"The pollination potential of bumblebee drones"

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The Pollination Potential of Bumblebee Drones

PhD – thesis

By

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Dedicated to

Vera & Vincent J. Wolf

and

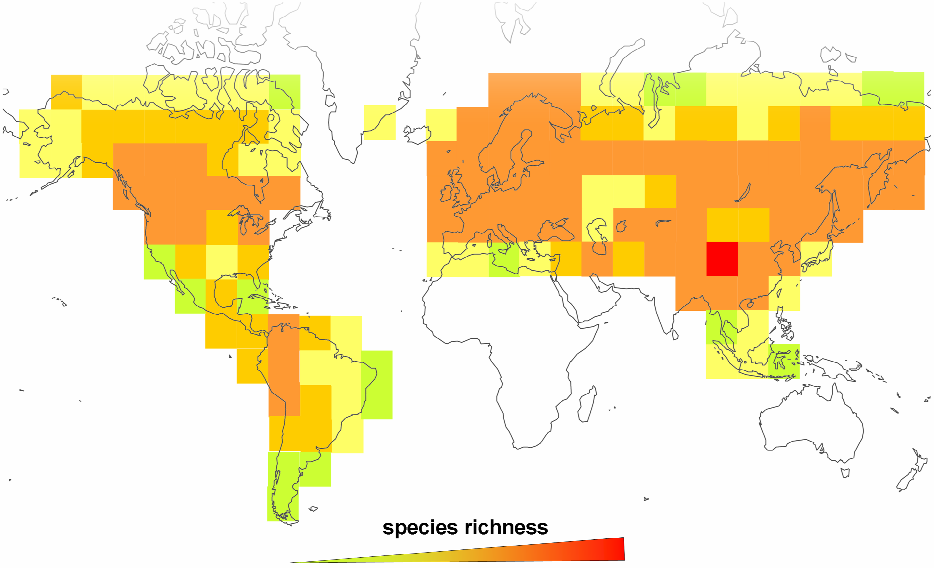
Gertrud Rast

Contents

Chapter I – General Introduction

Bumblebees are large, colourful and hairy bees that belong to the family Apidae (true bees). Within the Family Apidae (true bees) they form the distinct tribe Bombini, comprising true bumblebees (*Bombus*) and cuckoo bumblebees (*Psithyrus*)(Mauss 1994), though there is an ongoing discussion about the classification within the tribe. Worldwide between 250 (Williams 1998, Cameron *et al*. 2007) and 300 (Pedersen 1996, 2002) species are recognized. Before anthropogenically introduced to Africa, Australia and New Zealand (Goulson 2003[b]) bumblebees were, except very few species found in the East Indies and South America (Cameron & Williams 2003), naturally distributed in the Northern hemisphere. Within this range they typically occupy arctic, temperate or alpine habitats (Pedersen 2002) having a centre of diversity in the Himalayan region (Williams 1994, Cameron & Williams 2003)(Fig. 1). In Europe there are about 53 species of true bumblebees and 10 species of cuckoo bumblebees, where they occur in almost every habitat type, with some preferably inhabiting open lands (e.g. *Bombus ruderarius*), woodland (*B. monticola*) or alpine habitats (*B. alpinus*). Other species, such as *B. terrestris*, *B. lucorum* or *B. hypnorum*, are ubiquitous, and can be found in a wide range of natural and man-made habitats, including parks and gardens (von Hagen 1991).

Figure 1: Natural distribution of bumblebees (re-drawn after Williams 1994)



Among all bees (Apidae), bumblebees have attracted an intensive scientific interest, which reaches back over 150 years (Newman (1851), cited in Goulson 2003[a]) (first monograph on bumblebees by Sladen in 1912). One factor driving this interest is their biology as such.

Bumblebees are primitively eusocial insects forming annual colonies that consist of numerous sterile workers, which are headed by a mostly singly mated queen (Estoup *et al.* 1995, Schmid-Hempel & Schmid-Hempel 2000, Strassmann 2001)(Fig. 2). Here *B. hypnorum* is an exception among European bumblebees having queens that do occasionally mate multiply (Estoup *et al.* 1995, Brown *et al.* 2002).

Life history

Typically queens mate in autumn and exclusively enter hibernation, whereas the entire old colony (i.e. the old queen and the workers) and the drones die by the end of the season (e.g. Alford 1969, Plowright & Laverty 1984). Queens emerge from hibernation in spring and start searching for a suitable nest site. The exact time of emergence and nest foundation, however, may vary substantially among and within species (Plowright & Laverty 1984, Goulson 2003[a]) starting as early as February or March (*B. terrestris*) and lasting up to May or June (*B. sylvarum*).

In this phase of the life cycle bumblebees may be regarded as almost solitary insects, with the queens being in charge of all tasks necessary to rear the colony, such as foraging, incubating the brood and producing new eggs. The transition towards the truly eusocial phase begins with the emergence of the first and second worker generation that increasingly take over colony maintenance from the queen, who now exclusively devotes herself to reproduction leading to an exponentially growing worker force (Plowright & Laverty 1984, Goulson *et al.* 2002). Presumably triggered by the density of workers in the colony, the queen at some point switches (i.e. switch point) to produce eggs destined to become reproductives. As bumblebees are haplodiploid fertilized eggs will develop into new queens yet have a prolonged larval phase in contrast to worker larvae. Unfertilized, haploid eggs evolve into males, the drones (Goulson 2003[a]). About one week after the switch point (Duchateau & Velthuis 1988) some of the typically unmated workers ("elite workers", van Honk *&* Hogeweg 1981, van Doorn & Heringa 1986, Owen & Plowright 1982, Duchateau & Velthuis 1989) may start to compete with the queen over the control of reproduction (i.e. competition point) by laying unfertilized eggs, that develop into drones. The rate of successfully reared worker-laid drones, however, may vary greatly among and within species, ranging from no worker reproduction to almost exclusively worker-produced drones (Owen & Plowright 1982, Fletcher & Ross 1985, Bourke 1988, Paxton *et al.* 2001, Brown *et al.* 2003, Alaux *et al.* 2004).

In general, the reproductive strategy of a colony depends mainly on the size of its workforce. Whereas small colonies may never reach reproductive stage and perish, medium sized colonies dominantly produce energetically "cheap" drones. Only strong colonies are able to produce both queens and drones (Müller & Schmid-Hempel 1992[b], Goulson 2003[a]). However, numerous other factors may affect the overall colony performance determining the colonies reproductive success, such as foraging conditions and efficiency (e.g. Bowers 1985, Müller & Schmid-Hempel 1992[a], Corbet *et al.* 1995, Schmid-Hempel & Schmid-Hempel 1998, Clavell *et al*. 2008), parasite and pathogen load (Schmid-Hempel 1998, Imhoof & Schmid-Hempel 1999, Clavell *et al.* 2008) and their genetic load (e.g. Gerloff & Schmid-Hempel 2005).

