out of 40 field hives, suggesting generally low infestation levels in Cape honeybee colonies. We also found evidence of encapsulation in these field colonies, but encapsulation was only seen in the two colonies with the highest numbers of live beetles, suggesting that encapsulation may be triggered when parasite loads reach a threshold.

At least four SHB managed to escape encapsulation at night, possibly because honeybees are generally less active at night (Moritz & Kryger 1994). The observed matings and the case of cannibalism among SHB could well enhance SHB survival in large prisons such as the one in observation colony 4 confining about 200 SHB. Nevertheless, encapsulation is clearly an effective defence mechanism of honeybees because beetles are prevented from successful reproduction. This seems especially important in light of the high reproductive potential of SHB (Lundie 1940, unpublished data). Even if some SHB manage to escape, encapsulation provides a prolonged time window for the colonies to prepare for absconding, which is very common in African honeybees and can be triggered by parasites (Hepburn & Radloff 1998). Since the SHB is non-phoretic, absconding leaves the parasites behind. Indeed, the heavily infested observation colony 4 absconded after 57 days. No SHB larvae appeared even in this nest until it absconded, indicating that the combination of aggression behavior (Elzen et al. 2001) and encapsulation was able to prevent SHB reproduction even under heavy infestation levels. We conclude that social encapsulation of SHBs is another striking example of the co-evolution between insect societies and their parasites.

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## REFERENCES

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L, Wilson WT (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking honey bees in the Western Hemisphere. Apidologie 30: 361-366

Elzen PJ, Baxter JR, Neumann P, Solbrig AJ, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida* Murray. J Apic Res, in press

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin

Lundie AE (1940) The Small hive beetle, *Aethina tumida*. Sci Bull 220, Union of South Africa, Department of Agriculture and Forestry

Moritz RFA, Kirchner M, Crewe RM (1991) Chemical camouflage of the death head hawkmoth, *Acherontia atropus*. Naturwissenschaften 78: 179-182

Moritz RFA, Kryger P (1994) Self organisation of circadian rhythms in groups of honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 34: 211-215

Schmolke MD (1974) A study of *Aethina tumida*: The small Hive Beetle, Project Report, University of Rhodesia

**Table 1.** Social encapsulation in observation hive and field colonies of the Cape honeybee. Only infested colonies are shown (*=The number of escaped and encapsulated SHB could not be precisely determined for one prison in observation colony 4, because about 200 SHB were confined in a small area; nd = not determined, n = number of observed beetles or confinements).

| | Colony | Free moving SHB (n) | Confinements (n) | Encapsulated SHB (n) | | Escaped SHB (n) |
|---|---|---|---|---|---|---|
| | | | | alive | dead | |
| Observation hives | 1 | 8 | 6 | 32 | 0 | 1 |
| | 2 | 10 | 3 | 3 | 0 | 3 |
| | 3 | 23 | 1 | 12 | 5 | 0 |
| | 4 | 21 | 5 | ~200 | 7 | nd* |
| Field colonies | 1 | 3 | 0 | 0 | 0 | - |
| | 2 | 1 | 0 | 0 | 0 | - |
| | 3 | 1 | 0 | 0 | 0 | - |
| | 4 | 6 | 3 | 3 | 2 | - |
| | 5 | 2 | 0 | 0 | 0 | - |
| | 6 | 12 | 2 | 2 | 1 | - |
| | 7 | 3 | 0 | 0 | 0 | - |
| | 8 | 4 | 0 | 0 | 0 | - |

# 2.6 Cape (*Apis mellifera capensis*) and European (*Apis mellifera*) honey bee guard age and duration of guarding small hive beetles (*Aethina tumida*)

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**Abstract -** The guard age and duration of European (*Apis mellifera*) and Cape (*A. m. capensis*) honey bees guarding small hive beetle (*Aethina tumida*) prisons was determined using 3-frame observation hives, noting the commencement and termination of prison guarding by individually labelled honey bees. European honey bees in the United States began guarding small hive beetle prisons significantly earlier (beginning age 18.55 ± 0.52 days; mean ± standard error), guarded prisons significantly longer (duration 2.36 ± 0.31 days), and stopped guarding prisons significantly sooner (ending age 19.91 ± 0.57 days) than Cape honey bees in South Africa (beginning age 20.61 ± 0.38 days; duration 1.43 ± 0.12 days; and ending age 21.04 ± 0.37 days). Although the timing of prison guarding behaviour between the two subspecies is significantly different, it does not explain the differential damage to European and Cape honey bee colonies caused by small hive beetles.

**Key words:** *Apis mellifera*, *Apis mellifera capensis*, Aethina tumida, guarding behaviour, guard age, small hive beetle, Cape honey bees, age related division of labour, propolis, small hive beetle prisons

## 1. INTRODUCTION

Small hive beetles (*Aethina tumida*) are scavengers of honey bee (*Apis mellifera*) colonies. They are native to sub-Saharan Africa (Hepburn and Radloff, 1998), where their populations usually are controlled by defensive behaviour of their honey bee hosts (Elzen et al., 2001, Neumann et al., 2001). In contrast, small hive beetle infestations in colonies of European-derived A. mellifera subspecies are often extremely damaging to host colonies in the United States (Elzen et al., 1999; Hood, 2000). This occurs despite European bee defensive behaviour that appears to be qualitatively (but not necessarily quantitatively) similar to that of African honey bees (Ellis, 2002).

In addition to direct aggressive behaviour (biting, stinging) directed at small hive beetles (Elzen et al., 2001), African honey bees construct propolis prisons in which small hive beetles are encapsulated (Neumann et al., 2001). Similar imprisoning behaviour has been documented in European honey bees (Ellis, 2002) but the efficacy of social encapsulation by European honey bee remains unknown. Regardless, both honey bee subspecies station guards, who keep the beetles imprisoned, around the prison perimeter (Neumann et al., 2001; Ellis, 2002; fig. 1). Despite being imprisoned, small hive beetles are able to remain alive because they are fed by their honey bee captors (Ellis et al. 2002).

In this study, we determine the age of European and Cape (*A. m. capensis*) honey bees that guard small hive beetle prisons and the duration of beetle prison guarding for each honey bee subspecies. These data show guarding differences between the subspecies, suggesting possible reasons African honey bee subspecies can cope with small hive beetle infestations while European honey bee subspecies cannot. Further, these data aid in describing the recently discovered phenomenon of propolis prisons that are used by honey bees as a defensive tactic against small hive beetles.

## 2. MATERIAL AND METHODS

The experiments were conducted at Rhodes University in Grahamstown, South Africa (January-April & November-December 2001) and in Warren County, Georgia, USA (August/September 2001). In both locations, three observation hives were used. Each hive contained three frames of bees, two frames of brood, one frame of honey, and a laying queen. Honey bees used in the United States were of mixed European origin, while Cape honey bees were used in South Africa.

Approximately 25-40 small hive beetles were added to each hive 2-3 days after the observation hives were established. Once small hive beetle imprisoning behaviour was apparent in each hive (Neumann et al. 2001), 150-400 newly emerged honey bees, from a mixture of colonies, were individually marked with collared, numbered labels (Opalithplättchen) and added to each colony. No two observation hives were given newly emerged bees from the same colony.

Hives were monitored daily at approximately 09:00, 14:30, and 20:00 h. Location of imprisoned small hive beetles and guarding behaviour of marked honey bees (described in South Africa by Neumann et al., 2001 and in the United States by Ellis, 2002) were documented noting the commencement and duration of beetle prison guarding behaviour (fig. 1). Data were collected until all marked bees had stopped guarding beetle prisons (ranging from 21-28 days).

**Fig. 1.** Two European honey bees (one labelled "yellow 71") guarding an imprisoned small hive beetle. Notice the row of propolis, forming a prison wall, at the bottom of the photograph.

The beginning age of honey bees guarding beetle prisons, number of days they guarded, and the last day they guarded were analysed by analysis of variance (Statistica, 2001). Colonies were nested within location. When colony and location interacted, analyses were run separately by location. Means were separated using Tukey's multiple range tests; differences were accepted at the $\alpha \leq 0.05$ level.

## 3. RESULTS

### 3.1 Beginning guard age

European honey bees began guarding small hive beetle prisons two days earlier than did Cape honey bees ($F = 10.99$; $df = 1$; $P = 0.0014$)(table 1). There were colony × location interactions for beginning guard age ($F = 4.21$; $df = 4$; $P = 0.0039$). In South Africa, workers in one Cape colony (colony 3) began guarding small hive beetle prisons significantly earlier than in the other two colonies ($F = 6.24$; $df = 2$; $P = 0.0040$; table 2). There were no significant differences with respect to the start of prison guarding in the European colonies ($F = 2.50$; $df = 2$; $P = 0.099$; table 2).

### 3.2 Ending guard age

European honey bees stopped guarding small hive beetle prisons one day earlier than did Cape honey bees ($F = 5.12$; $df = 1$; $P = 0.027$)(table 1). Colony × location interactions occurred for this variable ($F = 4.83$; $df = 4$; $P = 0.0016$). Workers in Cape colony 3 stopped guarding beetle prisons earlier than in the other Cape colonies ($F = 9.33$; $df = 2$; $P = 0.00040$; table 2). There were no significant differences among the European colonies with respect to ending guard age ($F = 2.06$; $df = 2$; $P = 0.15$; table 2).

### 3.3 Duration of prison guarding

European honey bees guarded beetle prisons almost one day longer than did Cape honey bees ($F = 4.30$; $df = 1$; $P = 0.041$)(table 1). There was no significant colony × location interaction for this variable ($F = 2.48$; $df = 4$; $P = 0.051$).

## 4. DISCUSSION

European honey bees begin guarding small hive beetle prisons earlier, guard for longer periods of time, and stop guarding sooner than Cape honey bees. This European bee behaviour may be in reaction to damage small hive beetles cause in European colonies (Elzen et al., 1999, 2000; Hood, 2000; Wenning, 2001; Ellis et al., in press). Because small hive beetles cause little or no damage in Cape bee colonies (Ellis et al., in press), Cape honey bees could be less inclined to begin guarding beetle prisons and then guard for shorter periods of time. This could imply that Cape honey bees are either remarkably efficient at small hive beetle prison guarding or that there are other factors besides imprisoning techniques that Cape bees use to control small hive beetle infestations. This difference between the bee subspecies could also reflect the differences in aggression towards free-running small hive beetles between African and European honey bee subspecies (Elzen et al., 2001). African workers vigorously attack free-running small hive beetles more often than European workers do. Thus prison guarding in African colonies may not have to be as efficient.

Further, it is possible that age-related division of labour is different between the two honey bee subspecies, with European honey bees advancing in age-specific tasks faster than their African counterparts. However, division of labour in Cape honey bees is poorly studied and therefore no further inferences on this point can be made.

Interestingly, the commencement of hive entrance guarding behaviour in European honey bees has been documented at 18-19 days of age (Winston, 1992). This is consistent with our findings that European bees began guarding small hive beetle prisons at 18.6 days of age (table 1) which implies that "guarding" behaviour is the same for honey bees whether they are doing so at the entrance of a hive or entrance of a beetle prison.

Winston (1992) also noted that guarding behaviour in honey bees chronologically overlaps with foraging behaviour, indicating that individuals from the same cohort could be doing either of the two tasks. In this study, labelled honey bees in all colonies in both locations were recorded foraging while other labelled bees were guarding beetle prisons. Therefore, one would expect that if beetle infestations in European honey bee colonies are large, colony foraging activity may be reduced because foraging age bees are guarding beetle prisons instead of foraging. Such reduction in the number of foraging bees for small hive beetle infested European colonies has been documented (Ellis et al. in press).

African honey bee subspecies south of the Sahara are sympatric with small hive beetles (Lundie, 1940; Schmolke, 1974; Hepburn and Radloff, 1998) and show considerable resistance towards infestations. However, the behavioural mechanisms regulating resistance that have been identified so far [aggression behaviour (Elzen et al., 2001) and prison building (Neumann et al., 2001)] are also present in European bees (Ellis, 2002). This strongly suggests that there are only differences in degree, but not in kind, between Cape and European subspecies with respect to resistance behaviour. Therefore, one could expect that there is some adaptive advantage to the degree of behaviour exhibited by Cape honey bee guards.

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## REFERENCES

Ellis, JD (2002) Life behind bars: why honey bees feed small hive beetles. American Bee Journal 142(4): 267-269.

Ellis, JD; Pirk CWW; Hepburn HR; Kastberger, G; Elzen PJ (2002) Small hive beetles survive in honeybee prisons by behavioural mimicry. Naturwissenschaften 89: 326-328.

Ellis, JD; Hepburn HR; Delaplane KS; Neumann P; Elzen PJ (2003) The effects of adult small hive beetles, *Aethina tumida* (Coleoptera: Nitidulidae), on nests and foraging activity of Cape and European honey bees (*Apis mellifera*). Apidologie in press.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L, Wilson WT (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking honey bees in the Western Hemisphere. Apidologie 30: 361-366

Elzen PJ; Baxter JR; Westervelt D; Randall C; Wilson WT (2000) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae), in Florida, USA. Apidologie 21: 593-594.

Elzen PJ, Baxter JR, Neumann P, Solbrig AJ, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida* Murray. Journal of Apicultural Research 40: 40-41.

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin

Hood, WM (2000) Overview of the small hive beetle, *Aethina tumida*, in North America. Bee World 81(3): 129-137.

Lundie, AE (1940) The small hive beetle Aethina tumida. Science Bulletin 220. Union of South Africa, Department of Agriculture and Forestry. 30 pp.

Neumann P; Pirk CWW; Hepburn HR; Solbrig AJ; Ratnieks FLW; Elzen PJ; Baxter JR (2001) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Schmolke MD (1974) A study of *Aethina tumida*: The small Hive Beetle, Project Report, University of Rhodesia. 178 pp.

Statistica (2001) System reference, version 6. Stat-Soft; Tulsa, Oklahoma; 1063 pp.

Wenning CJ (2001) Spread and threat of the small hive beetle. American Bee Journal 141(9): 640-643.

Winston ML (1992) The honey bee colony: life history. In Graham, J (ed) The Hive and The Honey Bee. Dadant and Sons; Hamilton, Illinois, USA; pg. 73-101.

**TABLE 1. Beginning guard age, ending guard age, and duration of guarding behaviour (days) for Cape and European honey bees guarding small hive beetle prisons. The two bee subspecies differed for each parameter at *P* ≤ 0.05.**

| | Cape honey bees mean ± s.e. (n) | European honey bees mean ± s.e. (n) |
|---|---|---|
| average beginning guard age | 20.61 ± 0.38 (49)a | 18.55 ± 0.52 (33)b |
| average ending guard age | 21.04 ± 0.37 (49)a | 19.91 ± 0.57 (33)b |
| average duration of guarding behaviour | 1.43 ± 0.12 (49)a | 2.36 ± 0.31 (33)b |

**TABLE 2. Location × colony interactions for average beginning guard age and ending guard age (days) of Cape and European honey bees guarding small hive beetle prisons. Because of the significant interaction, colony analyses were run separately by location for these variables. Row totals followed by the same letter are not different at the α ≤ 0.05 level. Means were separated using ANOVA's and Tukey's multiple range tests.**

| | **Cape honey bees** | | |
|---|---|---|---|
| | Colony 1 mean ± s.e. (n) | Colony 2 mean ± s.e. (n) | Colony 3 mean ± s.e. (n) |
| average beginning guard age | 22.83 ± 1.51 (6)a | 20.97 ± 0.36 (29)a | 18.93 ± 0.73 (14)b |
| average ending guard age | 23.17 ± 1.45 (6)a | 21.59 ± 0.29 (29)a | 19.00 ± 0.74 (14)b |
| | **European honey bees** | | |
| | Colony 1 mean ± s.e. (n) | Colony 2 mean ± s.e. (n) | Colony 3 mean ± s.e. (n) |
| average beginning guard age | 18.2 ± 1.71 (5)a | 17.65 ± 0.69 (17)a | 20.09 ± 0.72 (11)a |
| average ending guard age | 18.2 ± 1.71 (5)a | 19.47 ± 0.73 (17)a | 21.36 ± 0.93 (11)a |

# 2.7 Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier)

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**Abstract -** The removal of small hive beetle [SHB] eggs and larvae was studied in seven *Apis mellifera scutellata* colonies. Because female beetles can protect their eggs by oviposition in small cracks we introduced unprotected eggs (N=7556 in seven colonies) and protected eggs (N=1612 in five colonies) into these colonies. Whereas all unprotected eggs were removed within 24 hours, 66±12% of the protected eggs remained, showing that SHB eggs are likely to hatch in infested colonies. However, all larvae (N=700) introduced into the same seven colonies were rejected within 24 hours. Workers responded quickly to the presence of SHB offspring in the colonies because 72±27% of the unprotected eggs and 49±37% of the larvae were removed within the first hour after introduction. The removal of SHB eggs and larvae was not correlated with colony phenotypes (size, amount of open and sealed brood, pollen and honey stores). Our data show that African colonies remove both SHB unprotected eggs and larvae within short periods of time. Therefore, we conclude that this removal behaviour plays an important role for the apparent resistance of African honeybees towards SHB infestations.

**Key words:** *Aethina tumida* / *Apis mellifera* / honeybee / parasite / small hive beetle

## 1. INTRODUCTION

The small hive beetle, *Aethina tumida* [SHB], is a honeybee parasite endemic to Africa, where it is considered only a minor pest (Lundie, 1940; Schmolke, 1974). In contrast, SHB can be harmful parasites in populations of European honeybees (Elzen et al., 1999). One possible explanation for differences in pest severity might be that honeybee subspecies sympatric with the SHB have evolved efficient resistance mechanisms. In particular, African honeybee colonies should remove efficiently SHB eggs and larvae.

It has been reported that African honeybee workers remove SHB eggs (Swart et al., 2001), but not a single study has quantified this behaviour yet. Likewise, little is known of the removal of SHB larvae. Lundie (1940) and Schmolke (1974) describe the “jetting” behaviour of the host bees (Fig. 1). Workers that get hold of a larva can carry it out of the colony at some distance (~20 meters; Lundie, 1940; Schmolke, 1974). Field observations indicate that larvae are efficiently rejected by such jettisoning workers (Lundie, 1940; Swart et al., 2001). This is supported by the observation hive study of Schmolke (1974), who reported that all introduced larvae are rejected within 24 hours. However, this jettisoning behaviour has never been rigorously quantified in field colonies yet. Moreover, the potential impact of colony phenotypes on the removal of SHB eggs and larvae has also never been quantified. Here we investigate the removal of SHB eggs and larvae by African honeybee colonies (*A. m. scutellata*).

**Figure 1.** A jettisoning worker is carrying a small hive beetle larva.

## 2. MATERIAL AND METHODS

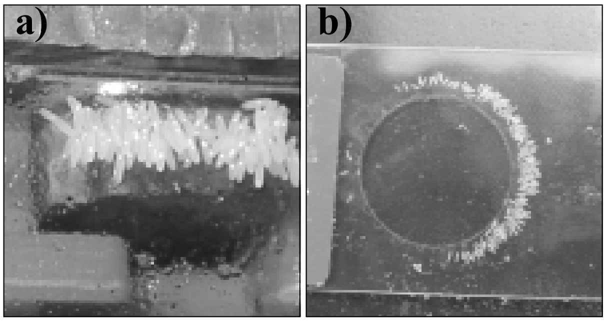
### 2.1 Experimental colonies and sampling of beetles

Seven unrelated colonies of *A. m. scutellata* were placed in 10-frame standard Langstroth hives with two boxes in a test apiary in Pretoria, South Africa. The bottom box contained honey, pollen and brood frames while the top box was empty. The colonies were given four days to settle down to prevent absconding before they were used as test colonies in the experiments. Adult SHB (N=491) were collected from the bottom board, outer frames and from closed prisons (Neumann et al., 2001b) of a single infested *A. m. scutellata* colony. Then, beetles were reared in the laboratory following standard protocols (Neumann et al., 2001a) with modifications as described below for each experiment.

### 2.2. Egg removal

Freshly collected beetles (N=371) were introduced into eight Apidea©-boxes containing pieces of comb with honey, pollen and brood of all stages. After 24 hours, the boxes were opened and the inner lids were removed. Because female beetles oviposit in small cracks (Lundie, 1940) we were able to obtain two kinds of eggs on these lids (Fig. 2): 1) unprotected eggs at the edges and 2) protected eggs around the inner circles. These lids were introduced into the test colonies (one lid into each colony) on top of the bottom box frames. After one, two, three, five, ten and 24 hours, the lids were briefly removed and remaining eggs were counted in the field using magnifying glasses [10×] before they were reintroduced into their respective test colony at the same within-hive location.

**Figure 2:** Unprotected **(a)** and protected **(b)** small hive beetle eggs that were laid onto the inner lids of the Apidea©-boxes.



### 2.3. Larva removal

Larvae that are reared on a mixed diet including honey are often coated with a sticky film (personal observations). Preliminary tests indicated that such larvae can easily escape from open petri-dishes. However, these tests also indicated that larvae that are reared using a “dry” approach on small amounts of sealed honeybee brood seem to have difficulties escaping from such open petri-dishes.

Freshly collected beetles (N=120) were introduced into three containers with frames containing only small patches of sealed brood [~10×15cm]. These pieces did not provide enough food for the larvae to reach maturity, so that all brood was consumed and larvae were not covered with sticky films. Larvae were collected from these containers and 100 larvae each were introduced into seven petri-dishes. Then, the dish were opened and introduced into each test colony on top of the bottom box frames. After one, two, four, seven and 24 hours, the dishes were briefly removed and remaining larvae were counted in the field before they were reintroduced into their respective test colony at the same within-hive location.

To control for the escape rate of larvae from the petri-dishes, three petri-dishes with 100 larvae each were introduced into containers and the number of remaining larvae in the open dishes was counted after one, three, five, nine and 24 hours.

### 2.4. Colony phenotype data

One day after the removal experiments were finished, colony phenotypes (size, amount of open and sealed brood, pollen and honey area) were evaluated for the seven test colonies using the standard Liebefelder method of colony estimation (Gerig, 1983; Imdorf et al, 1987).

### 2.5. Data analysis

Mann-Whitney U-tests were performed to test for differences in the removal rates of protected and unprotected eggs and to test for differences between the controls and the removal rates of larvae. Simple correlations (r-matrix) were performed between the colony phenotype data and the removal rates for SHB eggs and larvae.

## 3. RESULTS

### 3.1. Egg removal

Time to removal was determined for 9168 eggs (N=1612 protected eggs and 7556 unprotected eggs). Because female beetles did not lay eggs around the inner circles in two rearing boxes egg removal rates for protected eggs were evaluated in five of the seven colonies (N=7 for unprotected eggs). The number of tested unprotected eggs varied naturally between 248 and 2479 per test colony (1079.43 ± 1123.08; protected eggs: 322.4 ± 253.75, range: 74 to 716). The percentages of remaining eggs in the seven test colonies are shown in Figure 3. Significantly more protected eggs remained in the colonies than unprotected eggs (after one hour: unprotected eggs: 28.30±26.47%, range 3.47 to 68.06%; protected eggs: 85.02±13.67%, range: 70.53 to 99.55%; Z=-2.84, P<0.001; after 24 hours: unprotected eggs: 0±0%; protected eggs: 65.88±11.54%, range: 47.39 to 77.97%; Z=-2.84, P<0.002; Fig. 3).

### 3.2. Larva removal

As previously described (Schmolke, 1974), workers investigated larvae and carried them out of the colony (Fig. 1). Time to removal was determined for 700 larvae in the seven test colonies. The percentages of remaining larvae and in the controls are shown in Figure 4. A significantly higher proportion of larvae remained in the controls, than in the test colonies (after one hour: Z= -2.39, P<0.02; after 24 hours: Z= -2.39, P<0.02). After 24 hours all larvae were removed in all test colonies (Fig. 4).

### 3.3. Colony phenotype data

The colony phenotypes are shown in Table 1 and the correlation matrix for the colony phenotypes with the removal of SHB eggs and larvae in Table 2. Colony sizes and honey areas were positively correlated (Table 2). Likewise, the removal rates for unprotected eggs after one and two hours were positively correlated (Table 2). Otherwise, no significant correlations were found.

## 4. DISCUSSION

All adult SHB used in this study (N=491) were obtained from a single colony neither showing SHB larvae nor any other signs of serious infestation such as damaged comb or fermented honey. This supports earlier observations that African colonies can cope with high infestation levels (Neumann et al., 2001b) and further indicates that the removal of SHB offspring by the host workers is efficient. Indeed, 72±26% of all unprotected SHB eggs were removed within one hour and all of them within 24 hours. However, a significantly larger proportion of the protected eggs remained after 24 hours (66±12%), indicating that eggs in such areas are likely to hatch. This shows that oviposition of female SHB in cracks is adaptive, because it significantly enhances the survival chances of eggs.

African honeybees use considerably more propolis than European subspecies (Hepburn and Radloff, 1998). It seems likely that this abundant use of propolis not only enhances prison building (Neumann et al., 2001b) but also minimizes the number of available cracks in a colony, thereby limiting the number of protected beetle eggs.

Our results for the removal of larvae confirm earlier reports that jettisoning workers efficiently remove SHB larvae from infested colonies (Lundie, 1952). Moreover, our data agree well with Schmolke (1974) who found that 50% of artificially introduced larvae were removed within 90 minutes and 100% within 24 hours. Such rapid removal rates indicate that workers react quickly to the presence of both SHB eggs and larvae in the colony. Since SHB larvae can cause substantial damage to combs (Lundie, 1940; Schmolke, 1974), rapid colony responses appear important.

Colony sizes and honey areas were positively correlated as known from routine beekeeping experience. However, there were no significant correlations of the colony phenotype data with the removal rates for SHB eggs and larvae. This suggests that all our test colonies were strong enough to remove SHB eggs and larvae and further indicates that the removal of SHB offspring is probably not triggered by the amount of brood and/or food storage. Because protected eggs are likely to hatch, the removal of larvae is a key element for resistance. Nevertheless, the removal of eggs is also relevant because it reduces the number of hatching larvae. It seems likely that the removal behaviour of eggs and larvae is also present in colonies of European subspecies. However, there might be quantitative differences between African and European subspecies similar to the aggression behaviour towards adult SHB (Elzen et al., 2001), e.g. African honeybees may remove faster and/or more efficient. We conclude that removal behaviour plays an essential part for the apparent resistance of African honeybees. Future control efforts for SHB infestations might consider the role of cracks for successful beetle reproduction.

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### REFERENCES

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L., Wilson WT (1999) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western hemisphere. Apidologie 30: 361-366.

Elzen PJ, Baxter JR, Neumann P, Solbrig AJ, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*. J. Apic. Res. 40: 40-41.

Gerig L (1983) Lehrgang zur Erfassung der Volksstärke. Schweiz. Bienen-Zeitung 106: 199-204.

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin, Heidelberg, New York.

Imdorf A, Bühlmann G, Gerig L, Kilchenmann V (1987) Überprüfung der Schätzmethode zur Ermittlung der Brutfläche und Anzahl Arbeiterinnen in freifliegenden Bienenvölkern. Apidologie 18: 137-146.

Lundie AE (1940) The small hive beetle *Aethina tumida*. Science Bulletin 220, Dep. Agr. Forestry, Government Printer, Pretoria, South Africa.

Lundie AE (1952) The principal diseases and enemies of honey bees. S. Afr. Bee J. 27: 13-15.

Neumann P, Pirk CWW, Hepburn HR, Elzen PJ, Baxter JR (2001a) Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera: Nitidulidae). J. Apic. Res. 40: 111-112.

Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001b) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.). Naturwissenschaften 88: 214-216.

Schmolke MD (1974) A study of *Aethina tumida*: the small hive beetle. Project Report, University of Rhodesia, pp. 178.

Swart JD, Johannsmeier MF, Tribe GD, Kryger P (2001) Diseases and pests of honeybees, In: Johannsmeier M.F. (ed) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp 198-222.

**Figure 3:** Removal of SHB eggs (mean ± SD) after 0, 1, 2, 3, 5, 10 and 24 hours in the seven *A. m. scutellata* test colonies (triangles = unprotected eggs, circles = protected eggs).

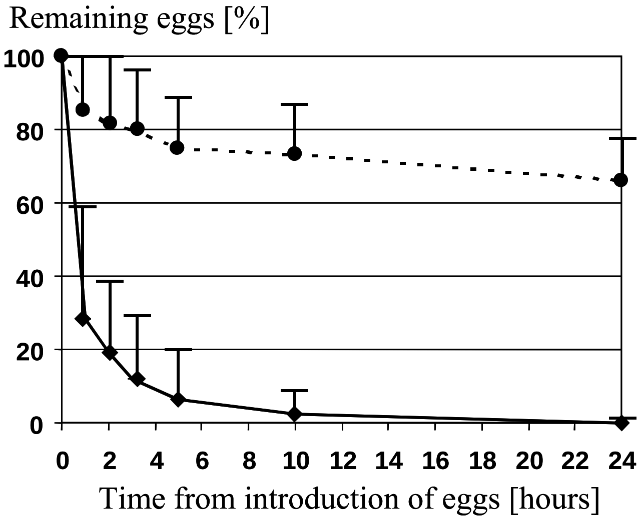

**Figure 4:** Removal of SHB larvae (mean ± SD) after 0, 1, 2, 4, 7, 10 and 24 hours in the seven *A. m. scutellata* test colonies (triangles = treatments, circles = controls).

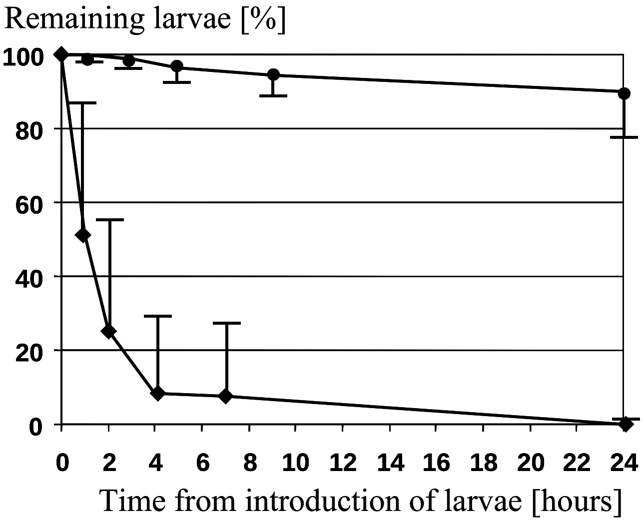

**Table I.** Colony phenotype data for the tested *A. m. scutellata* colonies. Colony size, sealed and open brood, pollen and honey are shown.

| Colony | Colony size [bees] | Brood [dm$^2$] Open | Brood [dm$^2$] Sealed | Pollen [dm$^2$] | Honey [dm$^2$] |
|---|---|---|---|---|---|
| 1 | 9035 | 13.25 | 22 | 8 | 44.25 |
| 2 | 8645 | 16 | 14.25 | 5.5 | 83.5 |
| 3 | 8450 | 14.75 | 13.25 | 9.5 | 32.5 |
| 4 | 5623 | 0 | 1.75 | 2.75 | 34 |
| 5 | 4290 | 0.5 | 0.5 | 8 | 29.25 |
| 6 | 7540 | 15.5 | 12 | 6.5 | 40 |
| 7 | 8125 | 15.25 | 11.75 | 7 | 39.25 |
| Mean | 7387±1765 | 10.8±7.23 | 10.8±7.5 | 6.8±2.2 | 43.3±18.5 |

**Table II.** Correlation r-matrix for the colony phenotype data and removal data for the tested *A. m. scutellata* colonies. Colony size, sealed and open brood, pollen, honey, removal of SHB protected and unprotected eggs after one and two hours and removal of SHB larvae after one and two hours were considered. The Bonferroni adjusted significance level is α= 0.0041. Significant correlations are indicated with * for p < 0.0041.

| | | | Colony Size | Brood | | Pollen | Honey | Egg removal | | | | Larva removal | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Open | Sealed | | | Unprotected | | Protected | | | |
| | | | | | | | | 1 hour | 2 hours | 1 hour | 2 hours | 1 hour | 2 hours |
| Colony size | | | 1 | | | | | | | | | | |
| Brood | Open | | 0.89 | 1 | | | | | | | | | |
| | Sealed | | 0.95 | 0.82 | 1 | | | | | | | | |
| Pollen | | | 0.28 | 0.44 | 0.47 | 1 | | | | | | | |
| Honey | | | 0.98* | 0.85 | 0.96 | 0.23 | 1 | | | | | | |
| Egg removal | Un-protected | 1 hour | -0.37 | 0.59 | 0.15 | 0.19 | 0.22 | 1 | | | | | |
| | | 2 hours | -0.30 | 0.54 | 0.07 | 0.14 | 0.15 | 1* | 1 | | | | |
| | Protected | 1 hour | -0.53 | -0.34 | -0.60 | 0.14 | -0.67 | 0.48 | 0.51 | 1 | | | |
| | | 2 hours | -0.18 | -0.35 | -0.28 | -0.14 | -0.30 | 0.30 | 0.30 | 0.68 | 1 | | |
| Larva removal | | 1 hour | -0.73 | -0.58 | -0.75 | -0.56 | -0.64 | -0.40 | -0.32 | -0.01 | -0.38 | 1 | |
| | | 2 hours | -0.48 | -0.35 | -0.53 | -0.62 | -0.37 | -0.36 | -0.29 | -0.27 | -0.55 | 0.95 | 1 |

# 2.8 The biology of the small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae): Gaps in our knowledge of an invasive species

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**Abstract -** Small hive beetles, *Aethina tumida*, are honeybee parasites native to Africa, where they are a minor pest only. In contrast, the beetles can be harmful parasites of European honeybee subspecies. Resistance of African subspecies to infestations is probably due to quantitative differences in a series of behaviours such as absconding, aggression, removal of parasite eggs and larvae and social encapsulation. The beetles use counter-resistance tactics such as defence posture, dropping, hiding, escape, egg laying in small gaps and trophallactic mimicry. Small hive beetles are efficient in long-range transportation (US: 1996, Australia: 2002) and can establish populations in temperate regions due to their overwintering capacity in honeybee clusters. Host shifts to other bee species may also occur. Thus, small hive beetles have the potential to become a global threat to apiculture and wild bee populations. However, our knowledge of the small hive beetle is still limited, creating demand for more research in all areas of its biology.

**Key words:** *Aethina tumida* / *Apis mellifera* / honeybee / invasive species / small hive beetle

## 1. INTRODUCTION

In recent decades, the frequency of biological invasions has increased to an unprecedented level, stimulating a multitude of research projects in population biology and community ecology (Hänfling and Kollmann, 2002). The small hive beetle, *Aethina tumida*, a nest parasite of honeybees, *Apis mellifera*, constitutes a recent example of such an invasive species in populations of European host subspecies. Here we summarize the literature on the biology and the current distribution of the small hive beetle. We will concentrate on examining the more proximate aspects of the biology of the beetle and the host that may contribute to the invasion process. This is particularly important because successful and sustainable control efforts require a detailed understanding of the invasion dynamics and of the biology of an invasive species.

## 2. THE SMALL HIVE BEETLE

The small hive beetle was first described by Murray (1867) and is native to Africa (Fig. 1). It belongs to the coleopteran family Nitidulidae, which contains ~2,800 described species in 172 genera worldwide (Habeck, 2002). The nitidulid beetles can be distinguished from other similar beetles by their transverse procoxal cavities, grooved metacoxae, dilated tarsal segments, small forth tarsi and three-segmented antennal club (Habeck, 2002). The nitidulid beetles can feed on fresh, rotten and dried fruits, plant juices, carrion, crops and on flowers (Lin et al., 1992; Fadamiro et al., 1998; Hepburn and Radloff, 1998; Smart and Blight, 2000; Wolff et al., 2001). The natural history and morphology of *A. tumida* were described by Lundie (1940) and Schmolke (1974).

### 2.1 Pest status and putative life cycle in Africa (Fig. 2)

Here we focus on the life cycle aspects necessary to understand and control the beetle. Other features are reported in more detail elsewhere (Lundie, 1940, 1951, 1952a,b; Schmolke, 1974; Hepburn and Radloff, 1998; Elzen et al., 2000c; Hood, 2000; Pettis and Shimanuki, 2000; Flügge, 2001; Neumann et al., 2001a,b; Swart et al., 2001; Ellis et al., 2002b,c). In its native range, the small hive beetle is usually a minor pest only, because successful reproduction appears most successful in weak, stressed colonies or in recently abandoned honeybee nests and is far less common in strong colonies (Lundie, 1940; Schmolke, 1974; Hepburn and Radloff, 1998; Fig. 2). In Africa, the main problems associated with the beetles are in the destruction of stored bee products (Lundie, 1940; Schmolke, 1974; Fig. 2), which most likely result from a lack of bee populations to guard against reproduction. However, neither the beekeeping terms "weak/stressed" vs. "strong/unstressed" colonies nor the actual levels of beetle reproduction in such colonies have been rigorously quantified yet. This appears of prime importance to understand the biology of *A. tumida*.

Strong African honeybee colonies, even if heavily infested (Neumann et al., 2001b; Neumann and Härtel, 2003), can usually prevent or postpone successful beetle reproduction (Hepburn and Radloff, 1998; Fig. 2). In such colonies small hive beetles usually have to wait until non-reproductive swarming ( = absconding or migration, Hepburn and Radloff, 1998; see 4.8) leads to recently abandoned nests (Fig. 2). Massive aggregations of small hive beetles and/or heavy infestations appear to induce absconding in Africa (Fig. 2). But neither beetle-induced absconding nor the potential effects of colony movements on levels of infestation and parasite population sizes are well understood (see 4.8). This seems highly relevant because parasite population sizes may trigger pest severity. The underlying reasons for the occurrence of beetle aggregations are also unclear (see 4.9).

Host finding (see 4.1) and intrusion into the colony (see 4.2) are most relevant for the invasion process (Fig. 2), but neither the actual cues nor the underlying mechanisms have been identified yet. Female beetles oviposit in the host colonies (see 4.7). The emerging larvae (see 4.7) develop until the wandering stage and then leave the nest for pupation in the soil (Fig. 2). While the adults have little impact on the colony, the larvae can cause severe damage to combs (Lundie, 1940; Schmolke, 1974), often resulting in the full structural collapse of the nest (Hepburn and Radloff, 1998). Newly emerged adults invade host colonies, thereby completing the life cycle of *A. tumida* (Fig. 2). In the laboratory, the life cycle can also be completed on fruits (see 2.2) and in bumble bee colonies (see 5; Fig. 2). However, the level of reproduction and feeding on fruits in the wild has not been studied, which seems important to investigate this potential transmission pathway. Likewise, the ability of small hive beetles to infest bumble bee colonies in the field is unknown. This should be investigated to evaluate the potential impact of small hive beetles on wild bumble bee populations.

### 2.2 Alternative food sources

Small hive beetles may use fruits as alternative food sources (Schmolke, 1974; Eischen et al., 1999; Ellis et al., 2002c) in the absence of honey bee colonies, e.g. following removal of colonies in migratory beekeeping (Eischen et al., 1999). Moreover, a complete life cycle can be achieved on fruits (Ellis et al., 2002c; Fig. 2). However, although larvae develop normally on avocado, cantaloupe, grapefruit and some other fruit with over 500 beetles observed in one cantaloupe (Eischen et al., 1999), the number of offspring per breeding couple is significantly lower than on bee products such as pollen (Ellis et al., 2002c). Furthermore, small hive beetles have never been observed to reproduce or even feed on fruits in the field in South Africa (MF Johannsmeier, personal communication). Likewise, there are no reports that small hive beetles are a crop pest in Southern Africa (MF Johannsmeier, personal communication). Therefore, reproduction on fruits appears to be rare if not absent in natural populations. This might be related to the different reproductive success on different diets (Ellis et al., 2002c). Although successful reproduction is in principal possible on other diets, small hive beetles should prefer honeybee colonies whenever possible to maximize their reproductive output. However, the actual amount of small hive beetle reproduction on fruits has never been rigorously investigated in the field. Therefore, we cannot completely exclude that the presence of an abundant food source other than honeybee colonies may serve as a refuge for the small hive beetle and as a source of further infestations.

## 3. Current distribution, pest status and putative life cycle in populations of European honeybees

### 3.1 Current distribution and pest status in the USA

The first confirmed detection of small hive beetles in the US was in St. Lucie, Florida in June 1998, as identified by the Florida Department of Agriculture and Consumer Services (Hood, 2000; Sanford, 2002). Earlier, unidentified specimen were collected in Charleston, South Carolina, in November 1996 (Hood, 1999a). The introduction of the small hive beetle into the USA was thought to have been through South Carolina and from there to Georgia and Florida (Hood, 2000). Since then, the small hive beetle has extended its range from 18 states by the end of 2001 (Hood, 2001), over 25 states in April 2002 (Evans et al., 2003), to 29 states in March 2003 (Fig. 3). This rapid spread is likely to result from natural range expansion and movement of infested honeybee colonies, migratory beekeeping, package bees and beekeeping equipment (Delaplane, 1998). Mt-DNA sequence analyses of the small hive beetle from the US and South Africa indicate that the populations on both continents belong to a single species, although it is not clear whether a single or multiple introductions occurred (Evans et al., 2000, 2003). Even strong colonies of European honeybee subspecies can be taken over and killed by small hive beetles in the US (Sanford, 1998; Elzen et al., 1999a,b). The state most severely affected by the small hive beetle has been Florida (Elzen et al., 2002; Fig. 3) and the damage to local apiculture can be serious (Elzen et al., 2000b). Indeed, only in 1998 in Florida losses were estimated to be in excess of $3 million (Ellis et al., 2002c).

### 3.2 Current distribution and pest status in Australia & Egypt

In July 2002 beetle damage was noticed in a nucleus colony in New South Wales (M Duncan, personal communication). The beetles were identified as *A. tumida* in October 2002 (Minister for Agriculture 2002). In March 2003, the small hive beetle is still fairly restricted in its occurrence (D Anderson [CSIRO], M Beekman, P Boland [Biosecurity Australia], L Cook [NSW Agriculture] and M Duncan, personal communications; Fig. 4). At present, the beetle is causing no noticeable losses (D Anderson [CSIRO], personal communication). In contrast to the US, strong colonies don't collapse with the beetle (D Anderson [CSIRO], M Duncan, personal communications).

In Egypt, small hive beetles were first detected in Etaie Al-Baroud (~110 km North-West of Cairo) in Summer 2000 (Mostafa and Williams, 2000). Since then, the small hive beetle was also found in other apiaries along the Nile Delta (AM Mostafa, personal communication). *A. tumida* is probably not endemic to Egypt (HR Hepburn, AM Mostafa and B Schricker, personal communications). In order to clarify whether the small hive beetle is native to Egypt or has been introduced, it seems necessary to investigate its distribution in upper Egypt, which is more close to its sub-Saharan endemic region (Fig. 1). At present, reports on the small hive beetle in both Australia and Egypt are largely anecdotal and more detailed studies are urgently required.

### 3.3 Putative life cycle in colonies of European honeybees (Fig. 2)

There seems to be two differences in the putative life cycle of small hive beetles in colonies of European honeybee subspecies in the US (Fig. 2):

**1) Overwintering capacity (Fig. 2):** European honeybee subspecies form a winter cluster in colder climates to survive longer periods of cold weather conditions (Gates, 1914; Corkins, 1930), a behaviour which is not expressed in African subspecies (Hepburn and Radloff, 1998). Despite its tropical origin, adult small hive beetles can overwinter within such clusters (Elzen et al., 1999a; Hood, 2000), where >300 beetles have been reported in small clusters (Pettis and Shimanuki, 2000). This is quite surprising, even in light of low aggression levels by the European bees (Elzen et al., 2001). It seems as if small hive beetles have adapted to temperate climates by exploiting the cluster behaviour of European subspecies. Thus, although -12°C for 24 hours is reported to kill all life stages of the beetle (Hood, 1999b), it is obvious that small hive beetles are able to survive in colder climates and have the potential to establish populations across a significant part of the US (Evans et al., 2003). Indeed, there are established beetle populations as far North as Ohio (Evans et al., 2003). More detailed studies are required to understand how small hive beetles can survive in the winter clusters. However, the establishment of beetle populations alone cannot explain the severe effects of infestations in US honeybee populations.

**2) Life history short-cut (Fig. 2):** In contrast to African subspecies, even strong colonies of European honeybee subspecies can be taken over and killed by small hive beetles in the US (Sanford, 1998; Elzen et al., 1999a,b; Fig. 2). Weakened and stressed colonies may even succumb within two weeks (Wenning, 2001). Thus, successful reproduction of the parasite seems to be more common in strong European colonies in the US (Fig. 2). It seems as if small hive beetles in European colonies in the US do not have to wait for recently abandoned nests or for favourable time windows (see Mutsaers, 1991). This constitutes a short-cut in the life history enabling successful reproduction more often than in African host populations.

What are the underlying reasons for such a life history short-cut? It might well be that European honeybee subspecies lack behavioural resistance mechanisms and therefore the small hive beetle is a serious threat. Indeed, the presence of large numbers of small hive beetles in African honeybee colonies does not significantly affect adult bee populations, brood area and foraging behaviour although small hive beetle presence significantly lowered all of these variables in European colonies (Ellis et al., 2003a). This indicates that behavioural characteristics are important to understand resistance towards small hive beetle infestations. In the following chapters we will address such behaviours in detail.

## 4. Behavioural interactions between host and parasite

### 4.1 Host finding (Fig. 2)

Adult small hive beetles are active flyers (Elzen et al., 1999b, 2000c) and individuals or occasionally swarms (Tribe, 2000) can infest honeybee colonies. It has been stated (Wenning, 2001), that small hive beetles can detect colonies under stress, e.g. due to disease or management techniques such as splitting, and that they are able to detect such colonies from a distance of about 13-16 km. Detection of stressed colonies might be adaptive in Africa, where reproduction is more likely in such colonies than in unstressed ones (Hepburn and Radloff, 1998). However, the actual mechanism which might allow small hive beetles to detect stressed colonies over large distances is unclear (see 4.9). Baited trap studies show that a combination of honey/pollen and adult bees is highly attractive to flying beetles, whereas adult bees alone are less attractive and brood, hive products and infested combs alone are not attractive (Elzen et al., 1999b). This indicates that an intact honeybee colony with food storage is most likely the preferred breeding place of small hive beetles.

### 4.2 Host intrusion (Fig. 2)

Honeybee colonies have highly specialized guard bees, that carefully scrutinize incoming individuals (Ribbands, 1953). However, the adult beetles can intrude strong honeybee colonies as well as weak ones with equal impunity (Lundie, 1940). Nevertheless, fewer beetles intruded into colonies with reduced entrances (Ellis et al., 2002a), suggesting that guard bees are capable of preventing intrusion at least to some degree. Beekeeping activities such as frequent inspections appear to facilitate beetle intrusion into host colonies. Some colonies have been reported to collapse after beekeepers have united them with other highly-infested supers (Sanford, 2002).

### 4.3 Aggression towards adult beetles

**1) host tactics:** *A. m. scutellata* and *A. m. capensis* protect themselves by active aggression towards both the adults and larvae (Lundie, 1940; Elzen et al., 2001; Neumann et al., 2001b). The bees try to bite or sting the adults but usually with only little success (Lundie, 1940; Elzen et al., 2001; Neumann et al., 2001b; Swart et al., 2001). In the few cases, when the adult beetles are decapitated (Neumann et al., 2001b) or extremities are removed (Schmolke, 1974), they are thrown out of the hive (Lundie, 1952b). Observations that small hive beetles can live for long periods of time even in strong colonies with relative impunity (Lundie, 1940) also suggest that aggression is not very effective in killing the beetles. This may be partly due to the hard exoskeleton (Lundie, 1940) but also due to the following defence tactics of the adult beetles.

**2) parasite tactics:**

**Defence posture:** When attacked, the adults can perform a turtle-like defence posture (Lundie, 1940; Schmolke, 1974; Neumann et al., 2001b). While exhibiting this defence posture the beetle stays motionless and tucks its head underneath the pronotum with the legs and antennae pressed tightly to the body (much like withdrawal in a turtle, Neumann et al., 2001b).

**Running:** Beetles usually move very quickly out of the range of bees (Schmolke, 1974; Neumann et al., 2001b).

**Dropping:** The beetles can deliberately drop from the combs to escape pursuit (Schmolke, 1974).

**Hiding:** Inside of the nest cavity, the adults typically hide in small cracks (Schmolke, 1974; Neumann et al., 2001b), under the bottom board of commercial hives (Lundie, 1940) or in cells (Schmolke, 1974; personal observations). While hiding in cells, small hive beetles usually stay motionless at the bottom (Lundie, 1940; Schmolke, 1974). When field colonies are inspected, the adult beetles are often seen moving from one hiding place to another one nearby (Lundie, 1940; Swart et al., 2001). This also happens on a regular basis in observation hives (Neumann et al., 2001b).

Nevertheless, although aggression is not very effective in killing the beetles, it may contribute to resistance. African honeybees show significantly more investigative contact and aggression behaviour to the adults than European ones (Elzen et al., 2001). About 1/3 of all encounters between African bees and adult beetles resulted in attacks by the workers, whereas this was only 1.4% in European bees (Elzen et al., 2001). Therefore, the adult beetles are probably under constant harassment in an African colony, which may minimize beetle reproduction.

### 4.4 Social encapsulation

**1) host tactics:** Sometimes the bees succeed in “corralling” (Elzen et al., 2000a,b) or “herding“ (Swart et al., 2001) the adult beetles into specific corners, preventing them from moving freely over the combs. When such beetles are corralled, or when they actively hide in small gaps (Schmolke, 1974; Neumann et al., 2001b), they are often encapsulated in propolis confinements (*A. m. scutellata*: Hepburn and Radloff, 1998; *A. m. capensis*: Neumann et al., 2001b; Solbrig 2001; Ellis et al., 2003b). This is not an artefact of observation hives because social encapsulation also occurs in normal field colonies (Neumann et al., 2001b). Corralling behaviour has never been observed in field colonies or natural nests. While it seems logically to assume that corralling occurs because it is a necessary part of social encapsulation, its occurrence can only bee inferred at this point.

During the encapsulation process, workers add propolis around detected hidden or corralled beetles and completely encapsulate most of them (Neumann et al., 2001b). The bees have a sophisticated tactic for limiting beetle escape during encapsulation (Neumann et al., 2001b). While some workers add propolis, one or more others continuously guard the beetles in both open and closed confinements day and night for up to 57 days (Neumann et al., 2001b). The guard workers continuously attack the beetles when they move to the edges of still open confinements and thus keep them imprisoned (Neumann et al., 2001b). Social encapsulation may be an additional factor for preventing or postponing successful reproduction of the parasite.

However, social encapsulation also occurs in susceptible European honeybee subspecies (Ellis et al., 2003c). Because the use of propolis is more abundant in African subspecies compared to European ones (Hepburn and Radloff, 1998) social encapsulation may be more efficient and/or more common in African honeybee colonies. Indeed, the number of confinements per colony and encapsulated beetles in these prisons were both lower in European colonies (Ellis et al., 2003c) than in African ones (Neumann et al., 2001b). Moreover, European honeybees guard prisons significantly longer than Cape honeybees (Ellis et al., 2003b). However, the underlying reasons for this or its effect on beetle survival and/or colony performance are unknown. African bees are more aggressive towards the small hive beetle (Elzen et al., 2001). Therefore, African prison guards may be more efficient in preventing beetle escape (Neumann et al., 2001b). Clearly, more detailed studies are necessary to evaluate to what extent social encapsulation triggers resistance towards small hive beetle infestations.

**2) parasite tactics:** Some beetles manage to escape encapsulation at night (Neumann et al., 2001b), possibly because honeybees are generally less active at night (Moritz and Kryger, 1994). Matings in prisons and cannibalism among small hive beetles were also observed (Neumann et al., 2001b), which might enhance their survival in large prisons. Despite no access to food in the combs, imprisoned beetles may survive for two months or longer (Neumann et al., 2001b). However, their survival is not due to metabolic reserves, because starved beetles die within a fortnight (Flügge, 2001; Ellis et al., 2002c). The beetles approach the prison guard bees, extend their heads towards and make antennal contact with the bees thus mimicking normal honeybee trophallaxis (Korst and Velthuis, 1982). Often workers respond with aggression, so it may take several attempts before the bees regurgitate food (Ellis et al., 2002d). Thus, long term survival of small hive beetles in prisons is probably also derived from behavioural mimicry (Ellis et al., 2002d).

### 4.5 Patrolling

Despite frequent searching, only few small hive beetles can be seen on the combs of strong colonies (Schmolke, 1974). This indicates that such colonies are able to prevent small hive beetle intrusion in the comb area at least to some degree by guarding this area. This comb guarding behaviour (= patrolling; Swart et al., 2001) seems to be more efficient in strong colonies due to the higher density of bees in the nest (Lundie, 1952b; Swart et al., 2001). Lundie (1952a) stated: "Any factor which so reduces the ratio of the population of a colony of bees to its comb surface that the bees are no longer able to protect the comb surface adequately is a precursor to the ravages of both the wax moth and *Aethina tumida*". The patrolling behaviour seems particularly well expressed in the brood area of the colony (Schmolke, 1974; Solbrig, 2001) but less well expressed in the outer frames and honey supers (personal observations). This might explain, why adult beetles may oviposit on outer frames and why larvae can appear on them after transport to the honey house. It appears as if the host becomes alerted by newly intruded beetles (Schmolke, 1974). We conclude that protection of the combs via patrolling/high bee density might contribute to resistance. However, this potential impact needs to be investigated in future studies.

### 4.6 Worker aggregation and cell content removal

When beetles manage to intrude into the comb area and hide in cells, African workers rapidly aggregate around them (S Härtel, personal communication; WRE Hoffmann, personal communication). Then, the workers remove the contents of nearby honey, pollen and brood cells to get access to the hidden beetles (Schmolke, 1974; personal observations). The bees get extremely agitated until the small hive beetle is finally removed from the comb area (personal observations) or deliberately shows the dropping behaviour (Schmolke, 1974). This behaviour may minimize small hive beetle oviposition on the combs.

### 4.7 Removal of small hive beetle eggs and larvae

**1) parasite tactics:** Female beetles oviposit in batches or irregular clutches (Lundie, 1940; Schmolke, 1974) of up to 210 eggs (mean = 14±20 eggs; S Härtel and PN, unpublished data) throughout the hive, but seem to prefer small gaps and the bottom of cells (Lundie, 1940; Schmolke, 1974). At initial stages of infestation, when no larvae are present, females significantly oviposit in cracks rather than on the combs (S Härtel and PN, unpublished data). Nevertheless, females can also oviposit on the combs, because super frames of infested colonies quickly show larvae after transport to the honey house (Lundie, 1940). On the combs oviposition seems to preferentially occur in pollen cells (>30 small larvae per cell; Lundie, 1940), probably because reproductive success can be very high on a pollen diet alone (Ellis et al., 2002c). The number of eggs laid per female is high in the first 24 hours after infestation (69±15 eggs; S Härtel and PN, unpublished data). Schmolke (1974) estimated about 1000 eggs per female in a three to four month period, after which oviposition declines. Oviposition of many eggs in gaps appears adaptive because survival chances for the offspring are enhanced (Neumann and Härtel, 2003).

**2) host tactics:**

**a) eggs:** It has been reported that African workers do remove small hive beetle eggs (Swart et al., 2001). This removal was recently studied in *A. m. scutellata* field colonies (Neumann and Härtel, 2003) by introduction of unprotected and protected eggs (laid in gaps). Whereas all unprotected eggs were removed within 24 hours, 66% of the protected eggs remained. This indicates that unprotected eggs are efficiently removed but also shows that eggs laid in gaps are likely to hatch (Neumann and Härtel, 2003).

**b) larvae (jettisoning behaviour):** Bees which get hold of a larvae can carry it out of the hive at some distance (~20 meters; Lundie, 1940; Schmolke, 1974). Sometimes there is a tug-of-war between two jettisoning workers tearing apart one larvae and resulting in both bees carrying out of the hive what they are holding (Schmolke, 1974). This jettisoning behaviour seems to be efficient (Lundie, 1952b) because all introduced larvae were removed within 24 hours in an observation hive study (Schmolke, 1974). Likewise, all larvae (N=700) introduced into seven *A. m. scutellata* field colonies were ejected within 24 hours (Neumann and Härtel, 2003). Field observations also indicate that larvae are efficiently ejected by jettisoning workers (Lundie, 1940; Swart et al., 2001).

African workers respond quickly to the presence of small hive beetle offspring because 72% of the non-protected eggs and 49% of the larvae were removed within one hour after introduction (Neumann and Härtel, 2003; see also Schmolke, 1974). The removal was not correlated with colony phenotypes (size, amount of open and sealed brood, pollen and honey area; Neumann and Härtel, 2003). However, Neumann and Härtel (2003) only studied relatively strong, unstressed colonies. Thus, these studies should be repeated with weak/stressed colonies. We conclude that removal behaviour plays an important role for the apparent resistance of African honeybees. However, it is unknown to what extent European bees remove small hive beetle eggs and larvae. Because prevention of beetle reproduction seems crucial, this behaviour should be more deeply investigated in the future.

### 4.8 Colony mobility: absconding and migration

African honeybee subspecies are much more mobile compared to European bees (Hepburn and Radloff, 1998). One can distinguish between two forms of non-reproductive swarming (Hepburn and Radloff, 1998):

**1) Absconding:** Absconding can be induced by severe disturbance, predation, and declining quality of the nest and/or nest cavity as well as by parasites (Hepburn and Radloff, 1998). The African subspecies are prone to absconding, queenright or not, and may abandon open and sealed brood and food stores as well (Hepburn and Radloff, 1998; Hepburn et al., 1999). African honeybee colonies can also respond to heavy small hive beetle infestations by absconding (Hepburn and Radloff, 1998; Fig. 2). However, it has also been shown that strong African colonies can tolerate large infestations with only minor colony level effects (Ellis et al., 2003a). Thus, it seems somehow contradictory that as if at times African colonies can tolerate large populations of small hive beetles, but at other times high infestation rates can induce abandonment of the nest. Several bees are probably involved in small hive beetle resistance (e.g. in prison guarding; Ellis et al., 2003b) when African colonies are heavily infested with hundreds of beetles (Neumann et al., 2001b; Neumann and Härtel, 2003). This might reduce colony efficiency in the long run because large scale infestations are a continuous and major predatory pressure on the honeybee nest (Hepburn and Radloff, 1998) and may favour beetle-induced absconding in Africa. Moreover, the occurrence of small hive beetle larvae and the resulting partial nest destruction and fermentation of the honey (Lundie, 1940) are also likely to play a role for beetle-induced absconding (M Duncan, personal communication; PN personal observations). There might be a seasonal pattern for beetle-induced absconding in Africa. Indeed, colony stress can be seasonal (Lundie, 1952b), e.g. it has been reported that small hive beetle damage may occur during the rainy season (Mutsaers, 1991). Finally, there might be an upper limit of infestation that can be tolerated, which is only exceeded in a few colonies due to massive beetle aggregations (see below). In any case, the underlying reasons for beetle-induced absconding are not fully understood yet and need further investigation.

**2) Migration:** Migration can be defined as a seasonally predictable phenomenon that may serve as an alternative to massive hoarding given the suitable flight temperatures and seasonal flowering of Africa (Hepburn and Radloff, 1998). Large scale migration of colonies without regard to reproduction are well documented for several African subspecies (Hepburn and Radloff, 1998). African colonies seem to prepare for migration by a reduction of egg-laying by the queen, waiting for the hatching of sealed brood, and consumption of stores (Hepburn and Radloff, 1998).

However, the distinction between the two forms is not absolute. There are also cases of “prepared” absconding (Hepburn and Radloff, 1998). This refers to only a few colonies in an apiary, while the majority stays behind, and can be regarded as migration on a small scale (Hepburn and Radloff, 1998). Similar to migration such colonies may also undergo preparation for nest desertion (see above). This behaviour has been recorded for several African subspecies (Hepburn and Radloff, 1998).

What are the effects of colony mobility on small hive beetle infestation levels and on parasite population densities? Any form of colony movement can be assumed to reduce colony levels of infestation with *A. tumida*, because the non-phoretic beetles are left behind. Moreover, the life cycle of the beetle is broken (Lundie, 1940). Reports that stationary hives were more vulnerable than hives that were moved on a regular basis (Lundie, 1940) point in that direction. On the other hand, recently abandoned nests after absconding, prepared absconding or migration are a breeding opportunity for small hive beetles because food stores and brood are not protected anymore. However, in such cases small hive beetles can be in strong competition with ants, which also exploit the recently abandoned nests (personal observations). While both absconding and seasonal migration may interrupt the small hive beetle’s life cycle, it seems that migration (in which food stores are consumed before departure) would have a much more serious limiting effect on beetle populations than disturbance induced absconding, in which large food reserves can be left behind. Thus, beetle population densities in Africa, where host colonies migrate seasonally, might be smaller compared to populations of European honeybee subspecies. This may partially explain different pest severities. However, not a single study has compared small hive beetle population sizes between the endemic and new ranges.

Although absconding is rare in European bees (Ruttner, 1986), it is also induced in infested European colonies (Ellis et al., 2003a). Because African subspecies are more prone to absconding than European bees (Hepburn and Radloff, 1998), another reason for better small hive beetle resistance/less pest severity may be that African bees are somehow more efficient in preparation for absconding and/or respond earlier with nest abandonment. We recommend more detailed studies on the effects of absconding and seasonal migration in future studies.

### 4.9 Small hive beetle aggregation pheromone?

Long range host finding of adults (Wenning, 2001) requires efficient cues. Furthermore, small hive beetle swarms can be occasionally observed in South Africa (Tribe, 2000). Massive aggregations of adult small hive beetles prior to the absconding of such heavily infested colonies can be found in Africa (Neumann et al., 2001b; Neumann and Härtel, 2003) and in the US (Elzen et al., 2002; Ellis et al., 2003a). In European honey bee hives, *A. tumida* infestations may consist of as many as 1,000 adults and several hundred larvae per hive (Elzen et al., 1999b). In a single *A. m. scutellata* colony 491 adult beetles were found, while all other colonies at the same apiary show low infestation levels (N = 7 colonies; mean infestation level = 14±12 beetles; S Härtel and PN, unpublished data). These colonies with large numbers of beetles are neither particularly weak nor have massive food stores (Neumann et al., 2001b), indicating that cues other than simple host colony size and food stores are responsible for their attractiveness. Indeed, aggregation pheromones have been described for a variety of Nitidulidae species and are widely used as control agents (Petroski et al., 1994; James et al., 2000). Such pheromones are produced by exceptional large specialized cells within the body cavity of nitidulid beetles (Nardi et al., 1996). We consider it very likely that a similar pheromone plays a role for long range host finding and aggregations of small hive beetles. Observations that males tend to infest before females (Elzen et al., 2000c) indicate that the aggregation pheromone might be male produced as in *Carpophilus obsoletus* and is attractive to both sexes (Petroski et al., 1994). Synergistic effects between food odours and aggregation pheromones for attracting small hive beetle might also play a role as shown for *Carpophilus lugubris* (Lin et al., 1992). However, in another nitidulid beetle, *Prostephanus truncatus*, the absence of upwind flight to food volatiles, or any synergism between pheromone and food volatiles suggests that the male-produced pheromone is the only known semiochemical for long-range host finding (Fadamiro et al., 1998). More research is needed to identify and evaluate the potential impact of different compounds such as aggregation pheromones, food volatiles, or any synergism between pheromone and food volatiles on the short and long-range dispersal and host selection of *A. tumida*.

## 5. Alternative hosts (Fig. 2)

Bumblebees do not occur in sub-Saharan Africa but are native to North America (Michener, 2000). Recent laboratory studies indicate that a host shift of *A. tumida* to bumblebees may occur in its new range (Stanghellini et al., 2000; Armbrose et al., 2000). Bumblebee colonies, *Bombus impatiens*, artificially infested with small hive beetles had fewer live bees, more dead adult bees and greater comb damage than controls (Stanghellini et al., 2000; Armbrose et al., 2000). The bees did not show any aggression either towards the adult beetles or to the larvae (Stanghellini et al., 2000), indicating a lack of behavioural resistance. However, nest defence of bumblebees against small intruders has been described and species vary in their reactions (Michener, 1974). For example, *B. atratus* (Sakagami et al., 1967; Sakagami, 1976) and *B. (Robustobombus) melaleucus* (Hoffmann et al., 2003) tend to be more aggressive than other species.

