The wild honeybees of Sudan: A test case for conservation, apiculture and invasive species

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Dedication

To my beloved family and wife

Dedication is also to all who work in the field of beekeeping

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Introduction

The honey bee genus *Apis* comprises a suite of different species including the cave breeding *Apis mellifera, A. cerana and A. koschevnikovi;* the free breeding dwarf honeybees *A. andreniformis, A. florea and A. nigrocincta* and the free breeding giant honeybees *A. dorsata* and *A. laboriosa.* The western honeybee *A. mellifera* is endemic to Europe, Africa and western Asia (Michener 1974; Ruttner 1988) and composed of more than 24 subspecies distributed in Europe, Asia and Africa. Non-managed colonies readily survive in the wild and large wild populations particularly occur in Africa. In addition *A. mellifera* has been introduced by man to all continents except Antarctica. The introduced *A. mellifera* for apiculture have been extremely successful in establishing feral populations in America and Australia (Moritz et al. 2005).

Wild and feral honeybees occur in colonies usually centred in natural cavities independently of managed hives that are maintained by beekeepers (Paton 1996). Honeybees, both feral and managed are frequent visitors at flowers and often remove 80% or more of the floral resources produced (Paton 1996, 2000). This can result in competitive displacement of native bees (Paton 1996; Schwarz and Hurst 1997; Schwarz and Hogendoorn 1999; Spessa 1999). Due to degradation and habitat loss as well as large scale bee breeding programs and commercial honeybee queen production wild honeybee populations have been claimed to be extinct in many European countries (Ruttner 1969; Ruttner 1988). Although large wild honeybee populations still exists in Africa (Fig. 1) and Asia large scale imports of colonies and queens has raised similar concerns (Moritz et al. 2005; Dietemann et al. 2009).

All other honeybee species are endemic to Asia (Ruttner 1988; Oldroyd and Wongsiri 2006) where they often occur in sympatry. However, more than two decades ago the dwarf honeybee *A. florea* had been detected out of its Asian endemic range in Africa. Lord and Nagi (1987) reported on an *A. florea* population detected in Khartoum (Sudan) in 1985 raising the additional concern of interspecific competition between native and the introduced novel honeybee species.

Honeybee biodiversity

Morphometric studies have provided a large amount of information on the structure of *Apis mellifera* L. subspecies (Ruttner 1988). Moreover during the last decades molecular markers such as mitochondrial and microsatellites have become the primary tool to analyse genetic diversity.

Morphometric studies

The honeybee *Apis mellifera* L. has been classified morphologically into 29 subspecies (Ruttner 1988; Engel 1999). These are grouped into four biogeographic branches M, C, A and O according to their distribution in Europe and Africa with an origin in the Middle East (Ruttner 1988). Genetic diversity in European honeybees (*Apis mellifera*) falls into two postglacial lineages. One lineage (M) includes two subspecies (*A. m. iberica, A. m. mellifera*) ranging from Gibraltar to Scandinavia and from Ireland to Poland. The other lineage (C) includes subspecies from central Europe and the northern Mediterranean *A. m. siciliana, A. m. ligustica, A. m. cecropia, A. m. macedonica* and *A. m. carnica.*

Fig. 1 Wild honeybee colonies are common in Africa. Photo was taken in Sudan (Sinjah state) during field work and samples collection of wild honey bees for this research.

Concerning the A and O lineage, six subspecies of *Apis mellifera* are taxonomically recognized in North and East Africa: 1) *A. m. lamarckii* in Egypt; 2) *A. m. intermissa* in Morocco, Algeria and Tunisia; 3) *A. m. sahariensis* in Morocco; 4) *A. m. litorea* in south-Somalia and eastern Africa; 5) *A. m. jemenitica* in Sudan, Somalia, Saudi Arabia, Yemen and Oman; 6) *A. m. syriaca* in Israel, Palestine, Syria, Jordan and Lebanon (Ruttner 1988). All of which belong to A lineage except *A. m. syriaca* which belongs to O lineage. Figure 2 illustrated the distribution of *A. mellifera* subspecies based on morphometry (Fuchs 1998b).

Molecular tools

In addition to morphometery phylogeographical studies have been conducted using molecular markers. In the 1980's allozyme studies based on malate dehydrogenase (*Mdh-1*) and hexokinase (*Hk-1*) polymorphisms have been used to characterize *A. mellifera* populations or to determine their racial composition (Sheppard and McPheron 1986; Spivak et al. 1988; Lobo et al. 1989; Lobo 1995). Also nuclear and mitochondrial DNA polymorphisms have been used to characterize African honeybees primarily aiming to classify the various subspecies (Smith and Brown 1988; Hall and Muralidharan 1989; Sheppard et al. 1991a; Clarke et al. 2001). In particular mitochondrial DNA (mtDNA) polymorphisms proved to be a powerful tool to classify honeybee subspecies (Cornuet and Garnery 1991; Smith 1991; Garnery et al. 1992; Moritz et al. 1994). The mtDNA variability in the coxI-coxII region became the standard tool to discriminate among the *A. mellifera* subspecies (Moritz et al. 1994, 1998; Garnery et al. 1995; De la Rúa et al. 1998). This region of the mtDNA harbours a highly variable non-coding repeat motive composed of a single copy motif P followed by a variable number of a repetitive motif Q. Because this region is highly AT-rich, it can be restricted with the enzyme *Dra* I to generate subspecies specific fragment patterns, which yield additional information for classification.