Reproducing colonies, however, may vary strongly in their reproductive strategy and sex ratio (Goulson 2003[a]). Here putatively influencing factors may be the duration of the hibernation-diapause (Beekmann & van Stratum 2000), the local resource availability (Bourke 1997), the timing (Beekman *et al.* 1998, Bourke & Ratnieks 2001) and the reproductive control hold by either workers or the queen, which results in differing sex-rations due to a haplodiploidy-induced asymmetric relatedness (Hamilton 1964, Trivers & Hare 1976, Owen & Plowright 1982, Duchateau & Velthuis 1988, Bourke & Ratnieks 2001, Beekman & Ratnieks 2002, Duchateau *et al*. 2004).

The overall timing of reproduction is, in contrast to temporal variations of colony founding (e.g. Goulson 2003[a]) more or less synchronized (Müller & Schmid-Hempel 2001[a]) with males typically emerging first (protandry)(Bulmer 1983, Bourke 1997, Beekman & van Stratum 1998). Since bumblebee males are, contrary to honeybee drones, able to mate multiply (Röseler 1973) it is thought that early emerging males have greater chance of contributing genes to the next generation (Bulmer 1983).

Figure 2: ***Bombus terrestris*** **queen on the nest** (© S. Wolf)

Ecological importance of bumblebees

In addition to a large suite of studies on the life cycle and colony traits of bumblebees, the scientific interest on these species is also driven by their outstanding ecological importance, namely the pollination service provided by the workers (e.g. Kevan & Baker 1983, Kevan *et al.* 1993, Kearns *et al.* 1998, Kevan 1999, Kevan & Phillips 2001, Packer & Owen 2001, Klein *et al*. 2007)(Fig. 3).

Bumblebees and Pollination

Pollination, i.e. the transfer of pollen from the anthers of one flower to the stigma of the same or another flower, is of fundamental importance to ensure the setting of seeds (Kevan & Baker 1983, Kwak & Jennersten 1991, Kearns *et al.* 1998, Goulson 2003[a], Knight *et al.* 2005[b]). Pollen dispersal is also of major significance in genetically connecting plant populations (e.g. Cresswell *et al.* 1995, 2002, Cresswell & Osborne 2004, Pasquet *et al.* 2008), potentially counteracting detrimental effects of habitat fragmentation on plant populations (e.g. Kearns *et al.* 1998, Aguilar *et al.* 2008).

The transport of pollen may be facilitated by various vectors such as wind (anemophily), water (hydrophily) and various animals (zoophily). The latter played a most significant role in the evolution and diversification of angiosperms (Kevan & Baker 1983, Crepet 1984, Galen 1996) leading to the wide spectrum in floral shape and size (Harder *et al.* 2001), colour (Chittka *et al.* 2001) and fragrance (Raguso 2001), which typically correspond to its pollinator-syndrome (Macior 1967, Galen 1996, Fenster *et al.* 2004). In contrast to wind and water mediated pollen-flow pollinating animals facilitate a by far more effective, directional and predictable transfer of pollen since they are attractable to rewarding flowers (e.g. Gegear & Laverty 2001, Waddington 2001). Consequently, the pollination of flowering plants by animals is of fundamental importance for ecosystem functioning (Regal 1982, Kevan & Baker 1983, Midgley & Bond 1991, Kevan *et al.* 1993, Batra 1995, Sargent & Ackerly 2008).

Insects represent by far the majority of pollinating animals (Kevan & Baker 1983, Kevan *et al*. 1993). Among the huge suite of insect pollinators, the genus *Bombus* has been recognized as one of the most efficient generalist (polylectic) pollinators, especially in the temperate and cold climate zones (Galen & Stanton 1989, Gauld *et al.* 1990, Campbell 1991, Westerkamp 1991, Bingham & Orthner 1998, Kearns & Thomson 2001, Cresswell & Osborne 2004, Hanley *et al*. 2009).

Because bumblebees are eusocial bees with a high local abundance, they out-compete most solitary insect pollinators. Their pollination efficiency even exceeds that of honeybees (*Apis mellifera*) for many wild and managed plants (Westerkamp 1991), which may be attributed to their ability for buzz-pollination and their generally more robust handling of flowers (Kevan *et al*. 1993, Morandin *et al.* 2001[a,b], Goulson 2003[a]). This has also been recognized in agriculture, and there is a rapidly growing and highly competitive market for rearing bumblebee colonies for pollination in greenhouses and orchards (Kearns *et al.* 1998, Morandin *et al.* 2001[a,b]; Dazgan *et al.* 2004, Velthuis & van Doorn 2006). Moreover, bumblebees are excellently adapted to harsh weather conditions and even forage under cold and rainy conditions, when honeybees refrain from foraging flights (e.g. Pedersen 2002, Goulson 2003[a]).

Bumblebees have an outstanding learning and memory capacity (Chittka 1998, Laloi *et al.* 1999, Fauria *et al.* 2002, Spaethe & Chittka 2003, Dyer & Chittka 2004, Stach *et al.* 2004, Dyer *et al.* 2005, Dyer 2006, Riveros & Gronenberg 2009) allowing effective foraging by selectively visiting profitable flowers. Initially innate (colour) preferences guide individual foragers to potentially suitable food resources (e.g. Lunau & Maier 1995, Gumpert 2000, Raine & Chittka 2009). These innate preferences become successively more specific and updated by individual foraging experiences. Foraging bees are mainly conditioned to visual and chemical cues, which are related to the flower itself (e.g. Neal *et al.* 1998, Chittka *et al.* 2001, Raguso 2001, Spaethe *et al.* 2001), but also to signals from prior visitors (Cameron 1981, Goulson *et al.* 1997, Stout *et al.* 1998, Dornhaus & Chittka 1999, 2004, Stout & Goulson 2001, Goulson 2009) or predators (Dukas 2001, Abbott 2006). Although bumblebee workers can exchange information on forage-quality and -quantity inside the colony (Dornhaus & Chittka 1999, 2004) foraging decisions in bumblebees are typically based on individual experiences (Thomson & Chittka 2001).

Figure 3: Pollen-dusted *Bombus terrestris* on willow (© S. Wolf)

The cognitive abilities in bumblebee workers are, however, not only beneficial on the pollinator side but have major consequences for the pollination efficiency (e.g. Plowright & Laverty 1984). “Flower constancy” is the essence of an efficient pollination service, where workers foraging on one type of flowers (majoring), typically disregarding alternative food sources (“minoring”) displaying different signals (Heinrich 1979a, Waser 1986, Goulson 2000, Gegear & Laverty 2001, 2005, Raine & Chittka 2005, 2007). Flower constancy depends on the bees' capacity for learning and memorizing sensory cues of a rewarding flower species that keeps them tracking as long as it provides a profitable nectar and/or pollen reward.