Small intruders are stung and carried outside by bumblebee workers (Michener, 1974) similar to the jettisoning behaviour of honeybees (Lundie, 1940; Schmolke, 1974). Moreover, social encapsulation of small intruders in wax or propolis confinements has also been described (Michener, 1974), but it is not known whether live intruders are also encapsulated. Colony defensiveness seems to be correlated with colony size, with smaller colonies being less defensive (Michener, 1974). Therefore, there might be considerable variance between bumblebee species and nests also with respect to small hive beetle resistance. More detailed studies on a variety of species and on a range of colony sizes are required to evaluate the susceptibility of bumblebees towards small hive beetle infestations.

Nevertheless, a new generation of small hive beetle was produced from adult to adult in each of the *B. impatiens* units which were held on soil (Stanghellini et al., 2000; Armbrose et al., 2000). Therefore, small hive beetles are in principle able to complete an entire life cycle in association with bumblebees. However, it is unclear whether adult beetles are able to find bumblebee colonies in the wild. We suggest bait trap studies (Elzen et al., 2000c) and studies of adjacent honeybee and bumblebee colonies (Whitfield and Cameron, 1993) to evaluate whether bumblebee colonies are attractive for adult beetles.

## 6. Discussion

The introduction of *A. tumida* in areas as far from its endemic range as North America and Australia illustrates the high anthropogenic transportation potential of this parasite. However, it appears difficult to trace back the actual transport mechanism into specific areas, especially if introduction is only detected after secondary spread. The small hive beetle is thought to have been transported to the USA aboard ship in 1996 (Wenning, 2001), because it first appeared near a major harbour (Hood, 2000). Successful alternate feeding on fruits suggests that the beetles may be transported on fruits (Ellis et al., 2002c). However, fruit shipments are usually subject to intensive quarantine and small hive beetles have not yet been detected in such shipments. It seems plausible to assume that the import of package bees, honeybee and bumblebee colonies, queens, hive equipment and or even soil (Brown et al., 2002) constitute potential invasion pathways of the small hive beetle. Nevertheless, at the current state of evidence it is still unclear how small hive beetles actually reached Australia and the US. The migratory nature of beekeeping is probably the greatest contributor of small hive beetle transmission within its new ranges (US: Delaplane, 1998; Australia: M Duncan, personal communication). Nevertheless, natural dispersal mechanisms may also considerably contribute. Thus, the small hive beetle most likely constitutes an example of a biological invasion that involves multiple dispersal processes such as long-range transport, migratory beekeeping and natural dispersal abilities. The pattern of small hive beetle spread is probably dominated by long-distance jump dispersal as in Argentine ants (Suarez et al., 2001). Detailed data and comparative studies on the invasion dynamics in the new ranges seem necessary to evaluate the contribution of individual processes to the spread of *A. tumida* and to improve the predictive power of future modelling efforts. Such studies are however still lacking.

The environmental requirements of the small hive beetle are readily met within a large range of the distribution of *A. mellifera* both in terms of survival and completion of its life cycle (Brown et al., 2002). Indeed, small hive beetles can establish populations in temperate regions (e.g. Ohio, Evans et al., 2003) due to their overwintering capacity. The requirement for lighter sandy soils during pupation can also be met within many areas (Brown et al., 2002). Thus, it is likely that, if introduced, the small hive beetle would swiftly become established in most of the range of the Western honeybee with major implications for apiculture. Also, the ability of small hive beetles to heavily infest the protected environment of honey houses may allow severe economic damage in any location worldwide.

A variety of control methods has been developed and discussed (e.g. Baxter et al., 1999; Ellis et al., 2002a; Elzen et al., 1999b; Hood, 1999b; Hood, 2000; Lafrèniere, 2000; Mostafa and Williams, 2000; Park et al., 2002 among others). They range from prevention through sanitation in apiaries and honey houses (Thomas, 1998), over trapping of larvae using fluorescent lights and adult beetles using nucleus hives (Sanford, 1998; Elzen et al., 1999b) to chemical control in the hive (Elzen et al., 1999b) and insecticide treatment of soil (Baxter et al., 1999; Lafrèniere, 2000). However, as in the case of *Varroa*, resistant strains may develop (Spreafico et al., 2001). Thus, the development of sustainable control methods seems desirable to avoid resistance to chemical treatments in the long run (e.g. pheromone trapping, biological control agents or breeding of resistant strains). In general, small hive beetle control should not overlook the control of other honeybee pests and *vice versa*. For example, grease/antibiotic patties used to control American foulbrood seem to worsen small hive beetle infestations because larvae readily accept the patties as food (Westervelt et al., 2001; Elzen et al., 2002).

The development of efficient control methods is likely for managed honeybees sooner or later, but appears difficult for wild bee populations. Thus, once established, small hive beetles may also pose a serious threat to wild bee populations with potential drastic ecological consequences. Several nitidulid species have a close association with social insects other than honeybees (Morse, 1998), e.g. Lundie (1940), reported about *Brachypeplus* species (*B. autitus*, *B. planus*, and *B. meyricki*) associated with stingless bees of the genus *Trigona*. Given that bumblebees may actually serve as an alternative host in nature and resistance is low (Stanghellini et al., 2000; Armbrose et al., 2000), small hive beetles may cause severe damage to bumblebee populations. Other bee species may also serve as alternative hosts (e.g. *Apis cerana*). Indeed, the reciprocal host shift of parasitic *Varroa* mites from *A. cerana* to *A. mellifera* has already proven to cause a global problem for apiculture and wild *A. mellifera* populations. However, there are differences when comparing *Varroa* and the small hive beetle. In case of *Varroa* an interspecific host shift has occurred between two species showing clear differences in their behaviour (e.g. hygienic behaviour) and nesting biology (e.g. drone cell construction). In case of the small hive beetle an intraspecific host shift has occurred between sympatric and non-sympatric host subspecies. Thus, rather quantitative differences seem to trigger resistance to this parasite (see Elzen et al., 2001) and breeding programs towards resistance may be more rewarding than in the case of *Varroa*.

Several potential reasons may be responsible for the difference between pest severity in Africa, in the US and in Australia:

**1) Different beekeeping techniques:** There are differences in beekeeping practices which may contribute to the damage caused by the small hive beetle. For example, African beekeepers tend to minimize the amount of honey stored in hives. However, no comparative data is available yet.

**2) Differences between introduced small hive beetle populations:** The Australian small hive beetle populations seem to be genetically different from those in the US and so may not cause the same problems as in the US (D Anderson, unpublished data). In this case one might expect a different beetle behaviour and/or reproductive potential in the US and Australia. Against this, small hive beetle behaviour appears to be very similar in the US and in Africa (Elzen et al., 2000b). Moreover, the small hive beetles found in North America are genetically very similar to beetles from Southern Africa (Evans et al., 2003). Thus, differences between beetle populations may explain divergent pest severity between Australia and the US but not between the US and Africa. However, detailed comparative studies on the behaviour and/or reproductive potential of small hive beetles in Africa and its new ranges are lacking.

**3) Enemy release hypothesis:** Invasive species such as the small hive beetle might have escaped from important parasites, predators or pathogens that limit populations in their native ranges (Keane and Crawley, 2002) and release from such enemies has been implicated in the success of invasive species (Huffaker and Messenger, 1997). Indeed, an average invasive species has more parasites in its native region than in the new range (Torchin et al, 2003). This point is entirely unclear because neither small hive beetle parasites nor predators or pathogens have been found yet.

**4) Climatic differences:** The number of beetle generations per year in temperate regions is likely to be smaller than in South Africa (five generations; Lundie, 1940) because temperature has an effect on beetle developmental time (Schmolke 1974; Neumann et al. 2001a). Thus, pest severity may be less too due to smaller beetle population sizes (see **6)** below). However, this has not been investigated yet. Very dry conditions may also limit beetle reproduction in its new ranges (Australia: M Duncan, personal communication; Egypt: AM Mostafa, personal communication). Thus, similar to Africa, where successful reproduction of the small hive beetle can be enhanced by hot and humid conditions (Swart et al., 2001), climatic differences may play a key role in damage because small hive beetle population growth is smaller (see **6)** below). This point may explain differences in pest severity between the US and Australia/Egypt but not between the US and Africa. However, the underlying reasons are still unclear and need further investigation.

**5) Different strains of honeybees:** Differences in African vs. European honeybee subspecies are numerous (see above). Therefore, we regard it as most likely that this is the major factor contributing to the different impact of small hive beetles on populations of African honeybees in Africa and European honeybees in the US. However, the bees which are apparently less affected in Australia are *A. m. ligustica* (M Duncan, personal communication), one of the predominant subspecies in the US (Schiff and Sheppard, 1995). Unless there are differences between Australian and US *A. m. ligustica* strains with respect to beetle resistance, this points in the direction that other factors are important for the apparent differences in beetle damage between Australia and the US.

In the US, the invasion of the Africanized honeybee may prove to be an advantage at least with regard to small hive beetle resistance because Africanized bees are likely to be resistant towards the small hive beetle. However, to our knowledge, the small hive beetle is not yet found in South America. Thus, Africanized bees have not encountered this parasite since their introduction to South America in 1956 (Kerr, 1957) and some resistance might have been lost.

Managed European honeybee populations are under strong selection pressures due to intense breeding over the past centuries. Traits such as absconding, aggression and abundant propolis usage have been selected against, which are undesirable from a beekeeping perspective but may trigger small hive beetle resistance. Therefore, the low resistance of managed European honeybees may not necessarily reflect actual susceptibility of wild European honeybee populations. It is possible that the susceptibility of managed European honeybees in the US, is a result of efficient breeding efforts in the past. This hypothesis remains to be tested with feral/wild colonies of European honeybee subspecies.

**6) Different densities of small hive beetle populations:** One potential reason, why Australia have had little small hive beetle damage so far, might be that it has only been there long enough to establish moderate numbers of adults in hives (M Duncan, personal communication). So, it might well be that the beetle populations will need some time to build up to a certain size before serious damage occurs (D Anderson [CSIRO], M Duncan, personal communications). In this case one might expect more severe problems in Australia in the nearby future when small hive beetle populations have build up. The higher mobility of African bees, in particular seasonal migration (see above), may also contribute to smaller parasite population sizes and consequent minor pest severity in Africa.

We conclude that at the current state of evidence it appears premature to decide which of these factors is important for the differences between beetle damage in the US and Australia. However, the differences between the US and Africa most likely result from behavioural differences between African and European subspecies, unless massive host shifts occur in the new range or unless important small hive beetle pests/parasites have not been identified yet. The known behaviours, which are probably involved in small hive beetle resistance of African bees, such as absconding (Hepburn et al., 1999), aggression (Elzen et al., 2001) and social encapsulation (Neumann et al., 2001b) also occur in susceptible populations of European honeybees (Ellis et al., 2003a,b,c). Therefore, it is obvious that the susceptibility of European bees is not due to a lack of behavioural resistance mechanisms. Resistance of African bees is probably due to quantitative differences in a series of behaviours such as absconding, aggression, removal of beetle eggs and larvae and social encapsulation. It is likely that general adaptations to higher predation and parasite loads are responsible for the apparent resistance of African honeybees rather than specific adaptations towards the small hive beetle. For example, African bees are in general more aggressive than European subspecies (Hepburn and Radloff, 1998). However, many of the behavioural mechanisms have only been qualitatively described, have not been tested in comparative studies between African and European bees or may even simply be unknown. Moreover, very important basic features like the number of beetle offspring per colony in the US and Africa and levels of infestation of African and European host populations have not been rigorously quantified yet. Therefore, more comparative studies between parasite and host populations in Africa, Australia and in the US are urgently required. In general, we still have a fragmentary knowledge of the small hive beetle, creating demand for more research in all areas of its biology. Joint research efforts of the scientific community seem necessary in the nearby future, because *A. tumida* has the potential to become a serious global problem for apiculture and natural bee populations.

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## References

Adjare SO (1990) Beekeeping in Africa, FAO, Rome, Italy.

Ambrose JT, Stanghellini MS, Hopkins DI (2000) A scientific note on the threat of small hive beetles (*Aethina tumida* Murray) to bumble bees (*Bombus* sp.) colonies in the United States, Apidologie 31, 455-456.

Anderson RH, Buys B, Johannsmeier MF (1983) Beekeeping in South Africa, Bulletin No. 394, Department of Agriculture, Pretoria, South Africa.

Aurelien RF (adapté Collart, E) (1950) Manuel d'Apiculture, Service de l'Agriculture, Léopoldville (Kinshasha), Democratic Republic of Congo.

Baxter JR, Elzen PJ, Westervelt D, Causey D, Randall C, Eischen FA, Wilson WT (1999) Control of the small hive beetle, *Aethina tumida*, in package bees, Am. Bee J. 139, 792-793.

Brown MA, Thompson HM, Brew M (2002) Risks to UK beekeeping from the parasitic mite *Tropilaelaps clareae* and the small hive beetle, *Aethina tumida*, Bee World 83, 151-164.

Castagné JB (1983) L'apiculture au Congo-Brazzaville, Bulletin Technique Apicole 10, 197-208.

Clauss B (1992) Bees and Beekeeping in the north western Province of Zambia, Mission Press, Ndola, Zambia.

Corkins CL (1930) The metabolism of the honey bee colony during winter, Bull. Wyoming Agricult. Exp. Station 175, 1-54.

Delaplane KS (1998) The small hive beetle, *Aethina tumida*, in the Southeast, Am. Bee J. 138, 884-885.

Dubois L, Collart E (1950) L'Apiculture au Congo Belge et au Ruanda-Urundi, Ministère des Colonies. Direction de L'Agriculture, de l'Élevage et de la Colonisation, Bruxelles, Belgium.

Eischen FA, Baxter JR, Elzen PJ, Westervelt D, Wilson W (1998) Is the small hive beetle a serious pest of U.S. honey bees? Am. Bee J. 138, 882-883.

Eischen FA, Westervelt D, Randall C (1999) Does the small hive beetle have alternate food sources? Am. Bee J. 139, 125.

Ellis JD, Delaplane KS, Hepburn HR, Elzen PJ (2002a) Controlling small hive beetles (*Aethina tumida* Murray) in honey bee (*Apis mellifera*) colonies using a modified hive entrance, Am. Bee J. 142, 288-290.

Ellis JD, Delaplane KS, Hood WM (2002b) Small hive beetle (*Aethina tumida* Murray) weight, gross biometry, and sex proportion at three locations in the south-eastern United States, Am. Bee J. 142, 520-522.

Ellis JD, Neumann P, Hepburn HR, Elzen PJ (2002c) Longevity and reproductive success of *Aethina tumida* (Coleoptera: Nitidulidae) fed different natural diets, J. Econom. Entomol. 95, 902-907.

Ellis JD, Pirk CWW, Hepburn HR, Kastberger G, Elzen PJ (2002d) Small hive beetles survive in honeybee prisons by behavioural mimicry, Naturwissenschaften 89, 326-328.

Ellis JD, Hepburn HR, Delaplane K, Neumann P, Elzen PJ (2003a) The effects of adult *Aethina tumida* (Coleoptera: Nitidulidae) on nests and foraging activity of African and European honey bees (*Apis mellifera*), Apidologie 34, in press.

Ellis JD, Holland AJ, Hepburn HR, Neumann P, Elzen PJ (2003b) African and European honey bee (*Apis mellifera*) guard age and duration of small hive beetle (*Aethina tumida* Murray, Coleoptera: Nitidulidae) prison guarding, J. Apic. Res. 42, in press.

Ellis JD, Hepburn HR, Ellis AM, Elzen PJ (2003c) Social encapsulation of small hive beetles (*Aethina tumida* Murray) by European honeybees (*Apis mellifera* L.), Insectes Soc., in press.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Cutts L, Wilson W, Eischen FA, Delaplane KS, Hopkins DI (1999a) Status of the small hive beetle in the U.S., Bee Culture 127, 28-29.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Delaplane KS, Cutts L., Wilson WT (1999b) Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae) attacking European honey bees in the Western hemisphere, Apidologie 30, 361-366.

Elzen PJ, Baxter JR, Allsopp M. (2000a) Small hive beetle experiences in South Africa, Bee Culture 128, 56.

Elzen PJ, Baxter JR, Neumann P, Solbrig AJ, Pirk CWW, Hoffmann WRE, Hepburn HR (2000b) Observations on the small hive beetle in South Africa, Am. Bee J. 140, 304.

Elzen PJ, Baxter JR, Westervelt D, Randall C, Wilson WT (2000c) A scientific note on observations of the small hive beetle, *Aethina tumida* Murray (Coleoptera, Nitidulidae) in Florida, USA, Apidologie 31, 593-594.

Elzen PJ, Baxter JR, Neumann P, Solbrig AJ, Pirk CWW, Hepburn HR, Westervelt D, Randall C (2001) Behavior of African and European subspecies of *Apis mellifera* toward the small hive beetle, *Aethina tumida*, J. Apic. Res. 40, 40-41.

Elzen PJ, Westervelt D, Ellis JD, Hepburn HR, Neumann P (2002) Method of application of tylosin, an antibiotic for American foulbrood control, with effects on small hive beetle (Coleoptera: Nitidulidae) populations, J. Econom. Entomol. 95, 1119-1122.

Evans JD, Pettis J, Shimanuki H (2000) Mitochondrial DNA relationships in an emergent pest of honey bees: *Aethina tumida* (Coleoptera: Nitidulidae) from the United States and Africa, Ann. Entomol. Soc. Am. 93, 415-420.

Evans JD, Pettis J, Hood WM, Shimanuki H (2003) Tracking an invasive honey bee pest: mitochondrial DNA variation in North American small hive beetles, Apidologie 34, 103-109.

Fadamiro HY, Gudrups I, Hodges (1998) Upwind flight of *Prostephanus truncatus* is mediated by aggregation pheromone but not food volatiles, J. Stored Products Res. 34, 151-158.

Flügge AM (2001) General physiological investigations of the small hive beetle, *Aethina tumida* M., a parasite of honeybees *Apis mellifera*, MSc thesis, FU Berlin, Germany.

Gates BN (1914) The temperature of the honeybee colony, Bull. USDA 96, 1-29.

Gorenz AM (1964) A start in bee-keeping in Ghana, Ghana Farmer 8, 108-114.

Habeck DH (2002) Nitidulidae. In: American Beetles, Volume 2, (Arnett RH, Thomas MC, Skelley PE and Frank JH eds.), CRC Press, Boca Raton, pp. 311-315.

Hänfling B, Kollmann J (2002) An evolutionary perspective of biological invasions, Trends Ecol. Evol. 17, 545-546.

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin, Heidelberg, New York.

Hepburn HR, Reece, Neumann P, Moritz RFA, Radloff SE (1999) Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of reproduction, Insectes Soc. 46, 323-326.

Hoffmann WRE, Torres A, Neumann P (2003) A scientific note on the nest and colony development of the Neotropical bumble bee *Bombus (Robustobombus) melaleucus* (Handlirsch 1888), Apidologie 34, in reviews.

Hood, WM (1999a) Small hive beetle, Clemson University Entomology Information Series; Clemenson, South Carolina, USA: EIIS/AP-2 (revised), 4pp.

Hood, WM (1999b) Small hive beetle, Clemson University Cooperative Extension Service, http://entweb.clemson.edu/caps/pestalrt/smhvbt/smhvbt.htm.

Hood, WM (2000) Overview of the small hive beetle *Aethina tumida* in North America, Bee World 81, 129-137.

Hood, WM (2001) Small hive beetle, *Aethina tumida*, control in North America, In: Abstracts of the 37th International Apicultural Congress, p40.

Huffaker C, Messenger P (1997) Theory and practice of biological control, Environ. Entomol. 26, 373.

James DG, Faulder RJ, Vogele B, Moore CJ (2000) Pheromone-trapping of *Carpophilus* spp. (Coleoptera: Nitidulidae) in stone fruit orchards near Gosford, New South Wales: Fauna, seasonality and effect of insecticides, Austr. J. Entomol. 39, 310-315.

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis, Trends Ecol. Evol. 17, 164-170.

Kerr WE (1957) lntroducao de abelhas africanas no Brasil, Brasil Apicola 3, 211-213.

Korst PJAM, Velthuis HHW (1982) The nature of trophallaxis in honeybees, Insectes Soc. 29, 209-221.

Lafrèniere R (2000) The small hive beetle: *Aethina tumida* Murray (Coleoptera: Nitidulidae), http://www.honeycouncil.ca/shbrevw.html.

Lepissier J (1968) L'Apiculture en Republique Centrafricaine, Ministère du Développement, Bangui, Central African Republic.

Lin HC, Phelan PL, Bartelt RJ (1992) Synergism between synthetic food odours and the aggregation pheromone for attracting *Carpophilus lugubris* in the field, Env. Entomol. 21, 156-159.

Lundie AE (1940) The small hive beetle *Aethina tumida*, Science Bulletin 220, Dep. Agr. Forestry, Government Printer, Pretoria, South Africa.

Lundie AE (1951) The principal diseases and enemies of honey bees, S. Afr. Bee J. 26, 15-16.

Lundie AE (1952a) The principal diseases and enemies of honey bees, S. Afr. Bee J. 27, 9.

Lundie AE (1952b) The principal diseases and enemies of honey bees, S. Afr. Bee J. 27, 13-15.

May AF (1969) Beekeeping, Haum, Cape Town, South Africa.

Michener CD (1974) The social behaviour of the bees, Harvard University Press. Cambridge.

Michener CD (2000) The bees of the world, The Johns Hopkins University Press, Baltimore Maryland.

Minister for Agriculture (2002) Small hive beetle confirmed in Sydney bee apiary: Amery, Agricult. media releases 31 October 2002, http://minister.agric.nsw.gov.au/reader/16058.

Moritz RFA, Kryger P (1994) Self organisation of circadian rhythms in groups of honeybees (*Apis mellifera* L.), Behav. Ecol. Sociobiol. 34, 211-215.

Mostafa AM, Williams RN (2000) New record of the small hive beetle in Egypt and notes on its distribution and control, Bee World 83, 99-108.

Morse R (1998) Nitidulids, Bee Culture 126, 17.

Murray A (1867) List of Coleoptera received from Old Calabar, Ann. Magazine Nat. Hist., London 19, 167-179.

Mutsaers M (1991) Bees in their natural environment in South-western Nigeria, Nigerian Field 56, 3-18.

Nardi JB, Dowd PF, Bartelt RJ (1996) Fine structure of cells specialized for secretion of aggregation pheromone in a nitidulid beetle *Carpophilus freemani* (Coleoptera: Nitidulidae), Tissue & Cell 28, 43-52.

N'diaye M (1974) L'apiculture au Sénégal. PhD thesis, University of Dakar, Dakar, Senegal.

Neumann P, Härtel S (2003) Removal of small hive beetle (*Aethina tumida* Murray) eggs and larvae by African honeybee colonies (*Apis mellifera scutellata* Lepeletier), Apidologie 34, in press.

Neumann P, Pirk CWW, Hepburn HR, Elzen PJ, Baxter JR (2001a) Laboratory rearing of small hive beetles *Aethina tumida* (Coleoptera: Nitidulidae), J. Apic. Res. 40, 111-112.

Neumann P, Pirk CWW, Hepburn HR, Solbrig AJ, Ratnieks FLW, Elzen PJ, Baxter JR (2001b) Social encapsulation of beetle parasites by Cape honeybee colonies (*Apis mellifera capensis* Esch.), Naturwissenschaften 88, 214-216.

Ntenga G (1970) Annual report of the beekeeping section for the year 1970, Ministry of Natural Resources, Dar-es-Salaam, Tanzania.

Ntenga GM, Mugongo BY (1991) Honey Hunters and Beekeepers, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Park AL, Pettis JS, Caron DM (2002) Use of household products in the control of small hive beetle larvae and salvage of treated combs, Am. Bee J. 142, 439-442.

Pettis J., Shimanuki H (2000) Observations on the small hive beetle, *Aethina tumida*, Murray, in the United States, Am. Bee J. 140, 152-155.

Petroski RJ, Bartelt RJ, Vetter RS (1994) Male-produced aggregation pheromone of *Carpophilus obsoletus* (Coleoptera, Nitidulidae), J. Chem. Ecol. 20, 1483-1493.

Phokedi KM (1985) Apiculture and its problems in Botswana, In: Proceedings of the 3rd International Conference on Apiculture in Tropical Climates, Nairobi, Kenya, pp 64-65.

Ribbands CR (1953) The behaviour and social life of honeybees, Bee Research Association Limited, London, UK.

Roberts E (1971) A survey of beekeeping in Uganda, Bee World 52, 57-67.

Rosário Nunes JF, Tordo GC (1960) Prospecções e ensaios experimentais apicolas em Angola, Junta de Investigacoes do Ultramar, Lisboa, Portugal.

Ruttner F (1986) Geographical variation and classification, In: Rinderer TE (ed.) Bee genetics and breeding. Academic Press Orlando, Florida, pp. 23-56.

Sakagami SF (1976) Specific differences in the bionomic characters of bumblebees. A comparative review, J. Fac. Sci. Hokkaido Univ. Zool. 20, 390-447.

Sakagami SF, Akahira Y, Zucchi R (1967) Nest architecture and brood development in a neotropical bumblebee, *Bombus atratus*, Insectes Soc. 14, 389-413.

Sanford MT (1998) *Aethina tumida*: a new beehive pest in the Western hemisphere, Apis 16, 1-5.

Sanford MT (2002) University of Florida Department of Entomology and Nematology: Featured Creatures, http://creatures.ifas.ufl.edu/misc/bees/small_hive_beetle.htm.

Schiff NM, Sheppard WS (1995) Genetic Analysis of Commercial Honey Bees (Hymenoptera: Apidae) from the Southeastern United States, J. Econ. Entomol. 88, 1216–1220.

Schmolke MD (1974) A study of *Aethina tumida*: the small Hive Beetle, Project Report, University of Rhodesia, pp. 178.

Smart LE, Blight MM (2000) Response of the pollen beetle, *Meligethes aneus*, to traps baited with volatiles from oilseed rape, *Brassica napus*, J. Chem. Ecol. 26, 1051-1064.

Smith FG (1960) Beekeeping in the Tropics, Longmans, London, UK.

Solbrig AJ (2001) Interactions between the South African honeybee *Apis mellifera capensis* Esch. and the small hive beetle *Aethina tumida*, MSc thesis FU Berlin, Germany.

Spreafico M, Eördegh FR, Bernadinelli I, Colombo M (2001) First detection of strains of *Varroa destructor* resistant to coumaphos. Results of the laboratory test trials, Apidologie 32, 49-55.

Stanghellini MS, Armbrose JT, Hopkins DI (2000) Bumble bee colonies as potential alternative hosts for the small hive beetle (*Aethina tumida* Murray), Am. Bee J. 140, 71-75.

Suarez AV, Holway DA, Case TJ (2001) Patterns of spread in biological invasions dominated by long-distance jump dispersal: insights from Argentine ants, Proc. Nat. Acad. Sci. USA 89, 1095-1100.

Svensson B (1984) Beekeeping in the Republic of Guiné-Bissau and the Possibilities for its Modernization, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Swart JD, Johannsmeier MF, Tribe GD, Kryger P (2001) Diseases and pests of honeybees, In: Johannsmeier M.F. (ed) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp 198-222.

Thomas MC (1998) Florida pest alert - the small hive beetle, Am. Bee J. 138, 565.

Torchin ME, Lafferty KD, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites, Nature, 421, 628-630.

Tribe GD (2000) A migrating swarm of small hive beetles (*Aethina tumida* Murray), S. Afr. Bee J. 72, 121-122.

Walter AR (1939a) Journal correspondence, S. Afr. Bee J. 13, 4-6.

Walter AR (1939b) Correspondence to AE Lundie, Government Apiary Library, Pretoria, South Africa.

Wenning CJ (2001) Spread and threat of the small hive beetle, Am. Bee J. 141, 640-643.

Westervelt D, Causey D, Neumann P, Ellis JD, Hepburn HR (2001) Grease patties worsen small hive beetle infestations, Am. Bee J. 141, 775.

Whitfield NB, Cameron SA (1993) Comparative notes on hymenopteran parasitoids in bumble bee and honey bee colonies (Hymenoptera: Apidae) reared adjacently, Ent. News 104, 240-248.

Wolff M, Uribe A, Ortiz A, Duque P (2001) A preliminary study of forensic entomology in Medellin, Colombia, Forensic Sci. Internat. 120, 53-59.

**Figure 1:** Records of the small hive beetle in Africa (March 2003): 1) South Africa: Walter (1939a,b); Lundie (1940, 1951, 1952a,b); May (1969); Anderson et al. (1983); 2) Botswana: Phokedi (1985); 3) Zimbabwe: Mostafa and Williams (2000); 4) Zambia: Clauss (1992); 5) Angola: Rosário Nunes and Tordo 1960; 6) Tanzania: Smith (1960); Ntenga (1970); Ntenga and Mugongo (1991); 7) Democratic Republic of Congo: Aurelien (1950); Dubois & Collart (1950); 8) Congo Republic: Castagné (1983); 9) Uganda: Roberts (1971); 10) Kenya: Mostafa and Williams (2000); 11) Ethiopia: Mostafa and Williams (2000); 12) Eritrea: Mostafa and Williams (2000); 13) Central African Republic: Lepissier (1968); 14) Nigeria: Mutsaers (1991); 15) Ghana: Gorenz (1964); Adjare (1990); 16) Guinea Bissau: Svensson (1984); 17) Senegal: N'diaye (1974); 18) Egypt: Mostafa and Williams (2000), probably recently introduced (see chapter 3.2).

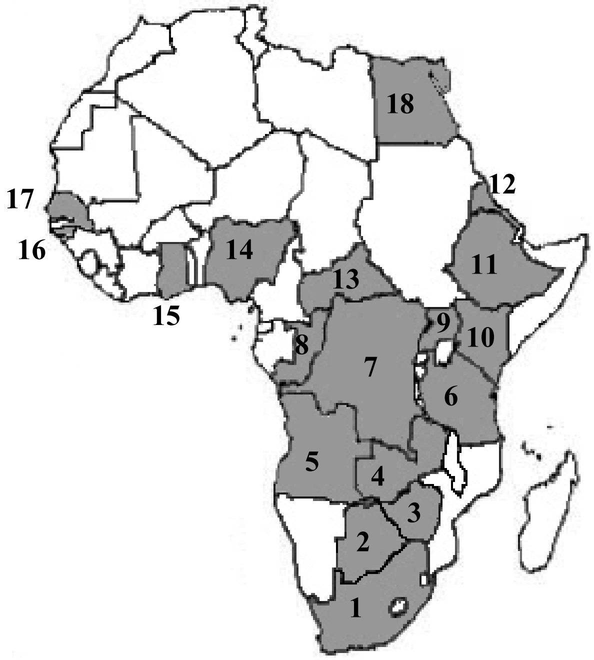


**Figure 2:** Putative life cycle of the small hive beetle (dotted lines = rare events or unclear; dashed lines and dashed box = colonies of European honeybee subspecies only).

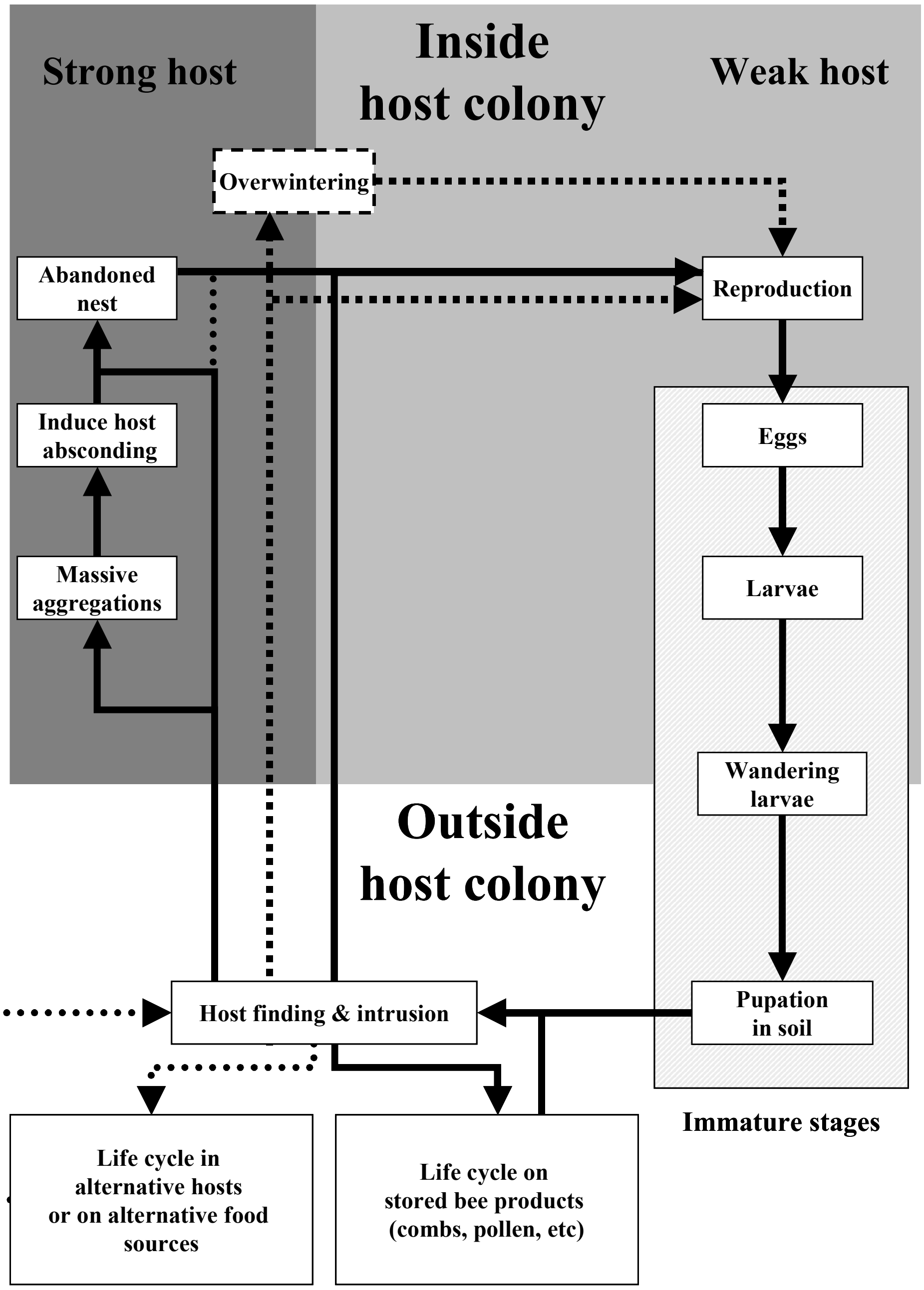
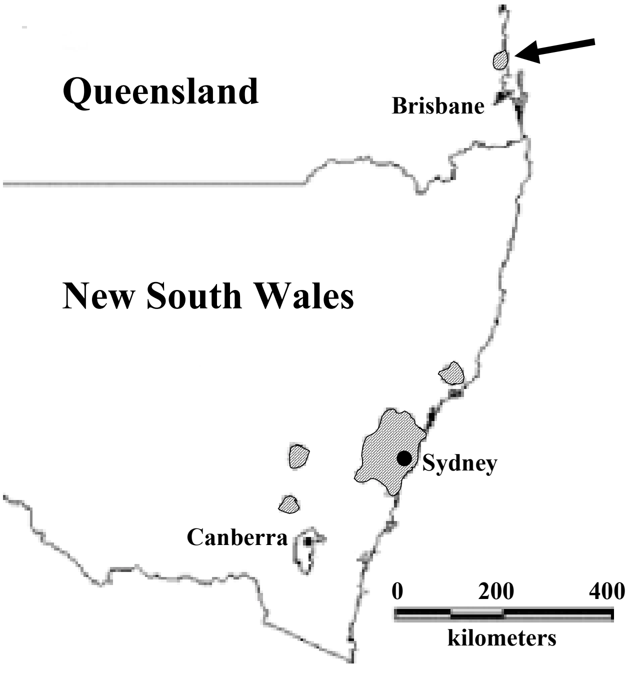


**Figure 3:** Current distribution of the small hive beetle in the USA (March 2003; J Pettis [USDA], personal communication). It has been reported in 29 states so far (year reported in brackets): 1) Florida (1998), 2) South Carolina (1998), 3) Georgia (1998), 4) North Carolina (1998), 5) New Jersey (1999), 6) Maine (1999), 7) Pennsylvania (1999), 8) Minnesota (1999), 9) Iowa (1999), 10) Wisconsin (1999), 11) Massachusetts (1999), 12) Ohio (1999), 13) Michigan (1999), 14) Louisiana (2000), 15) New York (2000), 16) North Dakota (2000), 17) Tennessee (2000), 18) Indiana (2000), 19) Vermont 2000, 20) Maryland (2001), 21) Virginia (2001), 22) Delaware (2001), 23) Illinois (2001), 24) Missouri (2001), 25) Mississippi (2001), 26) Arkansas (2002), 27) Alabama (2002), 28) Kentucky (2002), 29) W. Virginia (2003); dark area = severe damage.

**Figure 4:** Current distribution of the small hive beetle in Australia (March 2003; shaded areas & arrow = small hive beetle infestations; Picture courtesy of P Boland, modified).



# 3 The Cape honeybee (*Apis mellifera capensis* Esch.)

The Cape honeybee, *Apis mellifera capensis*, is native to the Eastern and Western Cape provinces of South Africa (Hepburn and Radloff, 1998) and is characterized by a unique set of traits related to worker reproduction. Honeybee workers don't usually reproduce, but they can activate their ovaries under queenless conditions to parthenogenetically produce haploid male sexuals (=arrhenotoky, Crozier and Pamilo, 1996). As an exception to this rule, laying workers of the Cape honeybee produce diploid female offspring (= thelytoky, Onions, 1912; Moritz and Haberl, 1994: Crozier and Pamilo, 1996). Although the thelytoky is via automictic parthenogenesis following meiosis, recombination through crossing over is rare (Moritz and Haberl, 1994). Thus, a worker's offspring are almost clonal (Moritz and Haberl, 1994) and usually develop into workers and sometimes into queens (Hepburn and Radloff, 1998). *A. m. capensis* workers can develop a so-called pseudoqueen phenotype with both a high ovarial development and a queenlike pheromonal bouquet. Such workers truly resemble queens and can e.g. suppress queen rearing and ovarial development in other workers and induce retinue behaviour in the host (Hepburn and Radloff, 1998; Neumann and Hepburn, 2002). Moreover, worker reproduction in the presence of queen is much more frequent in Cape honeybees than in European honeybees (Moritz et al., 1999). Finally, Cape honeybee workers show a great longevity (3-5 months Velthuis et al. 1990; up to five months and more Tribe and Allsopp 2001b). Therefore, these pseudoqueens have a high reproductive potential and are predisposed for reproductive conflicts. This led to a facultative social parasitic pathway of laying workers as part of the life history of the Cape honeybee (Neumann and Moritz, 2002). This pathway is particularly expressed, when susceptible host colonies of other honeybee subspecies are available. Because the parasitic workers do not participate in normal hive duties (Neumann and Hepburn, 2002), such as brood rearing, an infested colony dwindles down to a few host workers and eventually dies. This social parasitism by Cape honeybee workers has raised considerable attention after the introduction of Cape honeybee colonies from the Western Cape province of South Africa into the area of the neighbouring subspecies *A. m. scutellata* (Allsopp and Crewe, 1993). An estimated number of 100,000 host colonies dies each year, leading to the so-called "capensis calamity" for South African bee keeping enterprises (Allsopp and Crewe, 1993). Over a very large area of *A. m. scutellata* in north-eastern South Africa all parasitic Cape honeybee workers apparently belong to a single so-called pseudo clone (Kryger 2001a,b), because with the exception of a few mutational events all DNA microsatellite loci studied showed a maximum of two alleles indicating that they are progeny of a single founder worker (Kryger 2001a,b, Solignac et al. 2001).

Some important pre-adaptations and ultimate mechanisms of the social parasitism by laying Cape honeybee workers, such as thelytoky and the development of pseudoqueens, are long known. In contrast, several proximate mechanisms of the facultative social parasitic pathway are poorly understood. Likewise, the possibility of the evolution of a queenless social parasitic honeybee has also not been considered yet. Therefore, both laboratory and field studies as well as reviews on Cape honeybees are included in this thesis, to gain further insight into the social parasitic pathway of laying Cape honeybee workers. These papers are addressed in detail in the following chapters.

## Research goals and conclusions:

### 3.1 A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis*

**Published in:** S.E. Radloff, H.R. Hepburn, P. Neumann, R.FA. Moritz, P. Kryger (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis*. Apidologie **33**: 129-137. **Own contribution:** project idea, genetic analysis, manuscript.

Thelytokous parthenogenesis in Cape worker honeybees was used to produce a series of clonal progeny that were reared in three different, queenless arrhenotokous *A. m. scutellata* host colonies. Each individual Cape worker bee was genotyped at four DNA microsatellite loci to verify its clonal status and measured for 36 morphological characters. The clonal workers were then analysed by multivariate analysis to determine the quantitative effects of environment on the morphological characters. This in turn allows the estimation of the natural variation in the phenotypic expression of morphological characters. Coefficients of environmental variation were calculated and the relative stability of the character set was, in decreasing order, body size, forewings, wing venation, hairs and pigmentation.

### 3.2 Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera* L.) colonies

**Published in:** P. Neumann, H.R. Hepburn, S.E. Radloff (2000) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (Apis mellifera L.) colonies, Apidologie **31**: 479-486. **Own contribution:** project idea, data analysis, manuscript.

It was evaluated whether the modes of worker reproduction have an impact on reproductive dominance and on brood cell construction in queenless honeybee colonies in the natural hybrid zone between *A. m. capensis* with thelytokous laying workers and *A. m. scutellata* with arrhenotokous laying workers. Colonies of *A. m. capensis*, *A.*

*m. scutellata* and their natural hybrids were de-queened and de-brooded. The ratio of worker/drone cell construction and the sex of laying worker offspring were determined for 26 colonies. All *A. m. capensis* laying workers were thelytokous and all *A. m. scutellata* arrhenotokous. 42.1% of the hybrid colonies produced only female offspring while none produced only male offspring. This shows a significant advantage for thelytokous laying workers to become reproductively dominant in hybrid colonies. *A. m. capensis* colonies built only worker cells and *A. m. scutellata* only drone cells. Hybrid colonies produced either both cell types or only worker cells according to the mode of laying worker reproduction. In all colonies where laying workers produced male offspring drone cell building was found. The data strongly indicate that the mode of worker reproduction holds important consequences for cell construction and reproductive dominance.

### 3.3 Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): Host finding and resistance of hybrid host colonies

**Published in:** Neumann P, Radloff SE, Moritz RFA, Hepburn HR, Reece SL (2001) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): Host finding and resistance of hybrid host colonies. Behav Ecol **12**: 419-428. **Own contribution:** project idea, experiments, data analysis, manuscript.

Possible host finding and resistance mechanisms of host colonies were studied in the context of social parasitism by Cape honeybee workers. Workers often join neighbouring colonies by drifting but long-range drifting (dispersal) to colonies far away from the maternal nests also rarely occurs. We tested the impact of queenstate and taxon of mother and host colonies on drifting and dispersing of workers and on the hosting of these workers in *A. m. capensis*, *A. m. scutellata* and their natural hybrids. Workers were colony-specific paint-marked and reintroduced into their queenright or queenless mother colonies. After 10 days 579 out of 12034 labelled workers were recaptured in foreign colonies. It was found that drifting and dispersing represent different behaviours, which were differently affected by taxon and queenstate of both mother and host colonies. Hybrid workers drifted more often than *A. m. capensis* and *A. m. scutellata*. However, *A. m. capensis* workers dispersed more often than *A. m. scutellata* and the hybrids combined and *A. m. scutellata* workers also dispersed more frequently than the hybrids. Dispersers from queenright *A. m. capensis* colonies were more often found in queenless host colonies and *vice versa*, indicating active host searching and/or a queenstate-discriminating guarding mechanism. The data show that *A. m. capensis* workers disperse significantly more often than other races of *A. mellifera*, suggesting that dispersing represents a host finding mechanism. The lack of dispersal in hybrids and different hosting mechanisms of foreign workers by hybrid colonies may also be responsible for the stability of the natural hybrid zone between *A. m. capensis* and *A. m. scutellata*.

### 3.4 Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of worker reproduction

**Published in:** Hepburn HR, Reece SL, Neumann P, Moritz RFA, Radloff SE (1999) Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of worker reproduction. Insectes Soc **46**: 323-326. **Own contribution:** project idea, experiments, manuscript.

Absconding frequency and latency for absconding in queenright and queenless honeybee colonies in thelytokous *A. m. capensis*, arrhenotokous *A. m. scutellata* and their natural thelytokous hybrids were investigated. There was no significant difference in frequency of absconding among any of the queenright colonies. Absconding was significantly greater in thelytokous queenless colonies than in the queenless arrhenotokous ones. Latency to absconding did not differ among the three groups of queenright colonies nor between the queenright and queenless colonies of *A. m. capensis* and *A. m. scutellata*. There were significant differences in latency between queenright and queenless hybrids and significant differences in latency among the three groups of queenless colonies. Among queenless colonies, *A. m. capensis* absconded twice as readily as did *A. m. scutellata* and the hybrids were intermediate. After absconding events include the fates of the absconding colony as well as nestmates left behind. One group of orphaned nestmates of *A. m. capensis* amalgamated with another queenright colony. In the case of *A. m. scutellata* either drones were produced or the residual queenless colony was joined by a queenless thelytokous group, subsequently reared a queen and then absconded. Differences in the rate and degree of ovarial development indicate that queenless thelytokous workers have the physiological capacity for reproduction, a trait that contributes to colony fitness.

### 3.5 A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis*).

**Published in:** Neumann P, Pirk CWW, Hepburn HR, Radloff SE (2001) A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis*). Apidologie **32:** 113-114. **Own contribution:** project idea, experiments, data analysis, manuscript.

The spatial distribution and division of labour of workers was studied following a natural merger of two Cape honeybee colonies (A, B). No significant differences in total activity (all tasks/idleness) and mean queen-worker distances of individuals bees were observed between the two worker cohorts A and B before and after merger. However, total activity decreased and queen-worker distances increased after merger for the individual bees of both cohorts. There were significant differences among and between tasks of cohorts A and B before and after merger. While some tasks increased and others decreased, the patterns of changes between cohorts differed. Daily counts of queen-worker distances were significantly different on four occasions before merger but only once 24 hours after merger (data not shown), demonstrating effective cohort integration. Also workers of both cohorts were similarly distributed throughout the nest after merger. On queen removal cohort B workers did not attempt to re-queen but immediately merged with colony A. This may seem puzzling from an evolutionary perspective because the inclusive fitness of queenless workers is zero in the new unit. However, mergers are frequent in tropical honeybees and could be adaptive because workers may gain direct fitness. The lower levels of activity and the immediate increase in colony size after merger probably reduce pro rata survival costs. The origin of merging bees may matter, because task shifts differed in the two cohorts. This might be partially ascribed to age-related division of labour; however, this does not explain the substantial shifts observed both within and between the cohorts before and after merger. Possibly, workers changed tasks as a result of different behavioural thresholds and task specialization. Thus, the possible acquisition of more efficient genetic specialists may also contribute to reduce pro rata costs in the new unit. The task shifts and worker distribution suggest that many bees responded to a different colony environment in the new unit, presumably necessary for social integration.

### 3.6 The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis* Esch.): predisposition for social parasitism?

**Published in:** Neumann P, Radloff SE, Pirk CWW, Hepburn HR (2003) The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis* Esch.): predisposition for social parasitism? Apidologie **34**: in press. **Own contribution:** project idea, experiments, data analysis, manuscript.

The behaviour of drifted Cape honeybee workers was studied. Accidental drifting into neighbouring colonies is one mode of transmission to new host colonies. The behavioural patterns and spatial distributions of drifted Cape honeybee workers differed from those of non-drifted workers of the same age cohort. Drifted workers were significantly more idle and were more often found in areas away from the queen compared to non-drifted workers. The data suggest that drifted Cape honeybee workers may be predisposed for social parasitism in host colonies.

### 3.7 Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits

**Published in:** Pirk CWW, Neumann P, Ratnieks FLW (2003) Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits. Behav Ecol **14:** 347-352. **Own contribution:** project idea, data analysis, manuscript.

Worker policing was studied in queenright colonies of *A. m. capensis* and *A. m. scutellata*. In the Cape honeybee workers lay diploid (female) eggs via thelytoky. In other *A. mellifera* subspecies workers lay haploid (male) eggs via arrhenotoky. When thelytokous worker reproduction occurs, worker policing has no relatedness benefit because workers are equally related to their sister workers' clonal offspring and their mother queen's female offspring. Worker policing in *A. m. capensis* and in the arrhenotokous African honeybee *A. m. scutellata* was evaluated by quantifying the removal rates of worker-laid and queen-laid eggs. Discriminator colonies of both subspecies policed worker-laid eggs of both their own and the other subspecies. The occurrence of worker policing, despite the lack of relatedness benefit, in *A. m. capensis* strongly suggests that worker reproduction is costly to the colony and that policing is maintained because it enhances colony efficiency. In addition, because both subspecies policed each others eggs it is probable that the mechanism used in thelytokous *A. m. capensis* to discriminate between queen-laid and worker laid eggs is the same as in arrhenotokous *A. m. scutellata.*

### 3.8 Spatial differences in worker policing facilitate social parasitism by Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies

**Published in:** Neumann P, Pirk CWW, Hepburn HR, Moritz RFA (2003) Spatial differences in worker policing facilitate social parasitism by Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies. Insectes Soc **50:** 109-113. **Own contribution:** project idea, data analysis, manuscript.

Spatial differences in worker policing were investigated in the context of social parasitism by Cape honeybee workers. Cape honeybee laying workers produce female diploid offspring and are facultative social parasites. In queenright host colonies, such workers have to evade worker policing (removal of worker-laid eggs by other workers) to successfully reproduce. One mechanism seems to be low removal rates of eggs laid by parasitic workers. However, because queenright colonies of other subspecies (e.g. the neighbouring *A. m. scutellata*) are susceptible hosts, social parasitic workers probably also use behavioural tactics to evade policing. Indeed, field observations of infested *A. m. scutellata* host colonies indicate that brood from parasitic workers initially appears away from the queen. The egg removal rates for queen and worker-laid eggs were recorded in the top and bottom boxes of three queenright *A. m. capensis* and *A. m. scutellata* colonies, where the queens were caged in the bottom boxes. The egg removal data show that both subspecies are able to police worker-laid eggs, because more queen-laid eggs remained than worker-laid eggs in the bottom boxes. However, fewer *A. m. capensis* worker-laid eggs remained in the bottom boxes than in the top boxes. Moreover, whereas *A. m. capensis* also polices in the top boxes, no significant differences between the removal rates of worker and queen laid eggs were found in the top boxes of the *A. m. scutellata* colonies. This indicates that worker policing is not always fully effective in *A. m. scutellata* and may explain why this subspecies is so susceptible to infestations. The results also show that worker policing is less likely away from the queen. Therefore, queen evasion by laying social parasitic *A. m. capensis* workers appears to constitute a behavioural tactic to achieve successful reproduction in queenright host colonies.

### 3.9 Egg laying and egg removal by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.)

**Published in:** Pirk CWW, Neumann P, Hepburn HR (2002) Egg laying and egg removal by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.). Apidologie **33:** 203-212. **Own contribution:** project idea, experiments, data analysis, manuscript.

Egg laying and egg removal by workers were studied in queenright Cape honeybee colonies. Queenright *A. m. capensis* colonies exhibit egg laying by workers in periods of both low and high egg removal. To reproduce workers should lay in times of low egg removal to increase survival of their eggs. Were this so, a negative correlation between egg laying and removal would be expected. Egg removal rates for queen and worker-laid eggs and egg laying by workers were tested in queenright colonies. Worker-laid eggs were removed significantly faster than queen-laid eggs; but significant differences in egg laying by workers occurred among colonies. Egg laying and removal are positively correlated and co-dependent. Egg removal appears triggered by the number of worker-laid eggs. Intercolonial variation for laying worker egg number and egg removal rates may explain the phenotypic variation in worker reproduction in queenright Cape honeybee colonies.

### 3.10 Parasitic Cape bees in the northern regions of South Africa: source of the founder population

**Published in:** Neumann P, Radloff SE, Hepburn HR (2002) Parasitic Cape bees in the northern regions of South Africa: source of the founder population. South African Journal of Science **98:** 404-406. **Own contribution:** project idea, experiments, data analysis, manuscript.

The source of the founder population of the parasitic Cape honeybee workers in the northern regions of South Africa was traced using morphometric analysis. Multivariate discriminant analyses of 9 standard morphometric characters of honeybee workers were used to track the origin of a social parasitic pseudo-clone of thelytokous laying workers invading colonies of *A. m. scutellata* in South Africa. Twenty social parasitic workers were sampled from each of two infested *A. m. scutellata* colonies at two distant apiaries (Graskop and Heilbronn, about 390km apart) and compared with data obtained from 80 colonies in four different zones (zone I: thelytokous *A. m. capensis* morphocluster; zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; zone III: thelytokous *A. m. scutellata* morphocluster; zone IV, constituting an arrhenotokous *A. m. scutellata* morphocluster). Thelytokous laying workers naturally occur in zones I-III. Highly significant morphometric differences were found between the four zones. The data support the conclusion that the social parasitic workers belong to the thelytokous *A. m. capensis* morphocluster. It is most likely that the social parasitic workers originated from the heart of the range of the Cape bee in the Western Cape region in zone I. Morphometric analysis makes it feasible to restrict the possible origin of the social parasitic workers from the natural distribution range of thelytoky (±240.000$\text{km}^2$) down to about ±12.000 $\text{km}^2$, which equals a resolution capacity of about 95%.

### 3.11 Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.)

**Published in:** Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). Apidologie **33:** 165-192. **Own contribution:** manuscript.

The literature on the behaviour of Cape honeybees is reviewed. The paper concentrates on the more proximate behavioural mechanisms which may contribute to the facultative social parasitic pathway of laying Cape honeybee workers. Cape honeybee workers show important pre-adaptations for social parasitism and can cause the dwindling colony syndrome of host colonies. Parasitic workers may drift or actively disperse into host colonies. They may also join absconding swarms, which can merge with host colonies. After transmission, parasitic workers have to establish themselves in the host, which is probably promoted by their spatial distribution, their readiness to gain trophallactic dominance and their ability to survive worker-worker aggression. Established parasitic workers have to evade egg removal by other workers in host colonies. The resulting offspring is preferentially fed, can be expected to be highly virulent and may show different behaviour in the course of infestation. It is unknown why and how the host queen is lost. High numbers of parasitic workers are reared until the host colony dies or absconds. This offspring can infest new host colonies, thereby completing the social parasitic life cycle of laying Cape honeybee workers.

### 3.12 The Cape honeybee phenomenon: the evolution of a social parasite in real time?

**Published in:** Neumann P, Moritz RFA (2002) The Cape honeybee phenomenon: the evolution of a social parasite in real time? Behav Ecol Sociobiol **52:** 271-281. **Own contribution:** manuscript.

The literature on the biology of the Cape honeybee and on social parasitism in honeybees is reviewed. This paper concentrates on the Cape honeybee phenomenon in South Africa and on the more ultimate aspects of the social parasitism by *A. m. capensis* laying workers. Honeybee workers, *Apis mellifera*, don't usually reproduce, but can activate their ovaries under queenless conditions to produce male offspring. As an exception to this rule, laying workers of the Cape honeybee, *A. m. capensis*, parthenogenetically produce diploid female offspring, usually developing into workers and occasionally into queens. Some of such workers can develop into pseudoqueens which show high ovarial development and a queenlike pheromonal bouquet. Because there is high genetic variance for these characters, this results in an extreme intracolonial selection. This process is governed by a competition for reproductive dominance among workers leading into a facultative social parasitic reproductive pathway as apart of the life history of the Cape honeybee. *A. m. capensis* workers show an increased potential for invading foreign colonies. Inside of the host colony, parasitic *A. m. capensis* workers produce queenlike pheromones and swiftly activate the ovaries despite the presence of a queen. Eventually they establish themselves as pseudoqueens and replace the host queen. The parasitic worker offspring is preferentially fed by the host workers leading to highly virulent intercastes and thereby completing the social parasitic life cycle of laying *A. m. capensis* workers. Recently, a particularly virulent parasitic strain of *A. m. capensis* workers has invaded the neighbouring subspecies *A. m. scutellata* ("*capensis* calamity"). Because male sexuals are completely lacking in this invasive strain and females reproductives are never reared in infested *A. m. scutellata* host colonies, this results in reproductive isolation of the parasitic clones from the host gene pool. This sets the stage for the evolution of a queenless social parasitic honeybee. The Cape honeybee may therefore constitute a unique subject for studying sympatric speciation of a social parasite.

**References**

Allsopp MH, Crewe R (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. Am Bee J 133, 121-123.

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford.

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin Heidelberg New York.

Kryger P (2001a) The Capensis pseudo-clone, a social parasite of African honey bees. In: Proceedings of the 2001 Berlin Meeting of the European Section of IUSSI, Sept. 25-29, p 208.

Kryger P (2001b) The pseudo-clone of *Apis mellifera capensis* – an obligate social parasite in honeybees. In: Proceedings of the XXXVII International Apicultural Congress, Durban South Africa, p 33.

Moritz RFA, Haberl M (1994) Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). Heredity 73, 98-102.

Moritz RFA, Kryger P, Allsopp M (1999) Lack of worker policing in the Cape Honeybee (*Apis mellifera capensis*). Behaviour 136, 1079-1092.

Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). Apidologie 33, 165-192.

Neumann P., Moritz R.F.A. (2002) The Cape honeybee phenomenon: the evolution of a social parasite in real time? Behav. Ecol. Sociobiol. 52, 271-281.

Onions GW (1912) South African "fertile-worker bees". S Afr Agric J 1, 720-728.

Solignac M, Kryger P, Allsopp M, Mougel F, Koeniger N, Baudry E, Cornuet JM, 2001. Thelytoky in Cape honey bees: a genomic perspective. In: Proc of the 2001 Berlin Meeting of the European sections of IUSSI; Sept. 25-29, 2001, Berlin, Germany, p. 147.

Velthuis HHW, Ruttner F, Crewe RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: Engels W (ed) Social Insects. Springer Verlag, Berlin Heidelberg New York, pp 231-243.

# 3.1 A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis*

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**Abstract -** Thelytokous parthenogenesis in Cape worker honeybees, *Apis mellifera capensis*, was used to produce a series of clonal progeny that were reared in three different, queenless arrhenotokous *A. m. scutellata* host colonies. Each individual Cape worker bee was genotyped at 4 DNA microsatellite loci to verify its clonal status and measured for 36 morphological characters. The clonal workers bees, all of the same thelytokous matriline, were then analysed by multivariate analysis to determine the quantitative effects of environment on the morphological characters. This in turn allows the estimation of the natural variation in the phenotypic expression of morphological characters. Coefficients of environmental variation were calculated and the relative stability of the character set was, in decreasing order, body size, forewings, wing venation, hairs and pigmentation.

**Key words:** *Apis mellifera capensis* / clone / honeybee / morphometrics / environmental variance / thelytoky

## 1. INTRODUCTION

Multivariate analyses of morphometric characters are used to delimit variation in natural populations of honeybees (*Apis mellifera* L.). The accuracy and predictive value of these procedures depend on measurement quality as well as the quantitative relationship between the genotype and phenotype as modified by environmental effects (Falconer, 1989). The susceptibility of morphological characters to effects of environment was recognised long ago (Alpatov, 1929) and remains under scrutiny (Daly et al., 1991; Nazzi, 1992). Environmentally induced variation vis-a-vis genetic control of character expression are not easily separated (Thorpe, 1976).

Moreover, the heritability of morphological characters is rather variable (Rinderer, 1977; Rinderer et al., 1990; Poklukar and Kezic, 1994), as confirmed by both sib analysis as well as parent offspring regression methods (Moritz and Klepsch, 1985; Oldroyd et al., 1991). Of particular interest in this context, Oldroyd et al. (1991) demonstrated that apparent values of heritability actually declined when bees were raised under cross-fostered conditions.

The parent-offspring method of analysis, based on thelytokous parthenogenesis (production of diploid females by unmated laying workers), is a particularly useful probe because geographical or environmental variation in morphometric characters can be deduced from the fact that the offspring are all clonal, isogenic offspring of a single laying worker. Thus, intercolonial variation among cloned honeybees must reflect whatever environmental influences are operative whether they can be specified or not (Sokal et al., 1980; Moritz and Klepsch, 1985). By experiment and calculation it is thus possible to derive both coefficients of environmental variation as well as genetic residuals of morphological variation for natural populations (Hepburn et al., 2002).

Although occasional recombination events may occur in thelytoky through crossing-over and segregation of genes (Slobodchikoff and Daly, 1971), they are highly improbable in thelytokously reproducing honeybees (Moritz and Haberl, 1994). Thus, rather precise control over the genotype can be achieved and measured for clonal offspring produced by such laying workers, making it possible to quantify both environmental and genetic components of morphometric variation (Hepburn and Radloff, 2002; Hepburn et al., 2002).

## 2. MATERIAL AND METHODS

### 2.1. Sampling

Three queenless colonies of *Apis mellifera scutellata* Lepeletier infested with *Apis mellifera capensis* Escholtz laying workers (for biological details see Calis et al., 2002; Martin et al., 2002; Moritz, 2002; Neumann and Hepburn, 2002; Pirk et al., 2002; Reece, 2002; Wossler, 2002) were obtained from Pretoria, South Africa. Once all of the sealed worker brood of the former *A. m. scutellata* host queens emerged as adults, the colonies were monitored for laying worker brood of *A. m. capensis*. After this thelytokously produced worker brood was sealed, brood frames were removed from the colonies, individually confined in gauze-covered cages and placed in an incubator until adult emergence. After a week of feeding *ad libitum* (so that expansion and hardening of the exoskeleton was complete) the newly emerged adults of the laying worker brood were individually coded and genetically analysed to establish whether they originated from the same laying worker matriline.

### 2.2. DNA analysis

DNA was extracted from 20 workers of each colony and genotyped at four DNA microsatellite loci A107, A24, A28, and A43 (Estoup et al., 1993, 1994, 1995) according to routine protocols Neumann et al. (1999a,b,c).

### 2.3. Morphological measurements

Thirty-five morphological characters (standard in honeybee morphometry (Ruttner, 1988; Hepburn and Radloff, 1998), were measured from workers of three colonies (n = 16, 17, and 19 respectively per colony) which were offspring of the same matriline (Tab. I). Their Ruttner (1988) numbers are given in brackets as follows: length of cover hair on tergite 5 (1),width of tomentum on tergite 4 (2), width of stripe posterior of tomentum (3), length of femur (5), length of tibia (6), metatarus length (7), metatarsus width (8), tergite 3 longitudinal (9), tergite 4, longitudinal (10), sternite 3, longitudinal (11), wax plate of sternite 3, longitudinal (12) wax plate of sternite 3, transversal (13), distance between wax plates, sternite 3 (14), sternite 6, longitudinal (15), sternite 6, transversal (16), forewing, longitudinal (17), forewing, transversal (18), cubital vein, distance a (19), cubital vein, distance b (20), wing angle A4 (21), wing angle B4 (22), wing angle D7 (23), wing angle E9 (24), wing angle G18 (25), wing angle I10 (26), wing angle I16 (27), wing angle K19 (28), wing angle L13 (29), wing angle N23 (30), wing angle O26 (31), pigmentation of tergite 2 (32), pigmentation of tergite 3 (33), pigmentation of tergite 4 (34), pigmentation of scutellum (35), pigmentation of scutellar plate (36). In addition, the wing angle MJI (cf. Ruttner, 1988; Fig. 6.9) was measured.