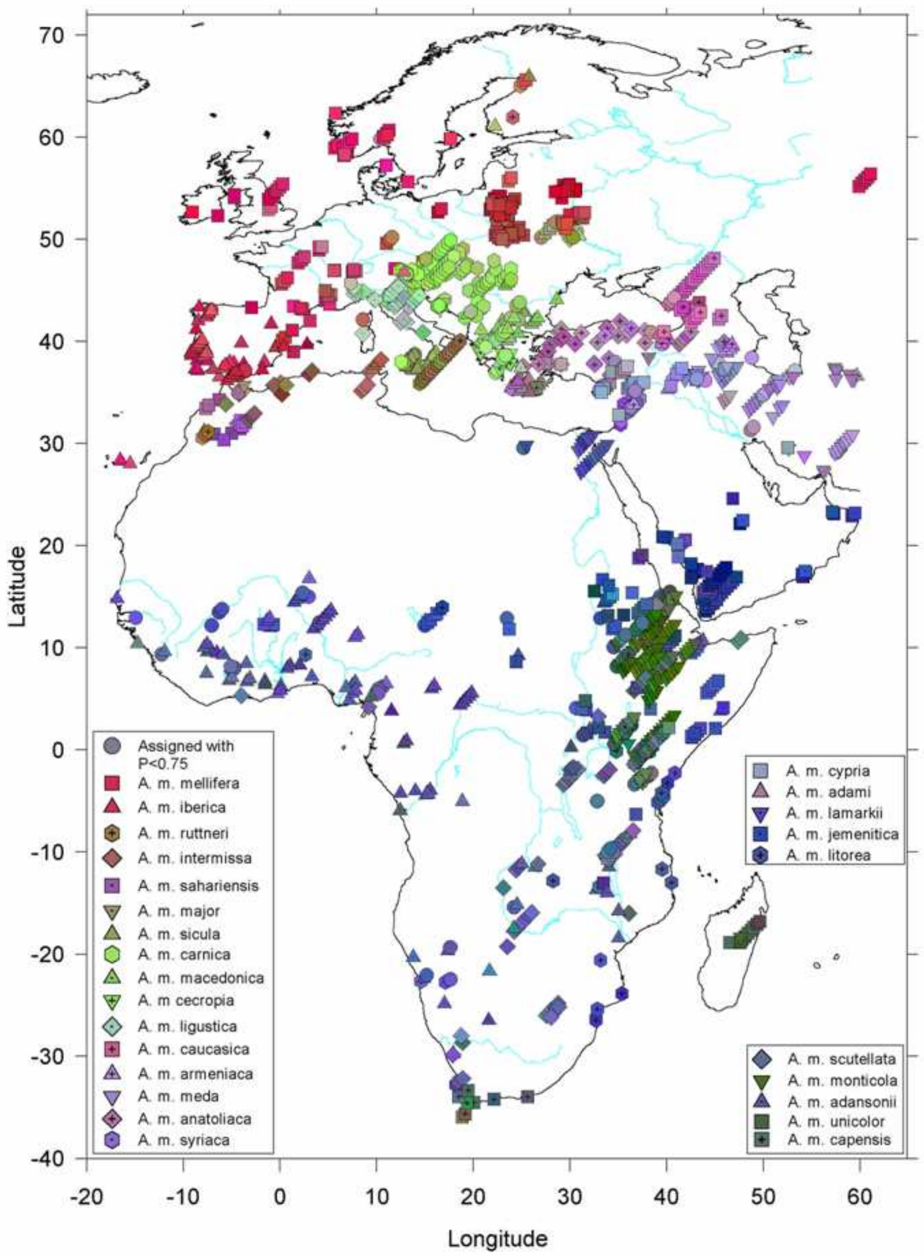

Fig. 2 Honeybees biodiversity and subspecies distribution. Data source (Fuchs 1998b).

Using mtDNA variability, the *Apis mellifera* subspecies could be classified into five major lineages: 1) lineage A including *A. m. intermissa, A. m. adansonii, A. m. scutellata, A. m. capensis* and *A. m. monticola* subspecies; 2) lineage M composed of the western European subspecies *A. m. mellifera* and *A. iberiensis* ;3) lineage C composed of *A. m. ligustica, A. m. carnica* and *A. m. caucasica* ;4) lineage O comprising *A. syriaca* and *A. lamarckii*; 5) lineage Y with *A. jemenitica* (Franck et al. 2001). The distribution of these first four lineages is very similar to the four evolutionary branches inferred from morphometric analysis by Ruttner (1988). Some minor differences concern the branches M and O the former includes also *A. m. intermissa* and *A. m. iberica* while the latter only includes the Near and Middle Eastern subspecies. Recently *A. mellifera* has also been characterized in both native and introduced ranges using 1136 SNP (single-nucleotide polymorphisms) marker (Whitfield et al. 2006). This study revealed that *A. mellifera* originated in Africa contrasting to earlier belief that they might have originated from Eastern honey bees (Ruttner 1988; Arias and Sheppard 2005).These techniques are however often to crude for detecting the impacts of introgression and competition at the population level. Bottlenecks, recent population declines and measuring gene flow can however be excellently analyzed with microsatellite DNA loci in honeybees (Estoup et al. 1994; Franck et al. 1998).Microsatellites were also used to show that African populations have high genetic variability, high effective population sizes, (Franck et al. 2001) and African honeybees were also found to be having the highest degree of multiple mating (polyandry) in comparison to other subspecies (Moritz et al. 1996; Franck et al. 2000; Kraus et al 2004). Moreover, with the advance of microsatellite DNA technologies, it is now not only possible to estimate the effective population size (Estoup et al. 1994) but also feasible to determine the actual numbers of colonies in any given area. Most suitable is an approach to use genotype data that has been obtained by taking advantage of the specific mating behaviour of honeybees with drone congregation areas (DCA) and highly polyandrous queens. Because wild honeybee colonies are extremely cryptic and hard to quantitatively detect in the field (Moritz et al. 2007; Shaibi et al. 2008) these tools based on drone genotypes allow for a precise detection of any introgression of introduced commercial populations into the wild.

The number of drone mates per queen honeybee can vary dramatically, ranging from single mating under extreme conditions of North Sea islands (Neumann et al. 1999a) to up to 45 male per queen in South-African populations (Neumann and Moritz 2000; Moritz et al. 1995, 1996). Since *A. mellifera* queens do not only have a high mean but also a high variance for the number of mates, they provide an excellent test system to study proximate factors affecting multiple mating.