Another important parameter for bee mediated gene-flow among plants is the distance over which pollen may be dispersed (Cresswell *et al*. 1995, Kwak *et al*. 1991, 1998, Pasquet *et al.* 2008). In bumblebees this has been primarily studied in the context of worker foraging distance applying a wide range of different methods. However, there is a great variance in foraging distance estimates among but also within species. Some estimates are as short as 312 m for *B. pascuorum* (Darvill *et al.* 2004), whereas other authors give 449 m (Knight *et al.* 2005[a]). Other species were found to forage equally short with 500m for *B. muscorum* (Walther-Hellwig & Frankl 2000[a,b]) and 450 m for *B. lapidarius* (Knight *et al.* 2005[a]), though larger foraging distances of up to 1500 m are also reported for the latter (Walther-Hellwig & Frankl 2000[a,b]). For *B. terrestris* workers, with enjoyed most scientific attention, foraging distance estimates range from few hundred meters to up to several kilometres depending on the methods used and the ecosystems tested (Dramstad 1996, Osborne *et al*. 1999, Cresswell *et al.* 2000, Walther-Hellwig & Frankl 2000[a,b], Osborne 2006, Darvill *et al.* 2004; Knight *et al.* 2005[a]; Chapman *et al.* 2003; Osborne *et al.* 2008; reviewed by Goulson & Osborne 2009). This inconsistency, especially within one species, indicates a high plasticity of the foraging behaviour depending, among others, on resource availability, quality and distribution (Heinrich 1975, Cartar 1991, Cresswell *et al.* 2000, Cresswell & Osborne 2004, Goulson 2003[a], Baude *et al*. 2008, Wolf & Moritz 2008). Thus, the range of potential pollen dispersal must be supposed to vary greatly with vegetation characteristics, challenging the scientific evaluation of bumblebee-mediated pollen-flow in the field.

Population ecology and population genetics

Based on their importance for the function of ecosystems studies on the population ecology of bumblebees are of particular importance, especially with respect to bumblebee conservation (e.g. Kearns *et al.* 1998, Chapman & Bourke 2001, Packer & Owen 2001, Brown & Paxton 2009, Grixti *et al.* 2009, Murray *et al.* 2009, Williams & Osborne 2009, Zayed 2009).

Bumblebee populations are particularly vulnerable to lose genetic diversity through genetic drift and small population sizes. Both haplodiploidy and sociality are associated with a generally reduced effective population size (N_e) due to reproductive division of labour (i.e. only a small proportion of the female population (queens) is reproducing) and the reduced number of gene-copies contributed by males (Graur 1985, Chapman & Bourke 2001, Packer & Owen 2001, Zayed 2009). In small and isolated populations, as found in fragmented landscapes, this aspect leads to a higher risk of losing genetic diversity by genetic drift and increases the probability of inbreeding (Gerloff *et al.* 2003, Gerloff & Schmid-Hempel 2005, Whitehorn *et al.* 2009). The latter is particularly detrimental in bumblebees because of their complementary sex-determination system (*csd*; e.g. Cook & Crozier 1995, Heimpel & de Boer 2008), where heterozygosity at the sex-locus is required to develop into females (Mackensen 1951). Unfertilized, thus haploid eggs inevitably develop into males. The same, however, is true for diploid eggs that are homozygote at the sex-locus (Duchateau *et al.* 1994, Duchateau & Mariën 1995). Such eggs may amount to 50 % of the queen-laid eggs, provided the queen and the drone, she mated with, share a sex allele, which is likely to occur under strong inbreeding (e.g. Cook & Crozier 1995). Since these eggs, destined to become workers, now develop into either unviable or sterile diploid drones. The colony suffers from a reduced worker-force, yet to the full costs. Such colonies are very likely not to reach reproductive stage further reducing genetic diversity in the next generation (Whitehorn *et al.* 2009). However, since the production of diploid drones reduces the entire female brood, even colonies that succeed to produce new queens will be negatively affected. Here the reduction of queen brood by up to 50% inevitably leads to a decrease in effective population size (Zayed 2004).

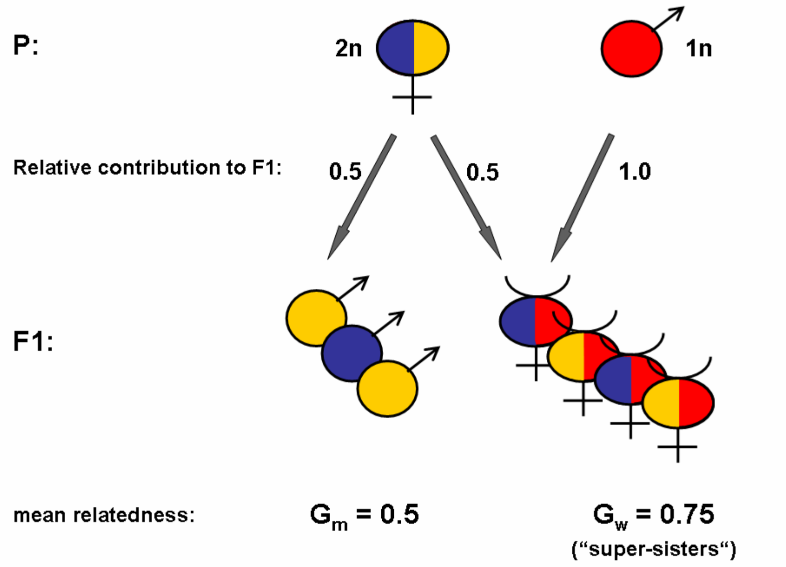
Multiple queen matings (polyandry) in social Hymenoptera are one strategy to raise the effective population size and to reduce the risk of inbreeding (e.g. Ratnieks 1990). Though the evolutionary implications of polyandry in social insects are controversially discussed in literature (e.g. Crozier & Page 1985, Moritz 1985, Keller & Reeve 1994, Strassmann 2000, Crozier & Fjerdingstad 2001, Brown & Schmid-Hempel 2003), it could be shown that the increase in genetic diversity may be associated with improved colony performance (e.g. Fjerdingstad & Crozier 2006). Even though this applies to artificially polyandrous bumblebee colonies either (Baer & Schmid-Hempel 1999), this strategy must be assumed to play a negligible role (Sauter *et al.* 2001) since bumblebees, with very few exceptions, are monoandrous (Schmid-Hempel & Schmid-Hempel 2000).

In sum, bumblebee populations comprise numerous traits negatively affecting effective population size, genetic variability and tolerance towards inbreeding (e.g. reviewed in Zayed 2009). Hence, they are exceptionally susceptible towards anthropogenic disturbance, such as habitat alteration and fragmentation, outlining the importance of studying bumblebees particularly on population level.

The population characterization in bumblebees, however, is rather challenging. As in all social insects, the population size is not determined by the number of workers but by the number of colonies (equally to the number of mated queens), which are typically highly cryptic and require great effort to get reliable estimates from nest counts (Osborne *et al.* 2007). The evaluation of the population size from worker counts is almost impossible since colonies may vary strongly in their contribution to a foraging population per site. Classical methods may therefore provide only limited information about the effective population size and nest densities. They do not allow any evaluation of the genetic profile of a population, including allele diversity, heterozygosity, inbreeding or population sub-structure.