### 2.4. Data analysis

Univariate analyses were carried out on the means, standard deviations and coefficients of variation for all morphological characters. Differences between colonies for the means and variances were tested using ANOVA with Scheffé multiple comparison tests and Levene's procedures (Rao, 1998). The Kolmogorov-Smirnov test was used to test for normality of the distribution of the coefficients of variation. Non-parametric Kruskal-Wallis, Wilcoxon matched pairs and Mann-Whitney tests were used to test for significant differences of the coefficients of variation between colonies and morphometric characters. Subsequent multivariate analyses included factor and discriminant analyses to determine morphometric differences between the colonies (Johnson and Wichern, 1998).

It must be noted that the coefficient of variation may not be stable nor representative for different kinds of measurements. While appropriate for length measures, in the case of pigmentation had the convention been to give high scores for "black" instead of "yellow" then any ranking of coefficients of variation might seem arbitrary. However, the reason for the measure of the coefficient of variation is to make measures of variation independent of the size (values) of the characters being measured, hence allowing comparisons. The drawbacks of the coefficients of variation do not directly nor indirectly bear on the calculation of the natural variances. If, for example, the bees were given codes so that for y values: "black" = 9 and "yellow" = 0 and then for x values: "black" = 0 and "yellow" = 9 (these are Ruttner, 1988 codes) the variances for both would be exactly the same; that is, $y = 9-x$ hence $var(y) = var(x)$. Of course the means would be different so that $mean(y) = 9 - mean(x)$ and hence the coefficients of variation would be different.

## 3. RESULTS

The genotypes of all workers (allele sizes in base pairs), which were included in the morphometric analysis, were determined by the analysis of four DNA microsatellite loci A107 (176 bp, 181 bp), A24 (90 bp, 94 bp), A28 (131 bp, 131 bp) and A43 (121 bp, 142 bp) and thus shown to derive from the same matriline.

Hypothesis tests for differences between colony means for each character showed that 25% (9/36) of them were significantly different ($P < 0.05$) and 75% (27/36) were not (Tab. I). Levene's tests for differences between colony variances for each character individually revealed that 3% (1/36) of the characters showed heterogeneous variation whilst 97% (35/36) were uniform and showed no significant differences (Tab. I).

The distribution of the coefficients of variation failed the test of normality and hence non-parametric procedures were used in the analysis ($d = 0.37$, $P < 0.01$). No significant differences were found between colonies for the coefficients of variation (Tab. II; c2 = 1.67, 2df, P = 0.8678; Wilcoxon matched pairs tests: C1 and C2, T = 305.0, P = 0.8698; C1 and C3, T = 296.0, P = 0.9795; C2 and C3, T = 258.00, P = 0.2387). Factor and discriminant analyses revealed a single morphocluster for the three colonies.

However, the susceptibility to environmental modification of different categories of morphological characters is variable, so that rank order may not necessarily be stable if environmental effects are variable. To consider this aspect, the 36 characters examined were classified in five categories of varying stability: A = angles of wing venation, S = size, H = hair, P = pigmentation and F = forewing and further analysed by groups. The means and standard deviations of these groups were: A = 0.0478 ± 0.0245, S = 0.0374 ± 0.0461, H = 0.1778 ± 0.0478, P = 0.3462 ± 0.3989 and F = 0.0394 ± 0.0214 respectively. Kruskal-Wallis test procedure shows that there is an overall highly significant difference among the five categories (H = 18.06, (4, n = 36), P = 0.0012) and that the pigmentation group (P) is indeed significantly more variable than the other character groups (Mann-Whitney U < 6.0, P < 0.05).

## 4. DISCUSSION

The experimental data clearly demonstrate that thelytokous parthenogenesis can unequivocally differentiate the various components of phenotypic variation. The data also show that the effect of environment on the different morphometric characters is significantly different among them. Thus, without knowing the precise environmental effect on each individual character, this would represent a non-conservative systematic error, resulting in a conflation of the population data. Coupled to multivariate analyses of morphometric characters, fine resolution of genetic components becomes possible.

It is of interest to consider the separate contributions of intra- and intercolonial variation in the phenotypic expression of morphometric characters. Table I clearly illustrates that intracolonial variation is considerably greater than intercolonial variation for all morphometric characters. Owing to the mathematical procedures to derive intercolonial values this could be expected to remain so were the number of colonies increased beyond the three discussed here.

The results further establish through the magnitudes of the coefficients of variation which of the 36 morphological characters are most conserved (least sensitive to environmental effects). This is highly important both to the analysis of natural populations as well as for honeybee intraspecific classification. Clearly the construction of population profiles and classification paradigms are mutually interdependent, the quality of any inferences being dependent on the characters considered.

In this respect the metrical characters for size are most useful in population analyses, the more subjective ones relating to pigmentation considerably less so. Of the 36 characters measured in this study 23 of them, all metrical, probably have generally high heritability values (Moritz and Klepsch, 1985; Cornuet and Garnery, 1991; Oldroyd et al., 1991). Morphological traits with low heritability (e.g. number of legs in adult worker bees) are constant and are therefore of no use for population studies. However, traits with high heritability will almost certainly be variable in a population. So for 23 traits the impact of environmental effects on the isogenic groups is small. Given the successfulness of thelytokous parthenogenesis as a genetic probe, in future it is possible to design experiments that simultaneously and quantitatively control all aspects of the genetic equation required to move from genotype through environmentally modulated phenotype.

## REFERENCES

Alpatov W.W. (1929) Biometrical studies on variation and races of the honey bee *Apis mellifera* L., Quart. Rev. Biol. 4, 1–58.

Calis J.N.M., Boot W.J., Allsopp M.H., Beekman M. (2002) Getting more than a fair share: Nutrition of worker larvae related to social parasitism in the Cape honey bee, *Apis mellifera capensis*, Apidologie 33, in press.

Cornuet J.M., Garnery L. (1991) Genetic diversity of *Apis mellifera*, in: Smith D.R. (Ed.), Diversity in the Genus *Apis*, Westview, Boulder, Colorado, pp. 103–115.

Daly H.V., Hoelmer K., Gambino P. (1991) Clinal geographic variation in feral honey bees in California, USA, Apidologie 22, 591–609.

Estoup A., Solignac M., Harry M., Cornuet J.M. (1993) Characterization of (GT)n and (CT)n microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*, Nucl. Acids Res. 21, 1427–1431.

Estoup A., Solignac M., Cornuet J.M. (1994) Precise assessment of the number of matings and of relatedness in honey bee colonies, Proc. R. Soc. London B. Biol. Sci. 258, 1–7.

Estoup A., Garnery L., Solignac M., Cornuet J.M. (1995) Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models, Genetics 140, 679–695.

Falconer D.S. (1989) Introduction to Quantitative Genetics, third edition, Longman, Harlow.

Hepburn H.R., Radloff S.E. (1998) Honeybees of Africa, Springer-Verlag, Berlin.

Hepburn H.R., Radloff S.E. (2002) *Apis mellifera capensis*: an essay on the subspecific classification of honeybees, Apidologie 33, in press.

Hepburn H.R., Radloff S.E., Neumann P. (2002) Genetic variation in a natural population of honeybees, *Apis mellifera capensis* Esch., Heredity (submitted).

Johnson R.A., Wichern D.W. (1998) Applied Multivariate Statistical Analysis, fourth edition, Prentice Hall, Upper Saddle River, New Jersey.

Martin S., Wossler T.C., Kryger P. (2002) Usurpation of *Apis mellifera scutellata* colonies by *A. m. capensis* workers, Apidologie 33, in press.

Moritz R.F.A. (2002) Population dynamics of the Cape bee phenomenon: the impact of parasitic laying worker clones in apiaries and natural populations. Apidologie 33, in press.

Moritz R.F.A., Haberl M. (1994) Lack of recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee), Heredity 73, 98–102.

Moritz R.F.A., Klepsch A. (1985) Estimating heritabilities of worker characteristics: a new approach using laying workers of the Cape honeybee (*Apis mellifera capensis*), Apidologie 16, 47–56.

Nazzi F. (1992) Fluctuation of forewing characters in hybrid honeybees from north-eastern Italy, J. Apic. Res. 31, 27–31.

Neumann P., Moritz R.F.A., Van Praagh J. (1999a) Queen mating-frequency in different types of honeybee mating apiaries, J. Apic. Res. 38, 11–18.

Neumann P., Van Praagh J., Moritz R.F.A., Dustmann J. (1999b) Testing reliability of a potential island mating apiary using DNA-microsatellites, Apidologie 30, 257–276.

Neumann P., Moritz R.F.A., Mautz D. (1999c) Maternity testing in honeybees using DNA-microsatellites, Apidologie 30, 505–512.

Neumann P., Hepburn H.R. (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis*), Apidologie 33, in press.

Oldroyd B.P., Rinderer T.E., Buco S.M. (1991) Heritability of morphological characters used to distinguish European and Africanized honeybees, Theor. Appl. Genet. 82, 499–504.

Pirk C.W.W., Neumann P., Hepburn H.R. (2002) Egg removal and egg laying by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis*), Apidologie 33, in press.

Poklukar J., Kezic N. (1994) Estimation of heritability of some characteristics of hind legs and wings of honeybee workers (*Apis mellifera carnica* Poll) using the half-sibs method, Apidologie 25, 3–11.

Rao P.V. (1998) Statistical Research Methods in the Life Sciences, Brooks/Cole Publishing Company, Pacific Grove, CA.

Reece S.L. (2002) A scientific note on the ovarial and pheromonal development of drifted and non drifted Cape honeybee workers (*Apis mellifera capensis*), Apidologie 33, in press.

Rinderer T.E. (1977) Measuring the heritability of characters of honeybees, J. Apic. Res. 16, 95–98.

Rinderer T.E., Daly H.V., Sylvester H.A., Collins A.M., Buco S.M., Hellmich R.L., Danka R.G. (1990) Morphometric differences among Africanized and European honey bees and their F1 hybrids (Hymenoptera: Apidae), Ann. Entomol. Soc. Am. 83, 346–351.

Ruttner F. (1988) Biogeography and Taxonomy of Honeybees, Springer-Verlag, Berlin.

Slobodchikoff C.N., Daly H.V. (1971) Systematic and evolutionary implications of parthenogenesis in the Hymenoptera, Am. Zool. 11, 273–282.

Sokal R.R., Bied J., Riska B. (1980) Geographic variation in *Pemphigus populicaulis* (Insecta: Aphididae) in eastern North America, Biol. J. Linn. Soc. 14, 163–200.

Thorpe R.S. (1976) Biometric analysis of geographic variation and racial affinities, Biol. Rev. 51, 407–452.

Wossler T.C. (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host A*pis mellifera scutellata* colonies, Apidologie 33, in press.

**Table I.** Means and intra- and intercolonial standard deviations (sd) of the 36 morphometric characters of workers from colonies C1-3.

| Character | C1 | | C2 | | C3 | | | Significance | level |
|---|---|---|---|---|---|---|---|---|---|
| | mean | intra sd sd | mean | intra sd | mean | intra sd | inter sd | mean | variance |
| (1) | 0.135a | 0.016 | 0.123a | 0.018 | 0.123a | 0.018 | 0.007 | ns | ns |
| (2) | 0.392a | 0.086 | 0.341b | 0.098 | 0.417a | 0.081 | 0.039 | * | ns |
| (3) | 0.711a | 0.111 | 0.687a | 0.148 | 0.749a | 0.092 | 0.031 | ns | ns |
| (5) | 2.515a | 0.049 | 2.556b | 0.044 | 2.556b | 0.039 | 0.024 | * | ns |
| (6) | 3.078a | 0.064 | 3.096a | 0.045 | 3.077a | 0.063 | 0.011 | ns | ns |
| (7) | 1.914a | 0.044 | 1.940a | 0.037 | 1.915a | 0.033 | 0.015 | ns | ns |
| (8) | 1.132a | 0.031 | 1.132a | 0.039 | 1.123a | 0.035 | 0.005 | ns | ns |
| (9) | 2.103a | 0.047 | 2.108a | 0.063 | 2.082a | 0.066 | 0.014 | ns | ns |
| (10) | 2.046a | 0.036 | 2.053a | 0.069 | 1.998b | 0.063 | 0.030 | * | ns |
| (11) | 2.659a | 0.039 | 2.646ab | 0.073 | 2.594b | 0.087 | 0.034 | * | ns |
| (12) | 1.203ab | 0.034 | 1.234a | 0.044 | 1.189b | 0.052 | 0.023 | * | ns |
| (13) | 2.118ab | 0.048 | 2.159a | 0.061 | 2.116b | 0.050 | 0.024 | * | ns |
| (14) | 0.304a | 0.061 | 0.311a | 0.047 | 0.293a | 0.056 | 0.009 | ns | ns |
| (15) | 2.681a | 0.049 | 2.676a | 0.045 | 2.652a | 0.038 | 0.016 | ns | ns |
| (16) | 2.848a | 0.059 | 2.847a | 0.037 | 2.797b | 0.044 | 0.029 | * | ns |
| (17) | 8.831a | 0.181 | 8.928a | 0.110 | 8.864a | 0.121 | 0.049 | ns | ns |
| (18) | 2.979a | 0.118 | 3.016a | 0.106 | 2.986a | 0.081 | 0.020 | ns | ns |
| (19) | 0.547a | 0.021 | 0.552a | 0.021 | 0.546a | 0.024 | 0.003 | ns | ns |
| (20) | 0.232a | 0.016 | 0.231a | 0.015 | 0.237a | 0.016 | 0.003 | ns | ns |
| (21) | 29.900a | 1.680 | 28.776a | 1.181 | 28.768a | 1.571 | 0.651 | ns | ns |
| (22) | 104.95a | 5.068 | 108.00a | 2.346 | 107.86a | 3.150 | 1.722 | ns | ns |
| (23) | 103.68a | 2.323 | 104.64a | 2.429 | 103.42a | 2.191 | 0.643 | ns | ns |
| (24) | 19.912a | 1.073 | 20.459a | 0.884 | 20.010a | 0.983 | 0.292 | ns | ns |
| (25) | 104.10a | 2.323 | 103.07a | 3.219 | 102.39a | 2.659 | 0.861 | ns | ns |
| (26) | 74.262a | 3.343 | 73.906ab | 3.640 | 71.637b | 2.505 | 1.424 | * | ns |
| (27) | 20.206a | 1.531 | 20.276a | 1.402 | 19.963a | 1.649 | 0.164 | ns | ns |
| MJI | 90.475a | 2.823 | 87.235b | 2.356 | 88.158b | 2.842 | 1.669 | * | ns |
| (28) | 80.212a | 1.433 | 79.559a | 1.255 | 80.600a | 3.690 | 0.526 | ns | ns |
| (29) | 12.181a | 0.830 | 12.070a | 0.822 | 12.663a | 1.398 | 0.315 | ns | * |
| (30) | 86.600a | 2.031 | 86.012a | 3.111 | 85.679a | 2.514 | 0.466 | ns | ns |
| (31) | 33.356a | 3.556 | 31.082a | 2.177 | 31.174a | 2.986 | 1.287 | ns | ns |
| (32) | 5.000a | 0.000 | 4.823a | 0.528 | 5.000a | 0.000 | 0.102 | ns | ns |
| (33) | 5.000a | 0.000 | 4.823a | 0.528 | 4.947a | 0.229 | 0.091 | ns | ns |
| (34) | 3.375a | 0.619 | 3.529a | 0.514 | 3.474a | 0.513 | 0.078 | ns | ns |
| (35) | 0.687a | 0.873 | 1.294a | 0.919 | 0.789a | 0.976 | 0.325 | ns | ns |
| (36) | 1.187a | 0.544 | 1.412a | 0.507 | 1.105a | 0.567 | 0.159 | ns | ns |

* $P < 0.05$; ** $P < 0.01$; ns = not significant; a,b = different letters within a row indicate significant mean differences ($P < 0.05$).

**Table II.** Coefficients of environmental variation of the 36 morphometric characters of workers from colonies C1-3.

| Character | Colony | | | Pooled |
|---|---|---|---|---|
| | C1 | C2 | C3 | |
| F(17) | 0.0205 | 0.0123 | 0.0136 | 0.0157 |
| S(15) | 0.0184 | 0.0168 | 0.0142 | 0.0165 |
| S(16) | 0.0207 | 0.0129 | 0.0156 | 0.0166 |
| S(5) | 0.0196 | 0.0173 | 0.0155 | 0.0174 |
| S(6) | 0.0208 | 0.0144 | 0.0205 | 0.0188 |
| S(7) | 0.0228 | 0.0190 | 0.0172 | 0.0193 |
| A(23) | 0.0224 | 0.0232 | 0.0212 | 0.0222 |
| S(13) | 0.0225 | 0.0284 | 0.0236 | 0.0249 |
| A(25) | 0.0223 | 0.0312 | 0.0259 | 0.0268 |
| S(11) | 0.0146 | 0.0278 | 0.0335 | 0.0269 |
| S(9) | 0.0225 | 0.0298 | 0.0319 | 0.0286 |
| S(10) | 0.0174 | 0.0337 | 0.0317 | 0.0288 |
| A(30) | 0.0234 | 0.0362 | 0.0293 | 0.0302 |
| MJI | 0.0312 | 0.0270 | 0.0322 | 0.0303 |
| A(28) | 0.0179 | 0.0158 | 0.0458 | 0.0309 |
| S(8) | 0.0278 | 0.0343 | 0.0311 | 0.0312 |
| F(18) | 0.0397 | 0.0352 | 0.0272 | 0.0341 |
| A(22) | 0.0483 | 0.0217 | 0.0292 | 0.0341 |
| S(12) | 0.0287 | 0.0358 | 0.0442 | 0.0372 |
| F(19) | 0.0386 | 0.0382 | 0.0441 | 0.0406 |
| A(26) | 0.0450 | 0.0493 | 0.0349 | 0.0433 |
| A(24) | 0.0539 | 0.0432 | 0.0491 | 0.0488 |
| A(21) | 0.0562 | 0.0410 | 0.0546 | 0.0512 |
| P(32) | 0.0000 | 0.1096 | 0.0000 | 0.0611 |
| F(20) | 0.0690 | 0.0633 | 0.0690 | 0.0673 |
| P(33) | 0.0000 | 0.1096 | 0.0464 | 0.0675 |
| A(27) | 0.0758 | 0.0692 | 0.0826 | 0.0762 |
| A(29) | 0.0681 | 0.0681 | 0.1104 | 0.0870 |
| A(31) | 0.1066 | 0.0700 | 0.0958 | 0.0927 |
| H(1) | 0.1163 | 0.1455 | 0.1488 | 0.1374 |
| P(34) | 0.1834 | 0.1458 | 0.1477 | 0.1583 |
| H(3) | 0.1569 | 0.2155 | 0.1227 | 0.1654 |
| S(14) | 0.2025 | 0.1509 | 0.1913 | 0.1821 |
| H(2) | 0.2200 | 0.2878 | 0.1949 | 0.2305 |
| P(36) | 0.4580 | 0.3593 | 0.5131 | 0.4396 |
| P(35) | 1.2701 | 0.7106 | 1.2367 | 1.0045 |

A = angles of wing venation, S = size, H = hair, P = pigmentation, F = forewing.

# 3.2 Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera* L.) colonies

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**Abstract -** Colonies of *A. m. capensis*, *A. m. scutellata* and their natural hybrids were dequeened and debrooded. The ratio of worker/drone cell construction and the sex of laying worker offspring were determined for 26 colonies. All *A. m. capensis* laying workers were thelytokous and all *A. m. scutellata* arrhenotokous. 42.1% of the hybrid colonies produced only female offspring while none produced only male offspring. This shows a significant advantage for thelytokous laying workers to become reproductively dominant in hybrid colonies. *A. m. capensis* colonies built only worker cells and *A. m. scutellata* only drone cells. Hybrid colonies produced either both cell types or only worker cells according to the mode of laying worker reproduction. In all colonies where laying workers produced male offspring drone cell building was found. Our data strongly indicates that the mode of worker reproduction holds important consequences for cell construction and reproductive dominance.

**Key words:** *Apis mellifera capensis* / *Apis mellifera scutellata* / honeybee / hybrids / laying worker / reproductive dominance / brood cell

## 1. INTRODUCTION

It has recently been shown that the mode of worker reproduction holds important consequences for the behaviour of honeybees. Indeed, thelytoky has significant effects on absconding behaviour of colonies [7] as well as on drifting [unpublished data] and policing behaviour of workers [13]. Two behaviours of honeybee workers which occur after queen loss are highly important for the reproductive capacity of these orphaned colonies (especially in recently absconded colonies): the reproductive dominance hierarchies among laying workers and the construction of brood cells.

Colony performance in queenless honeybees depends on the proportion of subordinate and dominant workers [8]. Because honeybee queens mate with many males [15] the colony consists of a large number of different patrilines among which there is reproductive competition under queenless conditions [12]. After a few weeks offspring of only some dominant patrilines is reared to the adult stage. If colonies simultaneously consist of patrilines capable of both thelytokous and arrhenotokous parthenogenesis (as is the case in the natural hybrid zone between *A. m. capensis* and *A. m. scutellata*), which patrilines become reproductively dominant after queen loss and, what are the consequences?

If the mode of worker reproduction exclusively determines reproductive dominance among workers, we would expect naturally occurring hybrid colonies between *A. m. capensis* and *A. m. scutellata* consisting of both arrhenotokous and thelytokous patrilines to produce either adult worker or drone offspring but not both. If, however, the mode of worker reproduction has only a modest impact on reproductive dominance hierarchies, we would expect successful reproduction of both arrhenotokous and thelytokous patrilines in queenless hybrid colonies.

In this paper we address the question: does the mode of worker reproduction have an impact on reproductive dominance and on brood cell construction in queenless honeybee colonies in the natural hybrid zone between *A. m. capensis* with thelytokous laying workers and *A. m. scutellata* with arrhenotokous laying workers?

## 2. MATERIAL AND METHODS

To assess the cell building pattern and subsequent production of laying worker offspring the following procedures were adopted. Ten queenright colonies were selected at each of seven localities along a transect extending from the natural distribution area of *A. m. capensis*, through the hybrid zone of *capensis*-*scutellata* which naturally occurs between them in the Eastern Cape area of South Africa, finally to the region of *A. m. scutellata* (*table I*). Localities were chosen to include pure *A. m. capensis* (Port Elizabeth [33.58S, 25.36E]), hybrids which are predominantly *A. m. capensis*, according to morphological analysis [3]; Addo [33.29S, 25.46E], Fort Beaufort [32.48S, 26.38E], Stutterheim [32.33S, 22.28E]) or predominantly *A. m. scutellata* (Queenstown [31.32S, 7.00E] and Molteno [31.22S, 26.22E]) and pure *A. m. scutellata* (Pretoria [25.46S, 28.12E]).

All of the experimental colonies were dequeened and completely stripped of any nesting material including brood, stores and combs. The remaining workers were then given only empty frames (without any wax foundation). Manipulations were done *in situ* in the different apiaries to preclude intersubspecific drifting, dispersing, introgression or amalgamation of the different populations [4, 7, 16]. This is highly important because *A. m. capensis* workers can invade other colonies and successfully reproduce [cf. 4]. Also, it must be noted that these bees are native honeybees in this region where there is no transport or migration of colonies nor is any bee breeding practised. Thus, the colonies studied are authentic samples of the natural wild population.

After 30 days the various localities were revisited and the full contents of all hives were collected and brought to our laboratory for the analysis of laying worker progeny and comb building behaviour. Thus, the ratios of worker cell to drone cell building were determined regardless of whether they contained any offspring or not and the progeny of laying workers was sexed. The occurrence of queen cell construction was also recorded.

It must be understood that the amount of new comb construction varied quite considerably among colonies ranging from about 0.5 of a Langstroth brood frame up to five frames of new comb. Naturally, the amount of brood produced also varied in a similar way from just a couple of dozens to several hundreds. Nonetheless all brood was sexed in all combs in the pharate adult stage (pre-hatching imagos) and all cells were assigned to either drone or worker type and expressed in ratio form. Clearly, some colonies contained more comb and brood than others, but despite the relative abundance or paucity of both comb and brood among the different colonies, each individual colony represents an independent sample.

We used $\chi 2$- and z-tests to test for the distribution of arrhenotokous and thelytokous reproducing workers and for the distribution of comb cell building among the hybrid colonies. To test the relationship between the taxa and the amount of queen cell construction we calculated a correlation across the transect.

## 3. RESULTS

As is often the case under experimental perturbations of this magnitude [7], 43 of our original 70 test colonies absconded (10% of *A. m. capensis*, 72% of the hybrids and 70% of *A. m. scutellata*) and one colony died, leaving a data base of 26 colonies (*table I*). Clearly, we do not know the actual fates of the absconded colonies. However, there is no statistical bias in the final results because analysis was restricted to these colonies that remained in the apiaries.

The sexes of offspring produced by queenless laying worker colonies of *A. m. capensis*, *A. m. scutellata* and their natural occurring hybrids are shown in *table I*. Pure *A. m. capensis* colonies produced only female offspring whereas the pure *A. m. scutellata* colonies produced only male offspring. In the natural hybrid zone 6 out of 14 colonies (43%) produced only female offspring whereas not a single colony produced only male offspring (*table I*). In 57% of the hybrid colonies offspring of both sexes was reared, however, hybrid colonies produced significantly more female offspring than male offspring (z = 7.82, p<0.0001). Interestingly, the laying workers laid female eggs in worker cells and male eggs in drone cells.

The ratios of worker to drone cell construction in our test colonies are shown in *table I*. The queenless, broodless *A. m. capensis* colonies built only worker cells while the *A. m. scutellata* colonies built only drone cells. The results clearly show that workers which are morphometrically and reproductively defined as pure *A. m. capensis* had a worker to drone cell ratio of 1:0 and indeed only worker brood was reared in those colonies. Moreover, the pure *A. m. scutellata* colonies produced a worker to drone cell ratio of 0:1 and only male offspring was reared. The ratios of worker to drone cell construction across the hybrid zone are variable (*table I*). All of the queenless, broodless hybrid colonies produced worker comb cells and 57% of them also produced drone cells, but in significantly fewer amounts ($\chi^2$ = 15.08, df = 2, p= 0.00053, *table I*). The ratios of workers to drones were converted into frequencies and then compared using a chi-square test.

All colonies which had only thelytokous reproducing laying workers built only worker cells while colonies which produced male and female worker offspring constructed both types of brood cells. This distribution was significantly different from random ($\chi^2$ = 11.0, df = 1, p= 0.0009, Fisher's exact test p= 0.003).

Interestingly, there is a significant correlation between the number of queen cells constructed per colony and the locality sampled. The more northerly the colonies were sampled (= more *A. m. scutellata*-like region of the hybrid zone), the fewer queen cells were constructed (r=-0.684, n = 26, p = 0.001, $r_s$=-0.683, n = 26, p = 0.000122). Colonies of pure *A. m. capensis* constructed significantly more queen cells ($\chi^2$ = 12.75, df = 2, p = 0.0017) than the hybrid colonies.

## 4. DISCUSSION

In the methods (see above) it is clear that the colonies had to build their own combs and were also native bees, including the hybrids. The native element is important because this means that the situation observed is likely to be the result of naturally occurring processes of gene flow, migration, etc. between the two races. The colonies were artificially made queenless and broodless. This may have affected the results in that the normal route to being queenless and broodless is via a period in which there is no queen, but in which brood usually occurs [cf. 4]. This may be of relevance to the competition among the racial types in hybrid colonies because non-*capensis* bees typically attempt to rear a new queen rather than going straight into worker reproduction as in *capensis* [cf. 4]. Moreover, *capensis* may have been favoured through a difference in the rate at which ovaries are activated or the proportion with previously activated ovaries [cf. 4]. Thus, the experimental design may have been somewhat stacked in favour of *capensis*.

Nevertheless, our results show that reproductive dominance in queenless honeybee colonies is strongly affected by the mode of worker reproduction. Although we found a significantly high amount of female offspring among the hybrid colonies, this dominance is not exclusive because within colonies from the natural hybrid zone both worker and drone offspring were simultaneously reared. However, the modes of worker reproduction and the construction of brood cells within queenless colonies are clearly co-dependent. In colonies with only thelytokous laying workers exclusively worker cells were constructed whereas colonies with only arrhenotokous laying workers built only drone cells. Colonies with both arrhenotokous and thelytokous reproducing workers constructed both types of brood cells.

Interestingly, the number of queen cells reared after queen loss seems to be dependent upon population structure. *A. m. capensis* colonies reared significantly more queen cells than the hybrids and we found a negative correlation within the hybrid zone from the more *capensis*-like region to the more *scutellata*-like region. In the lack of any quantitative comparative data on the number of queen cells built in this region, it may be that eggs derived from thelytokous laying worker patrilines occurring in the south may stimulate more queen cell building compared to the north. A role for brood pheromones in modulating the feeding behaviour of workers has been shown [9].

It might well be that thelytokous patrilines, presenting a more *A. m. capensis* like genotype, are innately predisposed to become dominant because only offspring of those patrilines can be used for re-queening the orphaned colonies. However, it might also well be that arrhenotokous reproducing patrilines are dominant in the hybrid zone, because high frequencies of thelytoky only occur in *A. m. capensis*.

That both worker and drone offspring were simultaneously reared in the hybrid colonies confirms previous observations [17] that successful reproduction of arrhenotokous and thelytokous workers in one honeybee colony is possible. Thus, it is obvious that arrhenotokous workers are also able to become reproductively dominant even if thelytokous laying workers are present in the queenless colony. We argue that the proximate reason for thelytokous laying workers being predisposed to become reproductively dominant is that thelytoky itself is closely linked with important traits leading to reproductive hierarchies among workers such as differing pheromonal bouquets and/or differences in ovariole development of the subordinate and dominant workers [cf. 4]. Every test colony within the hybrid zone tried to rear queens from laying worker offspring supporting the idea that the primary function of thelytoky is to replace lost queens [10, 11]. This may provide the ultimate reason for the dominance of thelytokous laying workers in the hybrid colonies.

In the test colonies workers constructed brood cells after queen loss according to the mode(s) of worker reproduction of the laying workers present in the colony. Cell construction and reproductive dominance may act synergistically. If no surrogate queen is present in a chain of building workers, drone cells are constructed and arrhenotokous laying workers may develop too because there has not been sufficient time for thelytokous workers to suppress them pheromonally. This may provide a parsimonious explanation for our findings and give support to the importance of decentralised decision making processes in honeybee colonies [18] as well as to the importance of the spatial distributions of workers among their colonies [14].

The thelytokous workers may dispense with building drone comb at all, even though they have a pseudoqueen. This is supported by other observations that *A. m. capensis* workers tend to respond to queenless conditions in a different way from other honeybee workers. For example the inapplicability of queen removal as a stimulus for new queen production [cf. 4] and the development of laying workers despite the presence of eggs and young larvae [1, 5] clearly show that Cape honeybee workers seem to have different behavioural mechanisms after queen loss. Given that the building period was only 30 days, it might well be that the absence of drone comb simply reflects that the workers were starting their combs from scratch, and concentrated on worker comb building first. Likewise, the presence of drone comb in the hybrids would reflect the fact that some arrhenotokous workers were not "convinced" by the pseudoqueen, and behaved as though hopelessly queenless.

In conclusion, our data give strong support to other studies showing that the mode of worker reproduction holds major consequences for other behavioural traits of honeybees [7, 13, 14].

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## REFERENCES

[1] Anderson R.H., The laying worker in the Cape honeybee, *Apis mellifera capensis*, J. Apic. Res. 2 (1963) 85-92.

[2] Hepburn H.R., Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch, Apidologie 25 (1994) 38-48.

[3] Hepburn H.R., Nefdt R.J.C., Whiffler L.A., Queen loss in the Cape honeybee: the interactions of brood, laying workers (false queens?) and queen cells, South Afr. J. Sci. 80 (1988) 363-368.

[4] Hepburn H.R., Crewe R.M., Portrait of the Cape honeybee, *Apis mellifera capensis*, Apidologie 22 (1991) 567-580.

[5] Hepburn H.R., Radloff S.E., Honeybees of Africa. Springer Verlag, Berlin, 1998.

[6] Hepburn H.R., Villet M.H., Jones G.E., Carter A.R., Simon U.I., Coetzer W., Winter absconding as a dispersal mechanism of the Cape honeybee, South Afr. J. Sci. 89 (1993) 294-297.

[7] Hepburn H.R., Reece S., Neumann P., Moritz R.F.A., Radloff S.E., Absconding in honeybees (*Apis mellifera*) in relation to queenstate and mode of worker reproduction. Ins Soc 46 (1999) 323-326.

[8] Hillesheim E., Koeniger N., Moritz R.F.A., Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers, Behav. Ecol. Sociobiol. 24 (1989) 291-296.

[9] Le Conte Y., Streng L., Poitout S.H., Brood pheromone can modulate the feeding behavior of *Apis mellifera* workers (Hymenoptera: Apidae), J. Econ. Entomol. 88 (1995) 798-804.

[10] Lenoir A., Cagniant H., Role of worker thelytoky in colonies of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae). Entomol. General. 11 (1986) 153-157.

[11] Moritz R.F.A., Two parthenogenetical strategies of laying workers in populations of the honeybee, *Apis mellifera* (Hymenoptera, Apidae). Entomol. General. 11 (1986) 159-164.

[12] Moritz R.F.A., Kryger P., Allsopp M.H., Competition for royalty in bees. Nature 384 (1996) 31.

[13] Moritz R.F.A., Kryger P., Allsopp M.H., Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). Behavior 136 (2000a) 1079 - 1092.

[14] Moritz R.F.A., Crewe R., Hepburn H.R., The role of the queen in the distribution of workers in the honeybee colony, *Apis mellifera*. Apidologie 31 (1) (2000b) in press.

[15] Neumann P., Moritz R.F.A., Van Praagh J., Queen mating frequency in different types of honeybee mating apiaries, J. Apic. Res. 38 (1-2) (1999) 11-18.

[16] Neumann P., Moritz RFA, Mautz D., Colony evaluation is not affected by the drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. Apidologie 31 (2000) 67-79.

[17] Pettey F.W., Worker laying in comb of extracting supers. J. Dept. Agric. Union South Afr. 4 (1922) 122-124.

[18] Pratt S.C., Decentralised control of drone comb construction in honey bee colonies, Behav. Ecol. Sociobiol. 42 (1998) 192-205

**Table I.** Construction of brood cells and sex of laying worker offspring in queenless colonies of *A. m. capensis*, *A. m. scutellata* and their natural hybrids. The total number of constructed queen cells per location are given (N = sample size of remaining colonies used for data analysis). Part of the numerical data was taken from another study [6] but has not previously been considered in terms of reproductive fitness.

| **Group** | | **Locality** | **N** | **Worker/drone cell ratio** | **Sex of offspring** | **Queen cells** |
|---|---|---|---|---|---|---|
| *capensis* | | Port Elizabeth | 9 | 1:0 | female | 81 |
| hybrids | predominantly *capensis* | Addo | 1 | 1:0 | female | 12 |
| | | | 1 | 2:1 | female/male | 13 |
| | | | 1 | 4:1 | female/male | 8 |
| | | Fort Beaufort | 1 | 1:1 | female/male | 5 |
| | | | 1 | 2:1 | female/male | 5 |
| | | | 1 | 20:1 | female/male | 5 |
| | | Stutterheim | 2 | 2:1 | female/male | 4 |
| | | | 1 | 1:0 | female | 1 |
| | predominantly *scutellata* | Queenstown | 2 | 1:0 | female | 20 |
| | | Molteno | 2 | 1:0 | female | 2 |
| | | | 1 | 10:1 | female/male | 1 |
| *scutellata* | | Pretoria | 3 | 0:1 | male | 0 |
| Total | | | 26 | | | |

# 3.3 Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): Host finding and resistance of hybrid host colonies

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**Abstract -** We studied possible host finding and resistance mechanisms of host colonies in the context of social parasitism by Cape honeybee (*Apis mellifera capensis*) workers. Workers often join neighbouring colonies by drifting but long-range drifting (dispersal) to colonies far away from the maternal nests also rarely occurs. We tested the impact of queenstate and taxon of mother and host colonies on drifting and dispersing of workers and on the hosting of these workers in *A. m. capensis*, *A. m. scutellata* and their natural hybrids. Workers were colony-specific paint-marked and reintroduced into their queenright or queenless mother colonies. After 10 days 579 out of 12034 labelled workers were recaptured in foreign colonies. We found that drifting and dispersing represent different behaviours, which were differently affected by taxon and queenstate of both mother and host colonies. Hybrid workers drifted more often than *A. m. capensis* and *A. m. scutellata*. However, *A. m. capensis* workers dispersed more often than *A. m. scutellata* and the hybrids combined and *A. m. scutellata* workers also dispersed more frequently than the hybrids. Dispersers from queenright *A. m. capensis* colonies were more often found in queenless host colonies and *vice versa*, indicating active host searching and/or a queenstate-discriminating guarding mechanism. Our data show that *A. m. capensis* workers disperse significantly more often than other races of *A. mellifera*, suggesting that dispersing represents a host finding mechanism. The lack of dispersal in hybrids and different hosting mechanisms of foreign workers by hybrid colonies may also be responsible for the stability of the natural hybrid zone between *A. m. capensis* and *A. m. scutellata*.

**Key words**: *Apis mellifera capensis*, *Apis mellifera scutellata*, honeybee, host finding, hybrid, social parasitism

## 1. INTRODUCTION

Social parasitism, where newly mated gynes seek host colonies and get adopted, is commonplace in insect societies (e.g. Wilson 1971; Hölldobler & Wilson 1990; Schmid-Hempel 1998); the adoption of foreign workers has also frequently been reported (e.g. Rauschmayer 1928; Neumann et al 2000c). At first glance, the latter seems to represent a true fitness gain for host colonies because new workers can support the offspring of the resident queens. However, such unmated workers can produce either males (arrhenotoky) or females (thelytoky) parthenogenetically. Such laying workers may compete with nestmate workers and queens for the production of sexuals in host colonies. Therefore, the adoption of foreign laying workers actually represents an intraspecific social parasitism, which is in contrast to the social parasitism by gynes (Bohart 1970; Roubik 1989; Field 1992) less well known in bees and wasps.

The reproductive cycles of social parasites must represent a skein of interacting variables/phenomena. For example, it is crucial for both host and parasite, where and how infection is established (Schmid-Hempel 1998): (1) A social parasite must find colonies of its host. (2) The social parasite must actually gain entrance into the host colony. Thus, the adoption or successful rejection of invading social parasites may represent important behavioural mechanisms of resistance and susceptibility for host colonies.

The Cape honeybee (*Apis mellifera capensis* Esch.) offers a prime test system to investigate the underlying behavioural mechanisms of both host finding by social parasites and behavioural resistance mechanisms of host colonies. Indeed, the social parasitism of Cape honeybee workers has been known since Onions (1912) first described *A. m. capensis* laying workers invading host colonies of *A. m. ligustica* far away from their maternal colonies. The adoption of *A. m. capensis* laying workers often results in the systematic usurpation of host colonies (Hepburn and Allsopp 1994). This social parasitism is expressed on the level of the host colonies' phenotypes as the so-called "dwindling colony" syndrome (Allsopp 1993; Greeff 1997), which has been documented for many thousand host colonies of the neighbouring subspecies *A. m. scutellata* (Allsopp and Crewe 1993; Hepburn and Allsopp 1994). While *A. m. capensis* laying worker brood is nurtured by host workers (Beekman et al. 2000), the host queen is somehow lost or killed and slowly the colony is taken over by the parasite (Hepburn and Radloff 1998). Then, the host colony dies, absconds (Hepburn et al. 1999) or a new *A. m. capensis* queen is raised (Allsopp 1992, 1993; Greeff 1997; Hepburn and Radloff 1998). It seems as if whole *A. m. scutellata* apiaries are systematically invaded by very few parasitic *A. m. capensis* workers (Kryger P. and Shyf A., personal communication). However, *A. m. capensis* social parasitism is not a beekeeping artefact, because laying honeybee workers contribute considerably to population fitness in natural South African populations (Moritz et al. 1998).

Obviously, intraspecific social parasitism by workers can only evolve in social insect species, where workers can reproduce. However, it seems more likely that social parasitism evolves in such species, where workers have the opportunity to maximize their own reproductive effort. Indeed, *A. m. capensis* workers show two important pre-adaptations for social parasitism:

1. The majority of laying workers of the Cape honeybee reproduces via thelytoky (Onions 1912; Hepburn & Crewe 1991), while some colonies may exhibit both arrhenotokous and thelytokous worker reproduction (Pettey 1922; Hepburn and Radloff 1998; Moritz et al. 1999). In contrast, the vast majority of laying workers of the neighbouring *A. m. scutellata* and of all other *A. mellifera* subspecies reproduces via arrhenotoky (Ruttner 1992), although very rare exceptions of thelytokous worker reproduction have also been reported (Mackensen 1953). Although in naturally occurring hybrid colonies between *A. m. capensis* and *A. m. scutellata*, both arrhenotokous and thelytokous worker reproduction occur, thelytokous laying workers show a significant reproductive dominance (Neumann et al. 2000a). Thelytoky appears to predispose a taxon for the evolution of aggressive worker reproduction (Greeff 1996, 1997) and consequently for social parasitism of workers because thelytokous laying worker offspring can immediately infest new host colonies.
2. Laying Cape honeybee workers may develop into pseudoqueens with a high ovarial development (Ruttner and Hesse 1981) and a queen-like pheromonal bouquet (Hemmling et al. 1979; Hepburn 1992; Hepburn 1994). Thus, pseudoqueens can inhibit the rearing of replacement queens in queenless *A. m. scutellata* host colonies as well as the ovarial development of *A. m. scutellata* host workers (Hepburn and Radloff 1998) and may induce retinue behavior in other workers (Anderson 1968). This represents an important pre-adaptation for a social parasite to gain reproductive dominance in host colonies.

However, in sharp contrast to the potential mechanisms of gaining reproductive dominance in host colonies, virtually nothing is known about host finding behavior of social parasitic honeybee workers and about potential resistance mechanisms of host colonies:

1. Host finding is an essential part of the life history of social parasites. It has been proposed that social parasitic *A. m. capensis* workers enter host colonies by passive "drifting" (Greeff 1997), resulting from slight orientation errors of young workers and sometimes of foragers (Rauschmayer 1928; Free 1958). Alternatively (but not mutually exclusive) *A. m. capensis* workers may perform active host finding, which cannot be explained by slight orientation errors (Johannsmeier 1983). Drifting of honeybee workers into neighbouring colonies is common and well established (Rauschmayer 1928; Ribbands 1953). However, “long-range” drifting (here termed dispersal) has less frequently, almost anecdotally been reported for other races of *A. mellifera*. Dispersing individuals of one colony joined a host colony 200m (Fresnaye 1963), 600m (Boyland-Pett et al. 1991) or 800m (Duranville et al 1991; Mossadegh 1993) away from their maternal colony and separated by patches of wood (Accorti 1991). We regard these very rare dispersal movements performed by only a tiny fraction of honeybee workers as a biological mechanism fundamentally different from simple drifting, but the underlying behavioural mechanisms and the possible significance for the host finding of social parasitic workers remain moot. Dispersal of *A. m. capensis* workers was first reported by Onions (1912) who designated such workers as "invaders", an often reported but never rigorously quantified behavioural feature of the Cape honeybee (Hepburn and Radloff 1998).
2. Parasite resistance in social insects often involves behavioural strategies (Schmid-Hempel 1998). In honeybees highly specialised guard bees of potential host colonies carefully scrutinise incoming individuals (Breed 1983) and may modify their acceptance-thresholds (Reeve 1989); e.g. guard bees attack workers infected with chronic bee paralysis virus more aggressively than healthy bees, especially during times of nectar flow (Drum and Rothenbuhler 1985). Thus, breaking into the fortress (Schmid-Hempel 1998) not only requires host finding by socially parasitic workers but also trespassing the host colony's guard force. In spite of the recent usurpations of many thousands of *A. m. scutellata* colonies (Allsopp and Crewe 1993; Hepburn and Allsopp 1994), the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* appears to be stable and, indeed, to exhibit a buffering capacity. Thus, we would expect the hybrid colonies to have special behavioural strategies to prevent the invasion of laying workers, representing a hybrid advantage (Barton and Hewitt 1985). One potential mechanism

could be that the natural hybrid colonies have a guard force, which is more efficient in rejecting social parasitic workers. The queenstate of mother and host colonies should also play a role because of rapid ovarial and pheromonal development in queenless workers (Hepburn and Radloff 1998) and because queenless host colonies seem to be more susceptible to infestations by laying *A. m. cap*ensis workers (Woyke 1995). Thus, queenless colonies of the natural hybrids should reduce their acceptance-thresholds (Reeve 1989).

In this study we specifically investigate: 1. The role of drifting and dispersing as host finding mechanisms of social parasitic *A. m. capensis* workers 2. Behavioural resistance mechanisms of host colonies using the apparent parasite resistance of natural hybrid zone colonies between *A. m. capensis* and *A. m. scutellata*.

## 2. MATERIAL AND METHODS

### 2.1 Sampling and experimental design

Honeybee colonies were simultaneously obtained from four locations in South Africa: *A. m. capensis* colonies from Port Elizabeth, *A. m. scutellata* from Steynsburg and naturally occurring hybrids from East London and Stutterheim (see Hepburn and Radloff 1998 for detailed information about the hybrid zone). It must be noted that virtually no transport or bee breeding is practiced in this region. Thus, the colonies are authentic samples of the natural wild population.

Six colonies each of *A. m. capensis*, *A. m. scutellata* and their hybrids were split into queenright and queenless parts of equal size. Each split contained two brood and three food frames in a white 5-frame hive. The colonies were transported to an apiary and arranged in three circles of 12 colonies each to equalize some of the effect apiary layouts may have on drifting (Jay 1966a,b, 1968). The colonies were arranged according to queenstate and taxonomic group to ensure equally possible movement permutations for all neighbouring colonies. The colonies were spaced 1m apart within each circle and the circles were placed 40 m apart (Fig. 1). All colonies were placed in the same sun-exposed environment and the position of the taxa towards the sun was changed clockwise between circles to control for sun position as a possible factor in the disorientation of honeybees (Jay and Warr 1984). Surplus colonies (N=7) were used to compensate for losses due to absconding (Hepburn et al. 1999). In these circumstances the majority of the colony absconded, leaving capped brood and a few hundred freshly emerged workers behind (Hepburn et al 1999). In one case, a queenless colony absconded, merged with another queenright colony (Neumann et al. 2000d) and subsequently the new unit absconded. The colonies were given a week to settle down before the experiments were started.

A major advantage of the experimental design is the similarity of the geometrical arrangements of the circles, enhancing orientation errors and increasing the apparent rate of dispersal. This design will greatly amplify the number of mistakes and of dispersal, which is necessary for studying the underlying behavioural mechanisms of dispersing, an otherwise very rare behavior of honeybee workers. The comparison between subspecies will be valid because the amplification is the same for all colonies.

### 2.2 Labelling and recapturing of workers

Sealed worker brood was taken from the experimental colonies and incubated until adult emergence. Freshly emerged workers of the same age cohort were individually paint-marked on the thorax using a colony-specific colour code and reintroduced into their mother colonies. Ten days later all labelled workers were recaptured. Workers recovered in their home circles were classified as "drifters", those in other than home circles "dispersers" (Fig. 1). Since handling of the workers was the same for those who remained, drifted and dispersed, a potential impact of handling would represent a conservative systematic error.

### 2.3 Data analysis

We used $\chi^2$-tests with Yates' correction and Fisher exact tests to determine variation in drifting and dispersing of workers with respect to queenstate and taxa. Z-statistics were used to test for differences in proportions between drifting and dispersing workers. Bonferroni adjustments were applied to the attained level of significance of the tests when paired comparisons were analysed. Correlations were tested using Spearman's rank correlation coefficient. For the drifters, weighted (adjusted) frequencies were calculated to take into account the design of the placing of the colonies in each circle because drifted bees usually prefer neighbouring colonies (e.g. Rauschmayer 1928). The probabilities $P_k$ that a worker drifted a distance of k colonies along the circumference of the hive clock at random were determined for k = 1 to 6 by proportionally dividing the circles into sectors. The adjusted frequencies were then calculated by dividing the observed frequencies by the appropriate $P_K$ values. To test whether dispersed workers showed a preference to disperse into the same sector in another circle as the one from which they originally came from, the mother and host circles were proportionally divided into eight sectors (Southwest, Southeast, Northwest, Northeast, South, West, North, East). Then, the overall distribution of dispersed workers among the same or different sectors was compared using a z-test. Statistical analyses were performed using Statistica©.

## 3. RESULTS

A total of 579 out of 12034 paint-marked workers were recovered from foreign colonies. The numbers of workers that drifted and dispersed between mother and host colonies are shown in Table 1, specific analyses of the various permutations are shown in tables 2 to 4. Here we only discuss the most significant results to ensure readability of the paper.

### 1. Drifting and dispersing

**1.1 Position**

The distribution of the observed frequencies of the drifted workers was significantly different from that of the expected frequencies, indicating that drifters prefer neighbouring host colonies ($\chi^2$ test: $\chi^2_5$=166.05, P<0.0001). The number of workers that drifted and their distances are significantly negatively correlated (Spearman's rank correlation: $r_s$= -0.98, N= 6, P=0.0275). 52% of the workers (253 out of 490) drifted by only the distance of a single colony and over 90% of the workers (451 out of 490) drifted within a three-colony distance of their mother colonies. Only 2% of the workers (12 of 490) drifted as far as six colonies away. However, 15.4% of the workers (89 of 579) dispersed into another circle (Fig. 1). Thus, significantly more workers dispersed the long distance into another apiary circle than expected from the distribution of the drifters (95% predicted frequency=0, P<0.0001; $\chi^2$ test: $\chi^2_2$=277.7, P<0.0001).

**1.2 Taxa**

Significant differences in the frequencies of workers that drifted or dispersed occurred both within ($\chi^2$ tests with Yates' correction: *A. m. capensis* queenright $\chi_4^2$= 65.7, P<0.0001; *A. m. capensis* queenless $\chi_{10}^2$= 269.8, P<0.0001; hybrid queenright $\chi_8^2$= 17.7, P= 0.0236; hybrid queenless $\chi_{10}^2$= 238.2, P<0.0001; *A. m. scutellata* queenright $\chi_8^2$= 84.2, P<0.0001; *A. m. scutellata* queenless $\chi_8^2$= 43.8, P<0.0001) and among the six groups ($\chi_{10}^2$= 305.3, P<0.0001, Tab. 1).

The impact of taxon on drifting and dispersing of workers is shown in Table 2a and in Fig. 2a. *A. m. capensis* and the hybrids drifted significantly more often than *A. m. scutellata*. Moreover, *A. m. capensis* significantly out-dispersed all of the other workers combined by 2:1 (chi-square test: $\chi_2^2$= 54.5, P<0.0001). But two colonies, C4+ and C4-, contributed 58.9% of all dispersers and 85.5% of all *A. m. capensis* dispersers. Excluding C4+ and C4-, they were no significant differences between the other *A. m. capensis* and the hybrid colonies (Z-test: z= 0.77, ns) nor between *A. m. capensis* and *A. m. scutellata* (Z-test: z= -1.12, ns). Interestingly, hybrid workers dispersed significantly less often than *A. m. scutellata* (Table 2a).

**1.3 Queenstate**

The effects of queenstate on drifting and dispersing is shown in Table 2b and in Fig. 2b. *A. m. capensis* workers drifted significantly more often from queenright than queenless colonies. In contrast, significantly fewer workers drifted from queenright than from queenless hybrid colonies.

### 2 Hosting of drifters and dispersers

**2.1 Taxa**

The weighted frequencies for all hosted drifted workers are given in Table 3a. The distribution of all dispersed workers among host colonies of the same and of different sectors in the new circles (Fig. 1) is shown in Table 3b. The impact of taxon on the hosting of drifters and dispersers is shown in Table 4a and in the Figures 3 and 4. Significant differences in the amount of hosting of drifters or dispersers were found within each of the six groups (*A. m. capensis* queenright z=6.5, P<0.0001; *A. m. capensis* queenless z=3.7, P=0.0001; hybrid queenright z=26.6, P<0.0001; hybrid queenless z=7.5, P<0.0001; *A. m. scutellata* queenright z=3.5, P=0.0002; *A. m. scutellata* queenless z=15.7, P<0.0001; overall: $\chi_5^2$ = 44.9, P<0.0001, Tab. 1).

Ignoring queenstate, there were no significant differences between *A. m. capensis* and *A. m. scutellata* in the numbers of drifters hosted (Fig. 3a, Tab. 4a). The hybrid colonies accepted significantly more drifters than the other taxonomic groups. But *A. m. capensis* colonies hosted significantly more dispersers than *A. m. scutellata* and the hybrid colonies. *A. m. capensis* colonies hosted proportionally more dispersers than drifters (z=5.8, P<0.0001), which was just the reverse for the hybrids (z=4.6, P<0.0001). No significant difference was found between the proportion of dispersers and drifters hosted by *A. m. scutellata* (z=0.5, ns).

### 2.2 Queenstate

The impact of taxon and queenstate on the hosting of drifters and dispersers is shown in Table 4 and in Figures 3 and 4. Queenright hybrid colonies hosted more drifters than queenless ones, whilst it was the reverse in *A. m. scutellata* (Tab. 3b and Fig. 3a). No significant difference was found in the number of drifters that were hosted by queenright and queenless *A. m. capensis* colonies.

The queenless *A. m. scutellata* colonies hosted more drifters than their queenright counterparts (Fig. 3). The hybrid queenright colonies hosted significantly more drifters, whilst queenright *A. m. capensis* and *A. m. scutellata* colonies hosted significantly more dispersed workers. Conversely, queenless *A. m. scutellata* colonies hosted significantly more drifted workers and queenless *A. m. capensis* colonies hosted significantly more dispersed workers.

Drifters from queenright *A. m. capensis* mother colonies were found significantly more often in queenright *A. m. capensis* host colonies ($\chi^2_5 = 83.8$, $P<0.0001$, Table 3). In contrast, drifters from queenright hybrid colonies were found significantly more often in queenless hybrid host colonies ($\chi^2_5 = 47.8$, $P<0.0001$) and *vice-versa* ($\chi^2_5 = 585.1$, $P<0.0001$). Likewise, drifters from queenless *A. m. scutellata* mother colonies were found significantly more often in queenright *A. m. scutellata* host colonies ($\chi^2_5 = 62.7$, $P<0.0001$).

Queenstate of the mother colony had no significant effect on the final destination of dispersers from hybrid or *A. m. scutellata* colonies. However, dispersers from queenright *A. m. capensis* colonies were found significantly more often in queenless *A. m. capensis* host colonies ($\chi_5^2 = 61.6$, $P<0.0001$) and *vice versa* ($\chi_5^2 = 45.4$, $P<0.0001$, Tab. 1, Fig. 4).

## 4. DISCUSSION

The test design involved a high density of colonies in our experimental apiaries, in order to amplify the frequency of drifting and dispersing. This is essential to study the underlying biological mechanisms of dispersing, a very rare behavior of honeybee workers. It is also important to reiterate that we tested entirely wild honeybees, including natural hybrids. Therefore, the results reflect the behaviour of workers from naturally occurring populations and are not artefacts of historical beekeeping origin.

Our results show that: 1. Drifting and dispersal represent entirely different behavioural phenomena. 2. Taxon and queenstate of mother and host colonies significantly affect drifting and dispersing as well as the hosting of foreign workers. 3. *A. m. capensis* shows a much higher dispersal frequency than *A. m. scutellata* and the hybrids, suggesting that dispersing constitutes a host finding mechanism of social parasitic workers. 4. The natural hybrids are not simply intermediate in behaviour but show dispersal behavior significantly less often than *A. m. capensis* and *A. m. scutellata*. Moreover, hybrid colonies accepted significantly fewer dispersed workers than *A. m. capensis* colonies. This may contribute to the stability of the natural hybrid zone. 5. There were significant differences for drifting and dispersing as well as for the hosting of drifters and dispersers among each of the tested groups indicating considerable inter-colonial variation.

### Drifting and dispersing

Clearly, the rather artificial experimental design overestimates the number of drifted and dispersed workers. Thus, a qualitative comparison with natural situations seems to be difficult. However, the underlying behavioural mechanisms for the behaviours under study remain the same regardless of the degree of amplification. Thus, the comparison between drifting and dispersing and between subspecies is still valid.

Only 4% (490 out of 12034) of all labelled workers drifted into another colony. Although our experimental design probably amplified the number of orientation errors, only a small proportion of workers actually drifted. Indeed, this result is similar to the proportion of drifted European *A. m. carnica* workers (5%, Neumann et al. 2000c), obtained in an test apiary especially designed to prevent orientation errors. This may either indicate that African honeybee workers are less prone to orientation errors or that African guard bees are more efficient in rejecting foreign workers. The latter seems more likely because African honeybee colonies accept by fare fewer drifted drones than European host colonies (male reproductive parasitism, Rinderer et al. 1985). Colony defensive behavior may be related to the efficiency of the guard force with respect to the hosting of foreign bees (Echazaretta 1988) and indeed colony defensive behavior is more readily expressed in African honeybees compared to European ones (Hepburn & Radloff 1998, personal observations). Dispersing was only performed by a tiny fraction of workers (0.74%, 89 out of 12034 labelled workers). This further underpins the need of an experimental design, which enhances the proportion of drifted and dispersed workers in order to study these behaviours of African honeybee workers.

We found that significantly more workers dispersed the long distance into another apiary circle than expected from the distribution of the drifters. That the latter preferred neighbouring hives has also been reported by others (e.g. Rauschmayer 1928). In contrast, dispersers did not only leave their own micro apiary but also did not prefer the same sector of the new circle, strongly suggesting that drifting and dispersing are not the same behavior. Moreover, there were significant differences in the distribution patterns of drifters and dispersers. While the hybrids drifted significantly more often than *A. m. capensis* and *A. m. scutellata*, they dispersed less often than the other groups. Given drifting and dispersing constitute the same behavior, one would expect a similar trend. Finally, whereas drifted *A. m. capensis* workers from queenright mother colonies were significantly more often found in queenright host colonies, *A. m. capensis* dispersers from queenright mother colonies were found significantly more often in queenless host colonies and *vice versa*. Were drifting and dispersal the same phenomenon we would have expected far fewer workers leaving their home circle and no differences between the tested groups. Therefore, dispersing is not simply long-range drifting but represents an entirely different behaviour of workers.

### Dispersal behavior as a host finding mechanism of social parasitic workers

Thelytokous worker reproduction in honeybees may well predispose the development of aggressive worker reproduction (Greeff 1997) and subsequently of social parasitism by workers. Indeed, the usurpation of *A. m. scutellata* colonies by *A. m. capensis* workers (Hepburn and Allsopp 1994) would appear as unequivocal support for this argument. However, without a suitable host finding mechanism the high incidence of infested *A. m. scutellata* colonies (Allsopp and Crewe 1993) is difficult to explain. It has been suggested that *A. m. capensis* workers enter colonies via drifting (Greeff 1997); but drifting is an accidental displacement into closely neighbouring colonies (Rauschmayer 1928) as confirmed by our results. Therefore, another host finding mechanism is required to explain the epidemic spread of *A. m. capensis* laying workers in the *A. m. scutellata* region (Allsopp 1992), especially between apiaries and in nature.

*A. m. capensis* dispersed significantly more than the hybrids and *A. m. scutellata* combined. Obviously, the greater the number of dispersers the higher the chance of colony take-over (Hepburn and Allsopp 1994) and the more effective spread of potential genes coding for social parasitic workers. Dispersing in *A. m. capensis* could be favoured as a result of thelytoky, reproductive dominance and rapid worker development in queenright and/or queenless mother and host colonies (Hepburn and Radloff 1998). Thus, the combination of thelytoky, rapid ovarial and pheromonal development and dispersing as a host finding mechanism may constitute a functionally related complex (rather than genetically linked, Greeff 1997), which could be expected to spread throughout the species. *A. m. capensis* colonies seldom re-queen from laying worker offspring (Allsopp and Hepburn 1997) and Cape honeybee queens show the highest yet reported degree of polyandry for *A. mellifera* (Neumann et al. 2000b). This weakens the argument that a "high mating risk" (Moritz 1986) is likely to explain the evolution of thelytoky in Cape honeybees. Thus, thelytoky in the Cape honeybee may as well be favoured as a result of this functionally related complex of worker reproductive traits (Neumann et al. 2000b).

The final destination of *A. m. capensis* dispersers in host colonies is clearly related to queenstate: Dispersers from queenright colonies were found significantly more often in queenless host colonies and *vice versa*. This clearly contrasts with the distribution pattern of the drifters and indicates that the hosting of drifted and dispersed workers are two different phenomena. Two non-exclusive interpretations of these observations are possible:

1. Dispersing workers actively "choose" host colonies. This would represent real host finding of social parasitic *A. m. capensis* workers.
2. Host colonies of different queen states deny access of dispersers from mother colonies of the corresponding queenstate indicating a queenstate-based discriminating guarding mechanism.

We also found a high variation for dispersing behavior at worker and colony levels. In particular, one *A. m. capensis* colony (C4) contributed 86.9% of all *A. m. capensis* dispersers. Striking examples for the dispersal of individual *A. m. capensis* workers were observed in the course of this experiment. An individually labelled *A. m. capensis* worker (Red 31) was recovered from a hybrid host colony 921m away from its mother colony separated by several high buildings and patches of wood. Likewise, another worker (Blue 10) was observed in an *A. m. capensis* host colony 500m away from its mother colony. Moreover, Blue 10 actually commuted between the host and mother colonies on four subsequent days.

These observations reinforce the point that orientation errors are inadequate to explain dispersing. They also strongly support observations of Onions (1912) and Johannsmeier (1983) that *A. m. capensis* workers were distributed among host colonies of *A. m. ligustica* and *A. m. scutellata* respectively in a manner which is not possible to explain by simple drifting. Our findings of colony and individual variation for dispersing are supported by Kryger and Van der Shyf (personal communication), who found that a single clone of *A. m. capensis* laying workers invaded a whole *A. m. scutellata* apiary. We argue that this intra- and intercolony variation may reflect a trade-off between reduced colony performance due to too many reproductive dominant workers (Hillesheim et al. 1989) and successful reproduction of social parasitic laying workers in host colonies leading to parasitic and non-parasitic strategies.

We cannot exclude that drifting and dispersing may well be equally important for the parasitism of *A. m. scutellata* apiaries. However, the situation experienced by workers in nature is very different from apiaries:

1. The range of topographical cues to finding the correct nest for honeybees in the wild is likely to be far greater than in an apiary with similar looking hives (as in our test design). Thus, drifting as a result of orientation errors seems highly unlikely to explain host finding of laying workers in nature.
2. Records for the population density of natural African honeybee populations range from 9 colonies/km$^2$ (McNally & Schneider 1996), over 100 colonies/km$^2$ (Hepburn HR, unpublished data) up to 328 colonies/km$^2$ (Quong 1993). Thus, natural distances between mother and potential host nests do not lay in the range of drifting but they do in the one of dispersing. There is evidence for non-progeny workers in feral Africanized honeybee colonies (Hung and Roubik 1992), suggesting that dispersing occurs between natural nests. Moreover, a foreign laying worker matriline appeared upon queen loss in a wild isolated *A. m. capensis* colony (Moritz RFA, unpublished data). This indicates that also *A. m. capensis* colonies can be infested and that the chance for social parasitic workers to have clone mates as new queens in host colonies is not zero under natural conditions. We conclude that dispersing behavior could be a host finding mechanism of social parasitic workers in nature supporting the rapid spread of laying Cape honeybee workers in the areas of *A. m. scutellata* in South Africa (Hepburn and Radloff 1998). Thus, dispersing behavior, which is performed by a minority of workers only, may represent an important part of the reproductive cycle of social parasitic *A. m. capensis* workers. Parasitism is common in other bees and wasps (Roubik 1989; Field 1992), suggesting that dispersing behavior is also present in these species. Our results provide another explanation for the long known (Onions 1912) and often reported (Hepburn and Radloff 1998) social parasitic character of *A. m. capensis* workers.