Introduced honeybees & biodiversity conflict

The native honeybee populations are arguably well adapted to local conditions including resistance to specific pest and diseases. For example, wild native African honeybee subspecies were found to be tolerant to the small hive beetle (Neumann and Elzen 2004) and showed enhanced resistance to many diseases in comparison to European honeybee subspecies (Dietemann et al. 2009). The Asian honeybee *A. cerana* established “equilibrium” with its parasite *Varroa jacobsoni* because, among other factors, it can physically remove mites and kill them. Carniolian honeybees *A. m. carnica* have been claimed to be resistant to *Nosema apis* and mites (Sanford 2003). *A. m. jemenitica* is well adapted to the hot dry climate (Ruttner 1988) and characterized by good hygienic behaviour (El-Niweiri and El-Sarrag 2006). Therefore it seems desirable to conserve the biodiversity of native honeybees in their native range by promoting native honeybees in apiculture (Moritz et al. 2005; Dietemann et al. 2009).

Apiculture is a most suitable enterprise to enhance family income in rural areas of developing countries. In many African countries developmental aid projects have therefore fostered the implementation of beekeeping as a tool to improve the socioeconomic structures of underprivileged communities (Illgner et al. 1998). Often these developmental projects are based on the importation of both colonies and queens of non-native honeybees' *A. mellifera.* Consequently, apicultural aid projects often bear considerable risks for the conservation of biodiversity of wild, endemic honeybee populations including three major mechanisms:

1) Imported bees may compete with wild colonies reducing local food availability.

2) Managed colonies may interbreed with wild populations and introgress into local gene pools.

3) Imported honeybees may spread novel diseases eradicating non-adapted regional wild populations.

4) Modern apiculture often relies on long distance migratory beekeeping locally enhancing biodiversity conflicts with the conservation of wild endemic honeybee subspecies.

Competition

Competitive displacement is a major biological driver to facilitate invasions. Invasive species appear to have specific traits or combinations of specific traits that allow them to outcompete native species (Kolar and Lodge 2001). Sometimes they just have the ability to grow and reproduce more rapidly than native species, but often invasion biology is more complex, involving a multiplex of traits and interactions (Thebaud et al. 1996; Reichard and Hamilton 1997). Because of the low density of beekeeping in Africa, the majority of honeybee colonies are still wild (Johannsmeier 2001) but this does not exclude that competition between imported and native bees are important factors in honeybee biology. The expansion and intensification of beekeeping efforts may enhance the competition, introgression and pathogen spill over between managed and wild honeybee populations (Paton 1996*;* Moritz et al. 2005)*.* Because managed hives are maintained throughout the year and fed in periods of dearth, the wild colonies are expected to experience considerable fitness disadvantages compared with the managed ones (Paton 1996)*.*

Introgression

The introgression risk of introduced honeybees is particularly large because the mating of honeybees cannot be controlled. Virgin queens conduct long distance nuptial flights and mate with drones from distant locations (up to 10 km) (Ruttner 1988; Koeniger and Koeniger 1991). Wild and managed populations can therefore easily interbreed causing the loss of local races. The best known example illustrating introgression of imported honeybees may be the case of the Africanized honeybee, where honeybee queens imported from Africa to Brazil in the 1950s spread as "Africanized" honeybee swarms within few decades to the southern states of the US (Visscher and Baptista 1996). In this process they almost completely displaced the European honeybee subspecies used before for apiculture in Latin America (Pinto et al. 2004; Collet et al. 2006; Schneider et al. 2004).

Another example of the negative impact of the introduced subspecies is known from South Africa where large scale migratory beekeeping caused the so called "capensis calamity" in beekeeping. Thousands of *A. m. scutellata* colonies were destroyed due to the invasion of parasitic *A. m. capensis* workers. These parasitic bees can enter a host colony, kill the queen and establish themselves as pseudo-queens to eventually destroy the colony (Neumann and Moritz 2002).

Pest and diseases

The global spread of the devastating *Varroa destructor* mite is probably the most dramatic example for the negative impact of imported stock and the man assisted spread of pests and diseases in apiculture (Morse and Flottum 1997). Imports of *A. mellifera* into Asia caused a spill over of *V. destructor* from the Asian honeybee *A. cerana* to *A. mellifera*. Subsequent colony transhumance of *A. mellifera* caused the spread of this deadly parasite across the world now being a global threat to both managed and wild *A. mellifera*.

Another example is the spread of the Small Hive Beetle (*Aethina tumida*) in North America forming a novel threat to the beekeeping industry in the USA (Neumann and Elzen 2004; Hood 2004). *A. tumida* is originally a harmless parasite of African honeybees south of the Sahara (Pettis and Shimanuki 2000; Neumann and Elzen 2004), but has devastating effects on European honeybees (Elzen et al. 1999; Neumann and Elzen 2004). The introduction of the small hive beetle into other regions is greatly facilitated by apicultural practice such as migratory beekeeping, dirty beekeeping equipment and queen imports (Brown et al. 2002). Recently the Small Hive Beetle was also reported in Ethiopia, Kenya, Zimbabwe and Egypt (Mostafa and Williams 2002).

Case Study

Due to extensive beekeeping activities in Europe, native honeybees have repeatedly been claimed to be extinct and replaced by commercial breeding lines (Ruttner 1969; Ruttner 1988). The impact of apiculture in Africa is, however, much weaker than in Europe (Hepburn and Radloff 1998), because of the much lower density of managed colonies and the much higher overall colony density of honeybees in Africa (Jaffé et al. 2009). Nevertheless, there have been repeated – and often massive - efforts to introduce European honeybee stock into Africa. These introductions are well documented for many African countries including South Africa, Zimbabwe, Zambia, Tanzania, Sudan, Senegal, Kenya, Ghana and Egypt (cited in Hepburn & Radloff 1998). We here focus on the honeybees of Sudan, a region with extremely high ecosystem diversity ranging from desert to rain forest, but with only one recognized honeybee subspecies, *A. m. jemenitica* (Ruttner 1988; Engel 1999).