Here the development of genetic tools such as microsatellites (Estoup *et al.* 1995, 1996, Reber-Funk *et al.* 2006) has revolutionized population ecology in general, but especially for social insects including bumblebees. As for the latter, the high intra-colonial relatedness among workers of G = 0.75 (super-sisters), resulting from a single mating (all workers in a colony share the same paternal allele)(Fig. 4), facilitates a reliable assignment of sampled workers to their maternal colony, provided a sufficient number of polymorphic markers is used.

Figure 4: Mating system with haplodiploidy and monoandry



Following population genetic principles (see Hartl & Clarke 1998) population sub-division and degrees of inbreeding may allow completely new insights into bumblebee population ecology. Consequently, molecular tools were applied in context of bumblebee conservation (Zayed 2009), revealing for instance low effective population sizes and alarmingly high degrees of inbreeding in some rare and declining bumblebee species (*B. muscorum:* Darvill *et al.* 2006; *B. sylvarum:* Ellis *et al.* 2006). In other species, however, population genetic analyses indicated vigorous, genetically diverse populations (e.g. Herrmann *et al.* 2007, Kraus *et al.* 2009), heating the debate why some bumblebee species seem to suffer from anthropogenic actions, whereas others remain abundant (e.g. Goulson *et al.* 2005, Fitzpatrick *et al.* 2007, Goulson *et al.* 2008[a,b], Grixti *et al.* 2009, Williams & Osborne 2009, Williams *et al.* 2009).

In addition to conservation issues population genetic tools enable to answer a wide spectrum of other scientific questions. So they are used to shed light into (co-)evolutionary processes (e.g. Schmid-Hempel 2001) and colonization pathways (Estoup *et al.* 1996, Widmer *et al.* 1998), to infer foraging distances and colony densities (Darvill *et al.* 2004, Knight *et al.* 2005[a], 2009) and to assess the effects of land use on bumblebee populations (Herrmann *et al.* 2007), just to name a few.

Irrespective of the huge diversity of scientific questions, methodological approaches and findings, the majority of the studies of bumblebee ecology have one thing in common: they are based on workers. On first sight this seems plausible, since workers are abundant over a long time and are normally easy to observe or to sample. Bumblebee males, which occur only a certain time period in the year and exhibit way less predictable behavioural patterns, have only rarely been addressed. This, however, disregards any function they may have in ecological context, apart from mating.

Bumblebee males

Males in social Hymenoptera have long been the “neglected gender” (Koeniger & Baer 2004, Apidologie special issue (2006)) and were disparaged as “flying sperm-containers”. In comparison to workers and queens, they have received limited scientific attention, which has been intensified just in recent years. The vast majority of the literature on bumblebee drones, however, focuses on their reproduction. On individual level studies concentrate mainly on drone larval development (e.g. Duchateau & Mariën 1995, Duvoisin *et al.* 1999), copulation motivation (Sauter & Brown 2001), sperm transfer (Duvoisin *et al.* 1999), evolutionary implications of copulation duration (Duvoisin *et al.* 1999, Brown *et al.* 2002, Brown & Baer 2005) and accessory gland secretions involved in copulation and female monopolization (Baer *et al.* 2000, 2001, Sauter *et al.* 2001, Brown *et al.* 2002; reviewed by Baer 2003). In this regard, bumblebee drones are even proposed as model organisms for studying sexual selection in social insects (Baer 2003). Individual drones have been also used for taxonomy using species specific pheromone secretions of the labial gland (Bertsch 1997, Bertsch *et al.* 2005) or for testing the hypothesized negative correlation between parasite susceptibility and ploidy (e.g. Ruiz-González & Brown 2006).

On colony level mainly queen mating frequency (Estoup *et al.* 1995, Baer & Schmid-Hempel 1999, Schmid-Hempel & Schmid-Hempel 2000, Strassmann 2001, Brown *et al.* 2002), diploid drone production (e.g. Duchateau *et al.* 1994) and colony sex ratio (e.g. Bourke 1997) have been studied. Population level studies predominantly address the conspicuous female attraction behaviour of drones. Reports on the mating patrol flight patterns of male bumblebees date back to Newman (1851, cited in Goulson 2003[a]) and Darwin (1886) and have since been subject of a number of studies unravelling the pre-copulatory behaviour in bumblebees.

Generally there are two main mate search strategies in bumblebees: “waiting” and “patrolling” (Alcock *et al.* 1978, Eickwort & Ginsberg 1980). Based on the choice of the rendezvous sites (sites where males encounter a female) bumblebee males relying on waiting for a receptive queen may be grouped in two categories: nest-entrance-waiting drones (e.g. *B. californicus* and potentially *B. rufocinctus*: Foster 1992, *B. muscorum*: Darvill *et al.* 2007) and perching drones (e.g. *B. confusus*: Hovorka *et al.* 1998, Kindl *et al.* 1999; *B. morrisoni* and *B. rufocinctus*: Bertsch *et al.* 2008). Nest-entrance waiting drones aggregate close to the entrance of a mature nest competing for access to emerging gynes (Foster 1992, Darvill *et al.* 2007). Drones exhibiting perching behaviour, on the other hand, base themselves on a prominent object in the landscape where they sit or over which they hover, waiting for virgin queens. From their outlook they dart after bypassing gynes or resembling objects, returning to the spot when being unsuccessful (e.g. Eickwort & Ginsberg 1980, Kindl *et al.* 1999, Bertsch *et al.* 2008).

The by far most common pre-mating behaviour in bumblebees is patrolling. Here drones repeatedly visit specific objects along a more or less circular route. In early morning each of these objects (e.g. leaves, tree trunks or prominent rocks, but almost never flowers (Goulson 2003[a])) has been pheromonally marked by the drone. The scent-marks are attractive to both, receptive queens and conspecific males. This leads to routes patrolled by numerous males at the same time. In temperate species typically less than 30 males occupy one patrol-route. In tropical bumblebee species, however, the number of drones per route may be as large as 720 (Stiles 1976, cited in Ayasse *et al.* 2001). Though this increases male-male competition, there is evidence, at least for *B. terrestris*, that queens prefer multiply marked spots, implying that routs patrolled by numerous males are more efficient in attracting queens (Ayasse *et al.* 2001).

Male pheromone composition involved in mate search has been studied in great detail (Stein 1963, Kullenberg *et al.* 1970, Svensson & Bergström 1977, 1979, Bergström *et al.* 1967, 1981, 1996, Bergman & Bergström 1997, Hovorka *et al.* 1998, Kindl *et al.* 1999, Bertsch *et al.* 2008), yet shall not be addressed here in detail. Generally, these pheromones are produced mainly in the labial and mandibular gland and are highly complex mixtures of numerous components.

Though patrolling is in parts well studied there is almost no information on the spatial and temporal dimension of bumblebee mating. Though Frank (1941) found one specific patrol route of *B. hortorum* to be 300 m long, there is almost no information on the area drones cover during their live-time. This includes the distance of a patrol-route to the natal nest of the founder drone, but also a potential switch from one to another patrol route. Both may potentially increase the mating area of a drone substantially, positively affecting effective population size. Here one can only speculate about how genetic diversity is maintained (or not).