### Resistance and susceptibility of host colonies

#### 1. Hybrid zone

Worker reproduction is an important aspect of the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* (Moritz et al. 1998) for several reasons. Although there is a morphometrically clearly defined zone of natural hybrid colonies, thelytoky has introgressed into the region (Hepburn and Radloff 1998). Since the hybrid zone seems to be stable, one could expect hybrid colonies to have behavioural strategies, explaining their apparent parasite resistance and consequently the stability of the natural hybrid zone. Indeed, the natural hybrids do not behave in an intermediate matter, but instead exhibit unique behavioural characteristics at worker and colony levels which are highly suggestive of a buffering capacity in the hybrid zone (Hepburn and Radloff 1998). Lack of dispersal by individual hybrid workers is one case in point. The hybrids dispersed less often than either *A. m. capensis* or *A. m. scutellata*. Given that dispersing represents the major host finding mechanism for social parasitic laying workers in nature, clearly fewer hybrid workers spread this gene than do workers of *A. m. capensis*. However, why do the hybrids lack this behaviour which is apparently favoured by natural selection in *A. m. capensis*?

One possibility to explain the lack of dispersal of hybrids might be the general clinal structure of the hybrid zone in which characteristics of *A. m. capensis* are gradually replaced by those of *A. m. scutellata* (cf. Hepburn and Radloff 1998). As a result, hybrid colonies may simultaneously consist of both arrhenotokous and thelytokous laying workers (Pettey 1922; Moritz et al. 1999, Neumann et al. 2000a). Since the population density in the drier parts of the hybrid zone is sparse and much lower than in *A. m. capensis* populations (Hepburn et al. 1994), the chance of finding a host colony in a dispersal event may be low. Moreover, thelytokous laying workers are more likely to become reproductively dominant in queenless hybrid colonies than arrhenotokous ones (Neumann et al. 2000a). The low dispersal frequency of the natural hybrids may therefore reflect a trade-off for thelytokous laying workers between the risk of death in the course of unsuccessful dispersal events against a high chance of successful reproduction in the mother colony with little risk after queenloss. This seems plausible in light of many *A. m. capensis* adaptations to the fynbos region, a macchia-like biome of the Cape region (Hepburn and Jacot-Guillarmod 1991).

Different hosting mechanisms of dispersed workers by hybrid colonies also come into play. In contrast to *A. m. capensis*, hybrid colonies hosted proportionally more drifters than dispersers. Moreover, queenless hybrid colonies hosted significantly fewer drifters than their queenright counterparts; and the former also hosted significantly fewer dispersers than queenright or queenless *A. m. capensis* colonies. These results are consistent with the supposition that hybrid colonies, especially queenless ones, scrutinize incoming individuals more carefully than *A. m. capensis*. If fewer dispersers were accepted by hybrid host colonies, especially by queenless ones, the chance of their usurpation should be smaller (Hepburn and Allsopp 1994). The results support earlier reports that natural hybrid colonies are somewhat resistant to *A. m. capensis* infestations (Greeff 1997). Given that dispersal of workers is typical for *A. m. capensis* and actually represents a host finding mechanism of social parasitic workers, these characteristics of the hybrids may partially explain the stability of the natural hybrid zone because of hybrid advantages (Barton and Hewitt 1985). This would also explain why the social parasitic trait of *A. m. capensis* workers did not spread through and beyond the natural hybrid zone (without human intervention).

### 2. *A. m. scutellata*

The recent usurpations of many thousands of *A. m. scutellata* colonies by *A. m. capensis* laying workers (Allsopp and Crewe 1993) strongly suggest that there is no effective resistance of *A. m. scutellata* to *A. m. capensis* infestations. In contrast to the hybrids, no significant difference was found between the proportion of dispersers and drifters hosted by *A. m. scutellata* colonies. Queenless *A. m. scutellata* colonies hosted more drifted workers than both queenless *A. m. capensis* and queenright *A. m. scutellata* colonies. However, queenright *A. m. scutellata* colonies hosted fewer drifters than queenright or queenless *A. m. capensis* and hybrid colonies. Similar to the hybrids, queenless *A. m. scutellata* colonies hosted fewer dispersers than both queenright and queenless *A. m. capensis* colonies. This indicates that although *A. m. scutellata* colonies may scrutinize incoming workers more carefully than do *A. m. capensis* colonies they nonetheless suffer massive usurpations (Allsopp and Hepburn 1994). Therefore other mechanisms must also play a role.

### 3. *A. m. capensis*

The hosting of foreign workers by *A. m. capensis* colonies may contribute to the understanding of host colony susceptibility to infestations by social parasitic workers. In contrast to the hybrids, *A. m. capensis* colonies hosted proportionally more dispersers than drifters. Moreover, queenright and queenless *A. m. capensis* colonies hosted more dispersed workers than queenless hybrid or *A. m. scutellata* ones, perhaps because invading laying workers may be less of a threat to *A. m. capensis* host colonies. In *A. m. capensis* many more workers are reproductively developed under queenright conditions than in other races (Hepburn and Radloff 1998) and *A. m. capensis* queens are apparently able to prevent take-over by parasitic workers. The role of the queens as one part of the within-hive parasite resistance mechanism of host colonies is also indicated by observations that *A. m. capensis* laying workers successfully invaded queenless European colonies but not queenright ones (Woyke 1995). Moreover, functional laying workers are present in queenright *A. m. capensis* colonies (Moritz et al. 1999). Therefore, invading social parasites must compete not only with the queen but also with well-developed functional laying host workers for reproductive dominance under queenless (Moritz et al. 1996) and queenright conditions, resulting in a lesser genetic threat to the host colony. The results for the hosting behavior of *A. m. capensis* colonies indicate that they are well adapted to the different pathways of worker reproduction (Hepburn 1994). We conclude that a combination of different hosting mechanisms and within-colony mechanisms may govern the resistance of host colonies to social parasitic *A. m. capensis* workers rather than guarding efficiency alone.

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## REFERENCES

Accorti M, 1991. Indagine preliminare sulla deriva delle api. Apicoltura 7: 1-15.

Allsopp MH, 1992. The *capensis* calamity. S Afr Bee J 64: 52-55.

Allsopp MH, Crewe R, 1993. The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. Am Bee J 133: 121-123.

Allsopp, M. H. & Hepburn, H. R. 1997 Swarming, supersedure and the mating system of a natural population of honeybees (*Apis mellifera capensis*). J Apic Res 36: 41-48.

Anderson RH, 1968. The effect of queen loss on colonies of *Apis mellifera capensis*. S Afr J Agric Sci 11, 383-388.

Barton NH, Hewitt GM, 1985. Analysis of hybrid zones. Ann Rev Ecol Syst 16: 113-148.

Beekman M, Calis JNM, Boot WJ, 2000. Parasitic honeybees get royal treatment. Nature 404: 723.

Boylan-Pett W, Ramsdell DC, Hoopinggarner RA, Hancock JF, 1991. Honeybee foraging behaviour, in-hive survival of infectious, pollen-borne blueberry leaf mottle virus and transmission of the virus in high bush blueberry. Pythopathology 81: 1407-1412.

Bohart GE, 1970. The evolution of parasitism among bees. - 41. Honour Lecture, The Faculty Association, Utah State University, 1-30.

Breed M, 1983. Nestmate recognition in honey bees. Anim Behav 31: 86-91.

Duranville C, Miniggio C, Arnold G, Borneck R, 1991. Dérive des butineuses et réinfestation des colonies d'abeilles par *Varroa jacobsoni*. Santé de l'Abeille 125: 228-235.

Drum NH, Rothenbuhler WC, 1985. Differences in non-stinging aggressive responses of worker honeybees to diseased and healthy bees in May and July. J Apic Res 24: 184-187.

Echazaretta CM, 1988. The relationship between drone drifting and aggression in honeybee colonies (*Apis mellifera* L.). MSc Thesis, University of Wales College of Cardiff, UK.

Field J, 1992. Intraspecific parasitism as an alternative reproductive tactic in nest-building wasps and bees. Biol Rev 67: 79-126.

Free JB, 1958. The drifting of honeybees. J Agric Sci 51: 294-306.

Fresnaye J, 1963. Les erreurs d'orientation des abeilles (dérive) dans la rucher moderne. Ann Abeil 6: 185-200.

Greeff MJ, 1996. Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee *Apis mellifera capensis*. Phil Trans R Soc Lon [B] 351: 617-625.

Greeff MJ, 1997. The Cape honeybee and her way north: an evolutionary perspective. S Afr J Sci 93: 306-308.

Hemmling C, Koeniger N, Ruttner F, 1979. Quantitative Bestimmung der 9-Oxodecensäure im Lebenszyklus der Kapbiene (*Apis mellifera capensis* Eschholtz). Apidol 10: 227-240.

Hepburn HR, 1992. Pheromonal and ovarial development covary in Cape worker honeybees. Naturw 79: 523-524.

Hepburn HR, 1994. Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch. Apidol 25: 38-48.

Hepburn HR, Allsopp MH, 1994. Reproductive conflict between honeybees: usurpation of Apis mellifera scutellata colonies by *Apis mellifera capensis*. S Afr J Sci 90: 247-249.

Hepburn HR, Crewe RM, 1991. Portrait of the Cape honeybee, *Apis mellifera capensis*. Apidol 22: 567-580.

Hepburn HR, Jacot Guillarmod A, 1991. The Cape honeybee and the fynbos biome. S Afr J Sci 87: 70-73.

Hepburn HR, Radloff SE, 1998. Honeybees of Africa. Berlin, Springer-Verlag.

Hepburn HR, Jones GE, Kirby R, 1994. Introgression between *Apis mellifera capensis* Escholtz and *Apis mellifera scutellata* Lepeletier: the sting pheromones. Apidol 25: 557-565.

Hepburn HR, Reece S, Neumann P, Moritz RFA, Radloff SE, 1999. Absconding in honeybees (*Apis mellifera*) in relation to queenstate and mode of worker reproduction. Ins Soc 46: 323-326.

Hölldobler B, Wilson EO, 1990. The ants. Berlin, Springer-Verlag

Hillesheim E, Koeniger N, Moritz RFA, 1989. Colony performance in honeybees depends on the proportion of subordinate and dominant workers. Behav Ecol Sociobiol 24: 291-296.

Hung ACF, Roubik WL, 1992. Biochemical evidence of non-progeny workers in feral Africanized honey bee (*Apis mellifera* L.) colonies (Hymenoptera: Apidae). Bee Science 2: 33-36.

Jay SC, 1966a. Drifting of honeybees in commercial apiaries. II. Effect of various factors when colonies are arranged in rows. J Apic Res 5: 103-112.

Jay SC 1966b. Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. J Apic Res 5: 137-148.

Jay SC, 1968. Drifting of honeybees in commercial apiaries. IV. Further studies on the effect of apiary layout. J Apic Res 7: 37-44.

Jay SC, Warr D, 1984. Sun position as a possible factor in the disorientation of honeybees in the southern hemisphere. J Apic Res 23: 143-147.

Johannsmeier MF, 1983. Experiences with the Cape bee in the Transvaal. S Afric Bee J 55: 130-138.

Mackensen O, 1953 The occurrence of parthenogenetic females in some strains of honeybees. J Eco Entomol 36: 465-467.

McNally LC, Schneider SS, 1996. Spatial distribution and nesting biology of colonies of the African honey bee, *Apis mellifera scutellata* Lepeletier, in Africa. Apidol 25: 547-556.

Moritz RFA, 1986. Two parthenogenetical strategies of laying workers in populations of the honeybee, *Apis mellifera* (Hymenoptera, Apidae). Entomol Gen 11: 159-164.

Moritz RFA, Kryger P, Allsopp MH, 1996. Competition for royalty in bees. Nature 384: 31.

Moritz RFA, Beye M, Hepburn RH 1998. Estimating the contribution of laying workers to population fitness in African honeybees (*Apis mellifera*) with molecular markers. Ins Soc 45: 277-287.

Moritz RFA, Kryger P, Allsopp MH, 1999. Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). Behav 136: 1079-1092.

Mossadegh MS, 1993. The biology of the tracheal mite *Acarapis woodi* (Acari: Tarsonemidae) and role of drone honey bees in intercolony spread of the mite, in North Carolina. Sci J Agric 16: 14-22.

Neumann P, Hepburn HR, Radloff SE, 2000a. Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera* L.) colonies. Apidol 31: 479-486.

Neumann P, Kryger P, Moritz RFA, 2000b. Testing the "high mating risk" hypothesis for the evolution of thelytoky in honeybees (*Apis mellifera capensis* Esch.). In review in Proc R Soc Lon [B].

Neumann P, Moritz RFA, Mautz D, 2000c. Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. Apidol 31: 67-79.

Neumann P., Pirk CWW, Hepburn HR, Radloff SE, 2000d. The natural merger of two honeybee (*Apis mellifera capensis* Esch.) colonies: A serendipitous case study. In review in Apidol.

Onions GW, 1912. South African ‘fertile worker bees’. Agric J Union S Africa 1: 720-728.

Pettey FW, 1922. Workers laying in comb of extracting super, Elsenberg Apiary. J Depart Agric Union S Africa 4: 122-124.

Quong A, 1993. The implication of traditional beekeeping in Tabora region, Tanzania for Miombo woodland conservation. School for international training, Tanzania.

Rauschmayer F, 1928. Das Verfliegen der Bienen und die optische Orientierung am Bienenstand. Archiv für Bienenkunde 9: 249-322.

Reeve HK, 1989. The evolution of conspecific acceptance thresholds. Am Nat 133: 407-435.

Ribbands CR, 1953. The behaviour and social life of honeybees. London, Bee Research Association Limited.

Rinderer TE, Richard LH, Danka RG, Collins AM, 1985. Male reproductive parasitism: A factor in the Africanization of European honey-bee populations. Science 228: 1119-1121.

Roubik DW, 1989. Ecology and natural history of tropical bees. Cambridge, Cambridge University Press.

Ruttner FR, 1992. Naturgeschichte der Honigbienen. München, Ehrenwirth Verlag.

Ruttner F, Hesse B, 1981. Rassenspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L. Apidol 12: 159-183.

Schmid-Hempel P, 1998. Parasites in social insects. New Jersey, Princeton University Press.

Wilson EO, 1971. The insect societies. Cambridge, Harvard University Press.

Woyke J, 1995. Invasion of Capensis bee. In: Proc. First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, (Magnuson P, ed), Pretoria, PPRI, 35.

**Table 1:** Drifting (Dr) and dispersing (Dis, bold) of workers between queenright (+) and queenless (-) mother and host colonies of *A. m. capensis* (C), *A. m. scutellata* (S) and their natural occurring hybrids (H).

| Colonies | Mother | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | Hosting | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Host | C4+ | C6+ | C10+ | C3- | C4- | C5- | C6- | C8- | C10- | H1+ | H4+ | H5+ | H6+ | H7+ | H1- | H2- | H4- | H5- | H6- | H7- | S1+ | S2+ | S3+ | S4+ | S6+ | S2- | S3- | S4- | S6- | S9- | Dr | Dis | ∑ |
| C2+ | 49 | | | 1 | | | | 3 | | | | | | 1 | | 3 | | | | | | | | | | | | | | | 57 | 0 | 57 |
| C3+ | | | 2 | | 7 | | | **1** | | | | | | | | | | 1 | | | | | | | | | | | | | 10 | 1 | 11 |
| C4+ | | | | | | | | | | 1 | | | | | | | | | | | | | | | | | | | | **2** | 1 | 2 | 3 |
| C6+ | **1** | | | | **8** | | | | | | | 1 | | | 1 | | | | | | | | | | | | | | | | 2 | 9 | 11 |
| C10+ | | **1** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 1 | 1 |
| C12+ | | | | | **10** | | 5 | | | | | | | | 2 | | | | | | | | | | | | | | | 1 | 8 | 10 | 18 |
| C3- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| C4- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| C5- | **9** | **1** | 7 | | 3 | | | | | | | | | | | | | | | | | | | | **4** | | | | | | 10 | 14 | 24 |
| C6- | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 0 | 1 |
| C8- | 2 | | | | | | | | | 4 | | | | | | | | | | | | | | | | | | | | | 6 | 0 | 6 |
| C10- | **10** | 21 | | | | **1** | | | | | | 9 | | | 7 | **1** | | | | | 1 | | | | 2 | | | | | | 40 | 12 | 52 |
| H1+ | 2 | | | 1 | **1** | | | 33 | | | | **1** | | 1 | | 31 | | | | | | | | | | | | | | | 68 | 2 | 70 |
| H2+ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| H4+ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| H5+ | **2** | 7 | | | **1** | **2** | | | | | | | | | 78 | | | | 11 | | | | **1** | **1** | | | | | | | 96 | 7 | 103 |
| H6+ | | | | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 0 | 1 |
| H7+ | 1 | | | 1 | | | | 2 | | 1 | | | | | | 17 | | **2** | | | | | | 12 | | | | | | | 34 | 2 | 36 |
| H1- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| H2- | 3 | | | | | | | 7 | | 7 | | | | 4 | | | | | | | | | | | | | | | | | 21 | 0 | 21 |
| H4- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| H5- | | | | | | 4 | | | | | | | | | | | | | | | | | | | | | | | | | 4 | 0 | 4 |
| H6- | | 1 | | | **7** | | | | | | | 16 | | | 1 | | | | | | | | | | 1 | 1 | | | | | 20 | 7 | 27 |
| H7- | **1** | | | | 1 | | | | | | | | | | | | | 1 | | | | **1** | | | **1** | | | | | | 2 | 3 | 5 |
| S1+ | **2** | | | | | | | | | | | | | **1** | 2 | | | | | | | **5** | | | | 1 | **2** | | | | 3 | 10 | 13 |
| S2+ | | | | | | | | | | | | | | | | | | | | | | | | 4 | | | 3 | | | | 7 | 0 | 7 |
| S3+ | | | | | | 1 | | **2** | | | | | | | | | | 1 | | | | | | | | **1** | | 16 | | | 18 | 3 | 21 |
| S4+ | 1 | | | | | | | | | | | | | 1 | | | 6 | | | | | | | | | | | | **1** | | 8 | 1 | 9 |
| S5+ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| S6+ | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| S1- | 20 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 20 | 0 | 20 |
| S2- | | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | 0 | 1 |
| S3- | 4 | | | | | | | | | | | | | | | | | | | | | 3 | | 11 | | | | | | | 18 | 0 | 18 |
| S4- | | | | | | | | | | | | | | | | | | 1 | | | | | | | | | | | 2 | | 3 | 0 | 3 |
| S6- | **1** | | | | 7 | 1 | | | | | | | 11 | | | | | 12 | | | | | | **1** | | | **2** | | | **1** | 31 | 5 | 36 |
| S9- | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | 0 | 0 | 0 |
| Dr | 82 | 31 | 9 | 3 | 19 | 6 | 5 | 45 | 0 | 13 | 0 | 26 | 11 | 7 | 91 | 51 | 6 | 16 | 11 | 0 | 1 | 3 | 0 | 27 | 3 | 2 | 3 | 16 | 2 | 1 | 4909 | 89 | 579 |
| Dis | 26 | 2 | 0 | 0 | 27 | 3 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 6 | 1 | 2 | 5 | 1 | 4 | 0 | 1 | 3 | 89 | | |
| ∑ | 108 | 33 | 9 | 3 | 46 | 9 | 5 | 48 | 0 | 13 | 0 | 27 | 11 | 8 | 91 | 52 | 6 | 18 | 11 | 0 | 1 | 9 | 1 | 29 | 8 | 3 | 7 | 16 | 3 | 4 | 579<br>489 | | |

**Table 2** Impact of taxon and queenstate on drifting and dispersing of *A. m. capensis*, *A. m. scutellata* and natural hybrid workers. The Z values are given. Relations between groups are indicated with < and > from left to top (C = *A. m. capensis*, H = hybrid, S = *A. m. scutellata*; + = queenright, - = queenless).

**a.** Impact of taxon (when ignoring queenstate). Significant results (P<0.0001) are indicated with *.

| Group | Dispersing | | | |
|---|---|---|---|---|
| | C | H | S | Overall $\chi_2^2$ |
| C | - | > 22.9* | > 4.6* | 54.5* |
| H | > 1.54 | - | < -4.4* | |
| S | < -10.6* | < -12.8* | - | |
| Overall $\chi_2^2$ | 105.1* | | | - |
| | Drifting | | | |

**b.** Impact of taxon and queenstate. Significant results (P<0.0017; Bonferroni adjusted level of significance) are indicated with *.

| Group | Dispersing | | | | | |
|---|---|---|---|---|---|---|
| | C+ | C- | H+ | H- | S+ | S- |
| C+ | - | < -0.6 | > 9.5* | > 7.6* | > 2.3 | > 3.6* |
| C- | < -3.2* | - | > 11.3* | > 9.0* | > 3.0* | > 4.5* |
| H+ | < -5.2* | < -1.8 | - | < -0.4 | < -4.5* | < -2.7 |
| H- | > 3.1* | > 6.6* | > 9.0* | - | < 3.5* | < -2.0 |
| S+ | < -8.5* | < -4.5* | < -2.5 | < -13.2* | - | > 1.1 |
| S- | < -10.9* | < -6.3* | < -4.0* | < -16.4* | > 1.3 | - |
| | Drifting | | | | | |

**Table 3a** Weighted frequencies of drifted workers grouped according to taxon and queenstate of their mother and host colonies (C = *A. m. capensis*, H = hybrid, S = *A. m. scutellata*; + = queenright, - = queenless).

| | | Host colonies | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mother colonies | | C | | H | | S | | |
| | | + | - | + | - | + | - | Total |
| C | + | 61 | 31 | 18 | 11 | 2 | 29 | 152 |
| | - | 16 | 4 | 44 | 34 | 1 | 44 | 143 |
| H | + | 5 | 13 | 2 | 27 | 1 | 11 | 59 |
| | - | 14 | 14 | 176 | 2 | 11 | 16 | 233 |
| S | + | 0 | 9 | 14 | 3 | 5 | 14 | 45 |
| | - | 1 | 0 | 0 | 6 | 20 | 2 | 29 |
| Total | | 97 | 71 | 254 | 83 | 40 | 116 | 661 |

**Table 3b** Distribution of dispersed workers among the host colonies' circles. Dispersed workers showed no preference to disperse into the same sector from which they originally came from (overall Z-test: z=0.24, ns).

| Mother colonies' | Host colonies' sector | |
|---|---|---|
| sector | same sector | different sector |
| Southwest | 0 | 13 |
| Southeast | 0 | 4 |
| Northwest | 3 | 23 |
| Northeast | 3 | 7 |
| South | 0 | 1 |
| North | 7 | 26 |
| West | 0 | 1 |
| East | 1 | 0 |
| Total | 14 | 75 |

**Table 4** Impact of taxon and queenstate on the hosting of drifted and dispersed workers by colonies of *A. m. capensis*, *A. m. scutellata* and their natural hybrids. The Z values are given. Relations between groups are indicated with < and > from left to top (C = *capensis*, H = hybrid, S = *scutellata*; + = queenright, - = queenless).

**a.** Impact of taxon (when ignoring queenstate). Significant results (P<0.0001) are indicated with *).

| Group | Dispersed | | | |
|---|---|---|---|---|
| | C | H | S | Overall $\chi_2^2$ |
| C | - | > 3.7* | > 4.1* | 18.8* |
| H | > 7.98* | - | < -0.3 | |
| S | < -0.7 | < -8.8* | - | |
| Overall $\chi_2^2$ | 93.0* | | | - |
| | Drifted | | | |

**b.** Impact of taxon and queenstate. Significant results (P<0.0017; Bonferroni adjusted level of significance) are indicated with *.

| Group | Dispersed | | | | | |
|---|---|---|---|---|---|---|
| | C+ | C- | H+ | H- | S+ | S- |
| C+ | - | < -0.4 | > 1.7 | > 3.1* | > 1.5 | > 4.4* |
| C- | < -2.0 | - | > 2.2 | > 3.6* | > 2.0 | > 5.1* |
| H+ | > 9.4* | > 12.3* | - | > 1.1 | < -0.2 | > 2.1 |
| H- | < -1.0 | > 1.0 | < -10.8* | - | < -1.3 | > 0.9 |
| S+ | < -5.4* | < -3.1* | < -18.2* | < -4.1* | - | > 2.3 |
| S- | > 1.3 | > 3.4* | < -7.7* | > 2.4 | > 7.0* | - |
| | Drifted | | | | | |

**Figure 1** Experimental design of the test apiary. The colonies are numbered and arranged in three circles of 12 colonies each. Hybrid colonies are underlined. All hive entrances (indicated with black bars) face the inner sides of the circles. The colonies within each circle are spaced 1 m apart. The circles were 40 m apart. The distances between the circles is not to scale (black colonies = *A. m. capensis*; shaded colonies = hybrid; white colonies = *A. m. scutellata*; + = queenright; - = queenless).

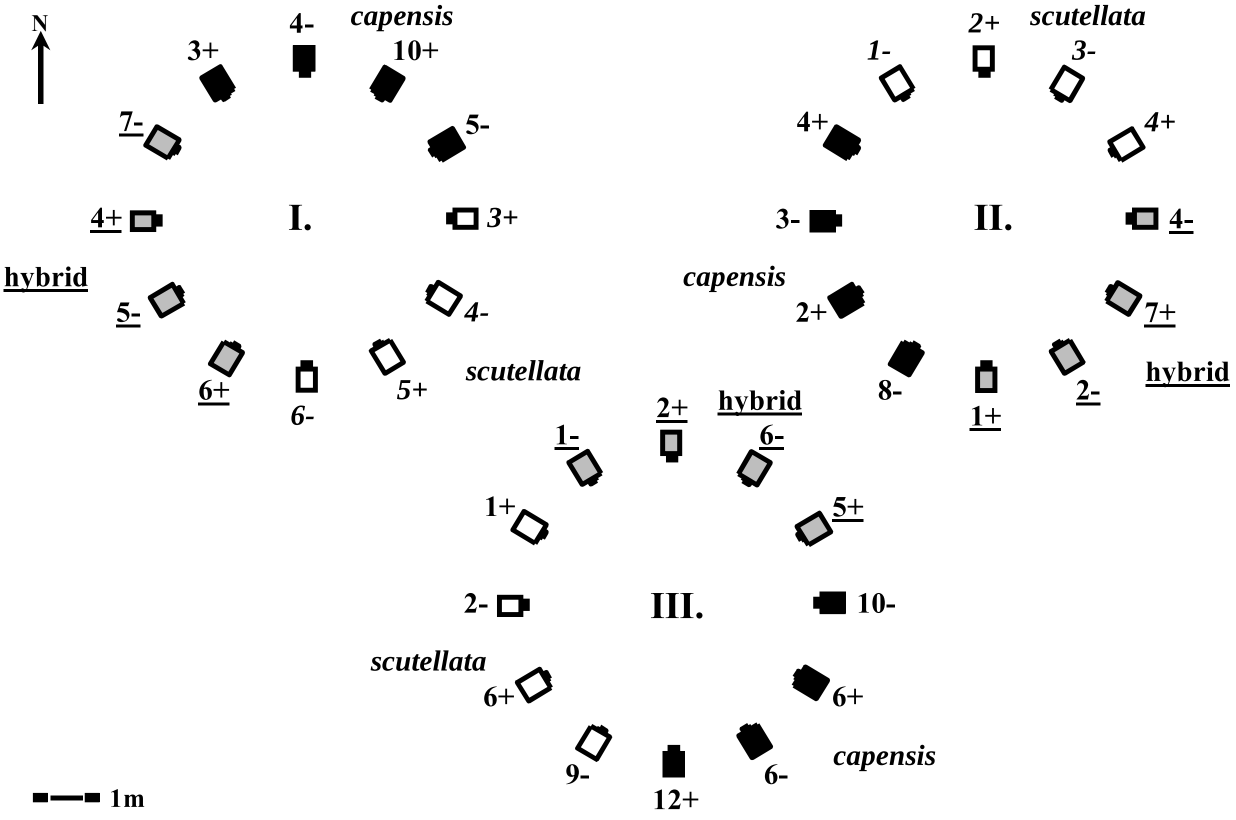


**Figure 2** Drifting and dispersing of workers from queenright and queenless mother colonies of *A. m. capensis*, *A. m. scutellata* and their naturally occurring hybrids as percentages of the total amount of individuals. Figure 2A shows the distribution of drifted workers among the mother colonies. Figure 2B shows the distribution of dispersed workers among the mother colonies. Two splits of one *A. m. capensis* colony (C4+ and C4-) contributed 59.6% of all dispersed workers.

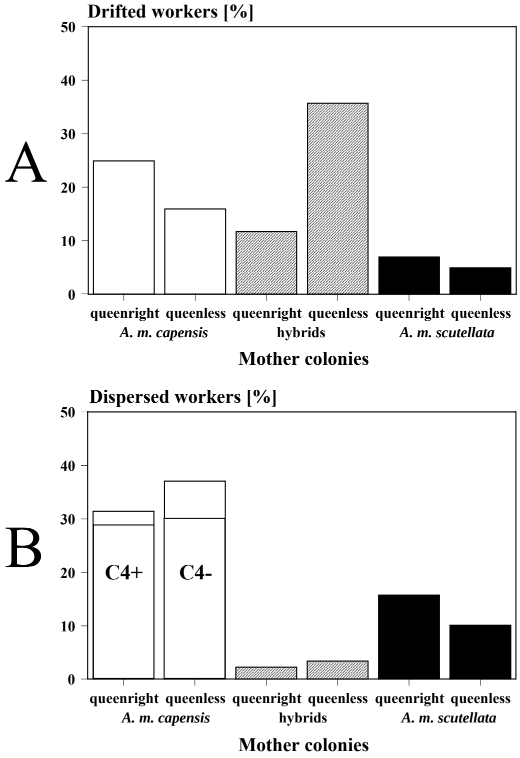


**Figure 3** Hosting of drifted and dispersed workers by queenright and queenless colonies of *A. m. capensis*, *A. m. scutellata* and their naturally occurring hybrids as percentages of the total amount of hosted drifted or dispersed workers. Figure 3A shows the distribution of drifted workers among the host colonies (derived from the weighted frequencies). Figure 3B shows the distribution of dispersed workers among the host colonies.

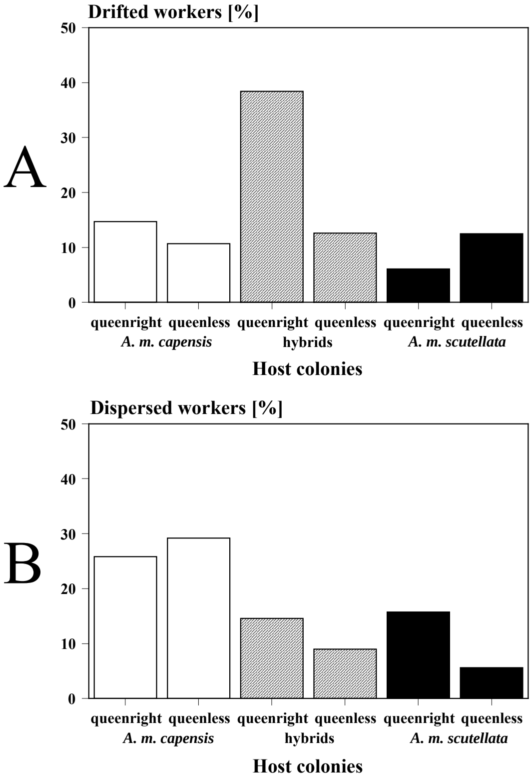
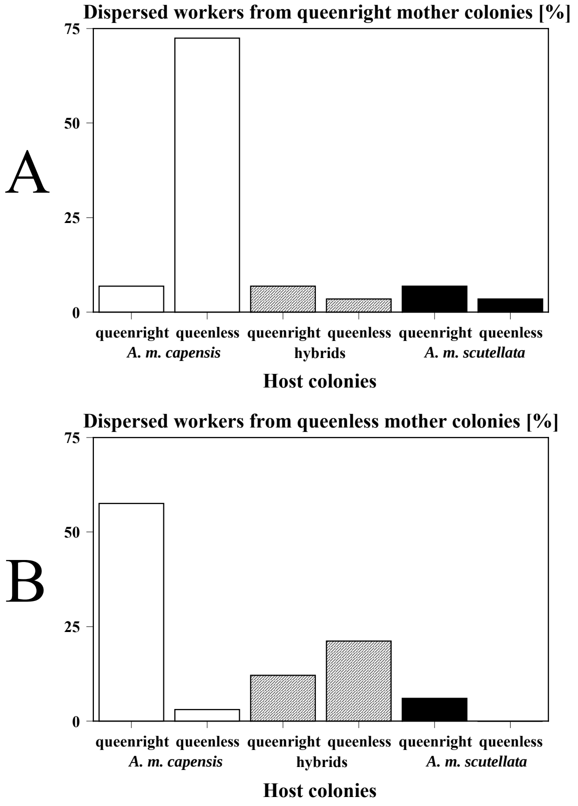


**Figure 4** Impact of queenstate of mother and host colonies on the final destination of dispersed *A. m. capensis* workers as percentages of the total amount of individuals. Figure 4A shows the distribution of dispersed workers from queenright mother colonies. Figure 4B shows the distribution of dispersed workers from queenless mother colonies.



# 3.4 Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of worker reproduction

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**Abstract -** We investigated absconding frequency and latency in queenright and queenless honeybee colonies in thelytokous *Apis mellifera capensis*, arrhenotokous *Apis mellifera scutellata* and their natural thelytokous hybrids. There was no significant difference in frequency of absconding among any of the queenright colonies. Absconding was significantly greater in thelytokous queenless colonies than in the queenless arrhenotokous ones. Latency to absconding did not differ among the three groups of queenright colonies nor between the queenright and queenless colonies of *A. m. capensis* and *A. m. scutellata*. There were significant differences in latency between queenright and queenless hybrids and significant differences in latency among the three groups of queenless colonies. Among queenless colonies, *A. m. capensis* absconded twice as readily as did *A. m. scutellata* and the hybrids were intermediate. After absconding events include the fates of the absconding colony as well as nestmates left behind. One group of orphaned nestmates of *A. m. capensis* amalgamated with another queenright colony. In the case of *A. m. scutellata* either drones were produced or the residual queenless colony was joined by a queenless thelytokous group, subsequently reared a queen and then absconded. Differences in the rate and degree of ovarial development indicate that queenless thelytokous workers have the physiological capacity for reproduction, a trait that contributes to colony fitness.

**Key words:** honeybees / absconding / arrhenotoky, thelytoky

## 1. INTRODUCTION

Absconding is a behavioural trait of all honeybees but is particularly well expressed in many African subspecies of *Apis mellifera* and other tropical species of *Apis* (Hepburn and Radloff, 1998; Ruttner, 1988; Punchihewa, 1994; Kevan, 1995; Hepburn and Radloff, 1998). Absconding in the same population may be resource-related and seasonal (Douhet, 1970; N'diaye, 1974; Rashad and El-Sarrag, 1978) or result from disturbances such as fire, predation (including beekeeping manipulations) and declining nest quality (Potiron, 1972; Castagné, 1983; Chandler, 1976). In disturbance related absconding a honeybee colony often abandons eggs, open and capped brood, food reserves, foragers afield and, sometimes, even the queen (de Villiers, 1883; Gough, 1928; Hepburn, 1988). In resource-related absconding it may cannibalise most of the nest contents before departure (Woyke, 1989; Mutsaers, 1994). From a beekeeping perspective absconding represents a loss in production so it has acquired a rather negative connotation.

However, from an evolutionary perspective, absconding is probably a major survival strategy in tropical climates where year-round conditions are equitable for some flowering and for honeybee flight. Honeybees of the temperate regions need to invest in large food stores in thermally secure nests to survive the harsh winter. Tropical honeybees are far less sessile than temperate bees and can follow honey flows or abscond to avoid nest predators. Although there is a potential benefit for absconding in a tropical environment, it is not free of costs. The brood cycle is interrupted and lost combs need to be constructed anew. Furthermore, investment in capped brood, newly emerged workers and foragers afield may also be lost.

The loss of the queen may have very different consequences for different African honeybee subspecies. If the workers cannot requeen themselves from the brood of a queen, laying workers develop. In most African subspecies these workers produce drones; but, in *A. m. capensis*, the workers produce through a thelytokous parthenogenesis female offspring which can develop into a queen. Thus the queenless colony with thelytokous laying workers has the potential to survive at its nest site whereas colonies with arrhenotokous laying workers are doomed once the drones are produced (Ruttner and Hesse, 1981; Ruttner, 1988). Here we report observations on absconding swarms of thelytokous *A. m. capensis*, arrhenotokous *A. m. scutellata* and their naturally occurring thelytokous hybrids, to evaluate the relationship between queen status and the mode of worker reproduction on absconding behaviour.

## 2. MATERIALS AND METHODS

Honeybee colonies used for the observations were thelytokous *A. m. capensis* from Port Elizabeth, arrhenotokous *A. m. scutellata* from the Steynsburg area and the thelytokous *A. m. capensis* x *A. m. scutellata* naturally occurring hybrids from the Stutterheim area (all localities within South Africa). Detailed descriptions of the biological characteristics and variations of these different honeybee populations are given elsewhere (Hepburn et al., 1998).

All colonies were collected from their home apiaries at the same time, each was transferred into a Langstroth-style 5-frame nucleus hive of 20*l* capacity and transported to an apiary prepared for them at Grahamstown, South Africa. The colonies were then subdivided to produce the following groups: (1) 12 queenright and 17 queenless colonies of *A. m. capensis*, (2) 10 queenright and 17 queenless colonies of *A. m. scutellata* and (3) 11 queenright and 16 queenless colonies of the natural hybrids. The colonies were given a week to settle and were then inspected on a weekly basis to monitor demographic changes and absconding events. The observations were made between January and March 1998 during which period there was a natural nectar flow. Thus the absconding observed is regarded as being disturbance-related (beekeeping manipulation) and not due to resource depletion.

ANOVA and non-parametric Kruskal-Wallis test procedures were used to test for significant differences in latency to absconding between *A. m. capensis*, *A. m. scutellata* and their natural hybrids. Chi-square and Fisher exact tests were carried out to test the magnitude of difference in absconding in relation to queen status and mode of worker reproduction. Choice of probability levels to constitute significance is arbitrary but in this study significance is defined as $P<0.10$.

## 3. RESULTS

### 3.1 Absconding events

The extent of absconding in 83 colonies of thelytokous *A. m. capensis* and *A. m. capensis* x *A. m. scutellata* hybrids and arrhenotokous *A. m. scutellata* in relation to queen status is shown in Table 1. Absconding occurs significantly more frequently in the queenless colonies of the two thelytokous groups than in their queenright counterparts (for *A. m. capensis*, $\chi^2$=5.12,1df, P=0.0237 with Yates correction, Fisher exact P=0.0140; for hybrids $\chi^2$=7.72, 1df, P=0.0054 with Yates correction, Fisher exact P=0.0034). However, the magnitude of difference in absconding between queenright and queenless *A. m. scutellata* is not significant ($\chi^2$=1.10, 1df, P=0.2943 with Yates correction, Fisher exact P=0.2365).

When the bees are grouped with respect to queen status and analysed in relation to subspecific categories, a different perspective is obtained. In these comparisons there are no significant differences in the extent of absconding among the queenright colonies of *A. m. capensis*, the *A. m. capensis* x *A. m. scutellata* hybrids and *A. m. scutellata* ($\chi^2$=0.61, 2df, P=0.7383). However, differences in the extent of absconding among subspecific categories approached significance among the queenless colonies ($\chi^2$=5.52, 2df, P=0.0632; *A. m. capensis / A. m. scutellata* comparison, Fisher exact P=0.0588).

### 3.2 Latency to absconding

Latency to absconding bears on colony fitness in terms of colony longevity and measures for this behaviour are summarised in Table 2. Subspecific comparisons demonstrate that there was no significant difference in the latency to abscond among any of the queenright colonies of *A. m. capensis*, *A. m. capensis* x *A. m. scutellata* and *A. m. scutellata* (F=0.451; 2,8df, P=0.6523, H=1.67, P=0.4338) . Queenlessness is a different matter: queenless colonies of *A. m. capensis* and *A. m. scutellata* reveal significant differences with respect to latency (F=5.386; 2,36df, P=0.0090, H=8.12, P=0.0172). In fact, queenless colonies of *A. m. capensis* absconded twice as readily as the queenless *A. m. scutellata* colonies. In this respect the *A. m. capensis* x *A. m. scutellata* hybrids were intermediate in absconding latency and did not differ significantly from either the queenless *A. m. capensis* or queenless *A. m. scutellata* colonies (Table 2).

When the bees were grouped with respect to queen status and analysed in relation to subspecific categories, there were no significant differences in absconding latency between queenright and queenless colonies of *A. m. capensis* (parametric P=0.4348; non-parametric P=0.4344) and *A. m. scutellata* (parametric P=0.4387; non-parametric P=0.5497) however, the hybrid group revealed a significant difference (parametric P=0.0700; non-parametric P=0.0316) in latency between the queenright and queenless colonies (Table 2).

### 3.3 Afterabsconding events

Here we adopt the term 'afterabsconding' by analogy with 'afterswarms' to refer to events subsequent to the departure of the main colony absconding from its nest. There are two aspects to afterabsconding: (1) the actual fate of the absconded colony as well as (2) the fate of fellow nestmates abandoned by the absconding colony following disturbance. Several observations were recorded as to the fate of abandoned nestmates usually consisting of eggs, larvae, capped brood and returning field bees left behind in the queenless maternal nest.

Four such colonies of the hybrids and one of *A. m. capensis* containing only a few hundred bees persisted as thelytokous laying worker colonies. One queenless colony of hybrids absconded and then amalgamated with another queenright hybrid colony, the new unit subsequently absconded. After several weeks one colony of abandoned *A. m. scutellata* nestmates began to produce drones. Another abandoned *A. m. scutellata* colony eventually produced a queen (presumably from the egg of an *A. m. capensis* or hybrid thelytokous laying worker) subsequent to which it absconded. No direct observations as to their eventual fate were made on any of the 'primary' absconding colonies in the present study, but this matter is addressed by inference from other studies in the discussion below.

## 4. DISCUSSION

The results unequivocally demonstrate that queenless colonies of the thelytokous *A. m. capensis* and their natural hybrids abscond at significantly higher rates than the arrhenotokous queenless *A. m. scutellata.* It can be noted that queenless colonies have a shorter latency to absconding than their queenright counterparts (Tables 1&2). The queenright colonies do not differ with regard to the extent or latency of absconding among themselves as a group. That the extent of and latency to absconding is significantly elevated in the queenless *A. m. capensis* x *A. m. scutellata* hybrids is consistent with their other *capensis*-like traits in the natural hybrid zone where they occur (Hepburn *et al.*, 1998). While absconding of queenright colonies is well documented for the African subspecies of honeybees (Hepburn and Radloff, 1998), it is the queenless units which are of particular interest simply because they retain reproductive fitness to varying degrees.

Given queenlessness, there is always some reproductive development among workers, but this varies greatly in relative latency and extent (Velthuis, 1970; Ruttner and Hesse, 1981). Previous measurements have shown that about 75% of the workers in a queenless, thelytokous *A. m. capensis* colony will have undergone some ovarial development within a fortnight and that 12% of workers will have ripe eggs (this figure is doubled if *A. m. capensis* is hosted in a queenless *A. m. scutellata* colony). In contrast, in *A. m. scutellata* only 30% of workers may show ovarial development and less than 1% will actually have ripe eggs, over the same period (Hepburn and Allsopp, 1994).

These differences in degree and rate of reproductive development among the thelytokous and arrhenotokous groups of queenless bees hold major consequences in terms of fitness. Queenless *A. m. capensis* workers have the physiological capacity for reproduction whether they remain queenless or amalgamate with other queenless or queenright bees (Hepburn, 1994). The high incidence of absconding in queenless *A. m. capensis* colonies in combination with the high reproductive capacity of laying workers and the amalgamation potential of absconded colonies could, in principle, provide a mechanism for social parasitism in honeybees.

It has been cogently argued that thelytoky is a more effective form of worker reproduction than arrhenotoky and that the frequency of queen-loss is a primary constraint in the spread of genes for thelytoky (Moritz, 1986; Greeff, 1996a,b, 1997). If the combination of thelytoky, rapid ovarial development, absconding and amalgamation or invasive drifting is a gene-related complex then it should spread throughout the species. Following the rules of natural selection, parasitic worker behaviour could establish itself through frequency dependent selection at a certain equilibrium (Moritz, 1989).

In any event, the possible fate of absconded colonies must be considered. Three different social development pathways have been documented in a study of 60 colonies of queenless, thelytokous *A. m. capensis* (and by inference to the thelytokous hybrids): (1) queens were eventually generated from the eggs of thelytokous workers in 19 colonies; (2) 16 colonies persisted as laying worker-led colonies; and (3) 17 others absconded yet again while 8 others dwindled and died (Hepburn, 1994). Comparable data for droneless, queenright colonies of *A. m. capensis* that abscond can be inferred from observations on such colonies caught in trap boxes. These colonies were winter, resource-related absconding units (Hepburn and Jacot-Guillarmod, 1991). Of 31 absconded colonies so caught, 5 starved to death, 11 others absconded yet again, and 15 colonies remained as stable, settled colonies. Thus the survival rate for queenright colonies was about 58% while that for queenless colonies was about 48% (not counting those that absconded yet again but which may nevertheless have survived in both cases). For thelytokous bees, survival of queenless colonies demonstrably contributes to fitness. Observations on the fate of nestmates abandoned and left behind by absconding colonies have not been previously reported. As we have seen, such handfuls of bees, often fewer than 300-400, may themselves abscond and amalgamate with other colonies or persist as laying worker colonies. The point is, even these small residual units are capable of reproduction and to a degree of fitness.

## REFERENCES

Castagné, J.B. 1983. L'apiculture au Congo-Brazzaville. *Bull. Tech. Apic. 10*(4): 197-208.

Chandler, M.T. 1976. The African honeybee - *Apis mellifera adansonii*: the biological basis of its management. *Proc. 1st Int. Conf. Apic. Trop. Climates*, London, UK, pp. 61-69.

de Villiers, J.H. 1883. Cape bees and 'animal intelligence'. *Nature* 28: 5-6.

Douhet, M. 1970. *L'Apiculture Sénégalaise*. Secrétariat d'État aux Affaires Etrangères, France.

Gough, L.H. 1928. Apistischer Brief aus Südafrika. *Der Bienenvater 60*: 30-32.

Greeff, J.M. 1996a. Thelytokous versus arrhenotokous worker reproduction in the Cape honeybee and other eusocial Hymenoptera. *Hereditas 124*: 99-103.

Greeff, J.M. 1996b. Effects of thelytokous worker reproduction on kin selection and conflict in the Cape honeybee, *Apis mellifera capensis*. *Phil. Trans. Roy. Soc.* London *B351*: 617-625.

Greeff, J.M. 1997. The Cape honeybee and her way north: an evolutionary perspective. *S. Afr. J. Sci. 93*: 306-308.

Hepburn, H.R. 1988. Absconding in the African honeybee: the queen, engorgement and wax secretion. *J. Apic. Res. 27*: 95-102.

Hepburn, H.R. 1994. Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch. *Apidologie 25*: 38-48.

Hepburn, H.R. and M.H. Allsopp, 1994. Reproductive conflict between honeybees: usurpation of *Apis* *mellifera scutellata* colonies by *Apis mellifera capensis*. *S. Afr. J. Sci. 90*: 247-249.

Hepburn, H.R. and A. Jacot Guillarmod, 1991. The Cape honeybee and the fynbos biome. *S. Afr. J. Sci. 87*: 70-73.

Hepburn, H.R. and S.E. Radloff, 1998. *Honeybees of Africa*. Springer-Verlag, Berlin, Germany.

Hepburn, H.R., S.E. Radloff and S. Fuchs, 1998. Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*. *Apidologie* (in press).

Kevan, P.G. (ed) 1995. *The Asiatic Hive Bee*. Enviroquest, Ltd. Cambridge, Canada.

Moritz, R.F.A. 1986. Two parthenogenetical strategies of laying workers in honeybee populations (*Apis mellifera* L.). *Entomol. Gen. 11*: 159-164

Moritz, R.F.A. 1989. Colony level and within colony level selection in honeybees. A two allele population model for *Apis mellifera capensis*. *Behav. Ecol. Sociobiol. 25*: 437-444

Mutsaers, M. 1994. Absconding of honeybee (*Apis mellifera adansonii*) colonies in Southwest Nigeria, related to the seasonal weight of colonies and combs. *Proc. 5th Int. Conf. Apic. Trop. Climates*, Cairo, Egypt, pp 8-9.

N'diaye, M. 1974. *L'apiculture au Sénégal*. PhD thesis, University of Dakar, Dakar, Senegal.

Potiron, R. 1972. Apiculture au Dahomey. *Gaz. Apic. 73*: 151-152.

Punchihewa, R.W.K. 1994. *Beekeeping for Honey Production in Sri Lanka*. Sri Lanka Department of Agriculture, Peradeniya, Sri Lanka.

Rashad, S.E. and M.S.A. El-Sarrag, 1978. Beekeeping in Sudan. *Bee Wld. 59*: 105-111.

Ruttner, F. 1988. *Biogeography and Taxonomy of Honeybees*. Springer-Verlag, Berlin, Germany.

Ruttner, F. and B. Hesse, 1981. Rassensspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Hongbiene *Apis mellifera L. Apidologie 12*: 159-183.

Velthuis, H.H.W. 1970. Ovarian development in *Apis mellifera* worker bees. *Ent. Exp. Appl. 13*: 377-394.

Woyke, J. 1989. Biology and management of African bees *Apis mellifera adansonii* in Africa. *32nd Int. Cong. Apic.*, Apimondia, Bucharest, Romania, pp 44-47.

**Table 1.** Absconding frequency in *A. m. capensis*, *A. m. scutellata* and their natural hybrids.

| Subspecies | Queenright | | Queenless | | Queenright/Queenless |
|---|---|---|---|---|---|
| | abscond | stay | abscond | stay | abscond |
| *A. m. capensis* | $5^{a}$ | 7 | $15^{a}$ | 2 | P<0.05 |
| hybrids | $3^{a}$ | 8 | $14^{a}$ | 2 | P<0.01 |
| *A. m. scutellata* | $3^{a}$ | 7 | $10^{b}$ | 7 | ns |
| total | 11 | 22 | 39 | 11 | |

* Means having the same symbol do not differ significantly.

**Table 2**. Latency in weeks (x±sd) to absconding in *A. m. capensis*, *A. m. scutellata* and their natural hybrids.

| Subspecies | Queenright | Queenless | Queenright/Queenless |
|---|---|---|---|
| ***A. m. capensis*** | $5.00 \pm 3.52^{a}$ | $3.60 \pm 3.03^{a}$ | ns |
| hybrids | $7.00 \pm 0.00^{a}$ | $4.71 \pm 1.91^{ab}$ | parametric P<0.1;<br>non parametric P<0.05 |
| ***A. m. scutellata*** | $5.67 \pm 1.25^{a}$ | $7.50 \pm 3.50^{b}$ | ns |

* Means having the same symbol do not differ significantly.

# 3.5 A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis* Esch.)

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**Key words:** *Apis mellifera capensis* / honeybee / merger / spatial distribution / division of labor

Natural mergers of honeybee colonies are commonplace in tropical Africa (Hepburn and Radloff 1998), but their consequences on organizational structure are unknown. Here we determine the spatial distribution and division of labor of workers following a merger of two colonies. Two unrelated colonies (each ~3000 bees) were placed in three-frame observation hives. When workers emerged from sealed brood of each colony, they were individually labeled and reintroduced into their respective mother hives. They are referred to as cohorts **A** and **B**, each comprising 300 workers of the same age. The behaviors and positions of all labeled workers and queens were recorded twice daily for 24 days (Kolmes 1989, Pirk et al. 2000). On day 14 colony **B** was dequeened, left its nest and merged with colony **A** on day 15.

4357 individual behavioral acts (48 different tasks) and 2263 queen-worker distances (1422 before and 841 after merger) were recorded for 360 labeled bees. Severe fighting initially occurred at the nest entrance when the merger began but no aggression occurred once the workers of colony **B** had entered the nest of colony **A**. No significant differences in total activity (all tasks/idleness) and mean queen-worker distances of individuals bees were observed between the cohorts **A** and **B** before and after merger (Tab I a). However, total activity decreased and queen-worker distances increased after merger for the individual bees of both cohorts (Tab I a). There were significant differences among and between tasks of cohorts **A** and **B** before and after merger (Table I b). While some tasks increased and others decreased, the patterns of changes between cohorts differed (Table I b). Daily counts of queen-worker distances were significantly different on four occasions before merger but only once 24 hours after merger (data not shown), demonstrating effective cohort integration. Also workers of both cohorts were similarly distributed throughout the nest after merger.

On queen removal cohort **B** workers did not attempt to requeen but immediately merged with colony **A**. This may seem puzzling from an evolutionary perspective because the inclusive fitness of queenless workers is zero in the new unit. However, mergers are frequent in tropical honeybees (Hepburn and Radloff 1998) and could be adaptive because workers may gain direct fitness. The lower levels of activity and the immediate increase in colony size after merger probably reduce *pro rata* survival costs (Hepburn and Radloff 1998). The origin of merging bees may matter, because task shifts differed in the two cohorts. This might be partially ascribed to age-related division of labor; however, this does not explain the substantial shifts observed both within and between the cohorts before and after merger. Possibly, workers changed tasks as a result of different behavioral thresholds and task specialization (Moritz and Page 1999). Thus, the possible acquisition of more efficient genetic specialists (Fuchs and Moritz 1999) may also contribute to reduce *pro rata* costs in the new unit. The task shifts and worker distribution suggest that many bees responded to a different colony environment in the new unit, presumably necessary for social integration.

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## REFERENCES

Fuchs S., Moritz R.F.A., Evolution of extreme polyandry in honeybees (*Apis mellifera*), Behav. Ecol. Sociobiol. 45 (1999) 269-277.

Hepburn H.R., Radloff S.E.. Honeybees of Africa, Springer, Berlin, 1998.

Kolmes S.A., An information-theory analysis of task specialisation among worker honey bees performing hive duties, Anim. Behav. 33 (1985) 181-187.

Moritz R.F.A., Page R.E., Behavioral threshold variability: costs and benefits in insect societies, in: Detrain C.J., Pasteels J. Deneubourg J.l. (Eds.), Information processing in Social Insects. Birkhäuser, Basel, 1999, pp. 203-215.

Pirk C.W.W., Polthier K., Neumann P., Preuss E., Estimating and visualizing the spatial distribution of individuals in social insect colonies (2000) http://www.sfb288.math.tu-berlin.de

**Table I.** Proportional comparisons for **(a)** individual workers and **(b)** whole cohorts **A** and **B** before and after merger. Differences in total activity and mean queen-worker distances for individual bees were analyzed with Mann Whitney U-tests. Z-tests of proportions were used to test for significant differences in the task performances of the whole cohorts **A** and **B**: (i) for each cohort and the new colony and (ii) to assess frequency changes of performances before and after merger between cohorts **A** and **B**. Only those behaviors are shown, where significant results have been obtained. Significant results are indicated with * for P<0.01 and ** for P<0.001 using Bonferroni adjustments (N= sample size, P= significance level, F= frequency, new colony = **A** + **B** combined).

| **(a)** Individual bees | Before merger | | | | | After merger | | | | | Before vs. After **A** | **B** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cohort **A** | N | Cohort **B** | N | P | Cohort **A** | N | Cohort **B** | N | P | P | P |
| Total activity | 0.51±0.33 | 170 | 0.51±0.28 | 180 | | 0.29±0.30 | 143 | 0.33±0.36 | 118 | | ** | ** |
| Mean queen-worker distance | 30.1±14.2 | 167 | 31.1±11.9 | 175 | | 43.3±13.9 | 132 | 44.7±16.4 | 106 | | ** | ** |

| **(b)** Whole cohorts | Cohort **A** | | | Cohort **B** | | | Changing patterns | | | New colony | Vs. **A** | **B** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before | After | | Before | After | | **A** | **B** | | | | |
| Task | F | F | P | F | F | P | | | P | F | P | P |
| Walk | 213 | 209 | * | 246 | 123 | * | -4 | -123 | * | 332 | * | * |
| Idleness | 478 | 903 | * | 612 | 681 | * | +425 | +69 | * | 1584 | * | * |
| Groom self | 49 | 15 | * | 78 | 14 | * | -34 | -64 | * | 29 | * | * |
| Inspecting empty/egg cell | 58 | 24 | * | 104 | 22 | * | -34 | -82 | * | 46 | * | * |
| Inspecting honey cell | 20 | 23 | | 38 | 9 | * | +3 | -29 | * | 32 | | * |
| Build comb | 1 | 8 | | 3 | 7 | | +7 | +4 | | 15 | * | |
| Groom other worker | 24 | 8 | * | 27 | 4 | * | -16 | -23 | | 12 | * | * |
| Get groomed | 4 | 0 | | 10 | 0 | * | -4 | -10 | | 0 | * | * |
| Lateral shake | 1 | 0 | | 5 | 1 | | -1 | -4 | | 1 | | * |
| Dorsoventral abdominal vibration | 5 | 0 | * | 2 | 0 | | -5 | -2 | | 0 | * | |
| Begging for food | 2 | 0 | | 2 | 22 | * | -2 | +20 | * | 22 | * | * |
| Attend queen | 6 | 0 | * | 1 | 0 | | -6 | -1 | | 0 | * | |
| Antennate with worker | 52 | 26 | * | 61 | 12 | * | -26 | -49 | * | 38 | * | * |
| Run (move faster ~3 cm/s) | 4 | 0 | | 12 | 1 | * | -4 | -11 | | 1 | * | * |
| Forage | 1 | 1 | | 0 | 4 | * | 0 | +4 | | 5 | | |
| Wax chain | 0 | 15 | * | 0 | 10 | * | +15 | +10 | | 25 | * | * |

# 3.6 The behaviour of drifted Cape honeybee workers (*Apis mellifera capensis* Esch.): predisposition for social parasitism?

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**Abstract -** Cape honeybee workers are facultative social parasites and drifting is one mode of transmission to new host colonies. The behavioural patterns and spatial distributions of drifted Cape honeybee workers differed from those of non-drifted workers of the same age cohort. Drifted workers were significantly more idle and were more often found in areas away from the queen compared to non-drifted workers. Our data suggest that drifted Cape honeybee workers may be predisposed for social parasitism in host colonies.

**Key words:** *Apis mellifera capensis* / drifting / honeybee / social parasitism / worker reproduction

## 1. INTRODUCTION

Laying workers of the Cape honeybee, *Apis mellifera capensis* Esch., are facultative social parasites (Neumann and Hepburn 2002; Neumann and Moritz, 2002) and individual workers can enter new host colonies by drifting (Neumann et al., 2001b). Drifting results from orientation errors of young workers during their orientation flights and of returning foragers (Rauschmayer, 1928; Free, 1958; Pfeiffer and Crailsheim 1998; Neumann et al. 2000). However, very few studies address the actual behaviour of drifted workers in host colonies. This is of prime importance with respect to social parasitism because the behaviour of drifted workers may govern whether an individual can reproduce in the host colony or not.

Drifted *A. m. carnica* workers are generally more inactive and seem to perform tasks of importance for the colony less frequently than those related to individual welfare such as receiving food or being groomed (Pfeiffer and Crailsheim, 1999). Similarly, reproductively dominant Cape honeybee workers do not participate as much in hive duties compared to subordinate ones (Hillesheim et al., 1989). Since the life expectancy of workers is related to work load (Woyke, 1984) and to individual reproductive success (Neumann and Moritz, 2002), inactivity of drifted workers might actually constitute a tactic to increase individual fitness (Pfeiffer and Crailsheim, 1999). This is especially the case, if successful reproduction of social parasitic *A. m. capensis* workers is more likely during certain time windows such as major pollen flows (A Schehle, personal communication), supersedure events or after queenloss (Härtel, Moltzer, Neumann and Hepburn, unpublished data).

Given that drifted Cape honeybee workers follow a tactic of “hopeful” reproductives in their new host colonies, other mechanisms also come into play. In particular, the development of queen-like pheromones appears to be an essential part of the social parasitic pathway of *A. m. capensis* workers (Wossler, 2002; Neumann and Hepburn 2002). However, the pheromonal bouquet of the host queen might suppress the ovarial development of the drifted workers unless they already show some pre-drifting ovarial development (Reece, 2002; Neumann and Hepburn, 2002). One mechanism to avoid the suppressive signals of the host queen may be simply to evade the queen’s mandibular gland signals and remain in areas of low 9-ODA concentration in the colony (Neumann and Moritz, 2002). Such behaviour is more readily expressed in *A. m. capensis* workers than in the neighbouring subspecies *A. m. scutellata* (Moritz et al., 2001) and those workers staying away from the queen show a more queenlike pheromonal bouquet (Moritz et al., 2002). However, it is unknown whether drifted Cape honeybee workers show a stronger tendency to stay away from the queen compared to non-drifted bees. Given that drifted *A. m. capensis* workers are less active and more frequently stay away from the queen, this would suggest that such workers are predisposed for social parasitism in their host colonies. Here we study the behaviour of drifted and non-drifted Cape honeybee workers.

## 2. MATERIAL AND METHODS

### 2.1 Sampling and experimental design

Five unrelated queenright colonies of *A. m. capensis* (each ~3000 bees) were placed in three-frame observation hives 1m apart from each other at Rhodes University, Grahamstown, South Africa. In order to reduce the potential impact of different hive environments on the drifting of workers, all observation hives were provided the same experimental set up. The middle frame in each hive contained brood and the top and bottom frames honey and pollen; while food and water were provided ad lib. Flight entrances were labelled with the same colour to amplify orientation errors (Rauschmayer, 1928). Parallel to the setting of the observation hives, frames with sealed worker brood were taken from each of the experimental colonies and placed in an incubator until adult emergence. Freshly emerged workers (N= 400 of the same age cohort for each colony) were individually marked using a combination of Opalithplättchen on the thorax and a colony specific colour code on the abdomen. Then, the marked workers were simultaneously reintroduced into their mother colonies.

### 2.2. Behavioural observations

Behavioural observations started 12 hours after the introduction of the labelled workers. A grid was drawn on the side panes of each observation hive, dividing the frames into 10 columns and 16 rows (5×5cm). The behaviours and positions of all labelled workers and of the resident queens were recorded twice daily for each colony from 09h00-12h00 and 15h00-18h00 for 24 days on both frame sides. Because honeybees show a daily activity rhythm being less active at night (Moritz and Kryger 1994), this might have interfered with data accuracy; however, all behavioural observations were performed during daylight when normal flight activity occurs. Moreover, all workers belonged to the same age cohort and cycling was the same for drifted and non-drifted workers. Finally, behavioural observations started with alternating colonies on subsequent days (Day one: colony 1, day two: colony 5; day three: colony 2, day four: colony 4, etc…). Task performances were allocated to two categories: (a) being idle (staying motionless on the comb) or (b) being active (performing any other task than being idle). The positions of individual workers in relation to the queens in the observation hive colonies were determined using a discrete geodesics model (Neumann et al. 2001a). Behavioural observations were performed blindly with respect to the objectives of the study.

### 2.4. Data analysis

Proportions of being idle and active were determined on a cohort level (drifted vs. non-drifted bees) and on an individual worker basis. We used mean queen-worker distances on an individual worker basis. Differences in total activity between drifted and non-drifted bees and mean queen-worker distances for individual bees were analysed with Mann-Whitney U tests. Z-tests of proportions were used to test for differences in the task performances of whole cohorts between drifted and non-drifted workers. Behavioural data for both worker activity and worker-queen distances were only analysed from experimental day 5 onwards because most workers performed their orientation flights and drifted within a five day time window. Differences between colonies with respect to activity levels and worker-queen distances were tested using Kruskal Wallis tests. In case colonies differed significantly, all calculations were performed excluding these particular colonies to ensure that differences between colonies were not affecting the results for the drifted and non-drifted bees.

## 3. RESULTS

A total of 332 labelled workers drifted into foreign colonies (Table I). The extent of drifting ranged from 5% to 32% with a mean of 17.8% ± 11.2%. A total of N=22897 individual behavioural acts and N=1732 mean queen-worker distances were recorded (Table II). When comparing whole drifted and non-drifted cohorts, drifted workers were significantly more idle (Table II). Similar results were obtained when comparing drifted and non-drifted workers on an individual basis (Table II).