Until today beekeeping is considered to be a highly beneficial activity and an important source of family income for many traditional beekeepers in Sudan (Fig. 3 top). There were about 200.000 honeybee hives in Sudan with a total number of about 50.000 beekeepers (El-Sarrag et al. 1988). The introduction of modern beekeeping into Sudan in 1928 was followed by vast imports of European honeybee stock including *A. m. ligustica, A. m. carnica, A. m. cypria* and *A. m. lamarkii* crosses. Since then there have been continued and repeated huge imports of foreign honeybees into the country (El-Sarrag and Nagi 1989). For example, during 1998-2003 a total of 16,953 commercial colonies have been introduced to further develop apiculture and implement modern techniques including migratory beekeeping in Sudan. As a consequence, modern apiculture (Fig. 3 bottom) in Sudan today exclusively depends on imported honeybee stock which may or may not pose a biodiversity risk for native honeybee subspecies. At the same time, the invasive dwarf honeybee, *Apis florea*, has been accidentally introduced to Khartoum in 1985 (Lord and Nagi 1987; Mogga and Ruttner 1988) and henceforth established extremely successful feral populations (Fig. 4), which continuously invading other states in Sudan (El-Niweiri et al. 2005).

Fig. 3 Traditional beekeeping in Sudan is based on traditional log hives (top) and native honeybee's subspecies whereas modern beekeeping used modern equipment hive (bottom) and often rely on imported honeybees.

Giving two introduced honeybee species, European *A. mellifera* and *A. florea,* Sudan provides a good model region to study the potential impact of non-native species on the wild native honeybees.

This study aims to assess the potential impact of the introduced honeybees species and beekeeping on the biodiversity of the wild native testing three hypotheses:

1) Introduced non-native honeybees (*A. mellifera* and *A. florea*) cause a competitive displacement with wild native *A. mellifera* colonies.

2) Managed *A. mellifera* colonies interbreed with wild populations and introgress into local gene pools.

3) Imported honeybees spread novel pest and diseases eradicating non-adapted regional wild populations.

Fig. 4 *Apis florea* has established extremely successful feral populations out of its Asian endemic range in Africa. Photo was taken in Sudan during field work and samples collection of *Apis florea* along the river Nile.

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Mitochondrial discrimination of honeybees (*Apis mellifera*) of Sudan

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Abstract

Sudanese honeybee populations are surrounded by a suite of various subspecies with different mitochondrial haplotypes, including the O-lineage in the north (Egypt), the Y-lineage in the east (Ethiopia) and the A-lineage in the south and west. Using *Dra* I analyses and the partial sequence of the tRNAleu COII region of 75 sampled colonies throughout Sudan, we never found the Y-lineage in Sudanese honeybees but instead seven different haplotypes from the A-, O-, and C-lineage (A_1, A_4, A_8, A_{13}, O_1', O_1 and C_2) suggesting that the Y-lineage is not common to Sudan. The mitochondrial haplotypes co-segregated with the highly diverse ecosystems in Sudan. Honeybees of the wet savannah and forest ecosystems showed the A-lineage, identical to *A. m. adansonii* and *A. m. scutellata*. The honeybees in the desert, semi desert, and dry savannah of Sudan have the O-lineage, similar to *A. m. lamarckii* and *A. m. syriaca*. Haplotype C_2 was found on apiaries with imported stock (*A. m. carnica*). This reclassification of the honeybees from Sudan has consequences for the interpretation of the biogeography of *A. mellifera* in the Maghreb and Mashriq regions.

Keywords: *Apis mellifera jemenitica* / subspcies / mitochondrial DNA / Sudan / biogeography.

The impact of apiculture on the genetic structure of wild honeybee populations (*Apis mellifera*) in Sudan

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Abstract

Apiculture often relies on the importation of non-native honeybees (*Apis mellifera*) and large distance migratory beekeeping. These activities can cause biodiversity conflicts with the conservation of wild endemic honeybee subspecies. We studied the impact of large scale honeybee imports on managed and wild honeybee populations in Sudan, a centre of biodiversity of *A. mellifera*, using as set of linked microsatellite DNA loci and mitochondrial DNA markers. The mitochondrial DNA analyses showed that all wild honey bees exclusively belonged to African haplotypes .However; we revealed non-native haplotypes in managed colonies on apiaries reflecting unambiguous evidence of imports from European stock. Moreover, we found significantly higher linkage disequilibria for microsatellite markers in wild populations in regions with apiculture compared to wild populations which had no contact to beekeeping. Introgression of imported honeybees was only measurable at the population level in close vicinity to apicultural activities but not in wild populations which represent the vast majority of honeybees in Sudan.

Keywords: *Apis mellifera* / Beekeeping / Conservation / Honeybee / Microsatellite DNA / Mitochondrial DNA / Introgression / Competition / DNA.

Mating in the rain? Climatic variance for polyandry in the honeybee (*Apis mellifera jemenitica)*

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Population Ecology (in revision)

Abstract

We tested the impact of colony density and climatic conditions on the level of queen polyandry in different wild populations of the honeybee (*Apis mellifera jemenitica*). We identified the mating frequency of queens by genotyping worker offspring (N=672) of 28 wild colonies with microsatellite DNA markers. The populations sampled in different climatic regions in Sudan showed a high variance for the queen mating frequency ranging from 9 to 23 estimated matings with an average of 14 ±3.94 (13.16 ± 4.87 effective matings). The degree of polyandry was highly significantly correlated with the rainfall in the various regions. In general, more rainfall resulted in lower mating frequencies. Polyandry did neither correlate with colony density nor with genetic diversity of the local sample populations. This suggests that variation in polyandry in wild honeybee populations of Sudan is primarily driven by climatic differences among ecosystems variation rather then by the absolute or effective local honeybee population size.