The behaviour of drones not on mate search is almost completely ignored, particularly when they are foraging on flowers to provision themselves and fuel the energy consuming patrol flights (Fig. 5). Only very few reports address flower visitation of bumblebee males (e.g. Svensson 1979, Gögler *et al.* 2009).

Figure 5: Nectar-feeding *Bombus terrestris* drone (© S. Wolf)

Only very limited information on the potential impact of the male flight behaviour on pollination is available (male-mediated pollination by *Psithyrus vestalis*: Gögler *et al.* 2009). This is surprising, because colonies used for commercial pollination are typically large colonies. These colonies often reach the reproductive state producing large numbers of drones. Hence, from both an ecological and economical aspects it seems highly rewarding to study the males' pollination potential:

1) Drones frequently feed on flowers to fuel their own energy demands (Svensson 1979). Given this time is lost for mate-search it is reasonable to assume that drones may selectively visit flowers potentially facilitating gene-flow among attractive, conspecific plants.

2) Workers groom their body to collect pollen in their corbiculae (Thorp 1979). This pollen, however, is lost for pollination (Thomson 1986) and causes a considerable discrepancy between workers foraging efficiency and their pollination efficiency (Westerkamp 1991).

Drones, in contrast, lack corbiculae, are often “pollen-powdered” when foraging for food, and may be highly efficient in mediating pollen flow.

3) Foraging in workers is limited by the distance to the nest to which they have to return repeatedly (central place foraging) (e.g. Cresswell *et al*. 2000). Drones, in contrast, never return to the colony once embarked on mate search. Therefore, drones foraging behaviour, independent of the maternal nest location, can be assumed to be less constrained in terms of foraging area and might qualify drones as important long distance pollen vectors. Consequently, the spatial and temporal dimensions of drones mating flights may well be relevant to determine the potential pollen dispersal through drones.

4) In late season drones may occur in very high densities that even exceed that of workers resulting in a high number of flowers potentially visited and pollinated by drones. Here a considerable impact on pollen-flow might be possible.

Aims of this thesis

The goal of this dissertation is to evaluate the pollination potential of bumblebee drones (*Bombus spp*.). Given the weak scientific record on drone flower visitation and flight distances, the presented studies in this thesis not only involve pollen-transport but also focus on the related traits important for a potential drone-mediated pollen-flow. I took advantage of both classical field methods and molecular methods (population genetics) to evaluate pollination-relevant traits of workers in comparison to that of drones, mainly focussing on the Buff-tailed bumblebee *Bombus terrestris* and the Red-tailed bumblebee *B. lapidarius*.

Hereafter the initial ideas behind each study conducted is presented in a concise form in order to justify their relevance for the topic.

Chapter II: My studies include classical methods and population genetic tools. Molecular markers, however, only provide reliable information when sampling is adequate. This not only refers to sample-size but also to the species identity. *B. terrestris* workers, however, are hard to distinguish from workers of *B. lucorum* using morphological traits. Since intermixed samples are very likely to affect any population genetic analyses, the reliability of species identity was evaluated from field samples of *B. terrestris / B. lucorum* in order to ensure reliable data for all further studies involving population genetics.

Chapter III: Pollen carried by either sex should shed light to the pollination potential *per se*. Here a comparison of both the quantity and the quality (type) of pollen carried on an individuals body was chosen to assess the number of carried pollen-grains potentially available for pollination. Moreover, flower choice behaviour (in terms of flower constancy), was evaluated by comparing the frequencies of pollen-types on the bodies of workers and drones.

In addition to the quantity and quality of pollen transferred by workers and drones, strong attention was paid to the spatial dimensions of potential pollen-flow.

Chapter IV: Foraging distance estimates in bumblebees are of great inconsistency. Here bumblebee foraging distance was determined using an exclusively forage providing transect, facilitating not only the recording of the flight distance but also an assessment of the factors mainly influencing foraging behaviour and flight distance of workers.

Chapters V & VI: Male flight distance in the field is particularly hard to measure due to their unpredictable foraging flight patterns. Therefore, I embarked on an indirect estimate of drone flight distances of *B. terrestris* and *B. lapidarius* to their maternal colonies using microsatellite markers in combination with information about worker dispersiveness.

Additionally the temporal dynamics of the drone population was evaluated to assess population sustainability and the spatial extent of potential pollen carry-over.

The presented work is a very first step towards a broadened view to pollination ecology and gives an initial and rough picture of the potential pollen-flow facilitated by bumblebee drones. It attempts to inspire researchers to do more detailed studies on this evidently rewarding and possibly surprising research topic.

Chapter II

The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae)

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Abstract

The bumblebees of the subgenus *Bombus sensu strictu* are a notoriously difficult taxonomic group because identification keys are based on the morphology of the sexuals, yet the workers are easily confused based on morphological characters alone. Based on a large field sample of workers putatively belonging to either *B. terrestris* or *B. lucorum*, we here test the applicability and accuracy of a frequently used taxonomic identification key for continental European bumblebees and mtDNA restriction fragment length polymorphism (RFLP) that are diagnostic for queens to distinguish between *B. terrestris* and *B. lucorum*, two highly abundant but easily confused species in Central Europe. Bumblebee workers were grouped into *B. terrestris* and *B. lucorum* either based on the taxonomic key or their mtDNA RFLP. We also genotyped all workers with six polymorphic microsatellite loci to show which grouping better matched a coherent Hardy-Weinberg population. Firstly we could show that the mtDNA RFLPs diagnostic in queens also allowed an unambiguous discrimination of the two species. Moreover, the population genetic data confirmed that the mtDNA RFLP method is superior to the taxonomic tools available. The morphological key provided 45% misclassifications for *B. lucorum* and 5% for *B. terrestris*. Hence, for studies on *B. terrestris* we recommend to double check species identity with mtDNA RFLP analysis, especially when conducted in Central Europe.

bumblebee/identification/morphology/mtDNA/population genetics

Chapter III

The pollination potential of bumblebee (*Bombus spp*.) drones (Hymenoptera: Apidae)

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***Animal Behaviour*: submitted**

Abstract

Bumblebees are among the most efficient pollinators. The pollination service of a colony is regarded to be tightly linked to foraging distance of workers. The role of flower visiting males as pollen-vectors, however, has been largely neglected. This disregards their long-distance patrolling flights and their less intensive grooming, which may well promote efficient pollen transfer. Here we compared quantity and quality of pollen on the body (pollination active pollen) of workers and drones of two bumblebee species (*Bombus lapidarius* and *B. terrestris*) to assess their flower constancy as measure of their pollination potential.

We show that males of both species visit flowers selectively and exhibit a flower constancy not significantly different to that of workers. However, we found highly significant differences in pollen-types collected by either sex with some types exclusively found in one sex. Although drones carried significantly less pollen than workers, their mean pollen loads exceeded 10.000 grains/individual, suggesting that drones contribute to the colony pollination service by spatially enhancing pollen-flow and expanding the range of pollinated plants. This may have substantial implications for the gene-flow of isolated plant populations in fragmented landscapes that depend on long-distance pollen-transfer.