Non-drifted workers had a mean distance of 33.59 ± 10.97 cm (N=1436) to the queens. However, drifted workers had a significantly higher distance of 37.03 ± 14.87 cm (N=296) to the resident queens (Mann-Whitney U-test: Z (approximation) = 3.47, P < 0.001). Significant differences were found in the proportions of being active/idle and queen-worker distances between the five colonies (active/idle: Kruskal Wallis H=136.7 with (4,1775)df, P<0.01; distance: H=81.1 with (4,1732)df, P<0.01).

Multiple comparison tests revealed, however, that only one colony (active/idle: colony 5; distance: colony 3) differed significantly from the other four colonies in the proportions of being active/idle and queen-worker distances. Repeated analyses on proportions of being active/idle and queen-worker distances using four colonies again found drifted workers to be significantly more idle and further away from the queen compared to non-drifted workers (Non-drifted workers (N=1185): idle 53.16%, drifted workers (N=295): idle 57.04%, Z=2.12, P<0.05; Non-drifted workers (N=1162): queen-worker distance 33.05 ± 11.17, Drifted workers (N=275): queen-worker distance 36.86 ± 14.77, Mann-Whitney U-test: Z (approximation) = 3.61, P<0.0001)..

### 4. DISCUSSION

A much higher proportion of workers drifted into foreign colonies than in previous studies on African bees (~5% Neumann et al. 2001b). Thus, our experimental design amplified the number of orientation errors to a great extent, suggesting that the underlying mechanisms for orientation errors are very similar between Cape bees and European honeybees.

Because all experiments were performed in observation hives, the results could have a limited interpretational range regarding the assessed behaviours due to the different architecture of human made hives and natural multi-comb nests. However, this appears less relevant for the present study which addresses drifting, a behaviour which is rare (if not absent) between natural nests but abundant in apiaries (Neumann et al 2001b).

Although the absolute differences were low for both activity levels and queen-worker distances, we nevertheless found highly significant differences between drifted and non-drifted workers. Thus, our data for Cape bees is consistent with earlier findings for *A. m. carnica* that drifted workers are less active in their new host colonies (Pfeiffer and Crailsheim, 1999). Behavioural activity of workers is related with their longevity (Woyke, 1984) and the reproductive output of individual workers (Neumann and Moritz, 2002). Moreover, drifted workers showed a higher mean distance towards the queen compared to non-drifted workers. Workers staying away from the queen produce more queenlike compounds in the mandibular gland secretions and this is consistent with the interpretation that they have further differentiated into reproductives than others (Moritz et al., 2002). Furthermore, worker policing (the removal of worker-laid eggs by other workers) is less well expressed in areas away from the queen in both queenright colonies of *A. m. scutellata* and *A. m. capensis* which appears to facilitate social parasitism by laying Cape honeybee workers (Neumann et al., 2003). Therefore, it appears as if drifted Cape honeybee workers are predisposed for social parasitism. Such a predisposition might be amplified by orders of magnitude when *A. m. capensis* workers drift into host colonies of susceptible subspecies such as the neighbouring one *A. m. scutellata*.

Given there is a true causative connection between drifting and subsequent behaviour, the question remains why drifted workers are less active and why there is a greater mean distance between drifted bees and the queen. One possible explanation for higher idleness could be that drifted workers are less stimulated by normally recognized signals in the colony (Seeley, 1998) and are rather less sensitive to stimuli cues in the colony (Seeley, 1998) than non-drifted workers. With respect to greater mean distance between drifted bees and the colony-right queen several explanations are possible. For example, it might well be that drifted workers actively avoid the queen to retain their reproductive capacity. Alternatively, but not mutually exclusive, it could well be that drifted workers try to go just far away from the outlet of the hive, or that drifted workers simply climb more up the combs in the hives than non-drifted ones. In any case, the actual mechanisms underlying the post-drifting behaviour of honeybee workers remain unclear and require further investigations.

We conclude that drifting constitutes not only one mode of host finding for social parasitic Cape honeybee workers (Neumann et al., 2001b; Neumann and Hepburn, 2002; Neumann and Moritz, 2002) but may also play an important role for the individual fate of such workers inside of the host colonies.

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## REFERENCES

Free J.B. (1958) The drifting of honeybees, J. Agric. Sci. 51, 294-306.

Hillesheim E., Koeniger N., Moritz R.F.A. (1989) Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers, Behav. Ecol. Sociobiol. 24, 291-294.

Moritz R.F.A., Crewe R.M., Hepburn H.R. (2001) Attraction and repellence of workers by the honeybee queen (*Apis mellifera* L.), Ethology 107, 465-477.

Moritz R.F.A., Crewe R.M., Hepburn H.R. (2002) Queen evasion and mandibular gland secretion of honeybee workers (*Apis mellifera* L.), Insectes Soc. 49, 86-91.

Moritz R.F.A., Kryger P. (1994) Self organisation of circadian rhythms in groups of honeybees (*Apis mellifera* L.), Behav. Ecol. Sociobiol. 34: 211-215.

Neumann P., Hepburn H.R. (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.), Apidologie 33, 165-192.

Neumann P., Moritz R.F.A. (2002) The Cape honeybee phenomenon: the evolution of a social parasite in real time? Behav. Ecol. Sociobiol. 52, 271-281.

Neumann P., Moritz R.F.A., Mautz D. (2000). Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary, Apidologie 31, 67-79.

Neumann P., Pirk C.W.W., Hepburn H.R., Radloff S.E. (2001a) A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis*), Apidologie 32, 113-114

Neumann P., Radloff S.E., Moritz R.F.A., Hepburn H.R., Reece S.L. (2001b) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies, Behav. Ecol. 12, 419-42.

Neumann P., Pirk C.W.W., Hepburn H.R., Moritz R.F.A. (2003) Spatial differences in worker policing facilitate social parasitism of Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies. Ins. Soc. 50, in press.

Pfeiffer K.J., Crailsheim K. (1998) Drifting of honeybees, Insectes Soc. 45, 151-167.

Pfeiffer K.J., Crailsheim K. (1999) The behaviour of drifted nurse honey bees, Insectes Soc. 46, 34-40.

Rauschmayer F. (1928) Das Verfliegen der Bienen und die optische Orientierung am Bienenstand, Archiv f. Bienenkunde 9, 249-322.

Reece S.L. (2002) A scientific note on the ovarial and pheromonal development of drifted and non-drifted Cape honeybee workers (*Apis mellifera capensis*). Apidologie 33 (2002) 213–214.

Ribbands C.R. (1953) The behaviour and social life of honeybees, Bee Research Association Limited, London, UK.

Seeley T. (1998) Thoughts on information and integration in honeybee colonies, Apidologie 29, 67-80.

Wossler T.C. (2002) Pheromonal deceit by honeybee workers leads to reproductive anarchy in *Apis mellifera scutellata* colonies, Apidologie 33, 139-163.

Woyke J. (1984) Correlations and interactions between population, length of worker life and honey production by honeybees in temperate region, J. Apic. Res. 23, 148-154.

**Table I.** Total distribution of drifted and non-drifted workers per host colony

| Colony | Non-drifted | Drifted | Total |
|---|---|---|---|
| 1 | 355 | 20 | 375 |
| 2 | 293 | 138 | 431 |
| 3 | 274 | 21 | 295 |
| 4 | 276 | 82 | 358 |
| 5 | 265 | 71 | 336 |
| Total | 1463 | 332 | 1795 |

**Table II.** Comparisons of percentages of being active/idle and mean queen-worker distances for drifted and non-drifted workers. Significant results are indicated with ** for P<0.001 (P= significance level).

|  | **Non-drifted** |  | **Drifted** |  | **Z** | **P** |
|---|---|---|---|---|---|---|
| **Whole cohorts** |  |  |  |  |  |  |
|  | No. of behavioural acts | % | No. of behavioural acts | % |  |  |
| Active | 9548 | 46.75 | 1070 | 43.23 | 3.32 | ** |
| Idle | 10874 | 53.25 | 1405 | 56.77 | -3.32 | ** |
| Total | 20422 | 100.00 | 2475 | 100.00 |  |  |
| **Individuals** |  |  |  |  |  |  |
|  | No. of workers | % | No. of workers | % |  |  |
| Active | 1459 | 49.82 | 316 | 43.86 | 3.81 | ** |
| Idle | 1459 | 50.18 | 316 | 56.14 | -3.81 | ** |

# 3.7 Cape honeybees, *Apis mellifera capensis*, police worker-laid eggs despite the absence of relatedness benefits

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**Abstract-** In the Cape honeybee, *Apis mellifera capensis*, workers lay diploid (female) eggs via thelytoky. In other *A. mellifera* subspecies workers lay haploid (male) eggs via arrhenotoky. When thelytokous worker reproduction occurs, worker policing has no relatedness benefit because workers are equally related to their sister workers' clonal offspring and their mother queen's female offspring. We studied worker policing in *A. m. capensis* and in the arrhenotokous African honeybee *A. m. scutellata* by quantifying the removal rates of worker-laid and queen-laid eggs. Discriminator colonies of both subspecies policed worker-laid eggs of both their own and the other subspecies. The occurrence of worker policing, despite the lack of relatedness benefit, in *A. m. capensis* strongly suggests that worker reproduction is costly to the colony and that policing is maintained because it enhances colony efficiency. In addition, because both subspecies policed each others eggs it is probable that the mechanism used in thelytokous *A. m. capensis* to discriminate between queen-laid and worker laid eggs is the same as in arrhenotokous *A. m. scutellata.*

**Key words:** *Apis mellifera*, egg removal, honeybee, thelytoky, worker policing, worker reproduction

## 1. INTRODUCTION

Inclusive fitness theory (Hamilton 1964a,b) has been successful in explaining and predicting social behaviour (Jarvis 1981, Crozier and Pamilo 1996, Ratnieks et al. 2001). Hamilton's rule, $Br > C$, shows the condition under which a social action is favoured in terms of the benefit to the recipient, the cost to the actor, and their genetic relatedness. Genetic relatedness is a key parameter and can now be measured with comparative ease and accuracy (Pamilo et al. 1997, Ross 2001). Many empirical studies confirm the importance of genetic relatedness in social evolution (e.g. Sundström 1994, Foster and Ratnieks 2000, among others). However, it is unlikely that any comparable technological breakthrough will facilitate the measurement of costs and benefits (Ratnieks et al. 2001). One solution to investigating the importance of costs and benefits in social evolution is to chose study systems in which relatedness is held constant but costs and benefits vary. Worker reproduction and worker policing in the Cape honeybee, *Apis mellifera capensis*, is one such system.

In most eusocial Hymenoptera workers cannot mate but retain ovaries and can lay eggs (Crozier and Pamilo 1996). Unfertilised worker-laid eggs are normally haploid (arrhenotoky, Crozier and Pamilo 1996) and develop into males if reared. However, in a few species (currently 6 ants and *A. m. capensis* are known; Wenseleers and Billen 2000) workers lay unfertilised diploid eggs which develop into females (thelytoky, Crozier and Pamilo (1996); see Mackensen (1943) and Tucker (1958) for rare cases of thelytoky in other subspecies of *A. mellifera*). *A. m. capensis* is native to the fynbos region (a macchia-like biome) in the Western and Eastern Cape provinces of South Africa (Hepburn and Radloff 2002). Thelytokous reproduction by Cape honeybee workers is long known (Onions 1912, reviewed by Hepburn and Radloff 2002) and many of the genetic details are also understood (Verma and Ruttner 1983, Moritz and Haberl 1994, Greeff 1996). Although the thelytoky is via automictic parthenogenesis following meiosis (Verma and Ruttner 1983), recombination through crossing over is rare (Moritz and Haberl 1994). Thus, a worker's offspring are almost clonal.

Workers' sons are rarely reared in queenright European honeybee colonies (Visscher 1989, Visscher 1996). This is because few workers have active ovaries (Ratnieks 1993) and because the eggs they lay are eliminated by worker policing (Ratnieks 1988). Honeybee workers in queenright colonies eat eggs laid by other workers (Ratnieks and Visscher 1989; Ratnieks 1993, Visscher 1996). Queen-laid and worker-laid eggs are probably discriminated by means of a queen-produced egg-marking pheromone (Ratnieks 1992, 1995).

Worker policing is selected for in a population of arrhenotokous social Hymenoptera on relatedness grounds alone when each colony has a single queen mated to more than two males (Ratnieks 1988; Foster and Ratnieks 2001). Honeybee queens, *Apis mellifera*, are typically mated to 5-30 males (Estoup et al. 1994, Fuchs and Moritz 1999, Neumann and Moritz 2000, Palmer and

Oldroyd 2000). As a result, worker policing is beneficial on relatedness grounds because workers are less related to other workers' sons (nephews r = 0.15, for an effective paternity of 10) than to the queen's sons (brothers r = 0.25). However, in *A. m. capensis* the situation changes significantly. In particular, workers lay female eggs and are as related to other workers' daughters as to the queen's daughters. But an individual laying worker is still more related to her own offspring (clonal daughter r = 1) than to the queen's offspring (r = 0.3, assuming an effective paternity frequency of 10). Therefore, on relatedness grounds alone one might expect that *A. m. capensis* workers would either not police each other or would do so less effectively than in other *A. mellifera* subspecies (Greeff 1996). Indeed, brood are frequently observed in hive boxes located above the queen excluder (a grid to small for the queen, but not the workers, to pass through, so that the queen cannot lay eggs in the upper boxes) in queenright Cape honeybee colonies (Pettey 1922, Hepburn and Radloff 1998, personal observations). Moritz et al. (1999) have shown that this brood is the female offspring of workers.

Hamilton's rule for worker policing in *A. m. capensis* can be written as follows:

$$Br_q > Cr_w$$
$$B/C > r_w / r_q$$
$$B/C > 1$$

where $r_q$ and $r_w$ are the relatedness of police workers to the queen's and other workers' female offspring, and B and C are the relative efficiencies (i.e., total reproduction) of colonies with and without worker policing. Clearly, worker policing is favoured if it increases the efficiency of the colony. Colony efficiency could be lowered, for example, if the laying of additional eggs in a cell reduces the overall efficiency of brood rearing. The existence of worker policing in *A. m. capensis* would support the hypothesis that worker reproduction is costly.

In this study we investigated whether worker policing occurs in *A. m. capensis* by quantifying the removal rates of worker-laid and queen-laid eggs of both *A. m. capensis* and of the neighbouring arrhenotokous subspecies *A. m. scutellata* in queenright colonies of both subspecies. Our results show that both *A. m. capensis* and *A. m. scutellata* police their own and the other subspecies' worker-laid eggs.

## 2. METHODS

### 2.1 Sampling colonies and experimental design

Queenright study colonies of *A. m. capensis* were obtained near Port Elizabeth, within the native range of *A. m. capensis* in the Eastern Cape province in southern South Africa. Queenright *A. m. scutellata* colonies were from the Pretoria area, within their native range. These localities were chosen because morphometrically and physiologically pure *A. m. capensis* and *A. m. scutellata* occur there (Hepburn and Radloff 1998, 2002). Hepburn and Radloff (1998) and Hepburn et al. (1998) review the distribution and biology of these two subspecies.

The colonies were placed in two study apiaries in Grahamstown, South Africa. The *A. m. scutellata* apiary was distant, >1km, from any other bee hives to minimize intersubspecific drifting and/or dispersing (Neumann et al. 2000b, 2001), which may result in social parasitism by *A. m. capensis* laying workers (Neumann and Hepburn 2002, Neumann and Moritz 2002). Both *A. m. capensis* and *A. m. scutellata* colonies were studied in order to compare African subspecies with arrhenotokous and thelytokous worker reproduction (Neumann et al. 2000a, Hepburn and Radloff 2002). The experimental set-up followed standard methods for investigating worker policing via egg removal (Ratnieks and Visscher 1989, Ratnieks 1995, Oldroyd and Ratnieks 2000). We used colonies of both subspecies as discriminator and egg-source colonies. Discriminator and egg source colonies were different colonies. All discriminator colonies and the source colonies for queen-laid eggs retained their original queens during the time they were used in the study. The source colonies for worker-laid eggs were made queenless two weeks before egg-removal trials were started. All colonies were housed in hives composed of two deep or medium Langstroth boxes with a queen excluder between the boxes and the queen in the bottom box.

### 2.2 Quantifying egg-removal rates

Our primary aim was to compare the removal rates of queen-laid and worker-laid eggs within each subspecies. Worker-laid eggs are either haploid or diploid in the two subspecies (male in *A. m. scutellata* or female in *A. m. capensis*). Therefore we compared these to queen-laid eggs of the same sex and used haploid, male, eggs laid by *A. m. scutellata* queens and workers and diploid, female, eggs laid by *A. m. capensis* queens and workers. Because *A. mellifera* queens lay fertilized eggs in worker cells and unfertilised eggs in drone cells (Ratnieks and Keller 1998), we were able to obtain unfertilised male eggs from drone cells in the queenright *A. m. scutellata* colonies and fertilized female eggs from worker cells in the queenright *A. m. capensis* colonies. For both the *A. m. capensis* and *A. m. scutellata* discriminator colonies we used test frames with both drone and worker cells, because *A. m. capensis* workers naturally lay diploid female eggs mainly in worker cells (Neumann et al. 2000a) and workers of arrhenotokous subspecies lay male eggs mainly in drone cells (Page and Erickson 1988, Ratnieks 1993). The drone cells were used for male eggs laid by *A. m. scutellata* workers and *A. m. scutellata* queens. The worker cells were used for female eggs laid by *A. m. capensis* workers and *A. m. capensis* queens. Following standard procedures (Ratnieks & Visscher 1989, Ratnieks 1995, Oldroyd & Ratnieks 2000) the test frames were placed above the queen excluder in each queenright discriminator colony and sandwiched between two frames containing brood of all ages (eggs, larvae and pupae). The other frames in this upper box contained a mixture of empty cells, honey and pollen. Queen-laid eggs were obtained from below the excluder in queenright source colonies (N=2 for *A. m. scutellata* and N=3 for *A. m. capensis*). Worker-laid eggs were obtained from the queenless source colonies (N=2 for *A. m. scutellata* and N=3 for *A. m. capensis*). For each discriminator colony (N=2for *A. m. scutellata* and N=3 for *A. m. capensis*) we used a single test comb which was initially placed into the hive two days before egg removal trials began.

Twenty queen-laid and 20 worker-laid eggs of *A. m. capensis* were transferred from the source colonies into worker cells of the test frames. Likewise, 20 queen-laid and 20 worker-laid eggs of *A. m. scutellata* were transferred from the source colonies into the drone cells of the test frames. Eggs were arranged in batches of twenty eggs each on the test combs. The test combs were then reintroduced into their discriminator colonies. After 2 and 4 hours the test combs were briefly removed and inspected to determine which eggs were still present. After 24 hours the remaining eggs were counted and removed. A new set of eggs was then transferred. Egg removal trials were made for five consecutive days for each discriminator colony. Moreover, removal rates of queen and worker-laid eggs from *A. m. capensis* and *A. m. scutellata* source colonies (N=2 *A. m. capensis* and N=2 *A. m. scutellata*) in queenright discriminator colonies of the same subspecies (N=3 *A. m. capensis* and N=3 *A. m. scutellata*) were also evaluated on three sequential days using the same experimental approach with the exception of the cross tests. In this study a total of 5 *A. m. scutellata* and 6 *A. m. capensis* were used as discriminator colonies, another 17 colonies supplied the different types of eggs for the trials (four queenright *A. m. capensis* and *A. m. scutellata* each for queen laid eggs and 4 queenless *A. m. scutellata* and 5 queenless *A. m. capensis* as source for worker-laid eggs). We compared the removal rates of worker-laid and queen-laid eggs both within and between subspecies using Kruskal-Wallis ANOVAs and Mann Whitney U-tests (with Bonferroni adjusted levels of significance) using Statistica.

## 3. RESULTS

Time to removal was determined for 2720 eggs. The percentages of remaining eggs per egg source in the two subspecies of discriminator colonies are shown in Figures 1 and 2. The results of the Mann-Whitney U-tests are shown in Table 1. *A. m. capensis* and *A. m. scutellata* discriminator colonies removed worker-laid eggs of their own subspecies significantly faster than queen-laid eggs of their own subspecies. Likewise, in both subspecies of discriminator colonies worker-laid eggs of the other subspecies were removed significantly faster than queen-laid eggs of the other subspecies. Worker-laid eggs of *A. m. scutellata* were removed significantly faster in *A. m. capensis* discriminator colonies than in *A. m. scutellata* colonies. However, *A. m. capensis* worker-laid eggs were not removed significantly faster in *A. m. scutellata* than in *A. m. capensis* discriminator colonies. Finally, there were no significant differences among the individual discriminator colonies of either subspecies in the removal rates of queen and worker-laid eggs of their own subspecies after 24 hours (*A. m. scutellata*: queen eggs H=7.56, ns., worker eggs H=6.99, ns.; *A. m. capensis*: queen eggs H= 5.61, ns., worker eggs H=5.72, ns.).

## 4. DISCUSSION

The data clearly show that queenright *A. m. capensis* and *A. m.* scutellata colonies both police worker-laid eggs of their own and of the other subspecies but accept a much larger proportion of worker-laid eggs than European honeybees. While *A. m. capensis* discriminator colonies remove worker-laid eggs of *A. m. scutellata* faster than *A. m. scutellata* colonies, *A. m. capensis* worker-laid eggs were not removed faster in *A. m. scutellata* discriminator colonies. Within each subspecies, there were no significant differences among discriminator colonies.

Our data suggest that the two African subspecies are similar in their general pattern to European bees, i.e. worker-laid eggs are less acceptable than queen-laid eggs, but there are differences in the details. In the *A. m. scutellata* discriminator colonies many more worker-laid eggs of both *A. m. scutellata* and *A. m. capensis* remained after 24 hours (17% of *A. m. scutellata* worker-laid eggs and 14% of *A. m. capensis* worker-laid eggs) compared to colonies of European subspecies (1%, Ratnieks & Visscher 1989) and even to *A. m. capensis* (1% of *A. m. scutellata* worker-laid eggs and 10% of *A. m. capensis* worker-laid eggs). Thus, it is possible, that either policing is lower and/or worker-laid eggs are more acceptable in African than European subspecies. The similar pattern in both subspecies suggests that the African origin of the tested subspecies may matter but not the genetics of worker reproduction. Moreover, less queen-laid eggs remained in the *A. m. capensis* colonies after 24 hours (20%) compared to earlier studies on European honeybees (45%, Ratnieks & Visscher 1989) and to *A. m. scutellata* (40%). This might be due to nestmate recognition for queen-laid eggs, which is well expressed in African honeybee subspecies (Pirk et al. 2001)

Our observations that worker policing in the thelytokous Cape honeybee is at least as effective as in the arrhenotokous *A. m. scutellata* (and possibly better) contrasts to theory, which shows no benefit of worker policing in *A. m. capensis* (Greeff 1996). In fact, the observed difference between the two African subspecies (*A. m. scutellata* with relatedness benefits and *A. m. capensis* without) is in the opposite direction to prediction (Greeff 1996). But this prediction is based on relatedness grounds alone (Greeff 1996). Our observation of worker policing in *A. m. capensis* is also partly in contrast to the other existing empirical study (Moritz et al. 1999) which showed that laying worker offspring does occur in queenright colonies. However, even if most worker-laid eggs had been removed some may have remained and it was these that were detected using DNA microsatellites (Moritz et al. 1999). Thus, Moritz et al. (1999) showed that some workers' eggs are reared but they do not show that worker policing is absent. The observed egg-removal rates in this study agree well with our casual observations of the study colonies which showed that no larvae were being reared above the queen excluders in the test colonies immediately prior to or during the experimental period. Since the occurrence of brood above the queen excluder seems to be frequent in Cape honeybees (Pettey 1922, personal observations) and in *A. m. scutellata* (T Wossler, personal communication, personal observations), it was quite possible that worker policing would prove to be less effective than we actually observed. Thus, the combined results of Moritz et al. (1999) and this study indicate that worker policing does occur in *A. m. capensis* but is sometimes not fully effective in preventing worker reproduction.

Why does worker policing still occur in *A. m. capensis*? Earlier theoretical studies (Greeff 1996) may have missed a critical piece in the cost benefit analysis for policing in the Cape honeybee. The occurrence of policing should be dependent on a trade off between the cost of policing and the cost of worker reproduction to overall colony efficiency and reproduction (Ratnieks 1988). Given that policing probably costs very little, because eggs are held in open cells which workers are regularly checking anyway, the costs derived from unhindered worker reproduction might easily be higher than the costs of policing. Thus, a large efficiency gain is not needed to favor worker policing. In the *A. m. capensis* situation the gain need only be marginal. Even if a queen is single mated, so that policing of worker-laid eggs has a relatedness cost, policing is still favored in colony efficiency increases by 20% (Ratnieks 1988). A recent theoretical study by Foster and Ratnieks (2001) on the European hornet, *Vespa crabro*, shows that worker policing can even more easily be selected for at a mating frequency of one as part of a sex allocation biasing strategy of workers. That is, workers want to eliminate males to cause a female biased sex ratio, and the only eggs they know to be male are workers' sons.

What are the possible costs of worker reproduction in honeybees? There are probably two main potential costs, reduced brood rearing efficiency and a reduced work rate of reproductive dominant workers, when worker reproduction occurs in queenright colonies:

1. Reduced brood rearing efficiency: Honeybee nests have a limited brood rearing area which constrains the number of eggs that can be laid by the queen, given that a queen typically will not lay an egg in a cell that already contains an egg (Ratnieks 1990). Worker egg laying, when common, is characterized by multiple eggs per cell because workers will lay additional eggs in cells that already contain an egg (Gary 2000). The earlier-laid eggs are often knocked down, squashed and killed by the abdomen of the laying worker. Only one larva can be reared to adulthood in a single cell and additional larvae are eaten by workers. This may lead to costs associated with cannibalism (Elgar and Crespi 1992). Such cannibalism costs are likely to be small because when two larvae occur in one cell, one is removed within a few days of hatching. Moreover, the larvae is cannibalized rather than thrown out of the colony, suggesting that some energy can be recycled. However, when many workers are laying eggs it may simply take longer for any cell to yield a worker, which is probably the main cost aspect. For example, if it took just one additional day to rear a worker per cell this would lead to a 5% reduction in the maximal rate of colony build up, given an egg-adult stage of c. 19 days in workers of the Cape honeybee (Hepburn and Radloff 1998).
2. Work rate of laying workers: Laying Cape honeybee workers in queenless and queenright groups do not participate as much in hive duties such as brood rearing compared to subordinate workers (Moritz and Hillesheim 1985, Hillesheim et al. 1989). Thus, a high frequency of laying workers may also reduce colony productivity (Hillesheim et al. 1989). This cost may not be greatly reduced by egg eating, because worker policing via oophagy does not directly stop or penalize egg-laying workers. It may cause a reduction in worker egg-laying over evolutionary time, but for it to be selected for there has to be an immediate benefit in the colony with policing.

Reproductive dominance seems to be strongly genetically determined (Moritz and Hillesheim 1985, Moritz et al. 1996). Therefore, almost clonal (Moritz and Haberl 1994) laying worker offspring are predisposed to develop into laying workers. Thus, worker policing via oophagy may limit the establishment of such laying worker matrilines in queenright colonies, constituting an immediate benefit for policing colonies. Alternatively, but not mutually exclusive, worker-worker aggression in queenright colonies, which is directed towards nestmates with activated ovaries (Visscher and Dukas 1995), might also restrict the establishment of such laying worker matrilines.

Worker reproduction in the Cape honeybee, if kept to a low level, will probably have almost zero efficiency cost. Therefore we can expect some worker reproduction even if there is policing. Indeed, there is considerable variation for egg-laying behavior in Cape honeybee workers (Neumann and Hepburn 2002). While in some laying worker colonies the brood nest is virtually indistinguishable from that of a queen, because there is only one egg per cell (Neumann and Hepburn 2002), others show the typical pattern of a queenless colony with laying workers such as multiple eggs per cell as the colonies in our study (personal observations). This suggests that it is possible in Cape honeybees to have a low level of worker reproduction, which does not interfere with brood rearing. Thus, the costs need not be high for a colony if the amount of worker reproduction is low.

The Cape honeybee example is a particularly convincing example. Thelytoky causes a change in kin structure rendering relatedness neutral with respect to worker reproduction (see above). Thus, if worker policing were not beneficial in *A. m. capensis* it should be evolutionarily lost. That is, workers should accept eggs laid by other workers. Loss of policing would be a simple adaptation. In fact, loss of policing already occurs in queenless *A. mellifera* colonies (Miller and Ratnieks 2001), which have failed to rear an emergency replacement queen. Worker-laid eggs are accepted and reared into a final cohort of males before the colony dwindles in population and dies (Page and Erickson 1988). The results also show that both races are able to police worker-laid eggs of the other subspecies, indicating that the same underlying mechanism is used for worker policing. Arrhenotoky (Crozier and Pamilo 1996), multiple paternity (Neumann and Moritz 2000, Palmer and Oldroyd 2000) and worker policing (*A. mellifera,* Ratnieks and Visscher 1989; *A. florea,* Halling et al. 2001; *A. cerana*, Oldroyd et al. 2001) appear to be ancestral in *Apis*, indicating that thelytoky is a derived condition in *A. m. capensis* and arose in a clade in which worker policing occurred.

In conclusion, we hypothesize that worker policing still exists in the Cape honeybee due to colony efficiency grounds. As predicted by Hamiltons' rule, this illustrates that relatedness alone cannot predict the reproductive characteristics of insect societies. The application of the inclusive fitness theory requires knowledge of costs, benefits and relatedness.

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## REFERENCES

Crozier RH, Pamilo P, 1996. Evolution of social insect colonies. Oxford University Press, Oxford.

Elgar MA, Crespi BJ, 1992. Ecology and evolution of cannibalism. In: Cannibalism, Ecology and Evolution among Diverse Taxa, Oxford University Press, Oxford.

Estoup A, Solignac M, Cornuet JM, 1994. Precise assessment of the number of patrilines and genetic relatedness in honeybee colonies. Proc R Soc Lond B 258*:* 1-7.

Foster KR, Ratnieks FLW, 2000. Facultative policing in a social wasp. Nature 407:692-693.

Foster KR, Ratnieks FLW, 2001. The effect of sex allocation biasing on the evolution of worker policing. Am Nat 158: 615-623.

Fuchs S, Moritz RFA, 1999. Evolution of extreme polyandry in honeybees (*Apis mellifera*). Behav Ecol Sociobiol 45: 269-277.

Gary NE, 2000. Activities and Behavior of Honey Bees. In: The Hive and the Honeybee, (Graham J.M. ed.). Dadant & Sons, Hamilton, Illinois; 289-372.

Greeff MJ, 1996. Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*. Phil Trans R Soc Lond B 351: 617-625.

Hamilton WD, 1964a. The genetical evolution of social behaviour I. J Theor Biol 7:1-16.

Hamilton WD, 1964b. The genetical evolution of social behaviour II. J Theor Biol 7:17-52.

Halling L, Oldroyd BP, Patimus B, Wattanachaiyingcharo W, Wongsiri S, 2001. Worker policing in *Apis florea*. Behav Ecol Sociobiol 49: 509-513.

Hepburn HR, Radloff SE, 1998. Honeybees of Africa. Springer-Verlag, Berlin.

Hepburn HR, Radloff SE, Fuchs S, 1998. Population and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*. Apidologie 29: 333-346.

Hepburn HR, Radloff SE, 2002. *Apis mellifera capensis*: an essay on the subspecific classification of honeybees. Apidologie 33.

Hillesheim E, Koeniger N, Moritz RFA, 1989. Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. Behav Ecol Sociobiol 24: 291-296.

Jarvis JUM, 1981. Eusociality in a mammal: co-operative breeding in naked mole rat colonies. Science 212: 571-573.

Mackensen O, 1943. The occurrence of parthenogenetic females in some strains of honeybees. J Econom Entomol 36: 465-467.

Miller DG, Ratnieks FLW, 2001. The timing of worker reproduction and breakdown of policing behaviour in queenless honey bee (*Apis mellifera* L.) societies. Insectes Soc 48: 178–184.

Moritz RFA, Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis* Esch.) Behav Ecol Sociobiol 17: 87-89.

Moritz RFA, Haberl M, 1994. Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). Heredity 73: 98-102.

Moritz RFA, Kryger P, Allsopp MH, 1996. Competition for royalty in bees. Nature 384: 31.

Moritz RFA, Kryger P, Allsopp MH, 1999. Lack of policing in the Cape honeybee, *Apis mellifera capensis*. Behaviour 136: 1079-1092.

Neumann P, Moritz RFA, 2000. Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.). Insectes Soc 47: 271-279.

Neumann P, Hepburn HR 2002. Behavioral basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch). Apidologie 33: 165-192.

Neumann P, Moritz RFA 2002. The Cape honeybee phenomenon: the sympatric speciation of a social parasite in real time? Behav Ecol Sociobiol, in press.

Neumann P, Hepburn HR, Radloff SE, 2000a. Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera*) colonies. Apidologie 31: 479-486.

Neumann P, Moritz RFA, Mautz D, 2000b. Colony evaluation is not affected by drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. Apidologie 31: 67-79.

Neumann P, Radloff SE, Hepburn HR, Moritz RFA, Reece SL, 2001. Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): Host finding and resistance of hybrid host colonies. Behav Ecol 12: 419-428.

Oldroyd BP, Ratnieks FLW, 2000. Anarchistic honey bee workers evade worker policing by laying eggs that have low removal rates. Behav Ecol Sociobiol 47: 268-273.

Oldroyd BP, Halling LA, Good G, Wattanachaiyingcharo W, Barron AB, Nanork P, Wongsiri S, Ratnieks FLW, 2001. Worker policing and worker reproduction in *Apis cerana*. Behav Ecol Sociobiol 50: 371-377.

Onions GW, 1912. South African 'fertile worker bees'. Agric J Union of S Afr 1: 720-728.

Page RE, Erickson EH, 1988. Reproduction by worker honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 23: 117-126.

Pamilo P, Gertsch P, Thoren P, Seppä P, 1997. Molecular population genetics of social insects. Ann Rev Ecol Syst 28: 1-25.

Palmer KA, Oldroyd BP, 2000. Evolution of multiple mating in the genus *Apis*. Apidologie 31: 235-248.

Pettey FW, 1922. Workers laying in comb of extracting super, Elsenberg Apiary. J Depart Agric Union S Africa 4: 122-124.

Pirk CWW, Neumann P, Hepburn HR, 2001. Nestmate recognition for eggs and worker policing in African honeybees (*Apis mellifera* L.). In: Proc. of the 2001 Berlin Meeting of the European sections of IUSSI; Sept. 25-29, 2001, Berlin, Germany; 106.

Ratnieks FLW, 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat 132: 217-236.

Ratnieks FLW, 1990. The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. Behav Ecol Sociobiol *26:* 343-348.

Ratnieks FLW, 1992. Evidence for an egg-marking pheromone in the honey bee. Am Bee J 132: 813.

Ratnieks FLW, 1993. Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. Behav Ecol Sociobiol 32: 191-198.

Ratnieks FLW, 1995. Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. J Apic Res 34: 31-37.

Ratnieks FLW, Keller L, 1998. Queen control of egg fertilization in the honey bee. Behav Ecol Sociobiol 44: 57-61.

Ratnieks FLW, Visscher PK, 1989. Worker policing in the honey bee. Nature 342: 796-797.

Ratnieks FLW, Monnin T, Foster KR 2001. Inclusive fitness theory: novel predictions and tests in eusocial Hymenoptera. Ann Zoo Fenn 38: 201-214.

Ross KG, 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Mol Ecol 10: 265–284.

Sundström L, 1994. Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. Nature 367:266-268.

Tucker KW, 1958. Automictic parthenogenesis in the honeybee. Genetics 43: 299-316.

Verma S, Ruttner F, 1983. Cytological analysis of the thelytokous parthenogenesis in the Cape honeybee (*Apis mellifera capensis* Escholtz). Apidologie 14: 41-57.

Visscher PK, 1989. A quantitative study of worker reproduction in queenright honey bee colonies. Behav Ecol Sociobiol 25: 247-254.

Visscher PK, 1996. Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. Behav Ecol Sociobiol 39: 237.

Visscher PK, Dukas R, 1995. Honey bees recognise development of nestmates' ovaries. Anim Behav 49: 542–544.

Wenseleers T, Billen J, 2000. No evidence for *Wolbachia*-induced parthenogenesis in the social Hymenoptera. J Evol Biol 13: 277-280.

**Table 1** Numbers of eggs from different subspecies, colony, and caste sources remaining after 24 hours in *Apis mellifera capensis* and *A. m. scutellata* discriminator colonies. Results of Mann Whitney U Tests are shown. The Bonferroni adjusted level of significance is p = 0.025 (DC = discriminator colony, C = *A. m. capensis*, S = *A. m. scutellata*, + = queenright, - = queenless, ns = not significant).

| Type of comparison | Egg source | DC (Number of DC, trials) | Trend | Egg source | DC (Number of DC, trials) | U- value | p- value |
|---|---|---|---|---|---|---|---|
| worker-laid vs. queen-laid | C- | C (6,24) | Faster | C+ | C (6,24) | 107.5 | <0.0002 |
| | S- | S (5,19) | Faster | S+ | S (5,19) | 78 | <0.003 |
| | S+ | C (3,15) | Slower | S- | C (3,15) | 47.5 | <0.001 |
| | C+ | S (2,10) | Slower | C- | S (2,10) | 13.5 | 0.019 |
| worker-laid vs. worker-laid | S- | S (2,10) | Slower | S- | C (3,15) | 46 | 0.003 |
| | C- | C (3,15) | Faster | C- | S (2,10) | 46 | ns |

**Fig. 1.** Numbers (mean, S.D) of queen-laid and worker-laid eggs of *A. m. capensis* and *A. m. scutellata* remaining after 0, 2, 4 and 24 hours in queenright *A. m. capensis* discriminator colonies.

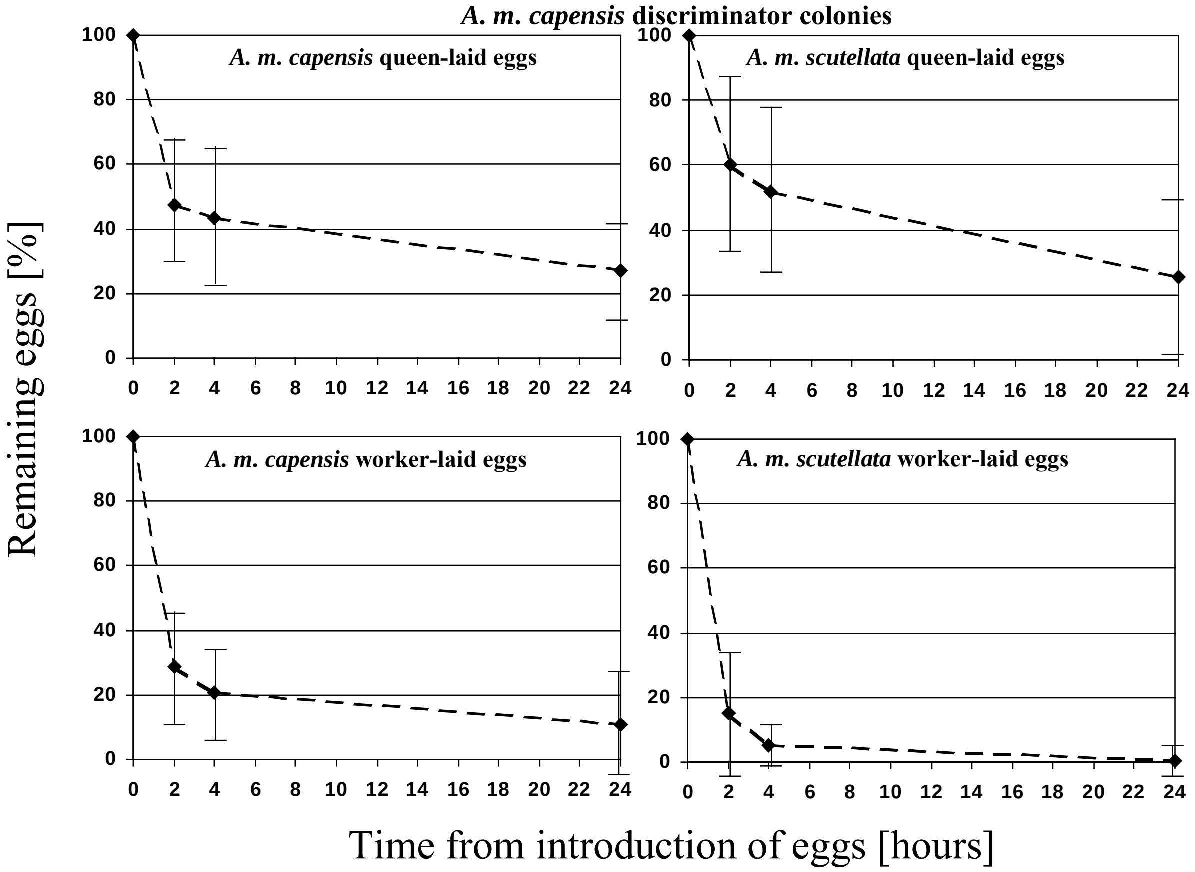
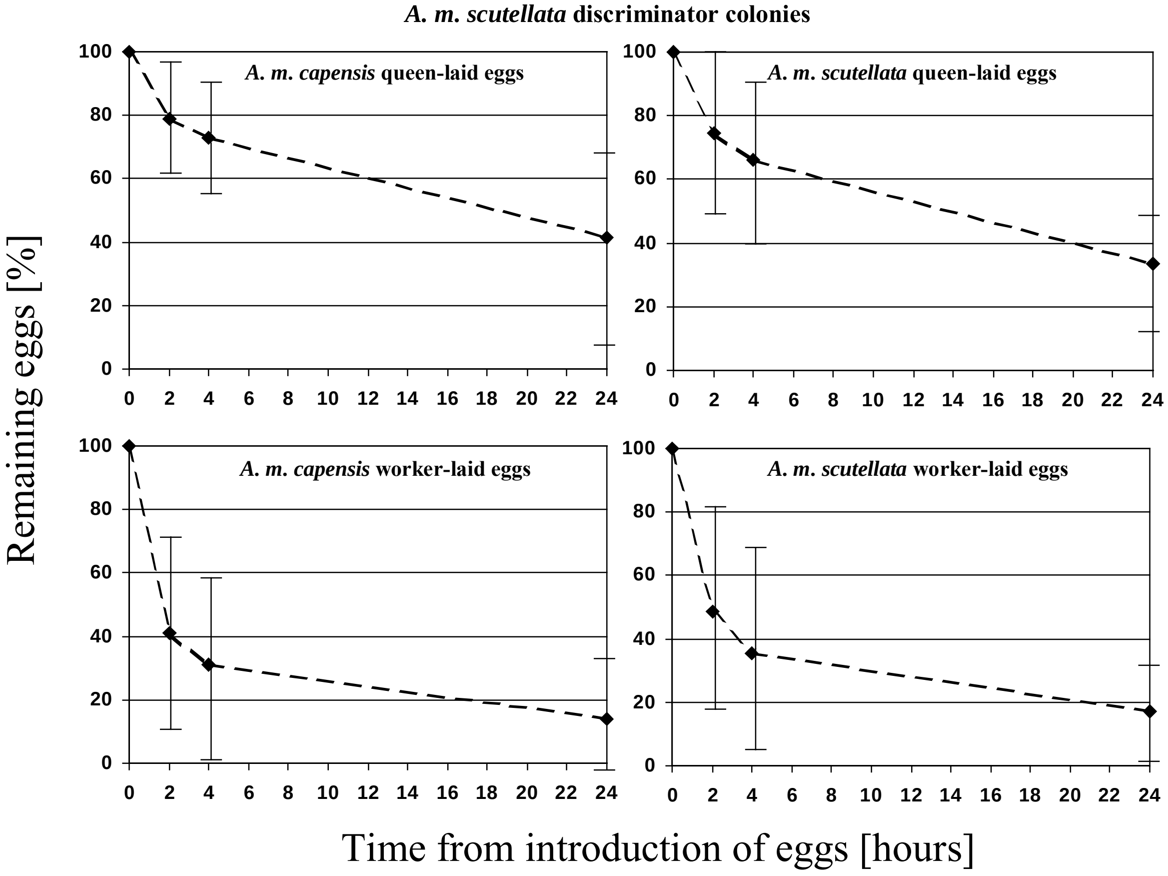


**Fig. 2.** Numbers (mean, S.D) of queen-laid and worker-laid eggs of *A. m. capensis* and *A. m. scutellata* remaining after 0, 2, 4 and 24 hours in queenright discriminator colonies of *A. m. scutellata*



# 3.8 Spatial differences in worker policing facilitate social parasitism of Cape honeybee workers (*Apis mellifera capensis* Esch.) in queenright host colonies

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**Abstract -** Cape honeybee laying workers (*Apis mellifera capensis*) produce female diploid offspring and are facultative social parasites. In queenright host colonies, such workers have to evade worker policing (removal of worker-laid eggs by other workers) to successfully reproduce. One mechanism seems to be low removal rates of eggs laid by parasitic workers. However, because queenright colonies of other subspecies (e.g. the neighbouring *A. m. scutellata*) are susceptible hosts, social parasitic workers probably also use behavioural tactics to evade policing. Indeed, field observations of infested *A. m. scutellata* host colonies indicate that brood from parasitic workers initially appears away from the queen. The egg removal rates for queen and worker-laid eggs were recorded in the top and bottom boxes of three queenright *A. m. capensis* and *A. m. scutellata* colonies, where the queens were caged in the bottom boxes. The egg removal data show that both subspecies are able to police worker-laid eggs, because more queen-laid eggs remained than worker-laid eggs in the bottom boxes. However, fewer *A. m. capensis* worker-laid eggs remained in the bottom boxes than in the top boxes. Moreover, whereas *A. m. capensis* also polices in the top boxes, no significant differences between the removal rates of worker and queen laid eggs were found in the top boxes of the *A. m. scutellata* colonies. This indicates that worker policing is not always fully effective in *A. m. scutellata* and may explain why this subspecies is so susceptible to infestations. Our results also show that worker policing is less likely away from the queen. Therefore, queen evasion by laying social parasitic *A. m. capensis* workers appears to constitute a behavioural tactic to achieve successful reproduction in queenright host colonies.

**Key words:** *Apis mellifera capensis* / honeybee / social parasitism / worker policing / worker reproduction

## 1. INTRODUCTION

Social parasitism in which mated gynes seek and invade host colonies and start reproducing at the expense of the host is widespread in social insects (Wilson, 1971; Wcisclo, 1981; Hölldobler and Wilson, 1990; Schmid-Hempel, 1998). In the eusocial Hymenoptera unmated workers of many species may reproduce parthenogenetically (Crozier and Pamilo, 1996), producing haploid male offspring (arrhenotoky), but in a few thelytokous species offspring are diploid females (Crozier and Pamilo, 1996). Thelytoky may predispose a taxon for the evolution of social parasitism by workers (Neumann et al., 2001; Neumann and Hepburn, 2002) as evidenced by laying workers of the Cape honeybee (*Apis mellifera capensis*, Onions, 1912; Hepburn and Crewe, 1990; Hepburn and Allsopp, 1994; Neumann et al., 2001; Neumann and Hepburn, 2002; Neumann and Moritz, 2002) which possess unique traits such as high pheromonal and ovarial development, high fecundity and high longevity (Neumann and Hepburn, 2002; Wossler, 2002). Such workers have been termed pseudoqueens (Velthuis et al., 1990) and are able to evoke retinue behaviour in other workers and to suppress the rearing of replacement queens (Anderson, 1968).

Nonetheless, there are potential defence strategies of honeybee host colonies against social parasitism by *A. m. capensis* laying workers. A variety of different behavioural strategies and tactics can be used by both parasite and host to achieve or counter successful parasite reproduction (Wilson, 1971; Wcisclo, 1981; Hölldobler and Wilson, 1990; Schmid-Hempel, 1998; Neumann and Hepburn, 2002). In queenright honeybee colonies, worker-laid eggs are eliminated by policing workers (Ratnieks, 1988; Ratnieks and Visscher, 1989; Ratnieks, 1993; Visscher, 1996) who may distinguish queen-laid and worker-laid eggs by a queen-produced egg-marking pheromone (Ratnieks, 1992; 1995; Oldroyd et al., 2002). Because both *A. m. capensis,* and *A. m. scutellata* are able to police their own as well as each others eggs (Pirk et al., 2002; 2003), the removal of worker-laid eggs appears to constitute an important resistance mechanism for queenright colonies in the context of social parasitism by laying workers.

However, the high incidence of queenright *A. m. scutellata* colonies parasitized (Allsopp and Crewe, 1993) by *A. m. capensis* laying workers following introduction of the latter into areas native for the former suggests that the parasitic workers are able to evade worker policing on a regular basis. Thus, it is very likely that laying workers of *A. m.*

*capensis* have evolved tactics and strategies to avoid worker policing in queenright colonies. Removal rates of worker-laid eggs is one case in point. Recently, it has been shown that eggs laid by *A. m. capensis* workers invading *A. m. scutellata* in its native range have lower removal rates compared to eggs laid by *A. m. scutellata* host workers (Martin et al., 2002). Moreover, laying workers may also show behavioural tactics to evade worker policing. In contrast to *A. m. scutellata*, workers of *A. m. capensis* seem to actually avoid queens (Moritz et al., 2001a,b) and such workers have a queen-like pheromonal bouquet (Moritz et al., 2002). Because brood commonly occurs above the queen excluder in queenright Cape honeybee colonies and their natural hybrids (Pettey, 1922; Tribe and Allsopp, 2001; personal observations) and up to 1/3 of the offspring in such colonies can be worker derived (Moritz et al., 1999). The spatial position of the queen may not only have an effect on the spatial distribution and pheromonal and ovarial development of workers (Moritz et al., 2001a;b; 2002), but also on worker policing and egg-laying behaviour.

Although queen substance is dispersed by messenger bees (Velthuis, 1972; Seeley, 1979) it is not evenly distributed in the colony. In very large colonies or in colonies, where the queen is restricted to a certain area of the hive (e.g. when using queen excluders in commercial beekeeping practice) workers may raise emergency queens (Müssbichler, 1952; Butler, 1960; Lensky and Slabezki, 1981; Swart et al., 2001). This strongly indicates that the queen signal is weakened in those areas. In commercial beekeeping the so-called modified Demaree technique, where young brood is being transferred above the queen excluder, is commonly used to raise new queens (Swart et al., 2001). If the removal of worker-laid eggs is affected by distance from the queen, then queen evasion by laying *A. m. capensis* workers could well constitute a behavioural tactic to achieve successful reproduction in queenright colonies. Here we investigate egg removal by workers in queenright *A. m. capensis* and *A. m. scutellata* colonies to test for spatial differences in egg removal.

## 2. MATERIAL AND METHODS

### 2.1 Experimental set up

Four queenright colonies and one queenless colony of *A. m. capensis* from its native range (Port Elizabeth) were placed at a test apiary in Grahamstown, South Africa. Likewise, four queenright colonies and one queenless colony of *A. m. scutellata* were used in another apiary near Pretoria, South Africa (see Hepburn and Radloff, 1998 for a review on the biology and distribution of the two subspecies). All colonies were housed in two 10 frame standard Langstroth boxes and given two days to settle down to avoid absconding (Hepburn et al., 1999). For both subspecies, the queens of the three queenright colonies were caged in containers (8x4x2.5 cm) with gauze on two sides, so that the workers had access to the queen (Pirk et al., 2002). The cages were fitted to a comb, by removing only as much wax as necessary and returned in the bottom boxes of their colonies (Pirk et al., 2002). These colonies with the caged queens were used as discriminator colonies for the experiments. No queen excluders were used in these colonies.

### 2.2 Removal rates for worker-laid and queen-laid eggs

One queenright and one queenless *A. m. capensis* colony were used as egg sources for the three queenright *A. m. capensis* discriminator colonies. Likewise, one queenright and one queenless *A. m. scutellata* colony were used as egg sources for the three queenright *A. m. scutellata* discriminator colonies. Egg removal rates for queen and worker-laid eggs were evaluated according to standard protocols (Ratnieks and Visscher, 1989) with the following modifications.

Two empty test combs for evaluating egg removal rates were placed into the bottom box (comb **1**) and top box (comb **2**) of each of the *A. m. capensis* and *A. m. scutellata* discriminator colonies. Test comb **1** was placed next to the frame with the caged queen and sandwiched between two brood frames in the bottom box. Test comb **2** was sandwiched between two brood frames in the top box. The test combs were introduced two days before the actual experiments started to avoid any potential influence deriving from the comb (Breed et al., 1995). The top and the bottom boxes were supplied with the same number of brood frames (eggs, unsealed and sealed brood) so that in both boxes the brood nest had the same size. All other frames contained honey and/or pollen. On 3 sequential days, 20 eggs of each egg source colony were transferred using special forceps (Taber, 1961) in empty worker cells of each test comb**.** After 24 hours all test combs were briefly removed from the colonies and all remaining eggs on them were counted.

### 2.3 Data analysis

Mann-Whitney U tests were performed using Statistica© to test for differences between the number of remaining queen and worker-laid eggs on the test combs.

## 3. RESULTS

Egg removal was evaluated for 720 eggs. Worker-laid eggs were more frequently removed from the test combs than queen-laid eggs in both the bottom and the top box of the *A. m. capensis* discriminator colonies (Tab 1). This was different in the *A. m. scutellata* discriminator colonies, where differential egg removal was only observed in the bottom boxes with the queen but not in the top boxes, where both worker- and queen-laid eggs had similar removal rates (Tab. 1).

Worker policing was more strongly expressed in the bottom box of the *A. m. capensis* discriminator colonies. Worker-laid eggs were removed more frequently from the bottom test comb than from the top test comb (Tab. 1). Workers in the *A. m. scutellata* colony showed no different policing efficiency in the top and bottom boxes of the colony (Tab. 1).

**Table 1:** Egg removal rates for queen and worker-laid eggs in the top and bottom boxes of queenright *A. m. capensis* and *A. m. scutellata* discriminator colonies. The mean ± sd of the numbers of queen-laid and worker-laid eggs remaining after 24 hours are shown. Trends for egg removal rates are shown from left to right within a row and from bottom to top within a column. The results of the Mann–Whitney U tests are given in brackets (Z-values). Significant results (using Bonferroni adjustments to the levels of significance ($p < 0.025$)) are indicated with * = $p<0.025$, ** = $p<0.01$ and *** = $p<0.001$.

| **Subspecies** | Egg type | Position (box) | | |
|---|---|---|---|---|
| | | bottom | | top |
| *A. m. capensis* | Queen-laid | 5.11±1.05 | < (1.03) | 6.89±1.96 |
| | | < (3.58)*** | | < (2.30)* |
| | Worker-laid | 0.44±0.73 | > (2.29)* | 3.22±3.30 |
| *A. m. scutellata* | Queen-laid | 5.89±2.73 | < (0.93) | 5.22±4.21 |
| | | < (2.78)** | | < (0.13) |
| | Worker-laid | 2.56±1.67 | > (1.38) | 4.22±3.00 |

## 4. DISCUSSION

The data clearly show that the removal of worker-laid eggs is reduced in the top boxes of the *A. m. capensis* colonies. Moreover, there are no significant differences between the removal rates of queen and worker-laid eggs in the top boxes of the *A. m. scutellata* colonies. Because worker policing is reduced in colony sections away from the queen, this facilitates social parasitism by laying workers. Therefore, host queen evasion may constitute a behavioural tactic of social parasitic Cape honeybee workers to achieve successful reproduction in queenright host colonies. The data suggest that the distance of workers from the queen not only affects their spatial distribution in the colony (Moritz et al., 2001a;b) and their pheromonal and ovarial development (Moritz et al., 2002) but also plays an important role for the removal of worker-laid eggs by other workers.

Our results support earlier findings (Pirk et al., 2002; 2003) that both *A. m. capensis* and *A. m. scutellata* are in principle able to police worker-laid eggs because significantly fewer worker-laid eggs than queen-laid eggs remained in the bottom boxes after 24 hours. Worker policing also occurred to a lesser degree in the top boxes of the *A. m. capensis* colonies, but not in *A. m. scutellata* suggesting that worker policing in the latter is not always fully effective. This is consistent with earlier findings for both subspecies (Pirk et al., 2002; 2003) and field observations that more queenright colonies of both *A. m. capensis* and *A. m. scutellata* show worker derived brood above the queen excluder (Pettey, 1922; Tribe and Allsopp, 2001; L. McGregor personal communication; personal observations) than colonies of European honeybee subspecies. Moreover, the number of *A. m. capensis* worker-laid eggs remaining in the bottom boxes was significantly lower than in the top boxes of the queenright *A. m. capensis* colonies. Because the brood nest was the same size in both boxes, this indicates that the distance from the queen has an impact on the egg removal behaviour of workers, and thus on the survival chances of worker-laid eggs. The data also suggest that evaluating the removal of worker-laid eggs above the queen excluder might actually underestimate the removal rates for worker-laid eggs in close vicinity to the queen.

What are potential reasons for this spatial effect? It has been suggested that normal queen pheromone transmission may be disrupted in an experimental set up using caged queens (Visscher, 1996). Restricting the queen from entering certain parts of the hive such as by using queen excluders (as it is typical in commercial beekeeping) or cages (this study) may interrupt normal queen pheromone transfer, especially if messenger bees (Seeley, 1985) are not fully effective or not as effective as the queen herself. Our experimental design probably amplified such an interruption, but the basic effects should be the same.

Because worker policing is reduced above the queen excluder (away from the queen), worker derived eggs in such areas may have enhanced survival rates. When such eggs hatch outside of the actual brood nest, brood pheromones may attract young nurse bees to the recently hatched larvae. Indeed, in the case of parasitic laying worker offspring it seems very likely that the hatching larvae can still be assured of nurse attention because *A. m. capensis* larvae are more attractive than those of other subspecies (Beekman et al., 2000). Because worker policing is not always fully effective above the queen excluder in queenright *A. m. scutellata* colonies, this may explain why *A. m. scutellata* colonies are so prone to invasions by *A. m. capensis* laying workers (Allsopp and Crewe, 1993; Hepburn and Allsopp, 1994). From this perspective, queen evasion by laying *A. m. capensis* workers may be highly adaptive for social parasitism in queenright host colonies. Indeed, our egg removal data agrees well with field observations. Brood of social parasitic Cape honeybee workers initially appears at the extreme outside frames, then closer to the actual brood nest and finally the brood nest of the host queen is flanked by brood frames with *A. m. capensis* worker-laid brood (Allsopp, 1995; Magnuson, 1995; Neumann and Hepburn, 2002). It would be interesting to test beekeeper's observations (A. Schehle, personal communications) that it takes considerably longer for social parasitic workers to take over host colonies lacking queen excluders.

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## REFERENCES

Allsopp, M.H., 1995 The *Capensis* problem 1992-1995. Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 5-30 June 1995, PPRI, Pretoria, South Africa, pp 10-31.

Allsopp, M.H. and R.M. Crewe, 1993. The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. *Am. Bee J. 133*: 121-123.

Anderson R.H., 1968. The effect of queen loss on colonies of *Apis mellifera capensis. S. Afr. J. Agr. Sci. 11:* 383-388.

Beekman M, J.N.M. Calis and W.J. Boot,, 2000. Parasitic honeybees get royal treatment. *Nature 404*: 723.

Breed M.D., M.F. Garry, A.N. Pearce, B.E. Hibbard, L.B. Bjostad and R.E. Page, 1995. The role of wax comb in honey bee nestmate recognition. *Anim. Behav. 50:* 489-496.

Butler C.G., 1960. The significance of queen substance in swarming and supersedure in honeybee (*Apis mellifera* L.) colonies. *Proc. Royal Entomol. Soc. L (A) 35*: 129-132.

Crozier R.H. and P. Pamilo, 1996. *Evolution of social insect colonies - sex allocation and kin selection.* Oxford University Press, Oxford, 365 pp.

Hepburn H.R. and M.H. Allsopp, 1994. Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis. S. Afr. J. Sci. 90:* 247-249.

Hepburn H.R. and R.M. Crewe, 1990. Defining the Cape honeybee: reproductive traits of queenless workers. *S. Afr. J. Sci. 86:* 524-527.

Hepburn H.R. and Radloff S.E., 1998. *Honeybees of Africa.* Springer Verlag Berlin, New York, 370 pp.

Hepburn H.R., S.L. Reece, P. Neumann, R.F.A. Moritz and S.E. Radloff, 1999. Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of worker reproduction. *Insectes soc. 46:* 323-326.

Hölldobler B. and E.O. Wilson, 1990. *The Ants.* Springer Berlin, Germany, 732 pp.

Lensky Y. and Y. Slabezki, 1981. The inhibiting effect of the queen bee (*Apis mellifera* L.): foot-print pheromone on the construction of swarming queen cups. *J Ins. Physiol. 27:* 313-323.

Magnuson P., 1995. Field observations. Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, pp. 5-9.

Martin S.J., M. Beekman, T.C. Wossler and F.L.W. Ratnieks, 2002. Self-replicating honey bees evade policing. *Nature 415:* 163-165.

Moritz R.F.A., R.M. Crewe and H.R. Hepburn, 2001a. Attraction and repellence of workers by the honeybee queen (*Apis mellifera* L.). *Ethology 107:* 465-477.

Moritz R.F.A., R.M. Crewe and H.R. Hepburn, 2001b. Clustering of related workers in the honeybee colony (*Apis mellifera* L.): adaptive process or inevitable pattern? *Apidologie 31*: 223-233.

Moritz R.F.A., R.M. Crewe and H.R. Hepburn, 2002. Queen evasion and mandibular gland secretion of honeybee workers (*Apis mellifera* L.). *Insectes soc. 49:* 86-91.

Moritz R.F.A., P. Kryger and M.H. Allsopp, 1999. Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). *Behaviour 136:* 1079-1092.

Müssbichler A., 1952. Die Bedeutung äusserer Einflüsse und der Corpora Allata bei der Afterweiselentstehung von *Apis mellfica. Z. vergl. Physiol. 34:* 207-221.

Neumann P. and H.R. Hepburn, 2002. Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). *Apidologie 33:* 165-192.

Neumann P. and R.F.A. Moritz, 2002. The Cape honeybee phenomenon: the evolution of a social parasite in real time? *Behav. Ecol. Sociobiol.* 52: 271-281.

Neumann P., S.E. Radloff, R.F.A. Moritz, H.R. Hepburn and S.L. Reece 2001. Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): host finding and resistance of hybrid host colonies. *Behav. Ecol. 12:* 419-42.

Oldroyd B.P., F.L.W. Ratnieks and T.C. Wossler 2002. Egg-marking pheromones in honey-bees *Apis mellifera*. *Behav. Ecol. Sociobiol. 51:* 590-591.

Onions G.W., 1912. South African 'fertile worker bees'. *Agr. J. Union S. Afr. 1:* 720-728.

Pettey F.W., 1922. Workers laying in comb of extracting supers, Elsenberg Apiary. *J. Dep. Agr. Union S. Afr. 4:* 122-124.

Pirk C.W.W., P. Neumann and H.R. Hepburn, 2002. Egg laying and egg removal by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.). *Apidologie 33:* 203-212.

Pirk C.W.W., P. Neumann and F.L.W. Ratnieks, 2003. Cape honeybees police worker-laid eggs despite the absence of relatedness benefits. *Behav. Ecol.*, in press.

Ratnieks F.L.W., 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat. 132:* 217-236.

Ratnieks F.L.W., 1992. Evidence for an egg-marking pheromone in the honey bee. *Am. Bee J. 132:* 813.

Ratnieks F.L.W., 1993. Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies. *Behav. Ecol. Sociobiol. 32:* 191-198.

Ratnieks F.L.W.,1995. Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. *J. Apic. Res. 34*: 31-37.

Ratnieks F.L.W. and P.K. Visscher, 1989. Worker policing in the honeybee. *Nature 342:* 796-797.

Schmid-Hempel P., 1998. *Parasites in social insects.* Princeton University Press Princeton, USA, 409 pp.

Seeley T.D., 1979. Queen substance dispersal by messenger workers in honeybee colonies. *Behav. Ecol. Sociobiol. 5:* 391-415.

Seeley T.D., 1985. *Honeybee ecology: a study of adaptation in social life.* Princeton University Press, Princeton, USA., 201 pp.