Keywords: Colony density / Ecosystems / Microsatellite DNA / Queen mating.

The invasion of the dwarf honeybee, *Apis florea* along the river Nile in Sudan

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Animal Ecology (in review)

Abstract

The spread of the dwarf honeybee, *Apis florea,* in Sudan along the River Nile in a linear fashion provides a good model to study the population dynamics and genetic analyses of a honeybee invasion. We use microsatellite DNA analyses to asses the population structure of both invasive *A. florea* and native *A. mellifera* along the river. The invasive *A. florea* had significantly higher population densities than the wild native *A. mellifera*. Nevertheless, we found no indication of competitive displacement, suggesting that although *A. florea* has a high invasive potential it coexists with native *A. mellifera* along the river Nile. On the other hand this high population of *A florea* in Sudan appeared to be originated from a single colony introduction more than two decades ago.

Keywords: *Apis florea* / *Apis mellifera* / Microsatellite DNA / Invasion / Competition / River Nile.

Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles

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Journal of Apicultural Research 47: 184–185

Abstract

In this study we report the results of the first systematic survey of honeybee colonies for SHB in Sudan. A total of 117 local honeybee colonies in modern and traditional apiaries (N = 81) and wild ones (N = 36) were inspected in 14 northern states of Sudan.Our surveuy demonstrate for the first time that SHB occur in Sudan. However, this distribution appears to be very limited both in its geographic coverage and in population because 3.42 % of colonies were infested at a low level in two of 14 investigated states. We did not find SHB in the central states (N = 48 colonies) or in the most northern one bordering Egypt (N = 26 colonies). The absence of SHB is likely due to the northern part of River Nile State being very dry and the river itself is mostly bordered by solid rock. Consequently, available pollen and nectar sources attractive to honey bees are exceptionally limited there. This lack of suitable feeding substrata certainly constrains the number of both wild colonies and those kept by beekeepers, thereby minimizing available host colonies for SHB. Moreover, very dry conditions also are likely constrain SHB reproduction, making the Sahara uninhabitable for the beetle. These observations support the view that the Sahara is the natural northernmost distribution limit of SHB. In conclusion, SHB most likely are endemic to sub-Saharan Africa only and have been introduced into Egypt.

Keywords: *Aethina tumida / Apis mellifera* / honey bee / small hive beetle.

Summary

Apiculture is often based on the introduction of non-native honeybee species and subspecies. However, any introduction of non-native species into novel ecosystems can cause extinctions of endemic wild species and hence there has been repeated concern about long distance transport of honeybees across continents. Nevertheless, the introduction of non-native species can be either beneficial or harmful depending on the species and where they are introduced. In this study I used the wild honeybees of Sudan as test case for conservation, apiculture and invasive species. The native honeybee (*Apis mellifera*) of Sudan has heen classified as a single morphocluster and taxonomic subspecies *A. m. jemenitica* (Ruttner 1988). The same subspecies is also characterized in Ethiopia by a specific mtDNA haplotype lineage called the Y-lineage (Franck et al. 2001). *A. m. jemenitica* is well adapted to the harsh and hot climate (Ruttner 1988 ; Hepburn and Radloff 1998), has good hygienic behaviour, free of foul brood disease and parasitic mites (El-Niweiri 2004) and like most African subspecies it is supposed to be adapted to the natural enemies such as the small hive beetle (Neumann and Elzen 2004).

Due to the repeated introductions of European *Apis mellifera* subspecies and the accidental introduction of *Apis florea* Fab., I tested the potential impact of these two different species on the native honeybees of Sudan in the course of three major mechanisms: 1) Managed colonies may interbreed with wild populations and introgress into local gene pools. 2) Imported bees may compete with wild colonies reducing local food availability. 3) Imported honeybees may spread novel diseases eradicating non-adapted regional wild populations

To detect any maternal introgression, I firstly discriminated the native wild honeybees of Sudan using a mitochondrial DNA marker tool. In this dissertation, I showed that honeybees of the Y lineage may represent only a very small fraction of the wild honeybees native to Sudan. *Dra* I analyses and the partial sequence of the tRNAleu COII region of the mtDNA of 75 sampled colonies throughout Sudan revealed that native honeybees of Sudan are much more diverse and are composed of six different haplotypes (A_1, A_4, A_8, A_{13}, O_1', O_1) corresponding to two different lineages, A and O instead of only one expected lineage (Y) (El-Niweiri and Moritz 2008)

Differences in climate and vegetation have been identified as the major causes of subspecies diversification (Potts and Behrensmeyer 1992). Our study confirms this view since the climatic and ecosystem differences fit well with the distribution of A and O haplotypes in Sudan. The O haplotypes were observed in the desert regions, whereas the A haplotypes were frequent in the more tropical climate. Mixed populations with A and O haplotypes were found in the transitional zones (between wet and dry ecosystems) (El-Niweiri and Moritz 2008).

Mixed populations were also found in managed apiaries where the mitochondrial DNA analyses also revealed non-native haplotypes. Since mtDNA analyses classified all wild samples exclusively to six different African haplotypes either beloging to the, A or the O lineage and we only found the European C_2 haplotype in colonies on two commercial apiaries, this represents unambiguous evidence of European imported honeybee stock. Although this is of principle concern from a conservation point of view, the import seems to have had no far reaching impact yet, because it was exclusively confined to the few

commercial apiaries sampled. Given the complete lack of European haplotypes in the wild colonies in spite of repeated and massive introductions over many decades (Mogga 1988; El-Sarrag and Nagi 1989), this may be another case where natural selection favours locally adapted *A. mellifera* and purges maladapted imported stock from the population (Moritz et al. 2005). The mal-adaption of imported stock to local conditions of Sudan may be driven by climatic conditions, which range from hot deserts to rain forests probably selecting for a high diversity of native honeybee subspecies (El-Niweiri and Moritz 2008, 2009) but against imported honeybees. Moreover the tolerances to specific pest and diseases, particularly the small hive beetle, which is endemic to Sudan and sub-Saharan Africa may select for native rather than imported honeybees (El-Niweiri et al. 2008). Particularly the small hive beetle which is detrimental to European honeybees (Elzen et al. 1999; Hood 2004; Neumann and Elzen 2004) causes little problems for African honeybees.