Bumblebee males, flight distance, flower constancy, pollen-load, fragmented landscapes

Chapter IV

Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae)

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***Apidologie* 39: 419 – 427 (2008)**

Abstract

A major determinant of bumblebees pollination efficiency is the distance of pollen dispersal, which depends on the foraging distance of workers. We employ a transect setting, controlling for both forage and nest location, to assess the foraging distance of *Bombus terrestris* workers and the influence of environmental factors on foraging frequency over distance. The mean foraging distance of *B. terrestris* workers was 267.2m ± 180.3m (max. 800m). Nearly 40% of the workers foraged within 100m around the nest. *B. terrestris* workers have thus rather moderate foraging ranges if rewarding forage is available within vicinity of the nests. We found the spatial distribution and the quality of forage plots to be the major determinants for the bees foraging decision-making, explaining over 80% of the foraging frequency. This low foraging range has implications for using *B. terrestris* colonies as pollinators in agriculture.

bumblebee/foraging/pollination/decision-making

Chapter V

Male flight distance and population sub-structure in the bumblebee *Bombus terrestris*

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Summary

1. Bumblebees are important pollinators in natural as well as agricultural ecosystems. Estimates of foraging range, population size and genetic population structure so far have been based on worker samples alone. Here we include both males and workers in a population genetic analysis to infer the contribution of males to these important ecological parameters.

2. The population genetic (microsatellite) analyses of *Bombus terrestris* L. populations on the island of Cabrera (Spain) and Halle (Germany) revealed high heterozygosities (0.60 ± 0.08 to 0.77 ± 0.13) and neither a deviation from Hardy Weinberg equilibrium nor linkage disequilibrium.

3. We detected five colonies (census population size) for the island population and 27 to 68 for the German mainland population. The genetic effective population sizes were $N_e = 7.5$ for the island and 40.5 to 102 for the mainland population respectively.

4. There was a significant genetic sub-differentiation between the male and the worker population samples, suggesting that males originated from different and/or more distant colonies than workers.

5. Based on the colony numbers we estimated the flight range of males, which ranged from 2.6 km to 9.9 km, much further than worker flight ranges. Bumblebee mediated pollen flow will therefore be much further then expected based on the foraging range of workers alone if males also contribute to pollination.

***Bombus terrestris*, flight distance, microsatellite, population structure, social insects**

Chapter VI

Temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae)

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***Molecular Ecology*: submitted**

Abstract

Bumblebees are of major ecological and economic importance. As in all social Hymenoptera, sociality and haplodiploidy leads to a generally reduced effective population size (N_e) associated to reduced population fitness. Using microsatellites on a large field sample of drones and workers of *Bombus terrestris* and *B. lapidarius* we tested two hypothetical scenarios of drone dispersal potentially increasing effective population size: 1) genetically distinct drone-cohorts sequentially pass through an area or 2) Drones belong to one large temporarily unstructured population with extended mating flight range. We used two different colony assignment approaches, deriving natal queen genotypes either from weekly separate drone sub-samples or from the overall sample, referring to the two Hypotheses. The majority of the drones in our sample-area originate from colonies farther away from the sampling location than the workers foraging range. Our results indicate a clear genetic differentiation between local, worker contributing, and foreign, only drone contributing colonies leading to an increase of the populations gene-pool. However, analysis of colony assignment variance and temporal distribution of drone-contributing colonies suggested Hypothesis 2 to be the more parsimonious one. Though queen dispersal remains to be studies, we argue that N_e is bumblebees may be increased through extended male mating flight ranges, estimated with 10.54 km^2 and 15.98 km^2, *B. terrestris* and *B. lapidarius*, respectively, almost doubling local colonies foraging range in both species. Accordingly, population genetic characterizations indicate strong, genetically highly diverse populations with no signs of inbreeding.

Chapter VII – Outlook

Bombus terrestris drone performance in learning and memorizing floral odours in comparison to workers

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In progress

Aims & Scope

The planned series of experiments will complement the studies on the pollination potential of bumblebee drones.

The aim here is to assess the cognitive capacities of drones in comparison to that of workers. Learning is the basis for efficient worker foraging behaviour, which is highly adaptive for colony functioning. Drones live a solitary life outside the colony and do not forage for pollen. Nevertheless, they also need to visit flowers for nectar take-up to cover their energy balance. Also for drones it should be highly adaptive to memorize rewarding food sources to optimize their net energy gain per time unit (e.g. Pyke 1978, Heinrich 1979[b], Waddington 2001), reduces their risks of predation (Dukas 2001, Abbott 2006), and minimize infection with pathogens and parasites (Dürrer & Schmid-Hempel 1994). We, therefore, hypothesize that the learning performance of drones should be similar to that of workers. This hypothesis is in line with the well developed learning capacity found in honeybees (*Apis mellifera*) drones (Benatar *et al.* 1995) even though they are independent of flower visitation because they are fed by workers in the colony.

I will use appetitive learning assays to evaluate the cognitive abilities in bees. This will include tests of short- and long-term olfactory memories using olfactory learning in drones and workers with the proboscis extension reflex (PER) paradigm. PER is a standard assay in which the bees learn to extend their proboscis towards a conditioned odour stimulus (CS) even if the unconditioned stimulus (US, a drop of sucrose) is not presented (Kuwabara 1957, Bitterman *et al.* 1983, Laloi *et al.* 1999, Laloi & Pham-Delègue 2004). In order to evaluate the cognitive potential for flower constancy, we will assess conditioning, extinction and reversal learning of drones and workers. The rate of extinction is a measure of how well the original association had been learnt (i.e. more constant animals should have slower extinction rates). In reversal learning, an animal, which has learnt that one odorant is rewarded and another is not, has to learn the opposite after inversion of the contingencies by the experimenter. More constant animals are predicted to be slower in reversing the original learning.

Detailed work plan

Study animals - We will use commercially bred *B. terrestris* colonies containing both workers and males for these experiments. In five replicate experiments with 20 drones and workers each, all individuals will be marked using numbered Opalith-tags in order to monitor their experimental treatments over time and to allow keeping all bees within their colony environment. Prior to the experiment, the individual bees will be separated, fed to satiation with sucrose solution (30% w/w), and then starved for 15-18 hours in order to increase the responsiveness towards the unconditioned stimulus (Riveros & Gronenberg 2009).

Floral odorants

We will test the learning abilities for a set of five different floral odours (including lavender, citral, geraniol (Thorn & Smith 1997, Laloi *et al.* 2001), linalool (Laloi *et al.* 1999, 2001), peppermint (Faber *et al.* 1999) following well established protocols for bumblebee workers (Laloi *et al.* 1999). For comparison, neutral (non-floral) odorants will be used (e.g. hexanol, nonanol) to ensure that no spontaneous responses are emitted before conditioning towards the test odorants.