Swart D.J., P. Kryger and M.F. Johannsmeier, 2001. Queen rearing. In: *Beekeeping in South Africa.* (M.F. Johannsmeier, Ed.) Plant Protection Research Institute, Pretoria, South Africa, pp 95-110.

Taber S., 1961. Forceps designed for transferring honey bee eggs. *J. Econom. Entomol. 54:* 247-250.

Tribe G.D., Allsopp M.H. (2001) Honeybee reproduction. In: *Beekeeping in South Africa.* (M.F. Johannsmeier, Ed.) Plant Protection Research Institute, Pretoria, South Africa, pp 40-46.

Velthuis H.H.W., 1972. Observations of the transmission of queen substances in the honey bee colony by the attendants of the queen. *Behaviour 41:* 105-129.

Velthuis H.H.W., F. Ruttner and R.M. Crewe, 1990. Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: *Social Insects: an evolutionary approach to castes and reproduction.* (W. Engels, Ed.). Springer Verlag, Berlin, Germany, pp. 231-243.

Visscher P.K., 1996. Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. *Behav. Ecol. Sociobiol. 39:* 237-244.

Wcisclo W.T., 1981. The roles of seasonality, host synchrony and behaviour in the evolutions and distributions of nest parasites in Hymenoptera (Insecta), with special reference to bees (Apoidae). *Biol. Rev. 62:* 515-543.

Wilson E.O., 1971. The Insect Societies. Harvard University Press Cambridge, USA, 548 pp.

Wossler T.C., 2002. Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *A. m. scutellata* colonies. *Apidologie 33:* 139-163.

# 3.9 Egg laying and egg removal by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.)

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**Abstract** – Queenright *Apis mellifera capensis* colonies exhibit egg laying by workers in periods of both low and high egg removal. To reproduce workers should lay in times of low egg removal to increase survival of their eggs. Were this so, a negative correlation between egg laying and removal would be expected. Egg removal rates for queen (N=240) and worker-laid (N=240) eggs and egg laying by workers were tested in queenright colonies. Worker-laid eggs were removed significantly faster than queen-laid eggs; but significant differences in egg laying by workers occurred among colonies. Egg laying and removal are positively correlated and co-dependent. Egg removal appears triggered by the number of worker-laid eggs. Intercolonial variation for laying worker egg number and egg removal rates may explain the phenotypic variation in worker reproduction in queenright Cape honeybee colonies.

**Key words:** *Apis mellifera capensis* / egg removal / laying workers / worker reproduction / worker policing

## 1. INTRODUCTION

With few exceptions (Oldroyd et al., 1994), worker-laid eggs are removed by other workers in queenright colonies of the European subspecies of *Apis mellifera* L. (Ratnieks and Visscher, 1989). This seems to be based on relatedness grounds (Ratnieks, 2000), because laying workers usually produce male offspring (Free, 1987). However, laying workers of the Cape honeybee *(Apis mellifera capensis* Eschscholtz) produce female offspring (Onions, 1912; Neumann et al., 2000; Hepburn and Radloff, 2002; Radloff et al., 2002), leading to predictions that egg removal is either not expressed at all, or is less expressed in this subspecies (Greeff, 1996). Nevertheless, queenright Cape honeybee colonies exhibit worker policing (Neumann, Pirk, Ratnieks, unpublished data), indicating that removal of worker-laid eggs can also be based on colony efficiency grounds.

Brood above the queen excluder is more frequently observed in queenright colonies of Cape honeybees (Pettey, 1922; personal observations) than in other subspecies of *A. mellifera* (Visscher, 1996). It has been shown that such brood is actually worker-derived (Moritz et al., 1999), indicating successful worker reproduction despite the presence of a queen and egg removal. Indeed, thousands of queenright colonies of the neighbouring subspecies *Apis mellifera scutellata* Lepeletier were taken over by laying *A. m. capensis* workers (Allsopp and Crewe, 1993; Martin et al., 2002), showing that *A. m. capensis* workers are facultative social parasites (for details see Hepburn and Allsopp, 1994; Neumann et al., 2001; Calis et al., 2002; Moritz, 2002; Neumann and Hepburn, 2002; Reece, 2002; Wossler, 2002). These observations strongly indicate that laying workers of *A. m. capensis* are able to evade worker policing, but what potential strategies, if any, could these laying workers use to increase the survival of their eggs?

Earlier observations showed (Pirk, Neumann, Hepburn, Radloff, unpublished data) that worker policing is subject to environmental variation within colonies of *A. m. capensis*, thus leading to periods of low egg removal rates under unfavourable weather conditions within a colony. Because worker policing is only exercised against eggs (Ratnieks and Visscher, 1989), worker-laid eggs need only survive three days after oviposition, which might fit well in a time window of low egg removal. So one potential strategy of laying *A. m. capensis* workers to evade worker policing, which we designate as hypothesis 1 could be that they are able to evaluate periods of low egg removal and lay their eggs during this time window. In this case one would expect a negative correlation between worker egg laying and the removal of worker-laid eggs by other workers, because workers should lay more eggs when there is less egg removal by other workers and *vice versa*.

But, the same environmental factors which affect egg removal behaviour may also reduce egg-laying activity (hypothesis 2). This might be simply due to the generally reduced activity of workers during periods with unfavourable weather conditions (Riessberger et al., 1998). Alternatively, periods with unfavourable weather conditions may also reduce the survival chances of worker-derived offspring because nurse bees change from the care of young larvae to the care of older larvae during such periods (Blaschon & Crailsheim, 2001). This may easily outweigh the risk of being removed by other workers. Thus, unfavourable weather conditions may not only reduce the activity of egg removal behaviour by workers but also of egg laying by workers. In this particular case one would expect a positive correlation between worker egg laying and removal of worker-laid eggs by other workers, because laying worker activity and egg removal behaviour are affected in the same way.

Alternatively a third hypothesis emerges, but not necessarily mutually exclusive of hypothesis 2, that laying workers may not be able to evaluate periods of low egg removal periods and egg removal is simply triggered by the number of worker-laid eggs present in the colony, leading to a positive correlation between egg laying and egg removal. Clearly, it is not possible to distinguish between hypothesis 2 and 3 because both predict a positive correlation between egg removal and number of worker-laid eggs.

Here we test these three hypotheses by evaluating egg laying and removal of worker-laid eggs by workers in queenright Cape honeybee colonies.

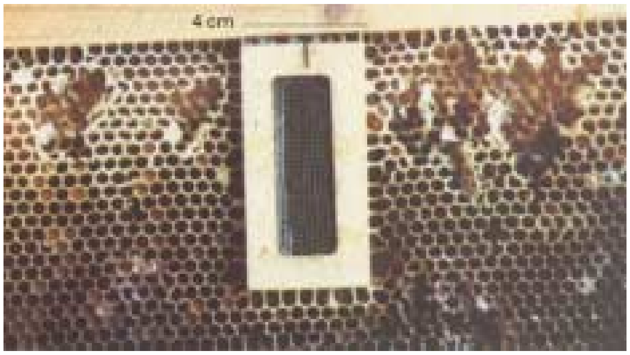
## 2. MATERIAL AND METHODS

Two queenless and four queenright *A. m. capensis* colonies were obtained from Port Elizabeth and placed in a test apiary in Grahamstown, South Africa. All colonies were unrelated to avoid any bias derived from nestmate recognition on egg removal behaviour (Pirk et al., 2001), and housed in 10-frame standard Langstroth hives with two brood boxes. The colonies were given two days to settle to avert absconding (Hepburn et al., 1999). Then, three test combs (A, B, C) were placed in the brood nest of each of three queenright test colonies two days before the experiments began to avoid any potential impact of the introduced comb (Breed et al., 1995) on egg removal behaviour.

The A test combs were used to evaluate the level of egg removal for queen and worker-laid eggs (Ratnieks and Visscher, 1989; Ratnieks, 1993). The level of egg removal was measured as the proportion of eggs removed from the total number of eggs that were transferred expressed as a percentage. One queenright and two queenless colonies were used as egg sources. Twenty queen and 20 worker-laid eggs were transferred daily for four days on each of the A test combs, which were then again sandwiched between two brood frames of the test colonies (Fig. 2) according to standard methods for evaluating egg removal rates (Ratnieks and Visscher, 1989; Ratnieks, 1993; Oldroyd and Ratnieks, 2000). After 24 hours the A test combs were briefly removed, all remaining eggs were counted and then removed before transferring a new set of eggs onto the comb.

The queens of the three test colonies were placed in small wooden cages [8 cm × 4 cm × 2.5 cm] with gauze mesh [mesh width = 2 mm], to allow feeding by workers but preventing the queens from egg laying (Fig. 1). These cages were attached in a frame of empty comb (B) and returned to the middle of the brood nest (Fig. 2). The B combs were not moved in any way during the experiment to keep the disturbance of the colony and the queen to a minimum.

**Figure 1.** The queen cage of frame B. The B frame was sandwiched between two brood frames in the bottom box next to empty combs A and C (Fig. 2), so that the queen was still present in the brood nest and workers had partial access to the queen.



Another empty test frame (C) was also placed in each of the colonies to evaluate egg laying by workers (Fig. 2). From the next morning onwards worker-laid eggs in each of the test colonies were counted once daily at 09:00 after removing the C test frames on four sequential days. During counting, sheets of transparent films were placed over the C combs and the position of each egg was recorded by using a reference grid to avoid double counting and to count only eggs laid by workers within a 24 hour period.

Simultaneously on four sequential days, the level of egg removal for queen and worker-laid eggs (A test combs) and the egg laying behaviour of workers were evaluated (C test combs). It is assumed that egg removal and egg laying could equally occur on both A and C combs, so that any difference in the numbers of egg between the combs would a be systematic error.

Mann Whitney U-tests were used to compare the level of egg removal of queen and worker-laid eggs on the A test combs after 24 hours. A c 2 -test was performed to test the difference in the number of worker-laid eggs between the colonies. The same test was used to compare the level of egg removal of worker laid eggs between the three colonies. Both tests were performed to investigate possible intercolonial variation. A Spearman rank order correlation was calculated for the number of worker-laid eggs and the level of egg removal of worker-laid eggs in the test cells.

## 3. RESULTS

On the A test combs a total of 240 queen-laid and 240 worker-laid eggs were tested for egg removal rates on a daily basis (Tab. I). Worker-laid eggs were removed significantly faster than queen-laid eggs (Mann-Whitney U-test: Z = 2.22, P < 0.026). There were no differences in the levels of egg removal of worker-laid eggs among the colonies (c 2 = 1.37; df = 2, n.s.), but there was variation within each test colony (Tab. I). A total of 57 eggs laid by workers were recovered from the C combs in the test colonies (Tab. I). Significant differences were found between the colonies in the number of worker-laid eggs (c 2 = 30.74; df = 2, P < 0.0001). The number of worker-laid eggs that were laid and the level of egg removal of worker-laid eggs were significantly positively correlated (Fig. 3).

## 4. DISCUSSION

The data confirm earlier findings that worker honeybees in queenright colonies of *A. m. capensis* are able to recognise and remove worker-laid eggs (Pirk et al., 2001). Moreover, this study shows a significant colony variation in egg laying by workers among the three discriminator colonies (Tab. I), but no significant differences for egg removal behaviour among them. The data also show that egg removal and egg laying by workers are positively correlated in the Cape honeybee (Fig. 3), indicating that egg removal and egg laying are co-dependent.

The standard method for evaluating egg removal behaviour in queenright honeybee colonies uses non-nestmate queen and worker-laid eggs (Ratnieks and Visscher, 1989; Ratnieks, 1993; Oldroyd and Ratnieks, 2000). Thus, nestmate recognition for eggs (Visscher, 1986) affects egg removal estimates by overestimating the removal rates of alien worker-laid eggs compared to native worker-laid eggs (Pirk et al., 2001). Although alien worker-laid eggs (transferred into the colony) were compared with native worker-laid eggs (laid in the colony) in this study, this would simply result in a systematic error that is the same for all colonies and would not affect the correlation between egg removal and egg laying.

The positive correlation between the number of worker-laid eggs and egg removal rates clearly indicates that Cape honeybee laying workers do not specifically lay eggs during periods of low egg removal rates. Hypothesis 1 can thus be rejected. Hypothesis 2 is based on the coincidence of periods of low egg removal rates with low egg laying rates and predicts a positive correlation between removal and egg laying. Also if egg removal, is triggered by the number of eggs found in the combs there would be a positive correlation between removal and egg laying (hypothesis 3). Thus, hypotheses 2 and 3 both fit our data but we cannot discriminate between the two. We consider hypothesis 3 as slightly more likely because less steps are involved to explain the observed correlation.

Our data show that the occurrence of worker-derived brood above the excluder is unlikely to reflect a specific strategy of laying workers to evade periods of high egg removal. We rather conclude that a combination of intercolonial variation in the number of eggs laid by workers, their egg removal rates and the ability of policing workers to remove worker-laid eggs may explain the phenotypic variation for successful worker reproduction in queenright Cape honeybee colonies.

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**REFERENCES**

Allsopp M.H., Crewe R.M. (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan, Am. Bee J. 133, 121–123.

Blaschon B., Crailsheim K. (2001) The impact of bad weather phases upon the brood care behaviour of nurse bees, Apidologie 32, 506–507.

Breed M.D., Garry M.F., Pearce A.N., Hibbard B.E., Bjostad L.B., Page R.E. (1995) The role of wax comb in honey bee nestmate recognition, Anim. Behav. 50, 489–496.

Calis J.N.M., Boot W.J., Allsopp M.H., Beekman M. (2002) Getting more than a fair share: nutrition of worker larvae related to social parasitism in the Cape honey bee *Apis mellifera capensis*, Apidologie 33, 193–202.

Free J.B. (1987) Pheromones of Social Bees, Chapman and Hall, London.

Greeff J.M. (1996) Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee *Apis mellifera capensis*, Phil. Trans. R. Soc. London B 351, 617–625.

Hepburn H.R., Allsopp M.H. (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*, S. Afr. J. Sci. 90, 247–249.

Hepburn H.R., Reece S.L., Neumann P., Moritz R.F.A., Radloff S.E. (1999) Absconding in honey-bees *(Apis mellifera*) in relation to queen status and mode of worker reproduction, Insectes Soc. 46, 323–326.

Hepburn R., Radloff S.E. (2002) *Apis mellifera capensis*: an essay on the subspecific classification of honeybees, Apidologie 33, 105–127.

Martin S., Wossler T., Kryger P. (2002) Usurpation of African *Apis mellifera scutellata* colonies by parasitic *Apis mellifera capensis* workers, Apidologie 33, 215– 232.

Moritz R.F.A., Kryger P., Allsopp M.H. (1999) Lack of worker policing in the Cape honeybee *(Apis mellifera capensi*s), Behaviour 136, 1079–1092.

Moritz R.F.A. (2002) Population dynamics of the Cape bee phenomenon: The impact of parasitic laying worker clones in apiaries and natural popu-lations, Apidologie 33, 233–244.

Neumann P., Hepburn H.R., Radloff S.E. (2000) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee *(Apis mellifera* L.) colonies, Apidologie 31, 479–486.

Neumann P., Radloff S.E., Moritz R.F., Hepburn H.R., Reece S.L. (2001) Social parasitism by honeybee workers *(Apis mellifera capensis* Esch.): Host finding and resistance of hybrid host colonies, Behav. Ecol. 12, 419–428.

Neumann P., Hepburn R. (2002) Behavioural basis for social parasitism of Cape honeybees *(Apis mellifera capensi*s), Apidologie 33, 165–192.

Oldroyd B.P., Smolenski A.J., Cornuet J.M., Crozier R.H. (1994) Anarchy in the beehive, Nature 371, 749.

Oldroyd B.P., Ratnieks F.L.W. (2000) Evolution of worker sterility in honeybees *(Apis mellifer*a): how anarchistic workers evade policing by laying eggs that have low removal rates, Behav. Ecol. Sociobiol. 47, 268–273.

Onions G.W. (1912) South African ‘fertile worker bees’, Agric. J. Union S. Afr. 1, 720–728.

Pettey F.W. (1922) Workers laying in comb of extracting supers, Elsenberg Apiary, J. Dept. Agric. Union S. Afr. 4, 122–124.

Pirk C.W.W., Neumann P., Hepburn H.R. (2001) Nestmate recognition of eggs and worker policing in the African honeybees *Apis mellifera capensis* (Esch.) and *Apis mellifera scutellata* (Lepeletier), in: Proceedings of the 2001 Berlin Meeting of the European Sections of IUSSI, September 25.-28., Berlin, Germany, p. 106.

Radloff S.E., Hepburn R., Neumann P., Moritz R.F.A., Kryger P. (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensi*s, Apidologie 33, 129–137.

Ratnieks F.L.W. (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies, Behav. Ecol. Sociobiol. 32, 191–198.

Ratnieks F.L.W. (2000) Worker policing in the honey-bee: basic facts and ideas, Insect Social Life 3, 3–10.

Ratnieks F.L.W., Visscher P.K. (1989) Worker policing in the honeybee, Nature 342, 796–797.

Reece S.L. (2002) A scientific note on the ovarial and pheromonal development of drifted and non-drifted Cape honeybee workers *(Apis mellifera capensi*s), Apidologie 33, 213–214.

Riessberger U., Blaschon B., Hrassnigg N., Crailsheim K. (1998) The behaviour of food storer *(Apis mellifera* L.) during good and bad weather conditions, Apidologie 29, 447–449.

Visscher P.K. (1986) Kinship discrimination in queen rearing by honey bee colonies *(Apis mellifer*a), Behav. Ecol. Sociobiol. 18, 453–460.

Visscher P.K. (1996) Reproductive conflict in honeybees: a stalemate of worker egg-laying and polic-ing, Behav. Ecol. Sociobiol. 39, 237–244.

Wossler T.C. (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *Apis mellifera scutellata* colonies, Apidologie 33, 139–163.

**Figure 2.** A schematic drawing of the arrangement of the test combs (A, B, C) in the bottom boxes of the three test colonies. The combs in the top box were empty or contained honey and/or pollen.

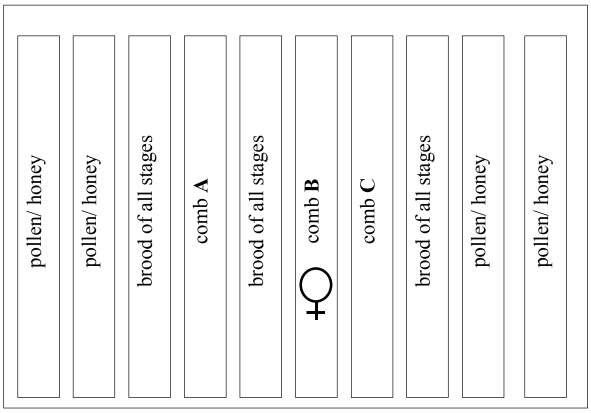
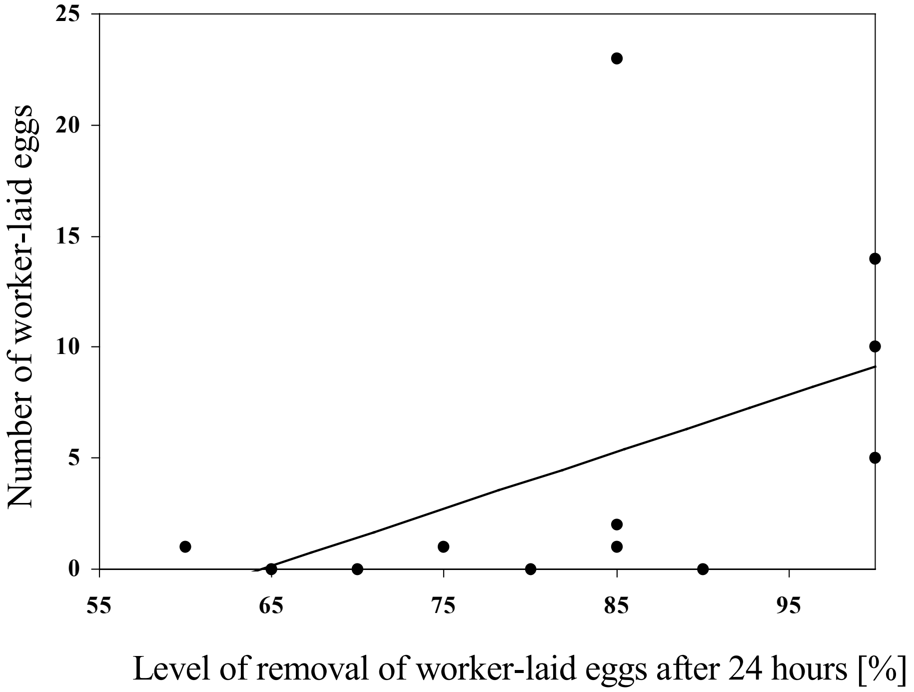


**Figure 3.** Number of worker-laid eggs (**C** comb) and removal rate (%) of worker-laid eggs **(A** comb) after 24 hours for three queenright *A. m. capensis* colonies on four sequential days. The number of worker-laid eggs and the level of egg removal are significantly positively correlated (Spearman Rank Order Correlation: r s = 0.6, *P* < 0.039).



# 3.10 Parasitic Cape bees in the northern regions of South Africa: source of the founder population

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**Abstract –** Multivariate discriminant analyses of 9 standard morphometric characters of honeybee workers were used to track the origin of a social parasitic pseudo-clone of thelytokous laying workers invading colonies of *A. m. scutellata* in South Africa. Twenty social parasitic workers were sampled from each of 2 infested *A. m. scutellata* colonies at two distant apiaries (Graskop and Heilbronn, about 390km apart) and compared with data obtained from 80 colonies in four different zones (zone I: thelytokous *A. m. capensis* morphocluster; zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; zone III: thelytokous *A. m. scutellata* morphocluster; zone IV, constituting an arrhenotokous *A. m. scutellata* morphocluster). Thelytokous laying workers naturally occur in zones I-III. Highly significant morphometric differences were found between the four zones. The data support the conclusion that the social parasitic workers belong to the thelytokous *A. m. capensis* morphocluster. It is most likely that the social parasitic workers originated from the heart of the range of the Cape bee in the Western Cape region in zone I. Morphometric analysis makes it feasible to restrict the possible origin of the social parasitic workers from the natural distribution range of thelytoky (±240.000km$^2$) down to about ±12.000 km$^2$, which equals a resolution capacity of about 95%.

**Key words:** *Apis mellifera*, honeybee, social parasite, morphometrics, thelytoky

## 1. INTRODUCTION

The recent human introduction of honeybee colonies with thelytokous (female producing) laying workers thought to be *Apis mellifera capensis*, into the region of the neighbouring arrhenotokous (male producing) subspecies *A. m. scutellata* in north-eastern South Africa resulted in the usurpation and subsequent loss of many thousands of colonies of the latter[1,2,3]. This resulted from intraspecific social parasitism of such thelytokous laying workers[4,5]. Recent genetic analyses[6,7,8,9,10] confirmed that a single matriline of thelytokous laying workers, constituting an almost genotypically identical so-called pseudo-clone[6,7] is invading large areas of the region of *A. m. scutellata*.

Because the social parasitic pseudo-clone workers are thelytokous and of black colour it was assumed that they are Cape bees[1,11]. This view is problematic because thelytoky naturally occurs in bees, which are morphometrically defined as *A. m. capensis*, *A. m. scutellata* and their natural hybrids[12]. Moreover, colour is a rather subjective and unreliable indicator of intrasubspecific categories[10].

We now report on the effectiveness of morphometric analysis as a forensic probe to track the founder population of the pseudo-clone of social parasitic thelytokous workers invading the neighbouring subspecies *A. m. scutellata*.

## 2. MATERIALS AND METHODS

Sealed brood frames containing social parasitic worker offspring were obtained from four *A. m. scutellata* colonies heavily infested with thelytokous laying workers, at two distant localities (Graskop and Heilbronn, about 390 km apart) in the natural distribution area of *A. m. scutellata* in South Africa. In a previous study[10] we have shown that the discriminant analysis produced only one cluster for the parasitic clone bees. That means that these bees are morphometrically inseparable despite very varied environmental conditions at the two sampling localities.

The samples were taken at late stages of infestation[5], when the host queen was already lost for more than two weeks. Thus, these frames definitely contained parasitic worker offspring because laying workers of *A. m. scutellata* produce drone offspring[13]. These brood frames were individually confined in gauze-covered cages and placed in an incubator until adult emergence. Twenty social parasitic workers from each of the 4 colonies were morphometrically analysed using 9 standard characters in honeybee morphometrics[14,15]. Their Ruttner[14] numbers are given in brackets as follows: length cover hair on tergite 5 (1); sternite 3, longitudinal (11); wax plate of sternite 3, transversal (13); wing angle 34 (22); wing angle N23 (30); wing angle O26 (31); pigmentation of tergite 2 (32); pigmentation of scutellum (35) and pigmentation of scutellar plate (36). The genotypic composition of the workers was verified by DNA microsatellite analysis[8].

These morphometric data were combined with previous data from 80 localities in southern Africa below 28° latitude south[16] and analyzed. This region consists of three zones with morphometrically different defined groups of honeybees where thelytokous worker reproduction naturally occurs (zones I-III; with a total of ±240,000 km$^2$) and a fourth zone with arrhenotokous worker reproduction (Figure 1). Thelytokous workers were collected from 24 localities in zone I (±70,000 km$^2$) for which the bees are morphometrically defined as *A. m. capensis*. Thelytokous bees were also collected from 5 localities in zone II (±80,000 km$^2$), a morphometrically defined natural hybrid area between *A. m. capensis* and *A. m. scutellata*, and from 19 localities in zone III (±80,000 km$^2$), which is morphometrically defined as *A. m. scutellata*. Finally, samples were taken from 32 localities in zone IV, which consists of arrhenotokous bees morphometrically defined as *A. m. scutellata* and which extends several million km$^2$ northwards into eastern Africa[15,16].

Multivariate discriminant analyses using 9 morphometric characters were carried out to determine cluster formations of the colonies at each locality and the parasitic clone workers. The Mahalanobis squared distances between the clusters of each locality and the parasitic clone cluster were calculated separately. The Mahalanobis distance is the distance between the centroids of the clusters in a multidimensional space[17]. The Mahalanobis squared distances failed tests of normality (Kolmogorov-Smirnoff, d=0.174, P<0.05) and hence the non-parametric Kruskal-Wallis test was used to test for significant differences in the distances between the four zones. Mann-Whitney U tests with Bonferroni adjustments to the levels of significance (i.e. $\alpha^* = 0.05/6 = 0.0083$) were used for the pair wise comparison of the distances between the zones. Box-and-Whisker plots were used to check for any extreme values of the Mahalanobis squared distances within the *A. m. capensis* morphocluster.

## 3. RESULTS

The Mahalanobis squared distances from the centroid of the parasitic clone cluster to each of the locality clusters are given in Table 1 and the distribution of which among the four zones is shown in Fig. 1. Highly significant differences were found between the four zones (H=62.60, 3 df, P<0.0001). Multiple pair wise comparisons revealed significant differences between zone IV and zones I, II and III (zone I: U=0.0, P< 0.0001; zone II: U=0.0, P=0.0004; zone III: U=42.0, P<0.0001). No significant difference (using Bonferroni adjustments) was found between zone II and III (U=17.5, P=0.0329) and between I and II (U=21.0, P=0.0244). The Mahalanobis squared distances between zones I and III were significantly different (U=26.0, P<0.0001). Three extreme outliers in the Mahalanobis squared distances were found within zone I at Piketberg (23), Mosselbaai (51) and Port Elizabeth (66, cf. figure 1). When the extreme values were removed from the analysis, a significant difference was found between zones I and II (U=6.00, P=0.0025). Four localities in the Western Cape (29, 34, 35 and 42) showed Mahalanobis squared distances below 6.

## 4. DISCUSSION

The data clearly show that the pseudo-clone of social parasitic thelytokous workers invading *A. m. scutellata* originates from the *A. m. capensis* morphocluster and further indicates that the pseudo-clone very probably originated from the Western Cape, the heart range of the Cape bee. Thus, we were able to confirm several earlier statements[1,3,11] that the social parasitic workers invading *A. m. scutellata* in its native range are indeed Cape bees and not thelytokous hybrids or *A. m. scutellata*. Our data also confirm that morphometric analysis can be used as an inexpensive forensic probe to track the origin of introduced honeybees[18]. In the particular case of South African bee populations, nuclear DNA markers are too variable to resolve the origin of introduced bees. For example, intrapopulation variation for several DNA microsatellite loci has been shown to be as high as interpopulation variation between *A. m. capensis* and *A. m. scutellata*[19]. However, unlike the thelytokous parasitic pseudo-clones, other introduced bees will reproduce with the local population and morphometric characters will become difficult to distinguish within a short period of time. So, morphometric analysis can be used for the originally introduced bees, but for later generations, the use may be difficult.

Obviously, the social parasitic honeybee workers could have only originated from the natural distribution area of thelytoky. Indeed, the data show that the parasites certainly do not originate from the region of arrhenotokous *A. m. scutellata* (Fig. 1). Moreover, smaller Mahalanobis squared distances were obtained from the pure *A. m. capensis* morphocluster as opposed to the natural hybrid zone and the more southerly *A. m. scutellata* localities, where thelytoky also naturally occurs (Fig. 1). Therefore it is evident that the parasitic workers originated from the *A. m. capensis* morphocluster and not from other areas, where thelytoky naturally occurs.

Within the *A. m. capensis* morphocluster the results indicate that it is unlikely that the parasitic worker clone originated from east of Heidelberg or north of Ceres/Malmesbury. Moreover, there is a region of 4 localities with Mahalanobis squared distances below 6

(Paarl, Riviersonderend, Hermanus and Bonnievale), which indicates that the social parasitic clone originates from the heart range of the Cape bee in the Western Cape region. Thus, morphometric analysis restricts the potential area of origin from the natural distribution range of thelytoky (±240.000 km$^2$) down to about ±12.000 km$^2$, which equals an resolution capacity for this approach of 95%.

Considering the clinal structure of the natural occurring hybrid zone between *A. m. capensis* and *A. m. scutellata*[16], it seems most likely that the social parasites with the highest within-host virulence[4,5] originated from the heart range of the Cape bee. In this region, almost all laying workers reproduce thelytokously and have high reproductive potential. Indeed, what is known of beekeeping activity seems to support our findings[3]. In any event, *A. m. capensis* colonies were brought from the western Cape to the Transvaal in about 1991 just before the usurpation of *A. m. scutellata* colonies by the social parasitic *A. m. capensis* reached epidemic levels[1,11].

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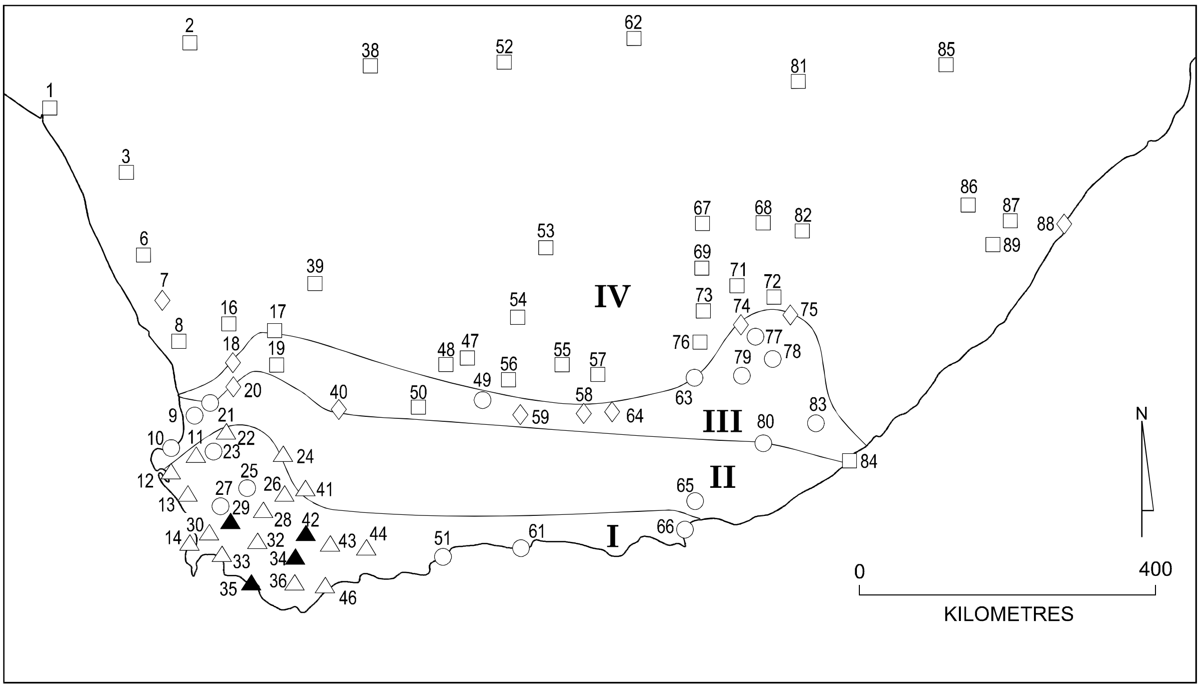
## REFERENCES

1. Allsopp M.H. and Crewe R. (1993). The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. *Am. Bee J.* **133**, 121-123.
2. Hepburn H.R. and Allsopp M.H. (1994). Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. *S. Afr. J. Sci.* **90**, 247-249.
3. Allsopp M.H. (1995). The *Capensis* problem 1992-1995. In *Proceedings, 1st International Electronic Conference on the Cape Bee problem in South Africa*, ed P. Magnuson, pp. 10-31, PPRI, Pretoria.
4. Neumann P., Radloff S.E., Moritz R.F.A., Hepburn H.R. and Reece S.L. (2001b). Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): Host finding and resistance of hybrid host colonies. Behav. Ecol. **12**, 419-428.
5. Neumann P. and Hepburn H.R. (2002). Behavioural basis for social parasitism by Cape honeybees (*Apis mellifera capensis* Esch.). *Apidologie* **33**, 165-192.
6. Kryger P. (2001a). The Capensis pseudo-clone, a social parasite of African honey bees. In *Proceedings, 2001 Berlin Meeting of the European Section of IUSSI*, p. 208, IUSSI, Berlin.
7. Kryger P. (2001b). The pseudo-clone of *Apis mellifera capensis* – an obligate social parasite in honeybees. In: *Proceedings, XXXVII International Apicultural Congress*, p.33, Durban South Africa.
8. Neumann P., Pirk C.W.W., Moritz R.F.A. and Hepburn H.R. (2001a). Trade off between dispersal capacity and virulence in a social parasitic Cape honeybee clone (*Apis mellifera capensis* Esch.). In *Proceedings, 2001 Berlin Meeting of the European Section of IUSSI*, p. 106, IUSSI, Berlin.
9. Solignac M., Kryger P., Allsopp M.H., Mougel F., Koeniger N., Baudry E. and Cornuet J.M. (2001). Thelytoky in Cape honey bees: a genomic perspective. In *Proceedings, 2001 Berlin Meeting of the European Section of IUSSI*, p. 147, IUSSI, Berlin.
10. Radloff S.E., Hepburn H.R., Neumann P., Moritz R.F.A. and Kryger P. (2002). A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis* (Esch.). *Apidologie* **33**, 129-137.
11. Allsopp M.H. (1992). The *capensis* calamity. *S. Afr. Bee J.* **64**, 52-55.
12. Hepburn H.R. and Radloff S.E. (2002) *Apis mellifera capensis* Eschholtz: concept or reality? *Apidologie* **33**, 105-127.
13. Neumann P., Hepburn H.R. and Radloff S.E. (2000). Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera*) colonies. *Apidologie* **31**, 479-486.
14. Ruttner F. (1988). *Biogeography and taxonomy of honeybees*, Springer, Berlin.
15. Hepburn H.R. and Radloff S.E. (1998) *Honeybees of Africa*. Springer, Berlin.
16. Hepburn H.R., Radloff S.E. and Fuchs S. (1998). Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*. *Apidologie* **29**, 333-346.
17. Johnson R.A., Wichern D.W. (1998) Applied Multivariate Statistical Analysis, 4th edition, Prentice Hall, Upper Saddle River, New Jersey.
18. Mogga G.B. and Ruttner F. (1988). *Apis florea* in Africa; source of the founder population. *Bee World* **69**, 100-103.
19. Moritz R.F.A., Beye M. and Hepburn H.R. (1998). Estimating the contribution of laying workers to population fitness in African honeybees (*Apis mellifera*) with molecular markers. *Insectes Soc* **277**, 277-287.

**Table 1.** Mahalanobis squared distances (D2) between the centroids of the parasitic clone cluster and the morphoclusters at each locality (locality numbers as in Hepburn *et al.* 1998; Zone I: thelytokous *A. m. capensis* morphocluster; Zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; Zone III: thelytokous *A. m. scutellata* morphocluster; Zone IV: arrhenotokous *A. m. scutellata* morphocluster; Hepburn *et al.* 1998; asterisks indicate extreme outliers found within Zone I).

| **Locality** | **D2** |
|---|---|
| **Zone I** | |
| 12. Langebaan | 9.41 |
| 13. Darling | 8.89 |
| 22. Citrusdal | 6.91 |
| 23. Piketberg* | 16.47 |
| 24. Tweeriviere | 7.43 |
| 25. Ceres | 11.31 |
| 26. Sandvlei | 6.38 |
| 27. Malmesbury | 10.52 |
| 28. Worcester | 7.15 |
| 29. Paarl | 5.64 |
| 30. Kraaifontein | 8.64 |
| 32. Villiersdrop | 9.24 |
| 33. Somerset West | 7.85 |
| 34. Riviersonderend | 5.57 |
| 35. Hermanus | 5.36 |
| 36. Napier | 7.42 |
| 41. Touwsrivier | 8.85 |
| 42. Bonnievale | 5.34 |
| 43. Swellendam | 7.61 |
| 44. Heidelberg | 8.25 |
| 46. Skipskop | 10.26 |
| 51. Mosselbaai* | 18.84 |
| 61. Wittedrif | 11.00 |
| 66. Port Elizabeth* | 16.58 |
| Mean ± s.d. | 9.21±3.59 |
| Mean ± s.d. without* | 8.05±1.84 |
| **Zone II** | |
| 9. Elandsbaai | 10.38 |
| 10. Velddrif | 14.68 |
| 11. Laaiplek | 10.30 |
| 21. Elandsvlei | 14.36 |
| 65. Addo | 11.55 |
| Mean ± s.d. | 12.25±2.13 |
| **Zone III** | |
| 17. Calvinia | 48.25 |
| 18. Botterkloof | 23.66 |
| 19. Sonop | 36.26 |
| 20. Clanwilliam | 10.85 |
| 40. Sutherland | 20.03 |
| 49. Beaufort West | 10.81 |
| 50. Middelwater | 51.25 |
| 58. Aberdeen | 29.34 |
| 59. Wiegenaarspoort | 28.49 |
| 63. Cradock | 11.32 |
| **Locality** | **D2** |
| **Zone III** | |
| 64. Kendrew | 23.22 |
| 74. Molteno | 23.99 |
| 75. Dordrecht | 26.47 |
| 77. Sterkstrom | 18.02 |
| 78. Queenstown | 14.21 |
| 79. Tarkastad | 12.58 |
| 80. Fort Beaufort | 11.55 |
| 83. Stutterheim | 13.10 |
| 84. East London | 36.06 |
| Mean ± s.d. | 23.70±12.41 |
| **Zone IV** | |
| 1. Alexander Bay | 71.08 |
| 2. Karasburg | 69.94 |
| 3. Nababeep | 50.74 |
| 6. Garies | 112.40 |
| 7. Bitterfontein | 23.01 |
| 8. Lutzville | 43.25 |
| 16. Nieuwoudtville | 39.28 |
| 38. Upington | 134.28 |
| 39. Tontelbos | 97.58 |
| 47. Booiskraal | 62.06 |
| 48. Vonkfontein | 88.63 |
| 52. Postmasburg | 132.23 |
| 53. Britstown | 66.41 |
| 54. Victoria West | 66.44 |
| 55. Murraysburg | 36.45 |
| 56. Nelspoort | 33.79 |
| 57. Boesmanskop | 63.14 |
| 62. Warrenton | 52.47 |
| 67. Springfontein | 51.74 |
| 68. Smithfield | 50.33 |
| 69. Venterstad | 55.51 |
| 71. Burgersdop | 37.55 |
| 72. Jamestown | 40.60 |
| 73. Steynsburg | 44.29 |
| 76. Hofmeyr | 33.68 |
| 81. Winburg | 79.90 |
| 82. Zastron | 110.89 |
| 85. Harrismith | 36.87 |
| 86. Underberg | 68.45 |
| 87. Richmond | 62.13 |
| 88. Durban | 27.50 |
| 89. Ixopo | 46.42 |
| Mean ± s.d. | 62.16±29.06 |

**Fig. 1** Distribution of the Mahalanobis squared distances among the four investigated zones Zone I: thelytokous *A. m. capensis* morphocluster; Zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; Zone III: thelytokous *A. m. scutellata* morphocluster; Zone IV: arrhenotokous *A. m. scutellata* morphocluster; Hepburn et al. 1998; open squares = 30+, open rhombi = 20-30, open circles = 10.5-20, open triangles = 6-10.5, filled triangles = <6).



# 3.11 Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.)

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**Abstract -** Cape honeybee workers show important pre-adaptations for social parasitism and can cause the dwindling colony syndrome of host colonies. Parasitic workers may drift or actively disperse into host colonies. They may also join absconding swarms, which can merge with host colonies. After transmission, parasitic workers have to establish themselves in the host, which is probably promoted by their spatial distribution, their readiness to gain trophallactic dominance and their ability to survive worker-worker aggression. Established parasitic workers have to evade egg removal by other workers in host colonies. The resulting offspring is preferentially fed, can be expected to be highly virulent and may show different behaviour in the course of infestation. It is unknown why and how the host queen is lost. High numbers of parasitic workers are reared until the host colony dies or absconds. This offspring can infest new host colonies, thereby completing the social parasitic life cycle.

**Key words:** *Apis mellifera capensis* / *Apis mellifera scutellata* / honeybee / social parasitism / worker reproduction

## 1. INTRODUCTION

The invasion of distant host colonies of *Apis mellifera ligustica* by *A. m. capensis* laying workers was first described by Onions (1912), but was only recently recognised as social parasitism (Velthuis et al. 1990). When colonies of the neighbouring subspecies *A. m. scutellata* and other subspecies were transported into the region of *A. m. capensis* (Moore 1911, Guy 1975, Tribe 1981, Moodie 1983) or *vice versa* (Lundie 1954, Johannsmeier 1983), this resulted in the widespread take over by *A. m. capensis* laying workers (Hepburn and Allsopp 1994). This was amplified by orders of magnitude when many *A. m. capensis* colonies were transferred into the area of *A. m. scutellata* (Allsopp 1992, Johannsmeier 1992, Lear 1992, Allsopp and Crewe 1993). An estimated 30.000 *A. m. scutellata* colonies were usurped by *A. m. capensis* laying workers in the Highveld of South Africa, resulting in the so-called "*capensis* calamity" for beekeepers (Allsopp 1993, Allsopp and Crewe 1993). These events established two important points: 1. Laying workers of *A. m. capensis* are facultative social parasites and 2. Colonies of *A. m. scutellata* are highly susceptible host colonies, because they apparently lack efficient resistance mechanisms to prevent the establishment of laying *A. m. capensis* workers.

Social parasitism by *A. m. capensis* laying workers is expressed at the level of the host colony phenotype by the dwindling colony syndrome (Allsopp 1995). This dwindling is characterized by an initial reduction in foraging, then fighting at the hive entrance and inside of the colony between host workers and *A. m. capensis* workers, the appearance of worker-laid brood, the loss of the host queen and finally the eventual (at least in the case of *A. m. scutellata* inevitable) death of the host colony and its replacement by *A. m. capensis* (Allsopp 1995, Magnuson 1995). This process lasts up to four months (Swart and Kryger unpublished data, cited in Martin et al. 2002b), some nine weeks on the average and may be even faster in the region of *A. m. scutellata* (Kryger 2001b).

Over a very large area of *A. m. scutellata* in north-eastern South Africa all parasitic Cape honeybee workers apparently belong to a single so-called pseudo clone (Kryger 2001a,b, Kryger et al. 2002), because with the exception of a few mutational events all DNA microsatellite loci studied showed a maximum of two alleles (Solignac et al. 2001) indicating that they are progeny of a single founder worker (Kryger 2001a,b, Solignac et al. 2001, Radloff et al. 2002).

The present review is focussed on what is known of the biology of *A. m. capensis* in its native fynbos region in South Africa (Hepburn and Jacot-Guillarmod 1991) and the naturally occurring hybrid zone between *A. m. capensis* and the neighbouring subspecies *A. m. scutellata* (Hepburn and Radloff 2002) but will draw parallels to the current situation of the *capensis* calamity and the *A. m. capensis* pseudo-clone population in the northern parts of South Africa were information has very recently become available. Here we will present a general pattern of the social parasitism by laying Cape honeybee workers. However, this does not imply that there are no differences with respect to certain aspects of this social parasitic pathway due to biological variations among different populations of laying *A. m. capensis* workers and/or their respective host colonies. Indeed, laying worker populations of *A. m. capensis* appear to show considerable differences with respect to social parasitism (Neumann et al. 2001b). For example, the *A. m. capensis* pseudo-clone population invading *A. m. scutellata* in its native range (Kryger 2001a,b) seems to be more virulent compared to a laying worker population from the native range of the Cape honeybee (Port Elizabeth, Neumann et al. 2001b). This might be a result of severe selection processes between different strains of parasitic *A. m. capensis* workers which occurred in the *A. m. scutellata* population (Neumann et al. 2001b, Wossler 2002). Such variability in *A. m. capensis* with respect to traits related to worker reproduction is well documented (Hepburn 1994, Hepburn and Radloff 1998).

Obviously, social parasitism by worker honeybees could have potentially evolved in all honeybee subspecies, because in principle they are able to reproduce parthenogenetically either via arrhenotoky (the production of drones, Ruttner 1992, Crozier and Pamilo 1996) or via thelytoky (the production of diploid females; Onions 1912, Crozier and Pamilo 1996). However, it seems more likely that social parasitism would evolve when individual workers have the opportunity to maximise their reproductive success. Indeed, individual fitness can be extremely high as shown by the wide spread of the *A. m. capensis* pseudo-clone (Kryger 2001a,b, Kryger et al. 2002). Moreover, *A. m. capensis* laying workers generally contribute considerably to population fitness in South African honeybee populations, because an *A. m. capensis* mitochondrial DNA type introgressed far into the *A. m. scutellata* population (Moritz et al. 1998). The latter observation indicates that worker reproduction in *A. m. capensis* is also very important in the native range of the Cape honeybee and is not only an artefact of migratory beekeeping as in the case of the *capensis* calamity.

Cape honeybee workers show a unique series of traits that reflect important physiological and genetic pre-adaptations for intraspecific social parasitism: high fecundity (ovarial development, Hepburn and Crewe 1990; high number of eggs laid, Velthuis et al. 1990 soon after queen loss, Ruttner and Hesse 1981, Hepburn and Radloff 1998), longevity (3-5 months Velthuis et al. 1990; up to five months and more Tribe and Allsopp 2001b), high and fast pheromonal development (Hepburn 1994, Simon et al. 2001, Wossler 2002, this issue) and thelytoky (Onions 1912, Hepburn and Crewe 1991).

Thelytoky appears to predispose for the evolution of aggressive worker reproduction (Greeff 1996, 1997) and consequently for social parasitism of workers (Neumann et al. 2001c), because self-replicating thelytokous laying worker offspring can immediately infest new host colonies, without an intervening sexual generation. Moreover, worker egg-laying (Pirk et al. 2002, this issue) and even successful worker reproduction (Pettey 1922, Moritz et al. 1999) is common in queenright colonies of *A. m. capensis*. This indicates that the effects of brood and queen pheromones on worker ovary inhibition is reduced (Wossler 2002, this issue) and that *A. m. capensis* laying workers are able to evade the removal of worker-laid eggs in queenright honeybee colonies (Martin et al. 2002a, Pirk et al. 2002, Neumann, Pirk, Hepburn and Moritz, unpublished data). Both ovary activation and escape of worker policing are essential features to explain the successful reproduction of laying *A. m. capensis* workers in queenright colonies of their own and of other subspecies. The high incidence of successful parasitized host colonies (Allsopp and Crewe 1993) appears as unequivocal evidence that both aspects happen on a regular basis at least in *A. m. scutellata* host colonies.

The pheromonal development of *A. m. capensis* workers can become much more queen-like (Crewe and Velthuis 1980, Velthuis et al. 1990, Wossler 2002, this issue) and the onset of pheromonal development more rapid (Hepburn 1992, Simon et al. 2001, Wossler 2002, this issue) than in European subspecies. Of particular importance is that ovarial and pheromonal development covary in *A. m. capensis* workers (Hepburn 1992). Although all combinations of reproductive traits are possible in laying *A. m. capensis* workers (Hepburn 1994), many more of them show the combination of both high ovarial development and a queenlike pheromonal bouquet (Hepburn and Radloff 1998) than other subspecies of *A. mellifera* (Velthuis 1970, Crewe and Velthuis 1980, Hepburn and Radloff 1998). Such workers have been termed surrogate (Hepburn 1992) or pseudoqueens (Crewe et al. 1990, Wossler 2002, this issue).

Taking into account the above mentioned pre-adaptations it seems not surprising that the adoption of *A. m. capensis* laying workers in host colonies of other honeybee subspecies (*A. m. ligustica* (Onions 1912), *A. m. mellifera* (Woyke 1995, personal communication), *A. m. scutellata* (Guy 1975, Lundie 1954, Johannsmeier 1983)) often results in their usurpation (Hepburn and Allsopp 1994, Hepburn and Radloff 1998). Since migratory beekeepers repeatedly moved *A. m. capensis* colonies from their native fynbos region (a macchia-like biome in the Eastern and Western Cape provinces of South Africa, Hepburn and Jacot-Guillarmod 1991) across the natural hybrid zone into *A. m. scutellata* populations in the Highveld of South Africa (Allsopp 1995) parasitic workers have spread widely throughout the summer rainfall regions of South Africa (Allsopp 1993). This strongly suggests that there is also a behavioural basis for social parasitism by Cape honeybees. Here we give an overview on the relevant literature dealing with behavioural traits of *A. m. capensis* related to social parasitism.

## 2. SOCIAL PARASITIC LIFE CYCLE OF CAPE HONEYBEE LAYING WORKERS

The social parasitic life cycle of *A. m. capensis* laying workers involves a series of critical steps and events associated with the two major aspects: transmission and virulence (Fig. 1). Transmission is the transfer of parasites from one host to another. Virulence is usually defined as the mortality caused by a parasite in a standard host (Schmid-Hempel 1998). In the context of social parasitism by Cape honeybee laying workers virulence is associated with the speed of host colony death by dwindling. The speed of colony dwindling is probably caused by a variety of factors such as the degree of reduction in foraging by host workers (Allsopp 1995), the chance of invaded parasitic workers to survive worker-worker aggression, the extent of intracolonial fighting (Allsopp 1995), the degree of losses of host workers (Allsopp 1995), the speed and extent of reproduction by parasitic laying workers and finally the timing of the loss of the host queen. Concomitantly there are substantial numbers of parasitic offspring that mainly focus on reproduction and which participate to a lesser extent in normal hive duties such as brood rearing (Hillesheim et al. 1989) or foraging (Martin et al. 2002b). If a given strain of social parasitic workers causes a host colony to die quicker, these workers can be considered to be more virulent. Detailed knowledge of both transmission and virulence is crucial to understand social parasitism of laying Cape honeybee workers because higher transmission increases parasite fitness, whereas higher virulence decreases parasite fitness because it damages the parasites' food supply. In the context of transmission and virulence two major questions emerge with respect to the behaviour of Cape honeybees: 1) What are potential behavioural mechanisms for the transmission of social parasitic workers? 2) What behavioural factors govern the virulence of social parasitic workers and consequently the resistance of host colonies?

We will now give a very brief overview of the reproductive life cycle of social parasitic laying *A. m. capensis* workers, which is shown in Fig. 1. Transmission may occur within one (horizontal) or between (vertical) generations (Schmid-Hempel 1998). Horizontal transmission of *A. m. capensis* workers may occur at the individual and at the colony level. Individual workers may spread into neighbouring hives by passive drifting (step 1 in Fig. 1, Neumann et al. 2000b, 2001c) or actively disperse into distant colonies (step 2 in Fig. 1, Neumann et al. 2001c). Both drifted and dispersed individual workers have to bypass the host colony's guard force (Lindauer 1952, Ribbands 1953) to successfully invade the host colony. Workers of *A. m. capensis* may also join absconding swarms (step 3 in Fig. 1, Hepburn et al. 1999), which can subsequently merge with other colonies (step 4 in Fig. 1, Hepburn and Radloff 1998, Hepburn et al. 1999, Neumann et al. 2001a). For vertical transmission *A. m. capensis* workers may join reproductive swarms (step 5 in Fig. 1). To successfully reproduce, *A. m. capensis* workers, which have successfully invaded a host colony must establish themselves as pseudoqueens (step 6 in Fig. 1, Hepburn 1992). Established pseudoqueens have to evade the removal of their eggs in host colonies (step 7 in Fig. 1, Ratnieks and Visscher 1989, Miller and Ratnieks 2001, Martin et al. 2002a, Pirk et al. 2002, this issue, Neumann, Pirk, Hepburn and Moritz unpublished data). The resulting parasitic offspring is preferentially fed by the host workers (step 8 in Fig. 1, Beekman et al. 2000, Calis et al. 2002, this issue), resulting in highly developed progeny. Occasionally an *A. m. capensis* queen may be reared (step 9 in Fig. 1; but see Swart et al. 2001 and Martin et al. 2002b). In the following sections, each of these points will be discussed in detail.

## 3. TRANSMISSION OF SOCIAL PARASITIC CAPE HONEYBEE WORKERS

Efficient transmission through beekeeping activity, or via the individual and/or colonial pathways is essential because a host colony of another subspecies that is taken over by *A. m. capensis* laying workers cannot remain alive on its own and eventually dwindles and dies (Swart et al. 2001, Martin et al. 2002b, Moritz 2002). These observations evoke a number of considerations. For example, the more virulent parasitic workers are and the faster host colonies die, the more efficient transmission must be in order to maintain such parasitic strains. Because the *A. m. capensis* pseudo clone (Kryger et al. 2002) inevitably spreads like a “disease“ in the region of *A. m. scutellata* (Swart et al. 2001, A. Schehle personal communication) and *A. m. scutellata* host colonies die rather rapidly (Kryger 2001b, Martin et al. 2002b) transmission of the parasite is apparently very efficient. In light of these observations two questions emerge: 1) What are potential mechanisms for individual workers or whole colonies (either pure *A. m. capensis* colonies or infested host colonies) to find and to successfully invade new host colonies? 2) To what extent is such transmission influenced by the development of the *A. m. capensis* workers? In the following sections we will address in detail these questions related to the potential transmission pathways at the level of the individual parasitic workers and at the colony level.

### 3.1. Pathway of horizontal transmission of individual workers

#### 3.1.1 Drifting and dispersing (steps 1 and 2 in Fig. 1)

What potential mechanisms allow individual workers to find new host colonies? It is well established that honeybee workers often join neighbouring colonies by accidental drifting (Rauschmayer 1928, Free 1958, Jay 1966a,b, 1968, Neumann et al. 2000b). Drifting is caused by slight orientation errors of young workers during their orientation flights and sometimes by returning foragers (Rauschmayer 1928, Free 1958) and also requires adoption of these workers by the guard force of the host colonies. The amount of drifting strongly depends on a variety of factors such as age of the bees (Ribbands 1953), race (Ruttner 1992) and the apiary layout (Jay 1966a,b, 1968). Long-range drifting of workers also rarely occurs to colonies far away from their maternal nests (Renz and Rosenkranz 2001, Neumann et al. 2001c).

Onions (1912) reported that *A. m. capensis* laying workers invaded colonies of *A. m. ligustica* far out of the range of young workers on their orientation flights. Likewise, Johannsmeier (1983) described a pattern of invasion by laying workers of *A. m. capensis* of an *A. m. scutellata* apiary, which was difficult to explain by simple drifting. When keeping colonies of *A. m. capensis* in areas of other honeybees (e.g. Germany), it was suggested that the Cape honeybee colonies be separated by 300 m from other colonies to prevent "drifting" (Koeniger and Wurkner 1992). Such a distance is difficult to explain by simple orientation errors, supporting the idea that *A. m. capensis* workers may actively seek host colonies over large distances.

Recently, the impact of queenstate and taxon of both mother and host colonies on short-range drifting and long range dispersing of workers and on the hosting of these workers in colonies of *A. m. capensis*, *A. m. scutellata* and their natural occurring hybrids was investigated in a mark-recapture experiment (Neumann et al. 2001c). Six colonies each of *A. m. capensis*, *A. m. scutellata* and their natural occurring hybrids were split into queenless and queenright parts and arranged in three circular micro-apiaries (Neumann et al. 2001c). 12034 workers of the same age cohort were colony specific labelled and reintroduced into their respective mother colonies. After 10 days all labelled workers were recaptured from either their mother colony or from foreign host colonies. 579 workers (~ 4%) drifted into other colonies within their apiary and 89 workers were recaptured in other apiaries than their home apiary (=dispersers, Neumann et al. 2001c).

It was established that drifting and dispersing represent entirely different behaviours (Neumann et al. 2001c) because: 1) Significantly more *A. m. capensis* workers dispersed into another apiary than expected from the distribution of the drifted workers. 2) Dispersers did not only leave their own apiary but also did not prefer the same sector of the new apiary, as expected from simple orientation errors involved in drifting. 3) There were significant differences in the distribution patterns of drifters and dispersers among the tested groups. Although the hybrids drifted significantly more often than *A. m. capensis* and *A. m. scutellata*, they dispersed less often than the other groups. Moreover, *A. m. capensis* workers dispersed more often than the hybrids and *A. m. scutellata* combined. If drifting and dispersing constituted the same behaviour, one would expect a similar trend. 4) Whereas drifted *A. m. capensis* workers from queenright mother colonies were predominantly found in queenright host colonies (as opposed to drifted workers from other subspecies), dispersers from queenright *A. m. capensis* colonies were more often found in queenless host colonies and *vice versa*.

Were drifting and dispersing the same phenomenon, one would expect far fewer *A. m. capensis* workers to leave their home apiaries and no differences between the tested groups (Neumann et al. 2001c). Thus, it is very likely that drifting is a result of slight orientation errors into closely neighbouring colonies whereas dispersing constitutes active host finding by *A. m. capensis* workers over large distances (from >40m up to c. 1km, Neumann et al. 2001c). It is of course very difficult to distinguish drifting and dispersing in the case of closely neighbouring colonies unless detailed behavioural studies reveal e.g. different flight patterns in front of the hive. It would be of prime interest to evaluate whether the predisposition for reproductive dominance in Cape honeybee workers (Moritz and Hillesheim 1985, Moritz et al. 1996) is linked with a high predisposition for invading other colonies.

Neumann et al. (2001c) found that a high proportion of dispersed *A. m. capensis* workers (>80%) originated from a single colony only. This suggests a high inter colony variability for this trait among *A. m. capensis* and may contribute to our understanding of the apparent differences between populations of *A. m. capensis* laying workers with respect to social parasitism, e.g. between the pseudo-clone invading *A. m. scutellata* (Kryger 2001a,b) and from the native range of the Cape honeybee (e.g. Port Elizabeth, Neumann et al. 2001c). When comparing these two populations of *A. m. capensis* laying workers with respect to transmission, Neumann et al. (2001b) found that pseudo clone workers from the Transvaal are less efficient in spreading compared to laying workers from Port Elizabeth. This might be related to the higher virulence, particularly to the more “queen-like” behaviour of the pseudo clone workers, which tend to stay on the combs when colonies are inspected (Magnuson 1995) and are less likely to leave their maternal colonies, e.g. during foraging (Martin et al. 2002b).

Breaking into a defended fortress (Schmid-Hempel 1998) not only requires host finding by potential social parasitic workers (either via “serendipitous” orientation errors or via active host finding) but also bypassing the host colony's guard force which carefully scrutinises incoming individuals (Lindauer 1952, Ribbands 1953). Thus, one might expect behavioural adaptations of social parasitic workers to facilitate the bypassing of guard bees. For example, drifted workers may facilitate the entrance to new colonies by offering droplets of food to the guard bees (Ribbands 1953, Free 1958). Recently, *A. m. scutellata* discriminator colonies that were split into queenright and queenless parts were used to test whether invading workers of *A. m. capensis* have special mechanisms to circumvent the *A. m. scutellata* guards compared to *A. m. scutellata* workers (M. Beekman, personal communication). Queenstate may play a role in this perspective because queenless colonies are more prone to infestations by laying *A. m. capensis* workers

(Woyke 1995), which may related to different guarding behaviour. However, neither race of the introduced bee nor presence or absence of the queen in the guarding colony affected the proportion of introduced workers accepted (M. Beekman, personal communication). Nonetheless, Tribe (1983) reported that invading *A. m. capensis* workers may be severely mauled by *A. m. scutellata* guard bees, but invariably are allowed to enter the hive. Mauling intensity of guard bees of other races towards intruding *A. m. capensis* workers has not been quantitatively investigated yet. Tribe (1983) further suggested that incidents of successfully intruding Cape workers may be related to their more queen-like pheromonal bouquet. So, one special way of *A. m. capensis* might be a fast pheromonal development (Simon et al. 2001) before the onset of ovarial development.

Guard bees may have different conspecific acceptance-thresholds (Reeve 1989) for workers with respect to their reproductive status. For example, recent data suggest that *A. m. capensis* colonies are able to prevent workers with developed ovaries from entering the colony (Reece 2002, this issue). This seems plausible because workers with developed ovaries can be discriminated against because they are attacked by other workers (Velthuis 1976, Visscher and Dukas 1995). This may form the behavioural basis for a queenstate-discriminating guarding mechanism, which was indicated by the hosting of drifted (Reece 2002) and dispersed *A. m. capensis* workers (Neumann et al. 2001c). However, the reproductive status of *A. m. capensis* workers actually invading host colonies is not yet known. Whether dispersing *A. m. capensis* workers also show alternative behavioural tactics to facilitate admission into the host colony is simply not known. However, if only very few workers are needed to initiate infestations it may well be that no special mechanisms are actually needed for *A. m. capensis* workers to get into host colonies.

We conclude that two basic mechanisms enable individual *A. m. capensis* workers to find new host colonies. Individual *A. m. capensis* workers may invade host colonies via passive drifting due to simple orientation errors. This appears to be the prevalent mode of individual host finding in apiaries with closely neighbouring hives but is unlikely in the wild population due to the low population density (Neumann et al. 2001c). Alternatively, but not mutually exclusive *A. m. capensis* workers may also actively seek host colonies (Johannsmeier 1983, Neumann et al. 2001c) and spread individually between apiaries and into the wild population. Whether invading workers show behavioural tactics to facilitate their invasion is not known. Likewise is not yet clear how the state of development of such workers affects their acceptance by the guard force of host colonies. Thus, more quantitative data are needed to evaluate the relative chance of invasion of *A. m. capensis* workers with or without developed ovaries and the pre- and post-invasion ovarial and pheromonal development of such workers.

An admittedly speculative interpretation would suggest that workers with undeveloped ovaries and worker-like pheromones are unlikely to enter other colonies. Workers with developed ovaries and worker-like pheromones would be most unlikely invaders (Visscher and Dukas 1995, Reece 2002). Workers with queen-like pheromones seem to be likely to enter (Tribe 1983). Workers with developed ovaries and queen-like pheromones (pseudoqueens) are also likely to successfully invade but are less likely to leave their host colonies because once they have developed into pseudoqueens and are also behaviourally queen-like do not fly up but rather stay on the combs in contrast to the *A. m. scutellata* host workers, when infested colonies are inspected (Magnuson 1995). This clearly indicates that already established pseudoqueens are less likely to spread. Thus, the combination of developed ovaries and queenlike pheromonal bouquet is likely to develop after a less queen-like worker has invaded another colony.