The introgression of honeybees through swarming can result in very different scenarios in honeybee populations and depends on the species and where it introduced. For instance the introduction of European honeybees *A. m. mellifera* and *A. m. ligustica* into northern Africa (Second 1975), did not cause maternal introgression into African honeybees (De la Rua et al. 1998). In contrast, the African honeybees introduced in the neotropics primarily spread by maternal introgression (=swarming) into the feral populations (Taylor 1988; Hall and Muralidharan 1989; Smith et al. 1989; Hall and Smith 1991), probably due to the extreme migratory behaviour, with multiple swarming and high adaptability of African honeybees to the tropics climate (Rashad and El-Sarrag 1978; Hepburn and Radloff 1998). Although I found no evidence of introgression through swarming, I did find evidence of paternal introgression through mating in locations with beekeeping activities. Linkage disequilibria were significantly enhanced in regions with apicultural activities. I can therefore not exclude that introgression through mating in locations with beekeeping activities is detectable at the population level (El-Niweiri and Moritz 2009). This kind of introgression is difficult to be managed, since the mating of honeybees queen is extremely promiscuous and cannot be controlled. Both wild and managed populations can easily interbreed, facilitating gene flow by sexual recombination. In this study, 1 found high variance for the native honeybee queen mating frequency ranging from 9 to 23 estimated matings with an average of 14±3.94 drones per queen. The degree of polyandry was highly significantly correlated with the rainfall in the sampled regions (El-Niweiri and Moritz 2009, Population Ecology, in review). This result suggested that variation in polyandry could be driven by climatic differences among ecosystems variation rather then by the absolute or effective local honeybee population density. Hence, in spite of the low density of imported stock, it may have an impact through paternal gene flow.

An introduced species might become invasive, if it can out-compete native species for resources such as nutrients and nesting sites. In this thesis, I assess the competition potential of the introduced honeybees in Sudan. I studied the impact of both introduced *A. mellifera* used in commercial apiculture and the introduced Dwarf honeybee (*A. florea*). The dwarf honeybees have been introduced to Sudan more than two decades ago and there is concern it might displace native bee species. Under a competitive displacement scenario a high density of introduced colonies should cause low native *A. mellifera* densities. Using microsatellite, I calculated the population densities based on the number of colonies detected and the mating flight range as in Moritz et al. (2007). I found no significant differences in population densities between sample locations with and without beekeeping, which often relies on the importation of non-native honeybees (*Apis mellifera*) (El-Niweiri and Moritz 2009). So if any competition between wild and managed populations had occurred, the effect was too small to be detected with our sample size. The dwarf honeybee *A. florea* had significantly higher population densities than the wild native *A. mellifera*. Nevertheless, we found no indication of competitive displacement, suggesting that although *A. florea* has a high invasive potential it coexists with native *A. mellifera* along the river Nile (El-Niweiri and Moritz 2009 submitted to Animal Ecology). Morphometric studies also did not show any impact of the imported bees on the native honeybee populations in Sudan (El-Sarrag 1977; El-Niweiri et al. 2007). In contrast to the introduction of the African honeybee into Brazil, where 50 introduced queens replaced the introduced European honeybees in America within a few decades, the repeated massive introductions of European honeybees into Africa do not seem to have caused any competitive displacement. This is also true for North Africa, where repeated large scale introductions of colonies into Tunisia (Lebdigrissa 1991) and Libya (Shaibi et al. 2009) had no or very little impact on the endemic honeybee populations. However, although there are no indications for competitive displacement, Franck (1999) reported on nuclear introgression of European alleles into Moroccan honeybee populations and also I detected indications of introgression in honeybee populations in Sudan (El-Niweiri and Moritz 2009) . This suggests that although invasions and competition within the genus *Apis* is well known (Moritz et al. 2005), the ability of non-native honeybees to compete with the native ones again seems to be highly subspecies specific and depends on where they have been introduced. Moreover, invasive species appear to have specific traits or combinations of specific traits that allow them to outcompete native species. For distance diseases are known to greatly facilitate invasive replacements, particularly if they are harmless to the invader but lethal to the resident species (Goulson 2003). This is also true in the Sudanese test case, since imported European bees found to be responsible of spreading *Varroa* mite in Sudan (El-Niweiri et al 2007). In contrast, A*. florea* colonies found to be free of pest and disease (El-Niweiri and El-Sarrag 2006) and even enhance the native pollinator spectrum (El Shafie et al. 2002). Nevertheless, I caution to see this as a story of success for *A. florea* invasions in general. I can definitely not exclude that in other parts of the world, with other *A. florea* populations, with other *A. mellifera* subspecies, with different parasite loads, in different ecosystems problems may appear like they did so often when man introduced non-native species.

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Zusammenfassung

Der Import nicht-heimischer Bienenarten oder –unterarten in neue Ökosysteme kann massive Veränderungen der heimischen Bienen-Populationen bis hin zur ihrer Verdrängung nach sich ziehen. Besonders deutlich wird dies an der Einführung afrikanischer Bienen in Brasilien, die sich als Hybride innerhalb von drei Jahrzehnten über fast den gesamten südamerikanischen Kontinent verbreitet hatten. Die Situation im Sudan ist möglicherweise ähnlich gelagert, weil Imker dort innerhalb von 10 Jahren mehr als 30000 Völker europäischer Bienenrassen einführten. Es stellte sich deshalb im Rahmen dieser Dissertation die Frage, ob die heimischen sudanesischen Honigbienen der Rasse *Apis mellifera jemenitica* durch den Import dieser Völker bedroht sind. Dabei sollte untersucht werden, ob die importierten Rassen den Genpool lokaler Honigbienen-Populationen beeinflussen, ob die putative Konkurrenz lokaler und importierter Bienenvölker um Nahrungsressourcen zu einer Reduktion der heimischen Bienenvölker führt und ob die importierten Bienenvölker neue Krankheiten oder Parasiten mit sich bringen, die zur Auslöschung nicht-adaptierter sudanesicher Bienenvölker beitragen könnten.