General experimental set-up

The basic experimental set-up consists of a harnessed bumblebee (Fig. 1), which is placed in front of an apparatus allowing standardized application of an air-odorant mixture towards the bees head. The unconditioned stimulus (US), which releases the proboscis reflex, consists of a drop of sucrose that will be manually presented to the bees' antenna while the odour (conditioned stimulus) is presented. Following the experimental setup of Riveros and Gronenberg (2009) individual bees will be trained with the following "training-sequence": 10s air-stream – 8s odoured air – 5 s air-stream. After 3s of the presentation of the odour the bee will additionally receive the unconditioned stimulus (sucrose solution ob the antenna) releasing the PER. Successful PER reflex will be rewarded with a drop of sucrose solution. Retention and extinction of the learned odour-reward-connection will be tested presenting the conditioned stimulus (odour) alone and omitting any reward for the bee (Komischke *et al.* 2002, Riveros & Gronenberg 2009).

Experiments

The experiment will be conducted in three specific experimental set-ups, covering the most relevant parameters for evaluating learning performances in relation to pollination potential:

Responsiveness: In an initial responsiveness test we will touch the antenna with a sugar solution (unconditioned stimulus: US) to release the proboscis extension reflex (U-PER) and test if the animal is responsive at all. Animals not responding to the US will be discarded.

Similarly animals will be confronted with the odour (CS) alone in order to ensure the bees naivety towards the conditioned stimulus. Bees that spontaneously react to an odour will be discarded from the experiment (Riveros & Gronenberg 2009).

Conditioning and learning: In the subsequent conditioning trials, we will pair an odour as the conditioned stimulus (CS) with the US. Individuals extending their proboscis will be fed with 5µl sucrose solution as reward. Five conditioning trials will be conducted for each individual followed by five trials, where we present the odorant without reward in order to measure extinction. The intertrial-intervals (ITI) will be 10min in all cases. Different odours will be tested using the same individuals.

Fig 1) Harnessed *B. terrestris* worker during the conditioning phase. Touching the antennae with sugar solution releases the unconditioned proboscis extension reflex (left) that can be associated to an olfactory stimulus. A positive reaction is rewarded with a sugar droplet (right) (photos: S. Wolf)

Reversal learning: Bumblebees forage in highly unpredictable environment with highly variable nectar/pollen availability (Heinrich 1976, Kevan & Baker 1983, Mačukanović-Jocić *et al*. 2004). This asks for an instantly accessible memory and high learning capacity similar to that of honeybees (Menzel 2001), which learn even reversal associations of stimuli and rewards (Chittka 1998, Komischke *et al*. 2002). Such swift learning bouts will reduce flower constancy because the bees will be able to quickly switch from one species to another more rewarding one. To evaluate such abilities in bumblebees, we will conduct an experiment on reversal olfactory learning again comparing workers and males. In the conditioning phase, two groups of naive bumblebee workers and drones (n (group and sex) = 30), respectively, will encounter either a sequence of four olfactory stimuli (CS+ and CS-) where one cue is reversely associated with a positive and negative stimulus (2 x A+/B- → B+/C-(reversions) or a non-reversed sequence of odours (2 x A+/B- → D+/C- (no reversion). In a final reversive test (C+/A-) the learning performances in both groups will be compared, revealing the capability of reacting to environmental changes such as variations in forage reward or availability. This experiment will be conducted precisely following the assay on honeybees given in Komischke *et al*. (2002) including

1. % responsiveness (PER) towards sucrose solution applied to the antenna
2. conditioning speed (trials needed to successfully associate a CS to a reward, learning curve)
3. Conditioning efficiency, measured in terms of the plateau reached after a fixed number of trials (n = 5)
4. short-term memory measured through the 2nd acquisition trial
5. Retention and extinction
6. Reversal performance

Chapter VIII - Synthesis

Pollination service by bumblebees is as important for ecosystem functioning as it is complex. It is characterized by a high plasticity of both flowering plants and pollinating species towards variations of numerous factors. Therefore, comprehensive assessment of pollination service, especially under natural conditions, is difficult due to many factors interplaying with efficient pollen transfer. This is illustrated by an impressive body of literature on bumblebee pollination. Though almost all aspects of this interaction have been addressed scientifically, these were exclusively assessed for bumblebee workers.

In contrast, bumblebee drones (except for drones of the cuckoo-bumblebee *Psithyrus vestalis* as pollinators of orchids, Gögler *et al*. 2009) to my knowledge never received attention in context of bee-mediated pollen-flow, which is surprising, since at least frequent flower visitation, longevity and the non-exploitation of pollen as protein resource, i.e. a reduced grooming behaviour and the lack of corbiculae, seem to qualify drones as efficient pollen vectors.

However, before embarking on any meaningful ecological research on *B. terrestris* or other bumblebee species, it was necessary to overcome a severe taxonomic problem. Species identification of the *Bombus sensu strictu* - complex in the field is extremely difficult if not impossible. Since this taxonomic problem includes two target species of my thesis, *B. terrestris* and *B. lucorum*, it was essential to resolve this issue. Although morphological keys are available, there are controversial claims about their reliability and I tested the reliability of morphological traits used for species identification of workers in the field with novel molecular tools (Murray *et al*. 2008). I could unambiguously show that the taxonomic keys are insufficient and that workers morphologically classified as *B. terrestris* definitely require confirmation of species status by using mtDNA digestion patterns (Chapter II).

Any evaluation of the pollination potential of bumblebee drones faces the same problems as studies on workers. A huge complexity of varying conditions, interacting environmental factors and behavioural plasticity will interfere with finding simple general rules as answers to any local pollination condition.

In order to assess the general relevance of drones for pollination I, therefore, focused in this thesis on three major drivers determining quality and quantity of pollination performance:

1) flower choice behaviour
2) flower constancy (i.e. subsequent visit of conspecific flowers due to learned preferences)
3) pollen dispersal distances

to evaluate the pollination potential of bumblebee drones compared to that of workers.

I compared the quantity and quality of pollen on the body (i.e. pollen available for pollination) of workers and drones from two common bumblebee species, *B. terrestris* and *B.*

lapidarius, all sampled in the same habitat at the same time (Chapter III). Drones of both species carried substantial amounts of pollen grains on their bodies (albeit less than conspecific workers), that compared well with the amounts of pollen reported for other pollinators. Moreover, the quality of the pollen loads (measured as pollen-type diversity) revealed that drones are as flower constant as workers are. Since flower constancy and, therefore, a highly selective sequential flower visitation is a major factor for efficient pollinators, this clearly indicates a high, yet so far unattended, pollination potential of male bumblebees. Even more surprising, drones of both species differed significantly from their workers in flower preference, which I could infer from pollen diversity analyses. Hence, male pollination not only quantitatively but also qualitatively complements the pollination by workers and the pollination service of a bumblebee colony is due to both workers and males (Chapter III). To also shed light into the mechanisms behind drone flower choice a study on their cognitive capacities towards floral traits is in progress and has potential to further improve our understanding of drones as pollen-vectors (Chapter VII).