### 3.2. Pathway of colonial horizontal transmission

#### 3.2.1 Absconding (step 4 in Fig. 1)

Absconding, a form of non-reproductive swarming (Hepburn 1988, Hepburn et al. 1999), is commonplace in colonies of African honeybees and can be triggered by a host of stimuli (Hepburn and Radloff 1998). In a natural population of Cape honeybees 20% of unmanaged colonies absconded (Allsopp and Hepburn 1997). Queen loss appears to promote absconding, which is extremely important with respect to the reproductive predisposition of queenless *A. m. capensis* workers (Hepburn 1994, Reece 2002). Indeed, queenless colonies of the Cape honeybee abscond twice as readily as queenless colonies of *A. m. scutellata* and their naturally occurring hybrids (Hepburn et al. 1999).

Moreover, the mode of worker reproduction may also play a role because colonies with predominantly thelytokous laying workers (*A. m. capensis*: Onions 1912, Hepburn and Crewe 1991; natural occurring hybrids: Neumann et al. 2000a) abscond significantly more often than *A. m. scutellata* colonies with arrhenotokous laying workers (Hepburn et al. 1999). Also *A. m. scutellata* colonies, which are highly infested with *A. m. capensis* laying workers in the Highveld of South Africa, frequently send out absconding swarms (Magnuson 1995). Since foraging activity is reduced by the host workers when infestations are initiated (Allsopp 1995, Swart et al. 2001), this may promote absconding of infested host colonies. Likewise, *A. m. capensis* remnants from recently collapsed *A. m. scutellata* host colonies also readily abscond (Allsopp 1998).

Johannsmeier (1983) reported that swarms of *A. m. scutellata* (1 queenless, two queenright) infested with *A. m. capensis* laying workers were caught 1 and 3 km away from an apiary where every colony infested with *A. m.*

*capensis* laying workers had been destroyed. Moore (1911) reported an *A. m. capensis* swarm migrating over 28 km, which fits well with estimates on the winter dispersal capacity of swarms in the fynbos region (Hepburn et al. 1993). A whole apiary of *A. m. scutellata* on an previously honeybee-free island, about 6 km away from the mainland and used as an *A. m. scutellata* island mating apiary similar to those of Europe (Neumann et al. 1999), was taken over by *A. m. capensis* (Anderson 1965). In light of the speed of dwindling observed in the Transvaal (Kryger 2001b) it is very unlikely that the *A. m. capensis* bees were introduced with the mating colonies because the problems with *A. m. capensis* laying workers occurred only after several months (Anderson 1965). This strongly indicates that a migrating swarm of *A. m. capensis* reached this island despite 6 km of open sea. Moreover, Hannabus (1945) reported that a migrating *A. m. capensis* swarm flew 11 km over open water. These observations clearly indicate that absconding and migration may be important mechanisms for the long-range transmission of parasitic *A. m. capensis* workers at the colony level. However, no qualitative assessments have been made so far regarding the importance of absconding in the spread of laying *A. m. capensis* workers (e.g. between apiaries and into the wild population).

### 3.2.2 Mergers (step 5 in Fig. 1)

After absconding, swarms may subsequently merge with each other in mid-air (Hepburn, personal observations), on tree congregations (up to 57 queenright swarms, Herman 1922; 11 queenright swarms, Hepburn and Whiffler 1988) or with established colonies (Walter 1939, Hepburn 1993, Hepburn and Radloff 1998, Neumann et al. 2001a). Such natural mergers require recruitment of colony parts or of a whole colony via scout workers to find a new nest site and/or host colony, just as in reproductive swarming (Seeley 1985), clearly constituting a colony phenomenon. With the exception of mergers in the open (mid-air or tree branches), the colony initiating the merger must subsequently invade the host colony, which might be associated with severe fighting at the host nest entrance (Anderson 1963, Neumann et al. 2001a) or not (Kigatiira 1988). The intensity of aggression, associated with invasion of the host colony appears to depend on food sources of the host and on size differences between the merging colonies (Kigatiira 1988). Surplus queens are usually eliminated within a short period of time after merger (Hepburn et al. 1988, but see Herman 1922 for the co-existence of two queens for a period of several years).

Such natural mergers of honeybee colonies are commonplace in tropical Africa (Hepburn and Radloff 1998), but their consequences on organisational structure of a colony and behaviour are largely unknown. The only reported study on this phenomenon strongly suggests that the origin of merging bees may matter, because task shifts in worker bees differed substantially between the colonies before and after they merged (Neumann et al. 2001a). Possibly, workers changed tasks as a result of different behavioural thresholds and task specialisation (Moritz and Page 1999). The task shifts and worker distribution observed in this study suggest that many bees responded to a different colony environment in the new unit. Depending on which reproductives dominate after such mergers, either the host and/or the invading workers are unrelated to the new offspring. In cases of successful invading and suppression of reproduction by the host, this constitutes a case of "slave making", analogous to that of some ant species (Hölldobler and Wilson 1990).

Thus, colonies which are well adapted to mergers and the resulting conflicts are predisposed to spread their genes (Kigatiira 1988). This has been claimed as one of the factors favouring the spread of *A. m. scutellata* in the Americas (Vergara et al. 1993). Since, mergers are particularly common among *A. m. capensis* (Herman 1922, Walter 1939, Hepburn 1988, Hepburn 1993, Hepburn et al. 1993), queens and workers of this subspecies should be well adapted in this regard. However, *A. m. scutellata* also shows frequent mergers (Silberrad 1976, Kigatiira 1988). Given that the fighting abilities of queens and queen-worker aggressive encounters are the same in both subspecies, worker-worker interactions with respect to reproductive dominance come into play. Because *A. m. capensis* workers are distinct gainers in this respect (see virulence below), they can be expected to become dominant after intersubspecific mergers with *A. m. scutellata* hosts.

We conclude that transmission at the colonial level may be important with respect to long range transmission between apiaries, introgression of parasitic workers into the wild population and the speed of colony dwindling because many parasitic host workers may enter simultaneously host colonies. Against this, *A. m. capensis* pseudoqueens appear to lack the ability to maintain swarm cohesion to the same extent as true queens, when placed with *A. m. scutellata* (Hepburn 1988). Moreover, mainly *A. m. capensis* workers were left behind, when artificially infested *A. m. scutellata* colonies absconded (Hepburn 1988, HMG Lattorff personal communication). Therefore, more data especially on the mergers of highly infested *A. m. scutellata* host colonies or of *A. m. capensis* remnants (Allsopp 1998) with non-infested host colonies are needed to further evaluate the importance and efficiency of absconding and colony mergers for the long-range transmission of *A. m. capensis* laying workers at the colonial level.

### 3.3 Pathway of colonial vertical transmission (step 6 in Fig. 1)

It is simply unknown whether parasitic *A. m. capensis* workers that have invaded *A. m. scutellata* or other subspecies join reproductive swarms cast out by their hosts at early stages of infestation or not. What is known from the fynbos region is that reproductive swarms frequently merge with each other (Hepburn, personal observations, Hepburn and Whiffler 1988), suggesting that this may also constitute a potential pathway for the transmission of parasitic *A. m. capensis* workers. However, this potential pathway is unlikely when the speed of host colony dwindling is fast (Martin et al. 2002b).

### 3.4 Beekeeping assisted transmission

Beekeeping assisted transmission appears to play a major role for the spread of parasitic *A. m. capensis* workers. Indeed, without human intervention the natural occurring hybrid zone appeared to be stable and "problems" with laying *A. m. capensis* workers were only reported after migratory beekeeping activities (Johannsmeier 1983, Allsopp 1992). Repeated observations that fixed site hobby beekeepers have lower infestation rates than migratory commercial beekeepers are numerous (Magnuson 1995, Swart et al. 2001). Large-scale beekeepers initiated the "*capensis* calamity" by transmission of colonies across the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* (see above). Moreover, hobby beekeepers often keep a very small number of colonies only and beekeeping scale probably also affects transmission because large-scale beekeepers more frequently move their hives than do hobby beekeepers.

In particular, hive management within apiaries, e.g. when colonies are inspected (Swart et al. 2001), may facilitate the invasion of *A. m. scutellata* colonies by social parasitic workers of *A. m. capensis* because the guard bees can be evaded. Indeed, invasion by *A. m. capensis* workers may successfully occur, when *A. m. scutellata* colonies are smoked during hive inspections (Tribe 1983) and colonies which were frequently inspected seem to show the signs of the "dwindling colony" syndrome faster than other colonies (A Schehle, personal communications). Moreover, the splitting of colonies, moving brood frames from one hive into another, transport and increasing the number of colonies for pollination purposes obviously facilitate the transmission of *A. m. capensis* workers. The dramatic differences of scale are reflected in the reports of Johannsmeier (1983) for small-scale apiaries and of Steinhobel (1977) for large-scale beekeeping by orders of magnitude. Whereas only 8 *A. m. capensis* colonies from Cape town were moved into an *A. m. scutellata* apiary with 40-50 colonies (Johannsmeier 1983), migratory beekeepers moved and opened at least 2000 colonies for making increase during pollination services (Steinhobel 1977).

We conclude that transmission of social parasitic Cape honeybee workers may occur via drifting and dispersing at the individual level and via absconding and colony mergers at the colony level. Very few data are currently available to evaluate the relative importance of these different transmission pathways for the actual spread of Cape honeybees among *A. m. scutellata* colonies. However, beekeeping activities seem to be crucial for transmission at least in case of the highly virulent *A. m. capensis* pseudo clone because mainly large scale beekeepers are affected by the *capensis* calamity. Infestation data for the wild population are urgently needed to evaluate the efficiency of long transmission of the social parasite besides migratory beekeeping.

## 4. VIRULENCE OF CAPE HONEYBEE LAYING WORKERS

It is well known, that when *A. m. capensis* workers are placed in queenright and or queenless *A. m. scutellata* colonies they readily adopt pseudoqueen status (induce retinue behaviour etc., Crewe and Velthuis 1980, Tribe 1983, Wossler 1991) which is related to the pheromonal status of such workers (Wossler 2002, this issue). Thus, *A. m. capensis* workers are apparently not reproductively regulated by the *A. m. scutellata* queen or by the host brood (Allsopp and Crewe 1993, see Wossler 2002 for a review). But which behavioural factors govern the virulence of *A. m. capensis* workers and consequently the susceptibility and the dwindling of host colonies?

We will now give a brief outline of the events following the successful invasion of an *A. m. capensis* worker into a host colonies and which are associated with virulence (see above for a definition) of the social parasites and the dwindling host colony syndrome (Fig. 2). When *A. m. capensis* workers have successfully invaded colonies either via drifting, dispersing, swarm mergers, joining of reproductive swarms or simply stayed in their host colonies they have to (further) develop their ovaries and establish themselves as pseudoqueens in the host colonies (Fig. 2). Such workers may or may not already show some pre-host development (see above). Due to the high variability for traits related to worker reproduction in *A. m. capensis* (Hepburn 1994), virulent laying workers may show two different phenotypes: high ovarial development and no queenlike pheromonal bouquet and a fully developed pseudoqueen phenotype with both high ovarial development and a queenlike pheromonal bouquet.

Pseudoqueen establishment (step 1 in Fig. 2) is based on a series of behavioural and pheromonal interactions between the host (queen and workers), invaded workers and their parasitic offspring. Inside of the host colony, *A. m. capensis* workers tend to avoid the host queen (step 2 in Fig. 2, Moritz et al. 2001a,b, 2002) and are prone to gain trophallactic dominance over host workers (step 3 in Fig. 2, Velthuis et al. 1990). These two mechanisms predispose the invaded workers to (further) develop their ovaries. However, workers with developed ovaries are attacked by other workers (Velthuis 1976, Visscher and Dukas 1995) and also *A. m. capensis* workers are attacked by host workers inside of the colony (Allsopp 1995). Thus, workers have to evade worker-worker aggression to successfully establish themselves as pseudoqueens (step 4 in Fig. 2, Tribe 1981, Allsopp 1995). *A. m. scutellata* host workers counter pseudoqueen establishment by aggressive behaviour towards *A. m. capensis* workers (step 5 in Fig. 2, Allsopp 1995, Magnuson 1995); but they also promote pseudoqueen establishment by being trophallactically subordinate (step 6 in Fig. 2, Velthuis et al. 1990). The host queen tends to pheromonally suppress the development of invaded *A. m. capensis* workers (Wossler 2002, this issue) and enhances the removal of worker-laid eggs, because egg removal eventually vanishes in queenless honeybee colonies (Miller and Ratnieks 2001).

Those workers surviving worker-worker aggression have successfully established themselves as parasitic pseudoqueens but have to evade the removal of worker-laid eggs in queenright host colonies (step 8 in Fig. 2) by preferentially laying eggs with low removal rates (Martin et al. 2002a) in parts of the hive where the removal of worker-laid eggs seems to be reduced (step 7 in Fig. 2, Neumann, Pirk, Hepburn and Moritz, unpublished data). Host workers promote this by removing fewer *A. m. capensis* worker-laid eggs above the queen excluder (step 9 in Fig. 2, Neumann, Pirk, Hepburn and Moritz, unpublished data).

The resulting parasitic offspring is preferentially fed by the host workers (step 10 in Fig. 2, Beekman et al. 2000, Calis et al. 2002, this issue) leading to highly developed progeny. Thus, parasitic offspring can be expected to be predisposed for further reproduction (step 11 in Fig. 2, Calis et al. 2002, this issue). This preferential feeding by the host workers (step 10 in Fig. 2) may also be responsible for the more “queen-like” behaviour of later generations of parasitic offspring compared to workers which have initially invaded the host (step 12 in Fig. 2, Magnuson 1995); especially when the host queen is lost (step 13 in Fig. 2, Allsopp 1995, Swart et al. 2001) and their pheromonal suppression vanishes in the course of infestation (Magnuson 1995). It is unknown which group (host workers, invaded workers or parasitic offspring) is responsible for the loss of the host queen (step 13 in Fig. 2).

Replacement queen rearing from the host queen offspring, recently after queenloss and the rearing of an *A. m. capensis* queen at later stages of infestation is pheromonally suppressed by the already established parasitic *A. m. capensis* pseudoqueens (Anderson 1968, Wossler 2002, this issue). Finally, *A. m. capensis* workers may infest new host colonies via the different transmission pathways, thereby completing the social parasitic pathway of laying *A. m. capensis* workers. In the following sections, these steps are discussed in detail.

**4.1 Pseudoqueen establishment: how to gain reproductive dominance in host colonies. (step 1 in Fig. 2):** After queen loss only a few pseudoqueens monopolize reproduction in queenless Cape honeybee colonies (Moritz et al. 1996), indicating strong competition for reproductive dominance among workers. Pheromones may play a key role in obtaining the position of a pseudoqueen since workers compete for producing the strongest pheromonal signal (Moritz et al. 2000). However, behavioural influences are also clearly shown to influence pseudoqueen status.

**4.1.1 Queen avoidance (step 2 in Fig. 2):** Worker avoidance of queen pheromonal suppression may be crucial to establish or maintain high pheromonal and ovarial development (Moritz et al. 2001a,b, 2002). Indeed, *A. m. capensis* workers tend to avoid the queen, while *A. m. scutellata* workers are more often found in their close vicinity (Moritz et al. 2001a). Workers with a queen-like pheromonal bouquet avoid the queen more than workers with a more worker-like pheromonal bouquet, which should reduce the suppressing effect on ovary development (Moritz et al. 2002). It is unclear whether the avoidance behaviour causes the more queen-like pheromonal bouquet or whether the queen-like pheromonal bouquet is associated with avoidance behaviourally. Moreover, there seems to be a spatial separation of pseudoqueens in queenless Cape honeybee colonies (Lattorff et al. 2001). Thus, workers may also avoid other highly developed workers to gain or maintain reproductive dominance. Alternatively, but not mutually exclusive such a spatial distance between pseudoqueens is needed to allow for more than one of such workers with a queen-like pheromonal bouquet. Since invading workers join a foreign host colony, the new colony environment may predispose them for gaining reproductive dominance. Indeed, even drifted workers participate less in hive duties (*A. m. carnica*: Pfeiffer and Crailsheim 1998, *A. m. capensis*: Neumann, Hepburn and Radloff, unpublished data) and tend to avoid the queen more than native control workers from the same age cohort (Neumann, Hepburn and Radloff, unpublished data). Thus, it appears as if foreign workers exhibit queen avoidance behaviour more readily, which probably predisposes them for gaining reproductive dominance in their host colonies.

**4.1.2 Trophallactic dominance (step 3 in Fig. 2):** It is well established that workers of *A. m. capensis* are prone to gain trophallactic dominance when caged with workers of other subspecies (Velthuis et al. 1990). Unless *A. m. capensis* workers do not feed themselves from pollen stores, which is unknown, this appears to be essential for ovary activation.

**4.1.3 Avoidance of worker-worker aggression (step 4 in Fig. 2)**: Honeybee workers with developed ovaries are attacked by other workers (Velthuis 1976, Visscher and Dukas 1995). Severe fighting occurs in some Cape honeybee colonies after queenloss (Stuart-Findlay 1953, Lundie 1954, Anderson 1963, 1968, 1977, Tribe 1981). Dead *A. m. capensis* workers can be found in large numbers in front of *A. m. capensis* colonies which recently lost their queens (Anderson 1977; up to one-third of the colony, Tribe and Allsopp 2001a,b). Also, *A. m. scutellata* workers in infested host colonies of *A. m. scutellata* show aggressive behaviour towards invaded *A. m. capensis* workers (Allsopp 1995). Indeed, severe fighting can be observed at the hive entrance of infested colonies (Swart et al. 2001). Thus, dead workers can be frequently found in front of infested *A. m. scutellata* colonies and can be used diagnostically to identify infestations with *A. m. capensis* laying workers at early stages (Allsopp 1995).

Clearly, only those workers surviving worker-worker aggression can successfully infest and reproduce in host colonies. Rapid development of a queenlike pheromonal bouquet (Simon et al. 2001) as indicated by observations of Tribe (1981) is one probable mechanism. When workers approach an *A. m. capensis* worker with a queenlike pheromonal bouquet, they may back away from her as much as from a queen (Tribe 1981). Alternatively, but not mutually exclusive, social parasitic workers may show behavioural traits to avoid aggressive encounters with host workers, such as hiding in small cracks in the nest (Tribe, personal communication). However, not a single study has been published in this regard. Interestingly, aggressive interactions among *A. m. capensis* laying workers have not been reported and laying *A. m. capensis* queens and laying pseudoqueens can be seen side by side (Tribe 1981). In contrast to *A. m. scutellata* workers, a high proportion of *A. m. capensis* pseudo clone workers survived which were artificially introduced into host colonies (50% Martin et al. 2002b). This shows good abilities to survive worker-worker aggression in host colonies and further indicates a high virulence of this particular strain.

Alternatively, *A. m. capensis* workers which have already a queen-like pheromonal bouquet and/or high ovarial development may enter host colonies (see above). This seems plausible in light of the fast ovarial and pheromonal development of *A. m. capensis* workers (Simon et al. 2001). In this case, trophallactic dominance can be easily established and worker-worker aggression inside of the colony easily avoided. Because almost all combinations of reproductive status are possible in *A. m. capensis* workers (Hepburn 1994), such workers with developed pheromones may have developed ovaries or not. In case of invading workers with already developed pheromones and ovaries, all steps leading to reproductive dominance can be omitted. It is likely, that such workers can immediately start egg laying within the host colonies. Initially most invaded *A. m. capensis* workers surviving worker-worker aggression should be able to develop the full pseudoqueen phenotype due to the inability of the *A. m. scutellata* queen and the host brood to suppress their development (Wossler 2002). At later stages it is likely that not all workers can develop into pseudoqueens because they are suppressed pheromonally by the already established *A. m. capensis* pseudoqueens.

**4.2 Evasion of worker policing (step 7 in Fig. 2)**

Eggs laid by workers are removed by other workers in queenright and in recently queenless colonies of European honeybees (worker policing, Ratnieks and Visscher 1989, Miller and Ratnieks 2001). Because both *A. m. capensis* and *A m. scutellata* are also able to police worker-laid eggs of their own and of the other subspecies (Neumann, Pirk and Ratnieks, unpublished data), policing of worker-laid eggs constitutes a host resistance mechanism and consequently parasitic *A. m. capensis* workers have to evade worker policing in queenless and queenright host colonies to successfully reproduce. Thus, the question emerges how *A. m. capensis* workers are able to evade worker policing.

Worker policing in the Cape honeybee is reduced during unfavourable weather conditions (Pirk et al. 2002, this issue, CWW Pirk unpublished data). So, one potential tactic of *A. m. capensis* laying workers, to achieve successful reproduction despite the presence of the host queen, could be to lay eggs when worker policing is reduced (Pirk et al. 2002). However, egg-removal and worker egg-laying are positively correlated in queenright Cape honeybee colonies, strongly indicating that laying workers do not use such a tactic (Pirk et al. 2002, this issue).

One mechanism seems to actually involve evasion of the queen and the brood nest of the host colonies by laying *A. m. capensis* workers (Neumann, Pirk, Hepburn and Moritz, unpublished data). Workers of *A. m. capensis* preferentially lay eggs above the queen excluder in queenright colonies, where worker policing is reduced (Neumann, Pirk, Hepburn and Moritz, unpublished data). Frequent field observations strongly indicate that this is expressed at the level of the host colony's phenotype as parasitic worker brood initially appearing at the extreme outside frames (Lundie 1954, Allsopp 1995, Martin et al. 2002b), followed by parasitic worker brood frames close to the actual brood nest of the host queen and finally flanking the brood frames of the host queen (Allsopp 1995). The eggs laid by pseudoqueens can escape policing and young nurse workers of the host colony are attracted by brood pheromones to the hatching larvae.

The second mechanism seems to be the removal rates of eggs laid by parasitic workers. Eggs laid by highly virulent parasitic workers of the *A. m. capensis* pseudo clone (Kryger 2001a,b, Kryger et al. 2002), which are invading *A. m. scutellata* colonies in the Highveld of South Africa, seem to have lower removal rates than laying workers of *A. m. scutellata* (Martin et al. 2002a) and intermediate between *A. m. capensis* queen-laid eggs and worker-laid eggs from the native range of the Cape honeybee (Port Elizabeth, Neumann et al. 2001b).

**4.3 Oviposition behaviour (step 8 in Fig. 2):** In the Cape honeybee worker egg-laying (Pirk et al. 2002, this issue) and successful worker reproduction in the presence of a queen (Pettey 1922, Moritz et al. 1999) is much more common than in European subspecies of *A. mellifera* (Ratnieks 1993, Visscher 1996). Moreover, laying worker colonies of *A. m. capensis* can persist for long periods without raising a new queen (up to 8 months, Hepburn, personal observations). These observations indicate that *A. m. capensis* laying workers may show specific adaptations for how many eggs are laid per cell, how and where eggs are laid in an individual brood cell and in which brood cell type workers preferentially oviposit (drone or worker cells), because inappropriate oviposition would cause drastic losses in the colony worker population within a short period of time causing a shorter life span of *A. m. capensis* laying worker colonies.

Indeed, thelytokous *A. m. capensis* workers preferentially lay eggs in worker cells (Neumann et al. 2000a), which have the appropriate cell size for rearing female offspring. Moreover, it is often very difficult to distinguish the brood nest of *A. m. capensis* pseudoqueens from that of a laying queen, because a single egg is laid at the bottom of the cell in a regular pattern (Tribe 1981, Tribe and Allsopp 2001b, personal observations). Such provisioning behaviour (in terms of number of eggs laid and how and where they are laid) might considerably enhance the longevity of queenless *A. m. capensis* colonies and the reproductive output of individual parasitic workers in the initial phase of infestation because only one larva per cell can be reared to adulthood and surplus offspring in one cell is cannibalised. Indeed, during usurpation of *A. m. scutellata* colonies by *A. m. capensis* pseudo clone workers, single worker-laid eggs appeared in worker cells (Martin et al. 2002b). This is in contrast to laying workers of other *A. mellifera* subspecies, which lay multiple eggs per cell (Hastings 1989; up to several dozens eggs in one cell in *A. m. scutellata*, personal observations) and may accidentally kill or even deliberately remove previously laid eggs.

**4.4 Rearing of parasitic offspring (Fig. 2):** The resulting parasitic offspring is preferentially fed by host colony workers of other *A. mellifera* subspecies (step 10 in Fig. 2, European *A. mellifera*: Beekman et al. 2000; *A. m. scutellata*: Calis et al. 2002, this issue). This results in highly developed workers (Calis et al. 2002, this issue), which can be expected to be highly virulent and predisposed for pseudoqueen establishment in the host (step 11 in Fig. 2). Thus, although such a preferential feeding may not be really needed for the social parasitic cycle to work it appears to clearly promote the dwindling of host colonies. The queenstate of the host colony may also play a role in this regard, because *A. m. capensis* workers reared in colonies headed by laying workers are bigger and have more ovarioles than those reared in a queenright colony (Woyke 1979).

However, in queenright (Hepburn et al. 1991) and queenless (Tribe 1981) *A. m. capensis* colonies only a few worker can actually develop the pseudoqueen phenotype, which might indicate that not all of these workers will establish themselves as pseudoqueens in the host colony. Although parasitic brood is preferentially fed by the host workers (Beekman et al. 2000, Calis et al. 2002), this picture might change in the course of infestation because the reduction in foraging causes a decrease in stored food. Moreover, fewer host workers have to nurse more and more hatching parasitic larvae. Finally, with the passage of time the size of the nursing cohort with developed hypopharyngeal glands quickly diminishes as well. Thus, the relative low ovarial development of *A. m. capensis* pseudo clone workers (Martin et al. 2002b) might not only be caused by pheromonal suppression of already established parasitic pseudoqueens but also a result of less efficient nutrition at later stages of infestation. Nevertheless, high numbers of adult *A. m. capensis* workers can be reared in an *A. m. scutellata* host colony (~3,000-10,000 Martin et al. 2002b).

Laying worker colonies of *A. m. capensis* in the fynbos region occasionally re-queen from laying worker offspring (c. 7% of all colonies, Allsopp and Hepburn 1997). However, this has not been reported yet from the colonies of *A. m. scutellata* which have been infested with *A. m. capensis* laying workers (Swart et al. 2001, Martin et al. 2002b). Indeed, recent genetic data also strongly indicates that there is no *A. m. capensis* queen rearing in infested colonies (Kryger et al. 2002). Such a lack of queen rearing in infested colonies (both of a new host queen recently after queenloss and of an *A. m. capensis* queen) is probably due to the pheromonal suppression by established *A. m. capensis* pseudoqueens (Anderson 1968).

**4.5 Behavioural changes in the course of infestation (step 12 in Fig. 2):** Field observations indicate, that there might be considerable differences between generations of parasitic workers reared in host colonies with respect to behaviour (Magnuson 1995). For example, in contrast to the *A. m. scutellata* host workers behaviourally "queen-like" parasitic workers of *A. m. capensis* do not fly up but rather stay on the combs when infested colonies are inspected (Magnuson 1995). Moreover, it has been reported that the pseudo clone bees are not capable of caring for themselves, because they are too queen-like in behaviour (Kryger 2001b). Especially after the loss of the host queen, when pheromonal suppression vanishes and parasitic offspring is reared in great numbers in the absence of the host queen, such "queen-like" behaviour may be even more readily expressed (Magnuson 1995). Likewise, oviposition behaviour changes in the course of infestation. Initially single eggs are laid per cell by parasitic workers (Martin et al. 2002b) but at later stages of infestation multiple eggs can be regularly found per cell (Allsopp 1995, Martin et al. 2002b).

There is severe competition for reproductive dominance among *A. m. capensis* workers (Moritz et al. 1996, Moritz et al. 2000), resulting in just a few pseudoqueens monopolising reproduction in a laying worker colony of *A. m. capensis* (c. 14 in a colony of 2000 bees, Tribe 1981). The pheromonal bouquet of pseudoqueens is apparently able to suppress queen rearing and development in other workers (Hepburn et al. 1988) and may also influence the behaviour of other parasitic workers. Therefore, it is likely that the traits "queen-like" behaviour and high ovarial and pheromonal development are not expressed by all *A. m. capensis* workers in a host colony at late stages of infestation (Hepburn and Allsopp 1994), even if they are all genetically predisposed for such a development (Moritz and Hillesheim 1985). This may be particularly interesting with respect to transmission to new hosts, assuming that workers with developed ovaries have fewer chances to invade host colonies (Reece 2002) and taking into account that pseudoqueens are unlikely to leave the hive (Tribe 1981). However this must be further investigated. For example, few *A. m. capensis* workers may even participate in foraging at later stages of infestation (Martin et al. 2002b), which is not expected from reproductive dominant workers.

**4.6 Loss of the host queen (step 13 in Fig. 2):** The host queen seems to play an important role in the context of social parasitism by laying workers (Tribe 1983, Woyke 1995, Neumann et al. 2001). However, given the current state of evidence, it is simply unclear what factors and which of the various groups in an infested colony (invaded *A. m. capensis* workers, *A. m. capensis* laying worker offspring reared in the host, *A. m. scutellata* host workers) actually cause the loss of the host queen and whether agonistic encounters are involved or not. Because, queen pheromone production in honeybees is associated with egg-laying (e.g. laying queens produce a stronger queen signal than virgin queens, Wossler 2002, this issue, and queens approaching supersedure, Allsopp and Hepburn 1997) a potential reduction in queen pheromone production resulting from reduced egg-laying by the host queen in the course of infestation may be relevant. This seems plausible in light of the massive egg laying by the parasitic *A. m. capensis* workers, simply resulting in less available space in the brood nest (see above Allsopp 1995). Alternatively, but not mutually exclusive a reduced nutrition of the host queen may also play a role (Kryger 2001). Finally the rapid lost of the host queen in some cases (Martin et al. 2002) indicates that immediate agonistic interactions should also be considered. However, not a single study has addressed this question to date.

We conclude that the spatial distribution of *A. m. capensis* workers in host colonies, their readiness to gain trophallactic dominance, the high chance of surviving worker-worker aggression and their oviposition behaviour are essential behavioural aspects for the virulence of social parasitic workers.

## 5. RESISTANCE AND SUSCEPITIBILITY OF HOST COLONIES

Queenless and queenright colonies of *A. m. scutellata* are highly susceptible hosts for invasion by Cape honeybees (Hepburn and Allsopp 1994). Likewise, queenless colonies of European subspecies are also susceptible (Woyke 1995). In general, queenless colonies are more prone to invasion by laying workers than queenright colonies (Tribe 1983, Woyke 1995), e.g. *A. m. scutellata* colonies may be successfully taken over when the virgin *A. m. scutellata* host queen departs on a mating flight or during swarming, when only sealed queen cells are left in a colony (Tribe 1983). In an apiary, where both *A. m. mellifera* and *A. m. capensis* were kept, problems occurred with queenless *A. m. mellifera* colonies rearing queens because *A. m. capensis* workers invaded such colonies, and the queen cells were destroyed (J. Woyke personal communication). However, it also happened that *A. m. mellifera* queens disappeared and laying *A. m. capensis* workers took over the colonies (J. Woyke personal communication). Nevertheless, queenright colonies of other subspecies than *A. m. scutellata* seem to be somehow resistant (*A. m. ligustica*, *A. m. carnica*, *A. m. caucasica* and *A. m. mellifera*, Woyke 1995). Moreover, the "dwindling colony" syndrome due to laying workers has never been reported from *A. m. capensis* colonies and there are reports that natural hybrid colonies between *A. m. capensis* and *A. m. scutellata* are somewhat resistant to *A. m. capensis* infestations (Greeff 1997; but see Reece 2002).

These observations strongly indicate that queenstate and race are important factors to explain the resistance of colonies towards infestations with *A. m. capensis* laying workers. So, why are *A. m. scutellata* colonies highly susceptible hosts, whereas queenright colonies of other subspecies seem to be somehow resistant and why was the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* stable without human intervention?

**5.1 Susceptibility of *A. m. scutellata* host colonies:** If *A. m. capensis* workers are not able to break into the fortress (Schmid-Hempel 1998) infestations cannot be initiated. Thus, efficient guarding by host colonies might constitute an important behavioural resistance mechanism in the context of social parasitism by *A. m. capensis* laying workers. However, although *A. m. scutellata* colonies may scrutinise incoming foreign workers more carefully than do *A. m. capensis* colonies, because *A. m. scutellata* colonies hosted fewer drifted and dispersed workers than did *A. m. capensis* colonies (Neumann et al. 2001c) nonetheless they are readily taken over by laying *A. m. capensis* workers (Hepburn and Allsopp 1994). This clearly indicates that efficient guarding behaviour is not a particular important aspect for host colony resistance.

Although the basic pattern of worker policing (worker-laid eggs are removed faster than queen-laid eggs) can be observed in queenright *A. m. scutellata* and *A. m. capensis* colonies, the actual number of remaining worker-laid eggs after 24 hours is much higher compared to European subspecies (~20% Neumann, Pirk and Ratnieks, unpublished data). This suggests that either policing is reduced and/or workers lay eggs which have lower removal rates compared to European subspecies. Given policing in *A. m. scutellata* is truly reduced, this might explain why queenright *A. m. scutellata* colonies may be more susceptible towards infestations by *A. m. capensis* laying workers compared to queenright colonies of European subspecies. Unfortunately, the relative importance of queenstate of *A. m. scutellata* host colonies and worker policing efficiency compared to queenright European host colonies for the success rate of *A. m. capensis* parasitic workers has not yet been investigated quantitatively.

The rejection of developed workers in queenless colonies seems to be more readily expressed in *A. m. capensis* than in *A. m. scutellata* (Anderson 1977). Clearly, this further contributes to our understanding of the susceptibility of *A. m. scutellata* host colonies to infestations by *A. m. capensis* laying workers because the more developed workers are rejected the fewer remain to successfully reproduce.

The high incidents of successful infestations of queenright *A. m. scutellata* colonies by social parasitic *A. m. capensis* workers (Allsopp and Crewe 1993) clearly indicate that *A. m. scutellata* queens are not able to suppress the development of invaded *A. m. capensis* workers. Thus, it is very likely that race specific ratios of compounds in the pheromonal bouquet of queens (see Wossler 2002 for a review on this particular issue) may play a key role in determining whether queens can suppress *A. m. capensis* laying workers or not. For example, virgin queens of *A. m. scutellata* were killed by *A. m. capensis* workers when kept in transport cages (Buys 1984) indicating that the workers did not accept the queens.

**5.2 Stability of the natural hybrid zone between *A. m. capensis* and *A. m. scutellata*:** Worker reproduction is an important feature in the natural occurring hybrid zone between *A. m. capensis* and *A. m. scutellata* (Moritz et al. 1998). Although there is a morphometrically clearly defined zone of natural hybrid colonies, thelytoky has introgressed into the region (Hepburn and Radloff 2002). Since the hybrid zone appeared to be stable, one could expect hybrid colonies to have behavioural features, explaining their resistance and/or the stability of the natural hybrid zone. Indeed, the natural hybrids do not behave in an intermediate matter, but instead exhibit unique behavioural characteristics at worker and colony levels which are highly suggestive of a buffering capacity in the hybrid zone (Hepburn and Radloff 1998, Neumann et al. 2001c).

First, hybrid workers disperse less often than either *A. m. capensis* or *A. m. scutellata* (Neumann et al. 2001c). Given that dispersing represents a host finding mechanism for social parasitic laying workers outside of apiaries, clearly fewer hybrid workers spread than do workers of *A. m. capensis*. However, why do the hybrids lack this behaviour? One possible explanation might be the general clinal structure of the hybrid zone in which characteristics of *A. m. capensis* are gradually replaced by those of *A. m. scutellata* (cf. Hepburn and Radloff 1998, Hepburn and Radloff 2002). As a result, hybrid colonies may simultaneously consist of both arrhenotokous and thelytokous laying workers (Pettey 1922; Moritz et al. 1999, Neumann et al. 2000a). Since the population density in the drier parts of the hybrid zone is sparse and much lower than in *A. m. capensis* populations (Hepburn et al. 1994), the chance of successful transmission may be low. Moreover, thelytokous laying workers are more likely to become reproductively dominant in queenless hybrid colonies than arrhenotokous ones (Neumann et al. 2000a). Thus, the low dispersal frequency of the natural occurring hybrids may reflect a trade-off for thelytokous laying workers between a low chance of successful transmission into a host colony against a high chance of successful reproduction in the mother colony after queenloss.

Second, different hosting mechanisms of dispersed workers by hybrid colonies may also play a role (but see above). In contrast to *A. m. capensis*, hybrid colonies host proportionally more drifters than dispersers (Neumann et al. 2001c). Moreover, queenless hybrid colonies host significantly fewer drifters than their queenright counterparts; and the former also host significantly fewer dispersers than queenright or queenless *A. m. capensis* colonies (Neumann et al. 2001c). If fewer dispersers were accepted by hybrid host colonies, especially by queenless ones, the chance of their usurpation should be smaller (Hepburn and Allsopp 1994). However, against this the hybrid colonies appeared unable to suppress post-drifting development of drifted *A. m. capensis* workers (Reece 2002), suggesting that they are not resistant with respect to infestations by laying *A. m. capensis* workers.

We conclude that a combination of worker policing inefficiency, less efficient rejection of developed workers in recently queenless colonies and queen inability to suppress worker development governs the susceptibility of *A. m. scutellata* host colonies to social parasitic *A. m. capensis* workers. In particular, race specific abilities of queen and or brood pheromones seem to play the key role to suppress the development and establishment of *A. m. capensis* workers in host colonies (Wossler 2002). However, colonies of other subspecies may have not yet encountered highly virulent strains of social parasitic *A. m. capensis* workers, such as the pseudo clone invading the Northern regions of South Africa. Thus, it remains to be tested whether other subspecies than *A. m. scutellata* are resistant against that particular strain or not. The lack of dispersal in the hybrids (Neumann et al. 2001c) and the very low population density in the hybrid zone (Hepburn et al. 1994) might have been responsible for the apparent stability of the natural occurring hybrid zone between *A. m. capensis* and *A. m. scutellata* before the *capensis* calamity occurred.

## 6. GENERAL CONCLUSIONS

*A. m. capensis* shows several behavioural traits favouring both transmission (e.g. dispersing behaviour, ready absconding of queenless units, frequent mergers) and virulence (e.g. queen avoidance, trophallactic dominance, oviposition behaviour, evasion of worker policing, "queen-like" behaviour) of social parasitic workers. However, while some behavioural aspects are well understood (e.g. trophallactic dominance), other factors associated with social parasitism by *A. m. capensis* laying workers are still unclear (e.g. loss of the host queen). Thus, although the *A. m. capensis* calamity occurred 10 years ago (Allsopp 1992, Johannsmeier 1992, Lear 1992) the behavioural aspects still need deeper investigation.

Here we suggest the reproductive cycle for social parasitism by laying Cape honeybee workers. *A. m. capensis* workers with undeveloped ovaries are predisposed to initially infest a potential host colony and to successfully establish themselves as pseudoqueens in host colonies when the host queen is still alive. Then, the first generation of highly developed parasitic workers emerges without the suppression of an *A. m. capensis* queen and/or pseudoqueens. This effect is probably amplified when the host queen dies. These workers are predisposed for pseudoqueen establishment (because they have better developed ovaries, Calis et al. 2002, this issue) and can take over a host colony until the host colony's queen dies. The high frequency of parasitic pseudoqueens in the host at later stages of infestation suppresses queen rearing and the development and gaining pseudoqueen status of newly hatching *A. m. capensis* workers. Such undeveloped *A. m. capensis* workers are more likely to successfully infest new host colonies via the individual or the colonial transmission pathways.

A recent model (Moritz 2002) indicates that infestations are likely to be fatal for apiary populations irrespective of beekeeping activities compensating for colony losses due to parasitation. Wild *A. m. scutellata* populations are however less likely to be affected by parasitic laying workers and stable equilibria between host and parasite occur over a wide range of the parameter space (Moritz 2002). Although it is unlikely that the parasitic pseudo clone represents a threat to the conservation of biodiversity, even low frequencies of parasitic *A. m. capensis* workers in wild honeybee population can cause a permanent threat to beekeeping activities (Moritz 2002).

It remains open whether social parasitism by laying workers is purely an artefact of beekeeping activity or a regular part of the life history of the Cape honeybee. Due to the high virulence of the *A. m. capensis* pseudo clone host colonies die quickly (Martin et al. 2002b). However, the transmission capacity appears to be reduced (Neumann et al. 2001b) and the problem is mainly restricted to large scale beekeepers. Thus, it appears as if the *capensis* calamity is probably maintained by large scale beekeeping. We conclude that more studies are needed to evaluate whether social parasitism by laying *A. m. capensis* workers is a peculiar sideline of reproduction reflecting a man-made problem or a general / regular lifestyle of the Cape honeybee.

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## REFERENCES

Allsopp M.H. (1992) The *capensis* calamity, S. Afr. Bee. J. 64, 52-55.

Allsopp M.H. (1993) Summarized overview of the *capensis* problem, S. Afr. Bee J., 65 127-136.

Allsopp M.H. (1995) The *Capensis* problem 1992-1995, in: (Magnuson P. (Ed.), Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, pp. 10-31.

Allsopp M.H. (1998) Capensis remnants do abscond, S. Afr. Bee J. 70, 156-160.

Allsopp M.H., Crewe R. (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan, Am. Bee J. 133, 121-123.

Allsopp M.H., Hepburn H.R. (1997) Swarming, supersedure and the mating system of a natural population of honeybees (*Apis mellifera capensis*), J. Apic. Res. 36, 41-48.

Anderson R.H. (1963) The laying worker in the Cape honeybee, *Apis mellifera capensis*, J. Apic. Res. 2, 85-92.

Anderson R.H. (1965) The island breeding station, S. Afr. Bee J. 37, 9-12.

Anderson R.H. (1968) The effect of queen loss on colonies of *Apis mellifera capensis*, S. Afr. J. Agric. Sci. 11, 383-388.

Anderson R.H. (1977) Some aspects of the biology of the Cape honeybee, in: Fletcher D.J.C. (Ed.), Proceedings of the Apimondia International Symposium on the Taxonomy, Biology and Economic Use of African Bees, Pretoria, pp. 107-114.

Beekman M., Calis J.N.M., Boot W.J. (2000) Parasitic honeybees get royal treatment, Nature 404, 723.

Buys B. (1984) Cape worker-bees dislike African virgin queens, S. Afr. Bee J. 54, 86-90.

Calis J.N.M., Boot W.J., Allsopp M.H., Beekman M. (2002) Getting more than a fair share: Nutrition of worker larvae related to social parasitism in the Cape honey bee, *Apis mellifera capensis*, Apidologie 33, in press.

Crewe R.M., Velthuis H.H.W. (1980) False queens: a consequence of mandibular gland signals in worker honeybees, Naturwissenschaften 67, 467-469.

Crewe R.M., Wossler T.C., Allsopp M.H. (1990) Workers in queen's clothing: why capensis workers become pseudoqueens, in: Anderson R.H., Buys B. (Eds.) Bees and Beekeeping in Southern Africa, Apimondia, Stellenbosch, pp. 83-89.

Crozier R.H., Pamilo P. (1996) Evolution of social insect colonies. Oxford University Press, Oxford.

Free J.B. (1958) The drifting of honeybees, J. Agric. Sci. 51, 294-306.

Greeff M.J. (1996) Effects of thelytokous worker reproduction on kin-selection and conflict in the Cape honeybee, *Apis mellifera capensis*, Phil. Trans. R. Soc. Lon. [B] 351, 617-625.

Greeff M.J. (1997) The Cape honeybee and her way north: an evolutionary perspective, S. Afr. J. Sci. 93, 306-308.

Guy R.D. (1975) General Moodie and the African Bee move south, S. Afr. Bee J. 47, 4-5.

Hannabus C.H. (1945) District notes. Paternoster, C.P, S. Afr. Bee J. 20, 16.

Hastings H. (1989) Two aspects of the biology of an African honeybee *Apis mellifera scutellata* (Hymenoptera, Apidae) laying workers and colony defense behaviour, PhD thesis, University of Natal, Pietermaritzburg, South Africa.

Hepburn H.R. (1988) Absconding in the African honeybee: the queen, engorgement and wax secretion, J. Apic. Res. 27, 95-102.

Hepburn H.R. (1992) Pheromonal and ovarial development co-vary in Cape worker honeybees, *Apis mellifera capensis*, Naturwissenschaften 79, 523-524.

Hepburn H.R. (1993) Swarming, absconding and migration in southern African bees, S. Afr. Bee J. 65, 61-66.

Hepburn H.R. (1994) Reproductive cycling and hierarchical competition in Cape honeybees, *Apis mellifera capensis* Esch., Apidologie 25, 38-48.

Hepburn H.R., Nefdt R.J.C., Whiffler L.A. (1988) Queen loss in the Cape honeybee: the interactions of brood, laying workers (false queens?) and queen cells, S. Afr. J. Sci. 80, 363-368.

Hepburn H.R., Whiffler L.A. (1988) Dwarfs and queenballs in swarms of the Cape honeybee. S. Afr J. Sci. 84, 851-852.

Hepburn H.R., Crewe R.M. (1991) Portrait of the Cape honeybee, *Apis mellifera capensis*, Apidologie 22, 567-580.

Hepburn H.R., Jacot-Guillarmod A. (1991) The Cape honeybee and the fynbos biome, S. Afr. J. Sci. 87, 70-73.

Hepburn H.R., Magnuson P., Herbert L., Whiffler L.A. (1991) The development of laying workers in field colonies of the Cape honeybee, J Apic Res 30, 13-16.

Hepburn H.R., Villet M.H., Jones G.E., Carter A.R., Simon U.I., Coetzer W. (1993) Winter absconding as a dispersal mechanism of the Cape honeybee, S. Afr. J. Sci. 89, 294-297.

Hepburn H.R., Allsopp M.H. (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*, S Afr J Sci 90, 247-249.

Hepburn H.R., Jones G.E., Kirby R. (1994) Introgression between *Apis mellifera capensis* Escholtz and *Apis mellifera scutellata* Lepeletier: the sting pheromones, Apidologie 25, 557-565.

Hepburn H.R., Radloff S.E., Honeybees of Africa. Springer Verlag, Berlin, 1998.

Hepburn H.R., Reece S., Neumann P., Moritz R.F.A., Radloff S.E. (1999) Absconding in honeybees (*Apis mellifera*) in relation to queenstate and mode of worker reproduction, Insectes Soc. 46, 323-326.

Hepburn H.R., Radloff S.E. (2002) *Apis mellifera capensis* (Eschscholtz): an essay on the subspecific classification of honeybees. Apidologie 33, in press.

Herman R.H. (1922) Monster swarm. S. Afr. Bee J. 2, 25.

Hillesheim E., Koeniger N., Moritz R.F.A. (1989) Colony performance in honeybees (*Apis mellifera capensis*

Esch.) depends on the proportion of subordinate and dominant workers, Behav. Ecol. Sociobiol. 24, 291-296.

Hölldobler B., Wilson E.O. (1990) The ants, Berlin, Springer-Verlag.

Jay S.C. (1966a) Drifting of honeybees in commercial apiaries. II. Effect of various factors when colonies are arranged in rows, J Apic Res 5, 103-112.

Jay S.C. (1966b) Drifting of honeybees in commercial apiaries. III. Effect of apiary layout. J. Apic. Res. 5, 137-148.

Jay S.C. (1968) Drifting of honeybees in commercial apiaries. IV. Further studies on the effect of apiary layout, J. Apic. Res. 7, 37-44.

Johannsmeier M.F. (1983) Experiences with the Cape bee in the Transvaal, S. Afric. Bee J. 55, 130-138.

Johannsmeier M.F. (1992) The Cape bee: a problem in the Transvaal. Plant Protection Research Institute, Pretoria, Leaflet.

Kigatiira K.I. (1988) Amalgamation in tropical honey bees, in: Needham G.R., Page R.E., Delfinado-Baker M., Bowman C.E., (Eds.) Africanized Honey Bees and Bee Mites, Ellis Horwood, Chichester, UK, pp. 62-71.

Koeniger N., Wurkner W. (1992) Die Kap Honigbienen (*Apis mellifera capensis*) Natürliche Verbreitung und die Schwierigkeiten der Haltung unter unseren klimatischen Bedingungen, Die Biene 128, 583-588.

Kryger P. (2001a) The Capensis pseudo-clone, a social parasite of African honey bees, in: Proceedings of the 2001 Berlin Meeting of the European Section of IUSSI, Sept. 25-29, p. 208.

Kryger P. (2001b) The pseudo-clone of *Apis mellifera capensis* – an obligate social parasite in honeybees, in: Proceedings of the XXXVII International Apicultural Congress, Durban South Africa, p. 33.

Kryger P., Solignac M., Lubbe A. (2002) The first honey bee social parasite is a parthenogenetic worker. Nature, in press.

Lattorff H.M.G., Neumann P., Moritz R.F.A. (2001) Spatial separation of pseudoqueens in queenless Cape honeybee colonies (*Apis mellifera capensis* Escholtz), in: Proceedings of the IUSSI – Meeting of the European sections, Berlin, Germany, p105.

Lindauer M. (1952) Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat, Z. vergl. Physiol. 34, 299-345.

Lear E. (1992) The *capensis* problem, S. Afr. Bee J. 64, 87-89.

Lundie A. E. (1954) Laying workers produce worker bees, S, Afr, Bee J. 29, 10-11.

Martin S., Beekman M., Wossler T.C., Ratnieks F.L.W. (2002a) Self-replicating honeybees evade worker policing, Nature in press.

Martin S., Wossler T.C., Kryger P. (2002b) Usurpation of *Apis mellifera scutellata* colonies by *A. m. capensis* workers. Apidologie 33, in press.

Magnuson P. (1995) Field observations, in: (Magnuson P. (Ed.), Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, pp. 5-9.

Miller D.G., Ratnieks F.L.W. (2001) The timing of worker reproduction and breakdown of policing behaviour in queenless honey bee (*Apis mellifera* L.) societies, Insectes Soc. 48, 178–184.

Moodie G. (1983) Reasons for the survival of the Cape bee, S. Afr. Bee J. 55, 34-37.

Moore W.E. (1911) What distances do swarms travel? S. Afr. Bee J. 63, 75-76.

Moritz R.F.A., Hillesheim E. (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis*), Behav Ecol Sociobiol 17, 87-89.

Moritz R.F.A., Kryger P., Allsopp M.H. (1996) Competition for royalty in bees, Nature 384, 31.

Moritz R.F.A., Beye M., Hepburn R.H. (1998) Estimating the contribution of laying workers to population fitness in African honeybees (*Apis mellifera*) with molecular markers, Insectes Soc. 45, 277-287.

Moritz R.F.A., Kryger P., Allsopp M.H. (1999) Lack of worker policing in the Cape honeybee (*Apis mellifera capensis*). Behavior 136, 1079 - 1092.

Moritz R.F.A., Page R.E. (1999) Behavioral threshold variability: costs and benefits in insect societies, in: Detrain C.J., Pasteels J. Deneubourg J.l. (Eds.), Information processing in Social Insects. Birkhäuser, Basel, pp. 203-215.

Moritz, R.F.A., Simon U., Crewe R.M. (2000) Pheromonal contest between honeybee workers, Naturwissenschaften 87, 395-397.

Moritz R.F.A., Crewe R.M., Hepburn H.R. (2001a) The role of the queen for the spatial distribution of workers in *Apis mellifera*, Ethology 107, 1-13.

Moritz R.F.A., Crewe R., Hepburn H.R. (2001b) Clustering of related workers in the honeybee colony (*Apis mellifera* L.): adaptive process or inevitable pattern?, Apidologie 31, 223-233.

Moritz R.F.A., Crewe R.M., Hepburn H.R. (2002) Queen evasion and mandibular gland secretion of honeybee workers (*Apis mellifera* L.), Insectes Soc. in press.

Moritz R.F.A. (2002) Population dynamics of the Cape bee phenomenon: The impact of parasitic laying worker clones in apiaries and natural populations, Apidologie 33, in press.

Neumann P., van Praagh J., Moritz R.F.A., Dustmann J. (1999) Testing reliability of a potential island mating apiary using DNA-microsatellites, Apidologie 30, 257-276.

Neumann P., Hepburn H.R., Radloff S.E. (2000a) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera*) colonies, Apidologie 31, 479-486.

Neumann P., Moritz RFA, Mautz D. (2000b) Colony evaluation is not affected by the drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary, Apidologie 31, 67-79.

Neumann P., Pirk C.W.W., Hepburn H.R., Radloff S.E. (2001a) A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis*), Apidologie 32, 113-114.

Neumann P., Pirk C.W.W., Moritz R.F.A., Hepburn H.R. (2001b) Trade off between dispersal capacity and virulence in a social parasitic Cape honeybee clone (*Apis mellifera capensis* Esch.), in: Proceedings of the IUSSI – Meeting of the European sections, Berlin, Germany, p106.

Neumann P., Radloff S.E., Moritz R.F.A., Hepburn H.R., Reece S.L. (2001c) Social parasitism by honeybee workers (*Apis mellifera capensis* Escholtz): Host finding and resistance of hybrid host colonies, Behav. Ecol. 12, 419-428.

Onions G.W. (1912) South African 'fertile worker bees', Agric. J. Union S. Afr. 1, 720-728.

Pettey F.W. (1922) Workers laying in comb of extracting supers, Elsenberg Apiary, J. Dept. Agric. Union South Afr. 4, 122-124.

Pfeiffer K.J., Crailsheim K. (1998) Drifting of honeybees, Insectes Soc 45, 151-167.

Pirk C.W.W., Neumann P., Hepburn H.R. (2002) Egg removal and egg laying by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.), Apidologie 33, in press.

Radloff S.E., Hepburn H.R., Neumann P., Moritz R.F.A., Kryger P. (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis* (Esch.), Apidologie 33, in press.

Rauschmayer F. (1928) Das Verfliegen der Bienen und die optische Orientierung am Bienenstand, Archiv f. Bienenkunde 9, 249-322.

Ratnieks F.L.W. (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honey bee colonies, Behav. Ecol. Sociobiol. 32 191-198.

Ratnieks F.L.W., Visscher P.K. (1989) Worker policing in the honey bee, Nature 342, 796-797.

Reece S.L. (2002) A scientific note on the ovarial and pheromonal development of drifted and non-drifted Cape honeybee workers (*Apis mellifera capensis* Esch.), Apidologie 33, in press.

Reeve H.K. (1989) The evolution of conspecific acceptance thresholds, Am. Nat. 133 407-435.

Renz M., Rosenkranz P. (2001) Infestation dynamics and reinvasion of *Varroa destructor* mites in honey bee colonies kept isolated and in groups. Apidologie 32, 492-494.

Ribbands C.R. (1953) The behaviour and social life of honeybees, Bee Research Association Limited, London, UK,.

Ruttner F., Hesse B. (1981) Rassenspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L, Apidologie 12, 159-183.

Ruttner F. (1992) Naturgeschichte der Honigbienen. Ehrenwirth Verlag, München.

Schmid-Hempel P. (1998) Parasites in social insects, Princeton University Press, New Jersey, USA.

Seeley T.D. (1985) Honeybee Ecology, Princeton University Press, New Jersey, USA.

Silberrad R.E.M. (1976) Bee-Keeping in Zambia, Apimondia, Bucharest, Romania.

Simon U., Moritz R.F.A., Crewe R.M. (2001) The ontogenetic pattern of mandibular gland components in queenless worker honey bees (*Apis mellifera capensis* Esch.), J. Insect Physiol. 47, 735-738.

Solignac M., Kryger P., Allsopp M.H., Mougel F., Koeniger N., Baudry E., Cornuet J.M. (2001) Thelytoky in Cape honey bees: a genomic perspective, in: Proceedings of the 2001 Berlin Meeting of the European Section of IUSSI, Sept. 25-29, p. 147.

Steinhobel F. (1977) Swarming on the Aloes and its utilization for making increase, in: Fletcher D.J.C. (Ed.), Proceedings of the Apimondia International Symposium on the Taxonomy, Biology and Economic Use of African Bees, Pretoria, pp. 152-156.

Stuart-Findlay M. (1953) Notes from the Western Province, S. Afr. Bee J. 28, 19-20.

Swart J.D., Johannsmeier M.F., Tribe G.D., Kryger P. (2001) Diseases and pests of honeybees, in: Johannsmeier M.F. (Ed.) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp. 198-222.

Tribe G.D. (1981) Cape honeybee behaviour, S. Afr. Bee J. 53, 10-12.

Tribe G.D. (1982) Do workers move eggs?, S. Afr. Bee J. 54, 18-19.

Tribe G.D. (1983) What is the Cape bee?, S. Afr. Bee J. 55, 77-87.

Tribe G.D., Allsopp M.H. (2001a) Honeybee activities and behaviour, in: Johannsmeier M.F. (Ed.) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp. 27-38.

Tribe G.D., Allsopp M.H. (2001b) Honeybee reproduction, in: Johannsmeier M.F. (Ed.) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp. 40-45.

Velthuis H.H.W. (1970) Ovarian development in *Apis mellifera* worker bees. Experimentalis et Applicata 13, 377-294.

Velthuis H.H.W. (1976) Egg laying, aggression and dominance in bees, in: Proceedings of the 15th International Congress of Entomology, Washington, pp 436-449.

Velthuis H.H.W., Ruttner F., Crewe R.M. (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honey bees, in: Engels W. (Ed.) Social Insects, Springer-Verlag, Berlin.

Vergara C., Dretz A., Percz de Leon A. (1993) Female parasitism of European honey bees by Africanized honey bee swarms in Mexico, J. Apic. Res. 32, 34-40.

Visscher P.K. (1996) Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing, Behav. Ecol. Sociobiol. 39, 237-242.

Visscher P.K., Dukas R. (1995) Honey bees recognise development of nestmates' ovaries, Anim. Behav. 49, 542–544.

Walter A.R. (1939) Notes from the districts. S. Afr. Bee J. 13, 4-6.

Wossler T.C. (1991) Social dominance of *Apis mellifera capensis* workers in *A. m. scutellata* colonies, in: Proceedings of the 8th Entomological Congress, Entomological Society of Southern Africa, Bloemfontein, p. 175.

Wossler T.C. (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *A. m. scutellata* colonies, Apidologie 33, in press.

Woyke J. (1979) New investigations on *Apis mellifera capensis*, Apiacta 14, 173.

Woyke J. (1995) Invasion of Capensis bee, in: Magnuson P. (Ed.), Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, Pretoria, PPRI, p. 35.

**Figure 1.** Minimum configurations for the reproductive cycle of social parasitic Cape honeybee workers. Shaded boxes and grey lines represent the normal sexual reproductive pathway of *A. m. capensis*. Blank boxes and black lines represent the social parasitic pathway of laying workers. Shaded/blank boxes represent steps, which are involved in both the sexual reproductive and parthenogenetical laying worker pathway. Dotted lines represent rare events (see Swart et al. 2001 and Martin et al. 2002b). For simplicity all behavioural interactions between individual steps have been omitted.

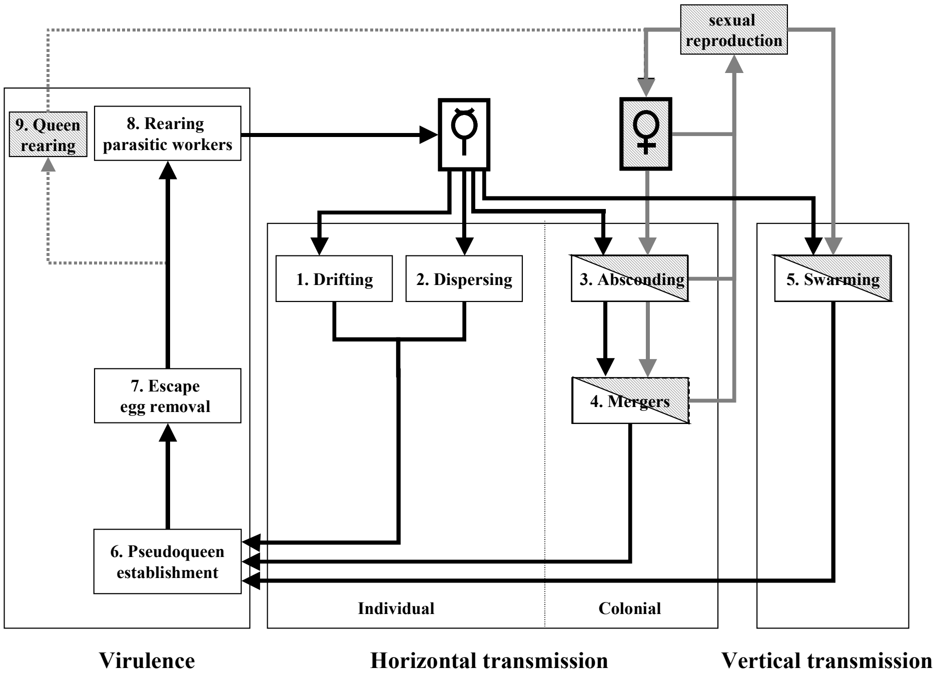
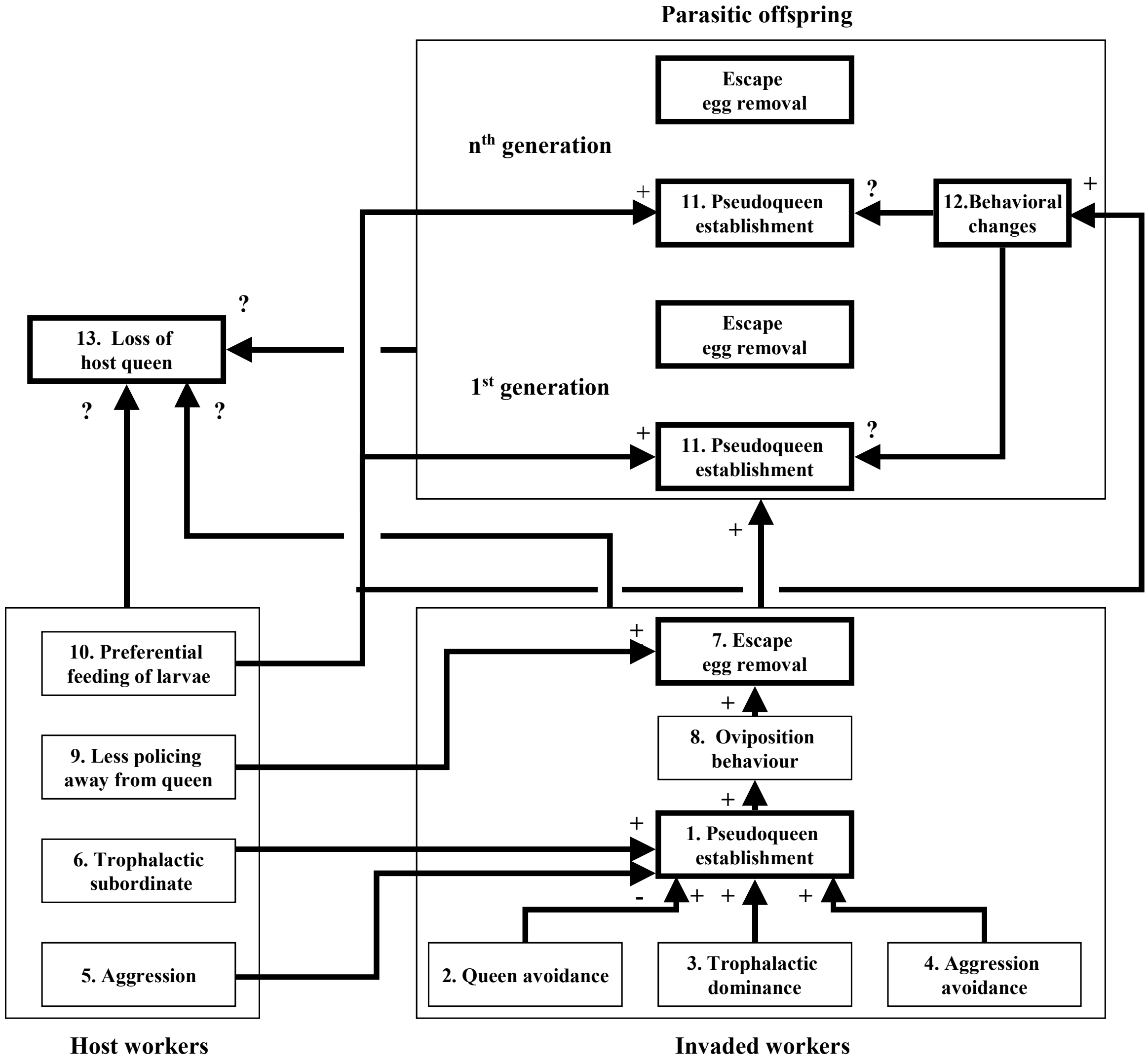


**Figure 2.** Virulence of social parasitic Cape honeybee workers following successful invasion, associated with parasitic pseudoqueen establishment and the "dwindling colony" syndrome of host colonies. The minimum number of behavioural interactions between host workers, invaded workers and parasitic offspring are shown. Positive and negative interactions are indicated with "+" and "-" respectively, unknown interactions with "?". The n<sup>th</sup> generation of parasitic offspring represents progeny reared after the loss of the host queen and is variable. For simplicity behavioural interactions related to the laying worker / pseudoqueen establishment and escape of egg-removal of parasitic offspring have been omitted.