Maternale Introgression in lokale Genpools wurde durch Genotypisierung der mitochondrialen (mt) DNA von 75 wilden Bienenvölkern aus dem ganzen Land verteilt erfasst. Die nativen Bienen im Sudan tragen sechs verschiedene mt-Haplotypen (A_1 , A_4, A_8 , A_{13}, O_1, O_1'). Dabei korreliert das Auftreten der O- und A-Haplotypen mit klimatischen und ökosystemaren Bedingungen: O-Haplotypen finden sich in Wüsten- und Halbwüsten-Zonen , A-Haplotypen kommen in den tropischeren Regionen des Sudans vor. Gemischte Populationen mit beiden Haplotypen treten in den Regionen zwischen den tropischen und trockenen Klimaregionen auf. In den Regionen, in denen Imkerei betrieben wird, finden sich auch Bienenvölker mit dem europäischen Haplotyp C_2. Allerdings weisen alle wilden Honigbienen-Völker in diesen Regionen die O- und A-Haplotypen auf, was bedeutet, dass sich die importierten Bienen nicht durch Schwärmen erfolgreich ausgewildert haben. Vermutlich sind die lokalen wilden Völker von *Apis mellifera* besser an ihre Umgebung adaptiert als die importierten Bienenvölker. Klimafaktoren mögen dabei eine zentrale Rolle spielen. Außerdem sind die afrikanischen Bienen besser an den kleinen Beutenkäfer *Aethina tumida* angepasst, der im Rahmen dieser Dissertation in den südlichen Regionen des Sudans erstmalig nachgewiesen wurde. *Aethina tumida* verursacht massive Schäden an Völkern europäischer Rassen und kann somit ihrer Auswilderung entgegenwirken.

Obwohl es keine Hinweise auf maternale Introgression durch Schwärmen von Bienenvölkern gibt, findet in Regionen mit kommerzieller Imkerei Introgression in lokale Genpools durch die Paarung von Königinnen mit Drohnen europäischer Herkunft statt. Hier herrscht verstärktes Kopplungsungleichwicht in den Völkern, die zur Analyse der Paarung von Königinnen an verschiedenen Orten im Sudan mit Imkerei aufgestellt wurden, im Vergleich zu Völkern, die an Standorten ohne Imkerei aufgestellt wurden. Die paternale Introgression kann kaum verhindert werden, weil sich Königinnen mit vielen Drohnen paaren. In den experimentellen Völkern betrug die Paarungsfrequenz zwischen 9 und 23 Drohnen mit einem Durchschnitt von 14 ± 4 Paarungen/Königin. Der Grad der Polyandrie ist hochsignifikant mit dem Niederschlag in den Regionen korreliert: Je höher der Niederschlag, desto niedriger ist der Grad der Polyandie. D.h., dass die

Variation in der Polyandrie eher durch die klimatischen Unterschiede als durch Variation der absoluten oder effektiven Populationsdichte an Bienenvölkern bedingt ist. Vergleicht man die Populationsdichten der nativen Bienenvölker in Regionen mit und ohne Imkerei, so ergeben sich keine nachweisbaren Unterschiede. D.h. zusammengefasst, dass die importierten europäischen Bienenvölker derzeit keine Bedrohung für die sudanesischen Wild-Populationen von *A. mellifera* darstellen, auch wenn ein Einfluss durch paternale Introgression über lange Zeiträume wahrscheinlich scheint.

Neben dem Import europäischer Bienen stellt die zufällige Einfuhr der südostasiatischen Zwerghonigbiene *Apis florea* im Jahr 1985 eine weitere putative Gefahr für die nativen Honigbienen dar. Deshalb wurde untersucht, ob *A. florea* die nativen Honigbienen in ihrem Invasionsgebiet entlang des Nils verdrängt. Die Zwerghonigbiene weist zwar eine hohes invasives Potential auf, da sie inzwischen etwa dreimal höhere Populationsdichten als die westliche Honigbiene hat, aber gibt bislang keinen Hinweis auf eine Verdrängung der heimischen Art. Beide Arten scheinen vielmehr in den agrarisch geprägten Regionen entlang des Nils zu koexistieren.

Obwohl innerhalb der Gattung *Apis* Konkurrenz, erfolgreiche Invasion und Verdrängung zwischen und innerhalb von Arten gut bekannt sind, zeigt sich bei den Studien im Sudan, dass die Fähigkeit nichtheimischer Unterarten und Arten mit den heimischen Unterarten erfolgreich zu konkurrieren, sehr von der ihrer Spezifität und den ökosystemaren Bedingungen abhängt.

Conclusion

The introduction of non-native honeybee species in Sudan suggested that non-native honeybees can be either beneficial or harmful depending on when and where they are introduced.

Mitochondrial RFLP studies allow for detecting of introduced and native honeybees and provide an important data base for developing coherent policies for the conservation of honeybees in their native range.

Climate variability plays major role for the diversification of honeybee's subspecies in Sudan.

The mitochondrial introgression of imported European honeybees into native African honeybees of Sudan is barely detectable. This may be due to the highly prolific life history profile of native honeybee subspecies with extreme migratory behaviour, multiple swarming and high adaptability to the new habitat and natural enemies.

Paternal introgression of imported European honeybees into native African honeybees of Sudan, can be detected regardless to the subspecies, population density and introduced location. Since the mating of honeybees cannot be controlled and both native and non-native populations can easily interbreed, gene flow will occur by sexual recombination.

Variation in polyandry of honeybees of Sudan could be driven by climatic differences among ecosystems variation rather then by the absolute or effective local honeybee population size.

Competition is one of the main biological factors that facilitate invasion. However, invasive species appear to have specific traits or combinations of specific traits that allow them to outcompete native species. For instance, the invasive *A. florea* in Sudan have the ability to adapt new habitat and the ability to grow and reproduce more rapidly than native species, whereas the imported *A. mellifera* found to be genetically introgressed in native gene pool and transfer diseases that could be lethal to the resident species.

The finding of the European C_2 haplotype, a high linkage disequilibria and the disease transfer in the vicinity of apiaries suggested that apiculture may not have a positive impact on the wild honeybee population in Sudan.

The best strategy to conserve native honeybee's population is to prevent imports of honeybees and promote the use of native honeybees in apiculture: a win-win scenario for all, the beekeeper, honeybee's health and biodiversity conservation of regional honeybee races.

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Appendix

Declaration on the Author Contributions

I. El-Niweiri MAA, Moritz RFA (2008) Mitochondrial discrimination of honeybees (*Apis mellifera*) of Sudan. Apidologie 39:566–573

First author: project idea, samples collection, DNA analysis, writing and preparing manuscript 95%
Second author: Supervision (5%)

II. El-Niweiri MAA, Moritz RFA (2009) The impact of apiculture on the genetic structure of wild honeybee populations in Sudan. Insect Conservation DOI: 10.1007/s10841-009-9231-4

First author: samples collection, experiments setting, DNA analysis, statistical data analysis, writing and preparing manuscript (90)
Second author: project idea and supervision (10%)

III. El-Niweiri MAA, Moritz RFA (2009) The invasion of the dwarf honeybee, *Apis florea* along the river Nile in Sudan. Submitted to Animal Ecology

First author: project idea, samples collection, experiments setting, DNA analysis, statistical data analysis, writing manuscript (95)
Second author: Supervision (5%)

IV. El-Niweiri MAA, El-Sarrag MS, Neumann P (2008) Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles. Journal of Apicultural Research 47(3):184–185

First author: field survey and samples collection, writing preparing manuscript (75%)
and identification of samples with the help of the second author (5%)
Third author: project idea, participation in writing of the manuscript and confirmation of specimens (20%)

V. El-Niweiri MAA, Moritz RFA (2009) Mating in rain? Climatic variance for polyandry in the honeybee (*Apis mellifera jemenitica*). Population Ecology (in revision)

First author: project idea, samples collection, DNA analysis, writing manuscript 95%
Second author: Supervision (5%)

Curriculum Vitae

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Education

- 1988-1992: Higher secondary school certificate, 24 El-Gorashi, Sudan
- 1993-1998: Bachelor of Science, honours degree, Option Entomology, Faculty of Agriculture, University of Khartoum , Sudan
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Conference contributions

- El-Niweiri MAA, El-Sarrag MSA (2004) Survey of the pests and diseases of honeybees in Sudan.The second national conference of pest management in Sudan, University of Gezeira ,Madeni, Sudan
- El-Niweiri MAA, Omer E, Moritz RFA (2007) Distribution of native and non-native honey bees in Sudan. OR at: 20th meeting of the German section of the IUSSI, 25th-28th Sept. 2007, Bochum, Germany.
- El-Niweiri MAA, Satti AA (2008) Status quo of honeybee (*Apis mellifera* L.) pests and diseases in Sudan. The 23rd International Congress of Entomology, 6 – 12 July 2008, International Convention Centre, Durban, South Africa.
- El-Niweiri MAA , El-Sarrag MSA, Satti AA (2005) Detection, distribution and incidences of insect pests and predators of honeybees (*Apis mellifera* L.) in Sudan. The 4th Arab International Apicultural Conference, 24-27 November 2005, Damascus, Syria.
- El-Niweiri MAA, El-Sarrag MSA, Satti AA (2007). Diseases and parasites of honeybees (*Apis mellifera* L.) in Sudan. The 5th Arab International Apicultural Conference, 25-28 November 2007, Tripoli, Libya.

- El-Niweiri MAA, Satti AA (2009) Detection of a New Threat Added to the Natural Impacts Hampering Spreading of Honeybees in Northern Sudan.The 6th Arab International Apicultural Conference, 25-28 November 2007, Abha, Saudi rabia
- El-Niweiri MAA, Moritz RFA (2008) The impact of beekeeping on the genetic structure of wild honeybee populations in Sudan. Third European Conference of Apidology EurBee, Belfast ,British, 10-14 Sept. 2008

Training courses

- Collection and Preservation of Biological Specimens. Faculty of Science, University of Khartoum, Sudan.
- Molecular Biology, Ministry of Health, Sudan.
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- Molecular Ecology, Institute of Zoology, Martin Luther University, Halle, Germany.

Work experiences

- 2000-06/2002: Research assistant, Biological control Department, Environment and Natural Resources Research Institute, National Centre for Research Khartoum, Sudan.
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- Member of Sudanese Agriculture Engineers Union
- Member of Sudanese Association for Environment Conservation

Publications

*El-Niweiri MAA, El-Sarrag MS, Neumann P (2008) Filling the Sudan gap: the northernmost natural distribution limit of small hive beetles. Journal of Apicultural Research 47(3): 184–185

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*El-Niweiri MAA, Moritz RFA (2009) Mating in rain? Climatic variance for polyandry in the honeybee (*Apis mellifera jemenitica*) Population Ecology (in revision)

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Publications denoted by * indicate the papers, on which the thesis is based.

Erklärung

Hiermit erkläre ich, dass diese Arbeit von mir bisher weder der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ich erkläre, dass ich mich bisher noch nicht um den Doktorgrad beworben habe.

Ferner erkläre ich, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe.

Halle (Saale), den 25. 08. 2009

Mogbel A A El-Niweiri

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