# 3.12 The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time?

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**Abstract -** Honeybee workers, *Apis mellifera*, don't usually reproduce, but can activate their ovaries under queenless conditions to produce male offspring. As an exception to this rule, laying workers of the Cape honeybee, *A. m. capensis*, parthenogenetically produce diploid female offspring, usually developing into workers and occasionally into queens. Some of such workers can develop into pseudoqueens which show high ovarial development and a queenlike pheromonal bouquet. Because there is high genetic variance for these characters, this results in an extreme intracolonial selection. This process is governed by a competition for reproductive dominance among workers leading into a facultative social parasitic reproductive pathway as apart of the life history of the Cape honeybee. *A. m. capensis* workers show an increased potential for invading foreign colonies. Inside of the host colony, parasitic *A. m. capensis* workers produce queenlike pheromones and swiftly activate the ovaries despite the presence of a queen. Eventually they establish themselves as pseudoqueens and replace the host queen. The parasitic worker offspring is preferentially fed by the host workers leading to highly virulent intercastes and thereby completing the social parasitic life cycle of laying *A. m. capensis* workers. Recently, a particularly virulent parasitic strain of *A. m. capensis* workers has invaded the neighboring subspecies *A. m. scutellata* ("*capensis* calamity"). Because male sexuals are completely lacking in this invasive strain and females reproductives are never reared in infested *A. m. scutellata* host colonies, this results in reproductive isolation of the parasitic clones from the host gene pool. This sets the stage for the evolution of a queenless social parasitic honeybee. The Cape honeybee may therefore constitute a unique subject for studying sympatric speciation of a social parasite.

**Key words:** *Apis mellifera capensis*, honeybee, social parasite, sympatric speciation, evolution

## 1. Social parasites in social insects

Social parasitism in social insects is defined as a relationship between two species where the parasite benefits in many ways from brood care or other socially managed resources at the expense of the host society (Schmid-Hempel 1998). Social parasitic tactics can also occur within a species (Roubik 1989). Social parasitism appears to be widespread and social parasitic species have been described in all major groups of social insects (ants, bees, wasps and termites, Schmid-Hempel 1998). Such species employ a wide variety of parasitic tactics and strategies (see Wilson 1971, Hölldobler & Wilson 1990, Schmid-Hempel 1998 for reviews). These include facultative (e.g. *Formica sanguinea*, Hölldobler & Wilson 1990) and obligate social parasites (e.g. *Polyergus rufescens*, Hölldobler & Wilson 1990). Social parasites can be permanent or temporary (e.g. in *Formica* ants, Hölldobler & Wilson 1990, Schmid-Hempel 1998). In temporary social parasitic species, a newly mated queen seeks a host colony and gets adopted. As her own brood is raised by the host, the host queens is killed and the host nest is progressively taken over by the parasite. Slave making species (dulotic) depend on the host species, e.g. because mandibles of the workers are not suitable for brood care (e.g. *Polyergus rufescens*, Hölldobler & Wilson 1990) and raid nests of the host species to obtain pupae of the host species (Hölldobler & Wilson 1990). In extreme social parasitic species such as *Teleutomyrmex schneideri* and *Polistes sulcifer* the worker caste is lost altogether and parasites conduct their entire life cycle in the nest of the host (inquiline parasites, Wilson 1971). Another well-known example for inquiline parasites, is the cuckoo bumblebee, *Psithyrus sp.* Females of these social parasites lay their eggs in a bumblebee nest and let workers of the host colony rear their offspring.

The classical theory for the evolution of social parasites is known as Emery's rule (Emery 1909, Le Masne 1956). It is based on a series of speciation steps from a true social species to a parasitic type parasitising on the social ancestor. As a consequence host and parasite should be phylogenetically closely related. Recently, molecular phylogenetic data on bumblebees gave strong empirical support for the above theory (Pedersen 1996). Many authors have expressed doubt whether sympatric speciation can actually occur in nature (Mayr 1963 among others). However sympatric evolution of social parasites has been repeatedly advocated by a series of authors (Hölldobler & Wilson 1990, Bourke & Franks 1991, Schmid-Hempel 1998). Buschinger (1986, 1990) suggested that there is a sympatric pathway for the evolution of social parasites through multiple queens (polygyny) and disruptive selection (parasitic and non-parasitic) within the population.

### 1.1 Social parasitism in honeybees?

Social parasitic species are not known for honeybees (*Apis* sp.). The only known cases of social parasitism refer to intraspecific social parasitic tactics. Best known is the so-called robbing behaviour, a form of cleptoparasitism, where workers of one colony steal the honey stores of another colony (Moritz and Southwick 1992). The process is simple and efficient: scouts enter a foreign colony and identify it as a highly rewarding food source. They then recruit foragers of their home colony to the target colony which, if insufficiently defended, will be completely depleted of its resources. Often the robbed colony will completely die in the process.

Not only workers but also sexual reproductives of a foreign colony can enter a host colony, and benefit from its resources at the expense of the host. Male sexuals (drones) in particular have been reported to drift among colonies (Neumann et al.. 2000b), which might constitute a social parasitic tactic. Rinderer et al.. (1985) suggested that a colony only produces a limited amount of drones which cannot be exceeded. This puts an adaptive facet to drifting of drones into foreign colonies, resulting from the poor orientation abilities of drones which are apparently less accurate in returning to their home colony than workers are (Free 1958, Neumann et al. 2000b, but see Moritz and Neumann 1996). The admission of the males by workers may therefore form a fitness disadvantage for the host colony. Rinderer et al. (1985) argued that this may be one explanation for the fitness advantage of Africanized honeybees over European stock in the Americas. Drones from Africanized colonies were found more frequently in European host colonies than *vice versa*. As a result the European colonies reduced their own drone production whereas the Africanized colonies could further increase the number of drones that they reared. However, it remains open whether this is an adaptive trait or simply a by-product of poor orientation abilities.

Queen usurpation is usually only considered as a within-nest affair and sister-queens fight over gaining access to the colony eventually leaving only one alive (Seeley 1985; Gilley 2001). This is not social parasitism, because the workers are closely related to the surviving queen, and workers benefit from queen reproduction as plausibly explained by inclusive fitness theory (Hamilton 1964a;b). There are however polygynous situations that do not match this situation and reflect a parasitic tactic (Roubik 1989). One prime example of social parasitism are the results of naturally occurring swarm mergers (Neumann et al. 2001a), following non-reproductive swarming (absconding, Hepburn et al. 1999), which are common in African subspecies of honeybees (Hepburn and Radloff 1998). Two or more unrelated swarms may merge into an initially polygynous unit (Hepburn & Radloff 1998). After a transient polygynous period, which can vary in time, eventually only a single queen survives re-establishing monogyny. Clearly the surviving queen profits from the resources provided by the usurped workers. If the usurped workers are unrelated to the queen, their inclusive fitness is clearly reduced. Therefore, this case reflects a typical temporary social parasitic tactic of the actively merging colony. This has been termed "female social parasitism" (Vergara et al. 1993) and was proposed to be another mechanism favoring the Africanization process of European honeybee populations in the Americas. It is analogous to a dulotic, slave raiding ant colony (Hölldobler & Wilson 1990). The main and crucial difference in all these social parasitic processes in honeybees compared to the case of cuckoo bumblebees (or any other social parasite) is that it is an intraspecific parasitic tactic and not an evolutionary strategy of an obligate socio-parasitic species.

### 1.2 Worker reproduction in honeybees

Honeybee workers cannot mate and are usually not reproductive. Their sterility is governed by a suite of pheromones, which are derived from the queen and the brood (Slessor et al. 1998). Nevertheless, there are occasions when laying workers can be found in the colony. Such workers develop regularly after queen loss, whenever the colony is unable to re-queen itself from remaining female brood ("hopelessly queenless"). Very few of these workers can develop into so-called false queens or pseudoqueens (Sakagami 1958; Velthuis 1970b) and not only activate their ovaries to a high degree but also possess a queenlike pheromone bouquet. Pseudoqueens can evoke retinue behaviour and suppress the ovary activation in other workers (Velthuis et al. 1965). These workers parthenogenetically lay unfertilized eggs developing into drones (arrhenotoky, Crozier and Pamilo 1996). The adaptive value for this behaviour seems obvious: rather then simply dying, workers increase their fitness by producing male sexuals that can significantly contribute to the population gene pool (Page & Erickson 1988).

Whereas worker reproduction is the rule in hopelessly queenless colonies throughout the species, laying worker activity in queenright colonies is less frequently observed (Visscher 1989; 1996). However, the potential effect of laying workers on reproduction is higher then one might expect in the light of rigid pheromonal control. Although there are only few workers in a queenright colony with developed ovaries, they can lay substantial numbers of eggs (Visscher 1996). However, these worker-laid eggs are efficiently removed by other workers (= "worker policing"; Ratnieks & Visscher 1989), which usually prevents that worker offspring is reared in a queenright colony (Page & Erickson 1988; Visscher 1996). Because honeybee queens mate with many males (polyandry; Lobo & Kerr 1993; Neumann and Moritz 2000; Palmer and Oldroyd 2000) workers are on average more closely related to the queen's male offspring than to the worker-laid drones making cannibalism of the latter an adaptive trait (Ratnieks 1988). Oldroyd et al. (1994) reported on a rare „anarchic“ trait, which leads to substantial worker reproduction even in the presence of a laying queen (Barron et al. 2001). Laying workers in anarchistic colonies lay eggs, which have low removal rates (Oldroyd & Ratnieks 2000) and contribute considerably to the drone production of such colonies. However, anarchic reproduction by worker honeybees is not equivalent to social parasitism. Parasitic tactics can only emerge if these laying workers get into foreign colonies to reproduce at the expense of unrelated individuals.

### 1.3 Worker reproduction in *Apis mellifera capensis*

The Cape Honeybee, *A. m. capensis*, forms a rather unique exception in the species *A. mellifera* (Hepburn and Radloff 2002). It is native to the Cape region of South Africa (Hepburn & Jacot-Guillarmod 1991; Hepburn and Radloff 2002) and is characterized by a unique set of genetic, behavioral and physiological traits related to worker reproduction (Hepburn & Crewe 1991; Hepburn 1994; Hepburn & Radloff 1998). Most workers of the Cape honeybee produce female offspring parthenogenetically (thelytoky, Onions 1912; 1914; Anderson 1963; Hepburn & Crewe 1991; Neumann et al. 2000a; Radloff et al. 2002). This reproductive strategy has been shown to be advantageous under a variety of environmental conditions (Moritz 1986, Greeff 1996). Recombination events are rare in thelytokous parthenogenesis resulting in almost clonal offspring (Moritz & Haberl 1994). However, workers of the neighboring subspecies *A. m. scutellata* (Hastings 1989; Hepburn & Crewe 1991; Hepburn & Radloff 1998; Neumann et al. 2000a) and of all other *A. mellifera* subspecies (Ruttner 1992) do not have this trait and thus only produce male offspring (although rare cases of thelytokous worker reproduction have been described; Mackensen 1953; Tucker 1958). As a consequence the theoretical assumptions for the policing trait do not hold for *A. m. capensis*. Greeff (1996) showed that worker policing should not be an adaptive trait in the Cape honeybee because workers are equally related to worker-laid female offspring and to the queens' female offspring irrespective of the degree of polyandry. Indeed, laying worker offspring was observed in the presence of a laying queen in a variety of studies (e.g. Pettey 1922; Moritz et al. 1999; Martin et al. 2002a;b; Pirk et al. 2002) and laying workers of *A. m. capensis* contribute considerably to reproduction in South African honeybee populations (Moritz et al. 1998). Thus, as predicted by theory (Greeff 1996), thelytoky is paired with a higher rate of reproductive success of laying workers.

The greater reproductive capacity of *A. m. capensis* workers in comparison to workers of other subspecies is striking. Workers typically have a queenlike spermatheca although they cannot mate (Anderson 1963; Ruttner 1976). Ovarial development of *A. m. capensis* workers is much higher compared to other races including the neighboring *A. m. scutellata* (Hess 1942; Hepburn & Crewe 1991) Furthermore, freshly-emerged workers can activate their ovaries within six days (Ruttner and Hesse 1981) and quickly produce a mandibular gland pheromone bouquet which can be similar to that of the queen (Hemmling et al. 1979; Crewe and Velthuis 1980; Velthuis et al. 1990; Simon et al. 2001). Finally, pseudoqueens are much more frequent in the Cape honeybee (Hepburn 1992; Hepburn & Radloff 1998) compared to other subspecies of *A. mellifera* (Velthuis 1970b; Velthuis et al. 1990). This series of potential pre-adaptations appears to be crucial for establishing social parasitism among laying Cape honeybee workers (Velthuis et al. 1990).

However, the frequency of reproductive dominant workers is low in natural populations of *A. m. capensis* due to a trade off between individual and colony level selection (Moritz 1989; Hillesheim et al. 1989). Colonies with high levels of reproductive dominant workers show decreased brood rearing reducing colony fitness (Hillesheim et al. 1989). ). During a phase of severe intracolonial selection only a few workers of distinct subfamilies (patrilines) become reproductively dominant after queenloss (Moritz et al. 1996). This further predisposes laying *A. m. capensis* workers for a social parasitic tactics if they successfully reproduce in foreign colonies (Moritz et al. 1999).

## 2 The Cape honeybee phenomenon

Reproductive *A. m. capensis* workers appear to be a constant factor of concern for practical beekeeping in southern Africa (Allsopp 1995; Magnuson 1995). Case reports of parasitic *A.m. capensis* “take-overs“ of other honeybee colonies persist in the beekeeping literature since 1912 (Onions 1912; Lundie 1954; Johannsmeier 1983; see Hepburn and Radloff (1998) for a review). In the last decade migratory beekeepers have repeatedly moved considerable numbers of Cape honeybee colonies across the naturally occurring hybrid zone (Hepburn & Crewe 1991; Hepburn et al. 1998) into the region of the neighboring *A. m. scutellata* (Allsopp 1995). This apparently has resulted in the “*capensis* calamity” since 1992 (Allsopp 1992; Allsopp & Crewe 1993; Allsopp 1995) affecting an estimated more than 30.000 *A. m. scutellata* colonies (Allsopp & Crewe 1993). These colonies served as hosts for invading social parasitic *A. m. capensis* workers (Hepburn and Allsopp 1994), resulting in substantial damage to beekeeping enterprises in South Africa (Allsopp 1992; Allsopp & Crewe 1993; Magnuson 1995).

The clinical symptoms of the infestation with social parasitic *A. m. capensis* workers are expressed at the level of the host colonies phenotypes as the "dwindling colony" syndrome (Allsopp 1993; Hepburn and Allsopp 1994; Greeff 1997). The "dwindling colony" syndrome dramatically changes the external and internal appearance of infested *A. m. scutellata* colonies (Allsopp 1993; Allsopp 1995; Magnuson 1995), enabling beekeepers to recognize infestations with parasitic *A. m. capensis* workers at various stages. A reduction in foraging and severe fighting at the hive entrance between *A. m. scutellata* host workers and *A. m. capensis* workers is accompanied by internal aggression and an increase in scattered brood patterns (Allsopp 1995). While *A. m. capensis* laying worker brood is preferentially nurtured by host workers (Beekman et al. 2000; Calis et al. 2002), the host queen is eventually lost or killed and the *A. m. scutellata* host colony is progressively taken over by the parasite (Hepburn and Radloff 1998; Martin et al. 2002b). Because the reproductive dominant *A. m. capensis* workers do not participate in brood rearing (Hillesheim et al. 1989) the infested colony dwindles down to a few *A. m. capensis* workers (Allsopp 1998) and eventually completely dies. Any raised parasitic *A. m. capensis* workers can infest new *A. m. scutellata* hosts and the parasitic life cycle is completed (Fig. 1). The high incidences of the "dwindling colony" syndrome in *A. m. scutellata* apiaries suggest that both transmission and virulence of the parasite are high (Neumann and Hepburn 2002). The interaction of two major factors, bee keeping practice and the unique set of traits relevant for the facultative social parasitic life cycle of laying *A. m. capensis*, appears to facilitate the magnitude of the "*capensis* calamity".

### 2.1 Transmission to new host

The prime mechanism resulting in the "*capensis* calamity" (Allsopp 1992) was beekeeping practice (Moritz 2002). Transport of colonies, exchange of frames and bees between colonies, splitting of colonies into queenright and queenless units, as well as placing potential host colonies in close proximity next to each other in apiaries clearly facilitates the spread of parasitic workers without involving any special host finding mechanism in the parasitic workers. This has been well documented (Johannsmeier 1983). In addition to these apicultural practices there are, however, also biologically relevant behavioral traits of the parasitic workers that considerably assist the spread of the parasite (Fig. 1).

#### Individual worker intrusion

The presence of foreign workers in honeybee colonies does not tell us how, and why these workers got there. True parasites actively search for host colonies, but these workers simply may have made orientation errors and have not actively invaded the host colony (drifting, Rauschmayer 1928; Neumann et al. 2000b). Although the homing ability of workers is better than that of drones, individual workers frequently do enter foreign hives by mistake (Free 1958; Neumann et al. 2000b). In contrast to robbing workers, drifted workers may prevent the rejection by the guard workers by offering droplets of nectar (Ribbands 1953). Regardless whether *A. m. capensis* workers show particular adaptations to gain access to the *A. m. scutellata* host colony (Tribe 1983) or not, invasion doesn't appear to be a critical step in the life cycle of the social parasite, if very low numbers of parasitic workers are sufficient to successfully initiate infestation (Neumann and Hepburn 2002). Indeed, although *A. m. scutellata* colonies have a very efficient guarding behaviour (Hepburn and Radloff 1998; Neumann et al. 2001c), they nevertheless suffer massive usurpations by laying *A. m. capensis* workers (Allsopp & Crewe 1993; Hepburn and Allsopp 1994). Usually the adoption by a foreign colony is to the disadvantage of the worker, because it now contributes its labor force to an unrelated colony and her inclusive fitness is reduced. If however the worker can establish herself as a pseudoqueen and usurp the colony, her fitness dramatically gains. As a consequence, those *A. m. capensis* workers with a combined phenotype of high drifting propensity and high queen pheromone production are pre-adapted to usurp foreign colonies and will be selected.

Although *A. m. capensis* workers have an increased propensity for drifting compared to *A. m. scutellata* (Neumann et al. 2001c), they also appear to have the potential for an active host finding mechanism. *A. m. capensis* workers revealed a high frequency for entering very distant host colonies (Neumann et al. 2001c), corroborating the notion of an active searching behaviour (Johannsmeier 1983; Neumann et al. 2001c) rather than just random drifting among colonies in close vicinity (Neumann et al. 2000b). It seems valid indeed to address *A. m. capensis* as a facultative social parasite.

#### Colony absconding and merger

Transmission not only occurs through individual workers but also at the colonial level (Fig. 1). *A. m. capensis* colonies have a high potential for absconding if environmental conditions become unfavourable (Hepburn & Radloff 1998; Hepburn et al. 1999). Hepburn et al. (1999) observed that queenless *A. m. capensis* colonies absconded on average after 3.6 weeks and twice as frequently as queenless *A. m. scutellata* ones. Since these swarms often merge with other colonies (Hepburn & Radloff 1998; Neumann et al. 2001a), chances for transmitting social parasitic workers are high. Likewise, *A. m. scutellata* colonies infested by parasitic *A. m. capensis* workers also frequently abscond, especially at late stages of infestation when the "dwindling colony" syndrome is fully expressed (Magnuson 1995; personal observations). Such absconding swarms are readily accepted into other hives at the same apiary (Magnuson 1995). The wild population of *A. m. scutellata* has been claimed to be highly infested with *A. m. capensis* laying workers (Allsopp 1995). Therefore, long-range dispersal mechanisms such as absconding and subsequent mergers of swarms (Allsopp 1998; Neumann et al. 2001a), are suspected to contribute substantially to the transmission of parasitic *A. m. capensis* workers (Fig. 1). However, despite frequent reports of beekeepers suffering from the "*capensis* calamity" (e.g. Magnuson 1995), no quantitative assessments on the actual numbers of mergers of infested *A. m. scutellata* colonies or *A. m. capensis* remnants (after the death of all *A. m. scutellata* host workers) with un-infested *A. m. scutellata* host colonies have been published so far. Clearly, more concrete data are needed here to critically evaluate the actual contribution of colony mergers to the spread of parasitic *A. m. capensis* workers.

### 2.2 Virulence in host colony

The successfully invading parasitic workers have to establish themselves as pseudoqueens within the host. A series of critical steps is essential for this development:

#### Pheromone secretion

The production of typical queen pheromones forms an important basis for the reproductive success of laying *A. m. capensis* workers. The fatty acid secretions of the mandibular gland pheromones have been best studied in the context of reproductive dominance in the honeybee. In queens the synthetic pathway leads to the so-called "queen substance" (9-oxo-2-(E)-decenoic acid; 9-ODA). The pathway is very similar in workers but the final secretion is dominated by 10-hydroxy-(E) 2-decanoic acid (10HDAA) and 10-hydroxy-(E) 2-decenoic acid (10HDA) (Plettner et al. 1996; 1998), the "worker substance". The ratio between queen and worker substances is a highly sensitive indicator of reproductive hierarchy status (Moritz et al. 2000; 2002). For example this ratio is higher in laying queens than in virgin queens in various African honeybee races (Crewe 1988). It is extremely high in laying queens of *A. m capensis* (9ODA/10HDA = 83.9) as compared to 7.7 in *A. m. scutellata* or 3.5 in *A. m. intermissa* (Crewe 1988). The signal of a pseudoqueen is also dominated by the queen substance, which among other pheromonal functions (Slessor et al. 1998; Wossler 2002) has been shown to be important for suppressing queen rearing and ovary development in other workers (Velthuis and van Es 1964; Velthuis 1970a; 1972). Consequently not only the queen's but also the laying worker's pheromonal mandibular gland signals inhibit ovary development (Velthuis et al. 1965) and the production of an own 9-ODA signal in other workers. Workers actually compete for the strongest signal (Moritz et al. 2000). Thus, pseudoqueens function as surrogate queens, pheromonally fulfilling the function of the true queen, which may be a possible mechanism for the take over process of host colonies.

Velthuis and van der Kerk (1988) studied the impact of age, environment and genes on the composition of the mandibular gland secretions in *A. m. capensis* workers. They concluded that "*the mandibular glands secretions express the progress an individual has made in the differentiation process leading to reproduction" (p86).* Although there is a strong genetic impact on the expression of the pseudoqueen phenotype in workers (Moritz and Hillesheim 1985), this does not imply that environmental cues are insignificant for the development of pseudoqueens. In order to establish a queen-like pheromonal signal, workers need to overcome the inhibitory effects of other queen pheromone sources (queen and pseudoqueens) and inevitably environmental factors such as nutrition, developmental stage, age will heavily interfere with the fatty acid secretions of the mandibular glands in both workers and queens.

#### Queen evasion

One way to avoid the suppressive queen signals may be to physically evade the queen's mandibular gland signals in areas of low 9-ODA concentration in the colony. Such behaviour has been observed, and is more strongly expressed in *A. m. capensis* workers than in other African honeybee subspecies (Moritz et al. 2001). A certain proportion of workers in the colony actively avoids the queen and can be found more often in regions of the colony away from the queen. At the same time these workers produce more queenlike compounds in the mandibular gland secretions suggesting that they have further differentiated into reproductives than others (Moritz et al. 2002).

There are two non-exclusive evolutionary explanations for this phenomenon:

1) Self-organized process selected through colony level selection: Assuming an initially random distribution of workers on the combs, there will be workers that are closer to the queen and others which are further away. Given that, pheromonal suppression is strongest in the close vicinity of the queen, workers more distant from the queen should be less suppressed, and more likely to express a queenlike signal. If this in turn reinforces queen evasion behaviour in such workers, we would expect a group of reproductive workers residing in areas of the colony which are infrequently visited by the queen or messenger workers loaded with the queen's pheromones (Velthuis 1972). If this were the only process involved in the development of laying workers, we would expect a random sample of workers to become reproductive. This however doesn't seem to be the case, since there is strong

genetic variance for reproductive dominance, and workers of specific subfamilies in the colony more frequently develop into reproductive workers than others (Moritz et al. 1996).

2) Adaptive behaviour of parasitic workers selected through individual selection: Since there is genetic variance for worker reproduction, selection should act upon the frequency of this trait in the population. Stable equilibria for the reproductive worker trait in the population (Moritz 1989; Greeff 1996) are possible because a high frequency of reproductive workers in the colony is detrimental to the colonial fitness (Hillesheim et al. 1989). This results in a balanced selection between colony level and individual level selection. Selection at the individual level favors the reproductive worker pathway, whereas selection at the colonial level favors high frequencies of subordinate workers.

Most likely both aspects, self-organization and selection, are important for the development of reproductive workers. For a pseudoqueen phenotype to develop, the right genotype is required, at the right time, at the right location in the host's nest. Thus, the interaction of individual intracolonial selection with self-organized environmental factors (= gene-environment interactions) is important, and pseudoqueens are a typical, but extreme case of phenotypic plasticity in life cycle strategies.

### Trophallactic dominance

When interacting with individuals of host races, *A. m. capensis* workers can be distinguished by their behaviour (Neumann and Hepburn 2002) and most important in this context seem to be behavioral elements belonging to the trophallactic system: food offering and food begging (Velthuis et al 1990). Since *A. m. capensis* workers are distinct gainers in food exchange, they seem to express more initiative in begging for food and more dominant in their responses. Thus, long before an *A. m. capensis* worker is able to elicit retinue behavior and to lay eggs, she starts to make a gain in food exchange with workers of host colonies (Korst and Velthuis 1982), easily gaining trophallactic dominance over host workers.

### Worker-worker aggression

In queenless and queenright *A. mellifera* colonies, workers with activated ovaries are often attacked by their nest mates (Sakagami 1958; Evers and Seeley 1986; Visscher & Dukas 1995). This constitutes a potential host defense mechanism in the context of social parasitism by laying workers. Also, in recently queenless colonies of *A. m. capensis* substantial aggression behavior among workers is strong and can cause the death of thousands of workers (Anderson 1968; Tribe 1981; 1983). Obviously, only those workers surviving worker-worker aggression can become reproductively dominant in host colonies. Tribe (1981) observed that attacking workers back away from laying *A. m. capensis* workers which have reached pseudoqueens status, similar to the way as they react to queens (Tribe 1981). Those workers, which are not fully pheromonally developed are eliminated by the host workers (Velthuis 1976). This strongly indicates that fast pheromonal and ovarial development which is important for competition among pseudoqueens (Moritz et al. 2000) may also reduce the time window of susceptibility to host worker aggression.

### Evasion of worker policing

Worker-laid eggs are removed by other workers (termed "worker policing", Ratnieks & Visscher 1989). Thus, established pseudoqueens have to counter worker policing in order to successfully reproduce. In fact, this ability is right at the heart of maintenance of parasitic lines and essential for an understanding of the "*capensis* phenomenon". Two non-exclusive mechanisms seem to be involved:

1. Highly virulent social parasitic workers lay eggs which have low removal rates (Neumann et al. 2001b; Martin et al. 2002a). Because honeybee queens have been claimed to mark their eggs with a queen-specific label (Ratnieks 1995), it might well be that the eggs-laid by social parasitic pseudoqueens are marked with a queen-label mimic. However, the actual mechanisms and compounds have not been identified yet and the mechanism is discussed highly controversial (Katzav-Gozansky et al. 2001; 2002; Oldroyd et al. 2002).

2. Laying workers appear to preferentially oviposit in areas of the nest, where worker policing is reduced (Neumann & Hepburn 2002). Thus, parasitic offspring initially appears in the extreme peripheral frames in newly infested colonies (Magnuson 1995). Queen-evasion behaviour (see above) of laying workers may facilitate this egg laying pattern.

### Rearing of parasitic worker offspring

*A. m. capensis* laying workers show a unique set of traits which maximizes the reproductive output. In contrast to laying workers of other subspecies, which lay multiple eggs per cell, preferentially on the side walls (Gary 2000; up to several dozens in *A. m. scutellata*, personal observations), laying workers of *A. m. capensis* typically lay only a single egg in the bottom center of a cell (Velthuis 1976; Tribe 1981; Hepburn & Radloff 1998; personal observations). Such a queen-like egg laying behavior might considerably contribute to the fitness of individual social parasitic workers because only a single larva can be reared per cell. The resulting capped brood of pseudoqueens is very often indistinguishable from that of a true queen (Tribe 1981). Furthermore, in contrast to laying workers of European subspecies, which may lay up to 15 eggs per day, *A. m. capensis* pseudoqueens may lay up to 200 eggs per day (Velthuis 1976). Moreover, the life span of an individual social parasitic worker appears to be a crucial factor for the reproductive success of such workers. Indeed, the duration of the reproductive period for pseudoqueens is 3-5 months compared to laying workers of other races which may reproduce up to 10 days (Velthuis et al. 1990). Thus, individual social parasitic *A. m. capensis* workers can produce between 60 and 200 times more offspring compared to laying workers of other races.

Recent data suggest that larvae of parasitic laying *A. m. capensis* workers are preferentially fed by workers of the host colonies (Beekman et al. 2000; Calis et al. 2002). The resulting workers almost represent intercastes, which appear to be nutritionally predisposed for high virulence (Calis et al. 2002). They can immediately infest new host colonies via the individual and colonial transmission pathway, thereby completing the reproductive cycle of social parasitic *A. m. capensis* workers.

**Loss of the host queen**

Because queenright host colonies appear to be more resistant than queenless ones (Tribe 1983; Woyke 1995), it seems as if the ability to displace the resident *A. m. scutellata* queen is an integral part of the invasion process. In particular, the loss of the host queen seems to occur inevitable at late stages of infestations (personal observations) similar to inquiline parasites (Schmid-Hempel 1998). However, at the current state of evidence it is completely unclear why and how the host queen is lost. Is the host queen actively killed by the parasitic pseudoqueens or by the *A. m. scutellata* host workers? Alternatively, do the pseudoqueen pheromone signals disrupt normal retinue behavior of the *A. m. scutellata* host workers, so that the host queen is ignored to death? Finally, because pheromone production by honeybee queens seems to be associated with the amount of brood production, a reduced egg-laying due to the massive reproduction of parasitic pseudoqueens at later stages of infestation may also contribute to the host queen loss (Neumann and Hepburn 2002). Detailed behavioral studies are necessary to identify the actual mechanisms.

## 3 Evolutionary perspective

The question remains if the Cape honeybee can evolve into an obligate social parasite at the species level. In order to obtain species status, two steps seem to be essential: selection of a virulent strain and separation of the social and the parasitic gene pools.

**1) Selection:** Laying *A. m. capensis* workers produce clonal female offspring (Moritz & Haberl 1994). Clonal lineages of parthenogenetic parasitic workers can theoretically be maintained infinitely without any recombination with the "social" gene pool. At the same time the intracolonial selection process for the most virulent laying worker operates at extreme selection intensities since only very few of the thousands of workers in a colony eventually develop into pseudoqueens (Moritz et al. 1996). The underlying mechanism of this extreme selection process seems well understood. Those workers which develop a queenlike pheromonal signal the fastest, suppress the development of a pseudoqueen phenotype in other workers (Velthuis et al. 1990; Moritz et al. 2000). The most virulent worker, will thus suppress competitors and eventually have the highest fitness in the colony and be favored by natural selection. Since she produces clonal offspring, selection at this level not only operates on specific genes for virulence, but actually on specific genotypes yielding high virulence. As a consequence, not only additive gene actions are selected, but also non-linear interactions between alleles (dominance interactions) and loci (epistatic interactions). The selectable genetic variance (heritability) for pseudoqueen development has been shown to be exceptionally high (up to $h^2 = 0.89$ Moritz and Hillesheim 1985).

**2) Isolation of gene pools:** In the natural hybrid zone between *A. m. capensis* and the adjacent *A. m. scutellata,* there is a considerable amount of introgression between the two honeybee subspecies (Moritz et al. 1998). Nevertheless, there is an extremely strong potential for separating a parasitic strain from the social honeybee gene pool that should not be overlooked. Specific gene and allele combinations that cause particular high virulence are maintained over generations because recombination through sexual processes are lacking (Kryger et al. 2001a;b). As a consequence drift and mutation will increase the genetic distance between a highly virulent parasitic lineage and the sexual reproducing host population. Eventually this lack of gene flow should cause a separation of a parthenogenetic, obligate parasite from the social honeybee population. Many facets of the life history of laying workers suggest that the gene flow from the parasitic *A. m. capensis* clone into the social host population is extremely reduced or perhaps completely lacking. The production of queens from pseudoqueen offspring does occur in *A.m. capensis* colonies (Moritz et al. 1996; Allsopp and Hepburn 1997) despite queen rearing is suppressed by the pseudoqueen mandibular gland pheromone (Anderson 1968). Parasitic pseudoqueens in infested *A. m. scutellata* colonies have been claimed to suppress queen rearing altogether (Neumann and Hepburn 2002). Indeed, successful re-queening has never been observed in infested *A. m. scutellata* colonies (Swart et al. 2001; Martin et al. 2002b; personal observations). Since the parasitic workers in the infested colonies do not produce drones (Velthuis et al 1990), there is a large potential that sexual reproductives are not produced at all in parasitized colonies. If the parasitic *A. m. capensis* clone never produces sexual reproductives, it cannot interbreed with its host, resulting in a separation of the two gene pools.

We currently do not know why parasitic workers do not have the same impact in *A. m. capensis* as they have in *A. m. scutellata.* Although the strong mandibular gland signal of *A. m. capensis* queens (Crewe 1988) might be one important factor, other yet unidentified mechanisms may be even more essential, clearly asking for more research in this field. Genetic analyses of the "*capensis* calamity" suggest that the separation of a social and a parasitic gene pool is currently taking place in infested *A. m. scutellata* populations. Apparently only a single worker clone has been invading different colonies of *A. m. scutellata* at very distant apiaries in Mpumalanga and Gauteng (Kryger et al. 2001a;b; Neumann et al. 2001b). Parasitic workers appeared to have identical allele combinations at several microsatellite DNA loci, strongly suggesting that they all belong to the same parasitic clone (Kryger et al. 2001a;b; Solignac et al. 2001). Because all workers are of clonal origin, there is no gene flow from the host population into the parasitic strain. Since workers of the *A. m. scutellata* host colonies did not share the alleles typical of the parasitic clone in the microsatellite DNA study of Kryger (2001a;b) it is also highly suggestive that potential gene flow from the parasitic clone into the host population is reduced and possibly completely lacking. Although the species concept is difficult to employ with clonal lines (Bock 1992), the Cape honeybee may therefore be a prime subject for students of sympatric speciation and the evolution of social parasites. We may be in the fortunate position to follow its evolution in real time.

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## REFERENCES

Allsopp MH (1992) The *capensis* calamity. S Afr Bee J 64: 52-55

Allsopp MH (1993) Summarized overview of the *capensis* problem. S Afr Bee J 65: 127-136

Allsopp MH (1995) The *Capensis* problem 1992-1995. In: Magnuson P (ed) Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, pp 10-31

Allsopp MH (1998) Capensis remnants do abscond. S Afr Bee J 70: 156-160

Allsopp MH, Crewe R (1993) The Cape honeybee as a Trojan horse rather than the hordes of Jenghiz Khan. Am Bee J 133: 121-123

Allsopp MH, Hepburn HR (1997) Swarming, supersedure and the mating system of a natural population of honeybees (*Apis mellifera capensis*). J Apic Res 36, 41-48

Anderson RH (1963) The laying worker in the Cape honey-bee *Apis mellifera capensis*. J Apic Res 2:85-92.

Anderson RH (1968) The effect of queen loss on colonies of *Apis mellifera capensis*. S Afr J Agric Sci 11: 383-388

Barron A, Oldroyd BP, Ratnieks FLW, 2001. Worker policing and anarchy in *Apis*. Behav Ecol Sociobiol 50: 371-377

Beekman M, Calis JNM, Boot WJ (2000) Parasitic honeybees get royal treatment. Nature 404: 723

Bock WJ (1992): The species concept in theory and practice. Japanese J Zool 9: 697-712

Bourke AFG, Franks NR (1991) Alternative adaptations, sympatric speciation and the evolution of parasitic, inquiline ants. Biol J Linnean Soc 43: 157-178

Buschinger A (1986) Evolution of social parasitism in ants. TREE 1:155-160

Buschinger A (1990) Sympatric speciation and radiative evolution of socially parasitic ants – Heretic hypotheses and their factual background. Z Zool Syst Evolutionsforsch 28: 241-260

Calis JNM, Boot WJ, Allsopp MH, Beekman M (2002) Getting more than a fair share: Nutrition of worker larvae related to social parasitism in the Cape honey bee, *Apis mellifera capensis*. Apidologie 33: 193-202

Crewe RM, Velthuis HHW (1980) False queens: a consequence of mandibular gland signals in worker honeybees. Naturwissenschaften 67:467-469

Crewe RM (1988) Natural history of honey-bee mandibular gland secretions: development of analytical techniques and the emergence of complexity. In: Needham GR, Page RE, Delfinado-Baker M, Bowman CE (eds) Africanized honeybees and bee mites. Ellis Horwood, Chichester, pp 149-158

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford

Emery C (1909) Über den Ursprung der dulotischen, parasitischen und myrmekophilen Ameisen. Biol Zentralbl 29: 352-362

Evers CA, Seeley TD (1986) Kin discrimination and aggression in honey bee colonies with laying workers. Anim Behav 34: 924-925

Free JB (1958) The drifting of honeybees. J Agric Sci 51: 294-306

Gary NE (2000) Activities and Behavior of Honey Bees. In: Graham JM (ed) The Hive and the Honeybee. Dadant & Sons, Hamilton, Illinois

Gilley DC (2001) The behavior of honey bees (*Apis mellifera ligustica*) during queen duels. Ethology 107: 601-622

Greeff JM (1996) Effects of thelytokous worker reproduction on kin selection and conflict in the Cape honeybee, *Apis mellifera capensis.* Phil Trans Roy Soc London B 351:617-625

Greeff JM (1997) The Cape honeybee and her way north: an evolutionary perspective. S Afr J Sci 93: 306-308

Hamilton WD (1964a) The genetical evolution of social behaviour I. J Theor Biol 7:1-16

Hamilton WD (1964b) The genetical evolution of social behaviour II. J Theor Biol 7:17-52

Hastings H (1989) Two aspects of the biology of an African honeybee, *Apis mellifera scutellata* (Hymenoptera: Apidae): Laying workers and colony defence behaviour. Ph.D. thesis. University of Natal, Pietermaritzburg

Hemmling C, Koeniger N, Ruttner F (1979) Quantitative Bestimmung der 9-Oxodecensäure im Lebenszyklus der Kaphonigbiene (*Apis mellifera capensis* Escholtz) Apidologie 10: 227-240

Hepburn HR (1992) Pheromonal and ovarial development co-vary in Cape worker honeybees, *Apis mellifera capensis*. Naturwissenschaften 79: 523-524

Hepburn HR (1994) Reproductive cycling and hierarchical competition in the Cape Honeybee, *Apis mellifera capensis.* Apidologie 25:38-48

Hepburn HR, Crewe RM (1991) Portrait of the Cape honeybee, *Apis mellifera capensis*, Apidologie 22: 567-580

Hepburn HR, Jacot-Guillarmod (1991) The Cape honeybee and the fynbos biome. S Afr J Sci 87: 70-73

Hepburn HR, Allsopp MH (1994) Reproductive conflict between honeybees: usurpation of *Apis mellifera scutellata* colonies by *Apis mellifera capensis*. S Afr J Sci 90:247-249

Hepburn HR, Radloff SE (1998) Honeybees of Africa. Springer Verlag, Berlin Heidelberg New York

Hepburn HR, Radloff SE (2002) *Apis mellifera capensis* (Eschscholtz): an essay on the subspecific classification of honeybees. Apidologie 33: 105-127

Hepburn HR, Radloff SE, Fuchs S (1998) Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*. *Apidologie* 29: 333-346

Hepburn HR, Reece, Neumann P, Moritz RFA, Radloff SE (1999) Absconding in honeybees (*Apis mellifera*) in relation to queen status and mode of reproduction. Insectes Soc 46: 323-326

Hess G (1942) Über den Einfluss der Weisellosigkeit und des Fruchtbarkeitsvitamins E auf die Ovarien der Bienenarbeiterin. Beih Schweiz Bienenztg 1: 33-110

Hillesheim E, Koeniger N, Moritz RFA (1989) Colony performance in honeybees (*Apis mellifera capensis* Esch.) depends on the proportion of subordinate and dominant workers. Behav Ecol Sociobiol 24: 291-294

Hölldobler B, Wilson EO (1990) The Ants. Springer Verlag, Berlin, Heidelberg, New York

Johannsmeier MF (1983) Experiences with the Cape bee in the Transvaal. S Afr Bee J 55: 130-138

Katzav-Gozansky T, Soroker V, Ibarra F, Francke W, Hefetz A (2001) Dufour's gland secretion of the queen honeybee (*Apis mellifera*): an egg discriminator pheromone or a queen signal? Behav Ecol Sociobiol 51: 76-86

Katzav-Gozansky T, Soroker V, Hefetz A (2002) Evolution of worker sterility in honey bees: egg-laying workers express queen-like secretion in Dufour's gland. Behav Ecol Sociobiol 51: 588-589

Korst PJAM, Velthuis HHW (1982) The nature of trophallaxis in honeybees. Insectes Soc 29: 209-221

Kryger P (2001a) The Capensis pseudo-clone, a social parasite of African honey bees. In: Proceedings of the 2001 Berlin Meeting of the European Section of IUSSI, Sept. 25-29, p 208

Kryger P (2001b) The pseudo-clone of *Apis mellifera capensis* – an obligate social parasite in honeybees. In: Proceedings of the XXXVII International Apicultural Congress, Durban South Africa, p 33

Le Masne G (1956) Recherces sur les fourmis parasites: *Plagiolepis grassei* et l'evolution des Plagiolepis parasites. C R Acad Sci 243:673-675.

Lobo JA, Kerr WE (1993) Estimation of the number of matings in *Apis mellifera*: extensions of the model and comparison of different estimates. Eth Ecol Evol 3: 337-345.

Lundie AE (1954) Laying workers produce worker bees. S Afr Bee J 29: 10-11

Mackensen O (1953). The occurrence of parthenogenetic females in some strains of honeybees. J Econom Entomol 36: 465-467

Magnuson P (1995) Field observations. In: Magnuson P (ed) Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, pp 5-9

Martin S, Beekman M, Wossler TC, Ratnieks FLW (2002a) Self-replicating honeybees evade worker policing. Nature 415: 163 - 165

Martin S, Wossler TC, Kryger P (2002b) Usurpation of *Apis mellifera scutellata* colonies by *A. m. capensis* workers. Apidologie 33: 215-232

Mayr E (1963) Animal Species and Evolution. Harvard University Press, Cambridge MA

Moritz RFA (1986) Two parthenogenetical strategies of laying workers in honeybee populations (*Apis mellifera* L.). Entomol Generalis 11, 159-164

Moritz RFA (1989) Colony level and within colony level selection in honeybees. A two allele population model for *Apis mellifera capensis*. Behav Ecol Sociobiol 25: 437-444

Moritz RFA (2002) Population dynamics of the Cape bee phenomenon: The impact of parasitic laying worker clones in apiaries and natural populations. Apidologie 33: 233-244

Moritz RFA, Haberl M (1994) Lack of meiotic recombination in thelytokous parthenogenesis of laying workers of *Apis mellifera capensis* (the Cape honeybee). Heredity 73: 98-102

Moritz RFA, Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis*). Behav Ecol Sociobiol 17: 87-89

Moritz RFA, Southwick EE (1992) Bees as superorganisms. An evolutionary reality. Springer Verlag, Berlin, Heidelberg, New York

Moritz RFA, Neumann P (1996) Genetic analysis of the drifting of drones in *Apis mellifera* using multilocus DNA-fingerprinting. Ethology 102: 580-590

Moritz RFA, Kryger P, Allsopp M (1996) Competition for royalty in bees. Nature 384: 522

Moritz RFA, Beye M, Hepburn RH (1998) Estimating the contribution of laying workers to population fitness in African honeybees (*Apis mellifera*) with molecular markers. Insectes Soc 45: 277-287

Moritz RFA, Kryger P, Allsopp M (1999) Lack of worker policing in the Cape Honeybee (*Apis mellifera capensis*). Behaviour 136: 1079-1092

Moritz RFA, Simon UE, Crewe RM (2000) Pheromonal contest between honeybee workers. Naturwissenschaften, 87: 395-397

Moritz RFA, Crewe RM, Hepburn HR (2001) The role of the queen for the spatial distribution of workers in *Apis mellifera*. Ethology 107: 1-13

Moritz RFA, Crewe RM, Hepburn HR (2002) Queen evasion and mandibular gland secretion of honeybee workers (*Apis mellifera* L.). Insectes Soc 49: 86-91.

Neumann P, Moritz RFA, (2000). Testing genetic variance hypotheses for the evolution of polyandry in the honeybee (*Apis mellifera* L.). Insectes Soc 47: 271-279

Neumann P, Hepburn HR (2002) Behavioural basis for social parasitism of Cape honeybees (*Apis mellifera capensis* Esch.). Apidologie 33: 165-192

Neumann P, Hepburn HR, Radloff SE (2000a) Modes of worker reproduction, reproductive dominance and brood cell construction in queenless honeybee (*Apis mellifera*) colonies. Apidologie 31: 479-486

Neumann P, Moritz RFA, Mautz D (2000b) Colony evaluation is not affected by the drifting of drone and worker honeybees (*Apis mellifera* L.) at a performance testing apiary. Apidologie 31: 67-79

Neumann P, Pirk CWW, Hepburn HR, Radloff SE (2001a) A scientific note on the natural merger of two honeybee colonies (*Apis mellifera capensis*). Apidologie 32: 113-114

Neumann P, Pirk CWW, Moritz RFA, Hepburn HR (2001b) Trade off between dispersal capacity and virulence in a social parasitic Cape honeybee clone (*Apis mellifera capensis* Esch.). In: Proceedings of the IUSSI – Meeting of the European sections, Berlin, Germany, p 106

Neumann P, Radloff SE, Moritz RFA, Hepburn HR, Reece SL (2001c) Social parasitism by honeybee workers (*Apis mellifera capensis* Esch.): Host finding and resistance of hybrid host colonies. Behav Ecol 12: 419-428

Oldroyd BP, Ratnieks FLW (2000) Anarchistic honey bee workers evade worker policing by laying eggs that have low removal rates. Behav Ecol Sociobiol 47: 268-273

Oldroyd BP, Smolenski AJ, Cornuet JM Crozier RH (1994) Anarchy in the beehive. Nature 371: 749

Oldroyd BP, Ratnieks FLW, Wossler TC (2002) Egg-marking pheromones in honey-bees *Apis mellifera*. Behav Ecol Sociobiol (2002) 51: 590-591

Onions GW (1912) South African "fertile-worker bees". S Afr Agric J 1: 720-728

Onions GW (1914) South African "fertile" worker-bees. Agric J Union S Afr 7: 44-46

Page RE, Erickson EH (1988) Reproduction by worker honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 23: 117-126

Palmer KA, Oldroyd BP (2000) Evolution of multiple mating in the genus *Apis*. Apidologie 31: 235-248

Pedersen BV (1996) A phylogenetic analysis of cuckoo bumblebees (*Psithyrus*, Lepeletier) and bumblebees (*Bombus*, Latreille) inferred from sequences of the mitochondrial gene cytochrome oxidase. I. Mol Phylogenet Evol 5: 289-297

Pettey FW (1922) Workers laying in comb of extracting supers, Elsenberg Apiary. J Dept Agric Union South Afr 4: 122-124

Pirk CWW, Neumann P, Hepburn HR (2002) Egg removal and egg laying by workers are positively correlated in queenright Cape honeybee colonies (*Apis mellifera capensis* Esch.), Apidologie 33: 203-212

Plettner E, Slessor KN, Winston ML, Oliver JE (1996) Caste-selective pheromone biosynthesis in honeybees. Science 271: 1851-1853

Plettner E, Slessor KN, Winston ML (1998) Biosynthesis of mandibular acids in honey bees (*Apis mellifera*): De novo synthesis, route of fatty acid hydroxylation and caste selective beta-oxidation. Insect Biochem Mol Biol *28*: 31-42

Radloff SE, Hepburn HR, Neumann P, Moritz RFA, Kryger P (2002) A method for estimating variation in the phenotypic expression of morphological characters by thelytokous parthenogenesis in *Apis mellifera capensis* (Esch.). Apidologie 33: 129-137

Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial insects. Am Nat 132: 217-236

Ratnieks FLW (1993) Egg-laying, egg-removal, and ovarian development by workers in queenright honeybee colonies. Behav Ecol Sociobiol 32:191-198

Ratnieks FLW (1995) Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. J Apic Res 34: 31-37

Ratnieks FLW, Visscher PK (1989) Worker policing in the honey bee. Nature 342: 796-797

Rauschmayer F (1928) Das Verfliegen der Bienen und die optische Orientierung am Bienenstand. Archiv f Bienenkunde 9: 249-322

Ribbands CR (1953) The behaviour and social life of honeybees, Bee Research Association Limited, London, UK

Rinderer TE, Hellmich RL, Danka RG, Collins AM (1985) Male reproductive parasitism. A factor in the Africanization of honeybee populations. Science 228:1119-1121

Roubik DW (1989) Ecology and natural history of tropical bees. Cambridge, Cambridge University Press

Ruttner F (1976) The problem of the Cape bee (*Apis mellifera capensis* Escholtz): parthenogenesis - size of population - evolution. Apidologie 8:281-294

Ruttner F, Hesse B (1981) Rassenspezifische Unterschiede in Ovarentwicklung und Eiablage von weisellosen Arbeiterinnen der Honigbiene *Apis mellifera* L. Apidologie 12:159-183

Ruttner F (1992) Naturgeschichte der Honigbienen. Ehrenwirth Verlag, München.

Sakagami SF (1958) The false queen: fourth adjustive response in dequeened colonies. Behav 8: 280-296

Schmid-Hempel P (1998) Parasites in social insects, Princeton University Press, New Jersey, USA

Seeley TD (1985) Honeybee Ecology, Princeton University Press, New Jersey, USA

Simon UE, Moritz RFA, MORITZ RFA, Crewe RM (2001) The ontogenetic pattern of mandibular gland components in queenless worker bees (*Apis mellifera capensis* Esch.). J Insect Physiol 47: 735-738

Slessor KN, Foster LJ, Winston ML (1998) Royal Flavours: Honeybee Queen Pheromones. In: Vander Meer RK, Breed MD, Winston ML, Espelie KE (eds) Pheromone communication in Social Insects. Westview Press, Boulder CO

Solignac M, Kryger P, Allsopp M, Mougel F, Koeniger N, Baudry E, Cornuet JM, 2001. Thelytoky in Cape honey bees: a genomic perspective. In: Proc of the 2001 Berlin Meeting of the European sections of IUSSI; Sept. 25-29, 2001, Berlin, Germany, p. 147.

Swart JD, Johannsmeier MF, Tribe GD, Kryger P (2001) Diseases and pests of honeybees. In: Johannsmeier M.F. (ed) Beekeeping in South Africa, 3rd edition, revised, Plant Protection Research Institute Handbook No. 14, Agricultural Research Council of South Africa, Pretoria, South Africa, pp 198-222

Tribe GD (1981) Cape honeybee behaviour. S Afr Bee J 53: 10-12

Tribe GD (1983) What is the Cape bee? S Afr Bee J 55: 77-87

Tucker KW (1958) Automictic parthenogenesis in the honeybee. Genetics 43: 299-316

Velthuis HHW (1970a) Queen substance from the abdomen of the honeybee queen. Z Vgl Physiol 70:210-222

Velthuis HHW (1970b) Ovarian development in *Apis mellifera* worker bees. Ent Exp Appl 13:377-394

Velthuis HHW (1972) Observations on the transmission of the queen substance in the honeybee by attendants of the queen. Behaviour 41:105-129

Velthuis HHW (1976) Egg laying, aggression and dominance in bees, in: Proceedings of the 15th International Congress of Entomology, Washington, pp 436-449

Velthuis HHW, Ruttner F, Crewe RM (1990) Differentiation in reproductive physiology and behaviour during the development of laying worker honeybees. In: Engels W (ed) Social Insects. Springer Verlag, Berlin Heidelberg New York, pp 231-243

Velthuis HHW, van der Kerk A (1988) Age, environment, and genes in relation to the mandibular gland secretion of pure and hybrid *Apis mellifera capensis* worker bees. In: Needham GR, Page RE, Delfinado-Baker M, Bowman CE (eds) Africanized honeybees and bee mites. Ellis Horwood, Chichester, pp 80-86

Velthuis HHW, van Es J (1964) Some functional aspects of the mandibular glands of the queen honeybee. J Apic Res 3:11-16

Velthuis HHW, Verheijen FJ, Gottenbos AJ (1965) Laying worker honeybee: Similarities to the queen. Nature 207: 1314

Vergara C, Dretz A, Percz de Leon A (1993) Female parasitism of European honey bees by Africanized honey bee swarms in Mexico. J Apic Res 32: 34-40

Visscher PK (1989) A quantitative study of worker reproduction in queenright honey bee colonies. Behav Ecol Sociobiol 25: 247-254

Visscher PK (1996) Reproductive conflict in honey bees: a stalemate of worker egg-laying and policing. Behav Ecol Sociobiol 39:237

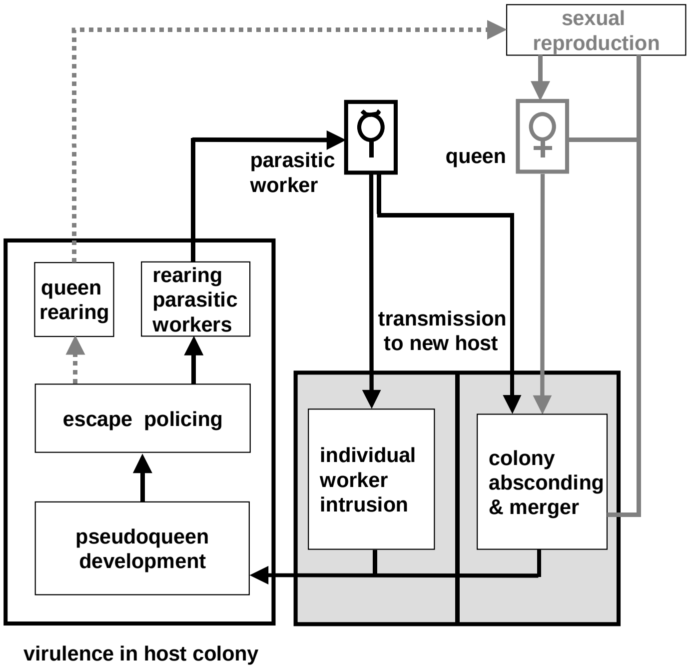
Visscher PK, Dukas R (1995) Honey bees recognise development of nestmates' ovaries. Anim Behav 49: 542–544.

Wilson EO (1971) The insect societies. Cambridge, Harvard University Press

Wossler TC (2002) Pheromone mimicry by *Apis mellifera capensis* social parasites leads to reproductive anarchy in host *A. m. scutellata* colonies. Apidologie 33: 139-163

Woyke J (1995) Invasion of Capensis bee. In: Magnuson P (ed) Proceedings of the First International Electronic Conference on the Cape Bee problem in South Africa, 05-30 June 1995, PPRI, Pretoria, p 35

**Figure 1.** Reproductive cycle of social parasitic Cape honeybee workers (*Apis mellifera capensis* Esch.). (Grey boxes and grey lines = bisexual reproductive pathway of *A. m. capensis*; black boxes and black lines = social parasitic pathway of laying workers; dotted lines = rare events). Transmission to new hosts (grey box) can occur via individual worker intrusion and/or via colony absconding and mergers. Successfully invading social parasitic workers need to establish themselves as pseudoqueens which is facilitated by a high virulence (white box) Their eggs escape worker policing in the host colonies. The host colony's worker preferentially feed the parasitic offspring so that parasitic workers with highly developed ovaries are reared which can be assumed to be highly virulent. High numbers of parasitic workers can be reared in the host, which can infest new hosts via the individual or the colony pathway, thereby completing the social parasitic life cycle of *A. m. capensis* workers. Queen rearing from laying worker offspring has never been observed in infested *A. m. scutellata* colonies.



# Curriculum vitae of
# Peter Neumann

## Personal data

| | |
|---|---|
| Nationality: | German |
| Born on: | 14.December 1967 |
| in: | Berlin |

## Education

| | | |
|---|---|---|
| Prim. school: | 1974-1980 | Robinson-Grundschule, Berlin |
| Highschool: | 1980-1987 | Lessing-Oberschule, Berlin |
| Study of Chemistry: | 1987-1989 | TU Berlin |
| Vordiplom: | 18.10.1988 | |
| Study of Biology: | 1989-1994 | FU Berlin |
| Vordiplom: | 14.11.1991 | |
| MSc thesis: | Title: "Untersuchungen mit DNA-fingerprinting zum Drohnenverflug bei der Honigbiene (*Apis mellifera* L.)" Principal investigator: Prof. Dr. B Schricker | |
| MSc: | 25.10.1994 | |
| Postgrad. study: | 1994-1998 | TU Berlin & MLU Halle-Wittenberg |
| PhD thesis: | MLU Halle-Wittenberg, Title: "The impact of polyandry and drifting on the genotypic composition of honeybee (*Apis mellifera* L.) colonies" Principal investigator: Prof. Dr. RFA Moritz | |
| PhD: | 16.07.1998 | |

## Professional Career

| Position | Period |
|---|---|
| Laboratory Assistant, at the FG Kernresonanz und Röntgen, Schering AG, Berlin, Germany | 20.02.-5.04.1991 |
| Volunteer, at the Zoologischer Garten Berlin, Berlin, Germany | 13.04.-8.05.1992 |
| Laboratory Assistant, at the Institut für Humangenetik, FU Berlin, Berlin, Germany | 1.01.-31.03.1993 |
| Teaching Student Assistant, at the Institut für Biologie, TU Berlin, Berlin, Germany | 1.04.1993-31.12.1994 |
| Teaching and Research Assistant; at the Institut für Ökologie und Biologie, TU Berlin, Berlin, Germany in a DFG funded research project,<br>Principal investigator: Prof. Dr. RFA Moritz | 1.01.1995-1.03.1998 |
| Teaching and Research Assistant, at the Institut für Zoologie, MLU Halle-Wittenberg, Halle/Saale, Germany in a DFG funded research project, Principal investigator: Prof. Dr. RFA Moritz | 2.03.1998-31.12.1998 |
| Temporary Visiting Researcher, at the Department of Genetics, Uppsala University, Sweden in a DAAD funded exchange project,<br>Principal investigators: Prof. Dr. P Pamilo & Prof. Dr. RFA Moritz | 1.01.1997-31.12.1998 |
| Visiting Researcher at the Ecological Research Station of Uppsala University, Öland, Sweden, Principal Collaborator: Dr. J Tengö | 1.05.1997-18.07.1997 &<br>1.07.-23.07.1998 |
| Visiting Researcher at the Department of Animal and Plant Science, Sheffield University, Sheffield, UK,<br>Principal Collaborator: Dr. FLW Ratnieks | 1.09.1997-16.09.1997 |

| Position | Period |
|---|---|
| Postdoctoral Research Fellow at the Department of Zoology & Entomology, Rhodes University, Grahamstown, Republic of South Africa, Principal Collaborators: Prof. Dr. HR Hepburn & Prof. Dr. SE Radloff | 1.01.1999 – 31.03.2001 |
| Visiting Researcher at the Institut für Zoologie, FU Berlin, Germany, Principal Collaborators: Prof. Dr. B Schricker & Dr. B Polaczek | 22.05.-29.05.1999 & 12.12.-17.12.1999 |
| Visiting Researcher at the Institut für Zoologie/Molekulare Ökologie, MLU Halle-Wittenberg, Halle/Saale, Germany, Principal Collaborators: Prof. Dr. RFA Moritz & Dr. P Bliss | 1.06.-4.07.1999 & 18.12.-23.12.1999 & 3.05.-20.06.2000 |
| Junior research group leader at the Institut für Zoologie, MLU Halle-Wittenberg, Halle/Saale, Germany | Since 1.04.2001 |
| Visiting Researcher at the Department of Zoology & Entomology, Rhodes University, Grahamstown, Republic of South Africa, Principal Collaborators: Prof. Dr. HR Hepburn & Prof. Dr. SE Radloff | 6.01.-07.04.2002 & 05.02.-14.02.2003 |
| Visiting Researcher at the Department of Sericultural and Apicultural Science, Zhejiang University, Hang Zhou, China, Principal Collaborator: Prof. Hu Fuliang | 4.08.-12.08.2002 & 22.08.-23.08.2002 |
| Visiting Researcher at the Eastern Bee Research Institute of Yunnan Agricultural University, Kunming, China, Principal Collaborators: Prof. Dr. He Sao Yu & Dr. Tan Ken | 13.08.-21.08.2002 |
| Visiting Researcher at the Department of Zoology & Entomology, University of Pretoria, Pretoria, Republic of South Africa, Principal Collaborators: Prof. Dr. RM Crewe & Dr. P Kryger | 2.01.-19.03.2003 |

**Memberships in scientific organisations**

International Union for the Study of Social Insects (IUSSI)

**Referee for international scientific journals**

Animal Behaviour / Annals of the Entomological Society of America / Apidologie / Bee World / Behavioural Ecology / Behavioural Ecology and Sociobiology / Ethology / Insectes Sociaux / Journal of Apicultural Research / Journal of Economic Entomology / Naturwissenschaften / Proceedings of the Royal Society [B]

**Guest editor**

Moritz RFA & Neumann P (2003) The Cape honeybee (*Apis mellifera capensis*): from laying workers to social parasites. Apidologie **33** (2)

**Awards and fellowships**

| | | |
|---|---|---|
| Book Award | German Chemical Industry | 1987 |
| Research Fellow | Rhodes University | 1999-2001 |
| Emmy Nöther Fellow | DFG | Since 2001 |
| Visiting Professor | Rhodes University | Since 2003 |
| Christian Wolff Award | MLU Halle-Wittenberg | 2003 |

| **Scientific activity** | **Number** | **Details** |
|---|---|---|
| **Co-operations (past & present)** | 21 | Columbia / Ethiopia / Finland / Germany / Jordan / Poland<br>PR of China / South Africa / Sweden / UK / USA |
| **Grants** | 3 | ~219781.90 € |
| **Congresses** | 28 | 13 international meetings |
| **Talks** | 49 | 2 plenary lectures, 20 invited talks |
| **Posters** | 16 | one poster awarded with Evenius prize |
| **Publications** | 86 | 38 articles in international peer reviewed journals,<br>42 conference proceedings, 5 popular articles,<br>one guideline for authorities (Brandenburg customs) |

Halle/Saale, Germany, Friday, 17. December 2004

Dr. Peter Neumann

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**Eidesstattliche Erklärung**

Hiermit erkläre ich an Eides statt, dass ich die Habilitationsschrift selbständig und ohne fremde Hilfe verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht wurden.

Halle/Saale den 17. Dezember 2004 Dr. Peter Neumann