In addition to the quantity and quality of pollen available for pollination also the spatial scale plays an important role in bee mediated pollen flow. Drones and workers differ substantially in their flight behaviour and flight range, which very likely affects pollination services. Worker flights are almost exclusively devoted to foraging for the colony and hence they are locally constrained in their foraging to be able to return to the colony (e.g. Cresswell *et al.* 2000, Cresswell & Osborne 2004). Drones, in contrast do not return to the colony and their flights primarily aim at successful mate search.

Using both classical mark and recapture and molecular methods, I determined flight ranges of drones in comparison to the foraging range of workers as a frame-setting measure of potential pollen dispersal. I firstly re-evaluated worker foraging distance to clarify the high inconsistencies and the still ongoing controversies on foraging distance estimates found in the literature (reviewed in Goulson & Osborne 2009). I estimated foraging distance along an exclusively forage providing linear transect (Chapter IV). The rather short foraging distances of *B. terrestris* workers were consistent with findings of other researchers that reported rather short foraging distances for this and other bumblebee species (e.g. Darvill *et al.* 2004, Knight *et al.* 2005[a]). More importantly, however, the experiment showed the strong relationship between resource quality and distribution and the foraging behaviour of bumblebee workers underlining the high plasticity of the behaviour towards these traits (Chapter IV).

The specific flight behaviour of drones (e.g. patrolling), colony-independence and self-provisioning on flowers (less frequent flower visits) render a direct measurement of the (mating) flight range by mark and recapture along a transect impossible. I used molecular tools instead to asses flight ranges of bumblebee males (Chapters V and VI). Since drone producing colonies are typically large and have many workers, these must forage within their foraging range around the colony. Hence, colonies, which exclusively contribute drones to a local sample of bees, must be located farther away from the sampling location than the workers foraging range (provided the non-sampling error is small). Male flight range, therefore, can be indirectly estimated from the worker and drone contributing colony density and the overall number of only-drone contributing colonies in a sample of drones and workers. This approach requires the use of sufficiently variable and reliable genetic markers that allow for accurate assignment of workers and drones to their mother colonies.

Assignment of sampled individuals to putative mother colonies by using highly variable microsatellite DNA markers became routine over the past ten years. Using a large suite of microsatellite markers and population genetic analyses, I firstly showed that the flight range of *B. terrestris* males significantly exceeds the foraging distance of workers (Chapter V). A more detailed study on the temporal structure of the drone population confirmed the extended male flight ranges for *B. terrestris* and for *B. lapidarius* indicating also large but temporally weakly structured drone populations (Chapter VI). Based on these results, drones of *B. terrestris* and *B. lapidarius* can be assumed to be highly dispersive while on mate-search, though the actual spatial and temporal dimensions of individual male patrol routes remain to be studied.

Conclusion

The presented body of work provides a first evaluation of the pollination potential of bumblebee drones under natural conditions. Inevitably this thesis draws a very general and rough picture of the characteristics and importance of drone-mediated pollen-flow. Nonetheless, my results demonstrate that bumblebee drones (*B. terrestris* and *B. lapidarius*) have an impressively high pollination potential and may complement the pollination service of workers quantitatively, qualitatively and at extended spatial scale. Hence, bumblebee male pollination biology opens a wide field of research, which may not only have relevance for natural pollination but also for pollination service in agricultural settings. Here, pollination in both the field and the greenhouse seem worthwhile to be ecologically and economically explored in more detail in the future.

Chapter IX – Zusammenfassung

Die Bestäubung durch Hummeln ist eine ebenso wichtige wie komplexe ökologische Interaktion. Sie zeichnet sich durch eine hohe Plastizität gegenüber Veränderungen in zahlreichen Faktoren, sowohl auf Seiten der blühenden Pflanze, als auch auf Seiten der Bestäuber aus. Die Vielzahl von Faktoren die einen effizienten Pollentransfer beeinflussen können, macht eine umfassende Bewertung der Bestäubungsleistung, vor allem unter natürlichen Bedingungen, schwierig. Dies wird auch durch die große Anzahl von wissenschaftlichen Arbeiten zu diesem Thema verdeutlicht. Obwohl dabei die meisten Aspekte dieser Interaktion wissenschaftlich bearbeitet wurden, fanden jedoch ausschließlich Hummelarbeiterinnen Beachtung.

Im Gegensatz dazu sind Drohnen (mit Ausnahme von Kuckuckshummel-Drohnen (*Psithyrus vestalis*) als Bestäuber von Orchideen, Gögler *et al.* 2009), nach meinem Kenntnisstand, nie im Bezug auf Bestäubung untersucht worden. Das ist überraschend, da häufige Blütenbesuche, ihre Langlebigkeit und die Irrelevanz von Pollen als Proteinquelle, und das damit verbundene verringerte Putzverhalten und das Fehlen von Pollenkörbchen, eine effiziente Pollenübertragung durch Hummeldrohnen wahrscheinlich erscheinen lassen.

Im Vorfeld von aussagekräftigen ökologischen Unteruchungen war es jedoch nötig, eine korrekte Artbestimmung sicherzustellen. Innerhalb des *B. sensu strictu* – Komplexes ist die Bestimmnug der Art im Freiland extrem schwierig, wenn nicht unmöglich. Da dies auch hier untersuchte Arten betrifft, *B. terrestris* und *B. lucorum*, war es unerlässlich dieses Problem zu lösen. Obwohl morphologie-basierte Bestimmungsschlüssel verfügbar sind, ist die Verlässlichkeit dieser Methode kontrovers diskutiert. Daher habe ich die die Verlässlichkeit von morphologischen Merkmalen zur Artunterscheidung mit Hilfe von neuen molekularen Methoden (Murray *et al.* 2008) getestet. Ich konnte dabei eindeutig zeigen, dass Bestimmungsschlüssel eine nur unzureichend genaue Artbestimmung zulassen und Hummlearbeiterinnen von *B. terrestris* eine Nachbestimmung durch mtDNA – Restriktionsmuster erfordern (Kapitel II).

Jegliche Bewertung des Bestäubungspotenzials von Hummeldrohnen stößt auf die selben Probleme, die schon bei Arbeiterinnen deutlich wurden. Die hohe Komplexität aus variierenden Bedingungen, interagierenden Umweltfaktoren und der Verhaltensplastizität behindert das Aufstellen von einfachen und allgemein gültigen Regeln als Antworten auf jegliche lokale Bestäubungssituation. Um die generelle Relevanz von Hummeldrohnen für die Bestäubung und das Bestäubungspotenzial der Drohnen im Vergleich zu Hummelarbeiteinnen zu bewerten, habe ich mich daher auf drei Haupteinflussgrößen konzentriert, welche die Quantität und Qualität der Bestäubungsleistung bestimmen:

1) die Auswahl von Blüten

2) die Blütenstetigkeit (d.h. der aufeinanderfolgende Besuch von artgleichen Blüten aufgrund von erlernten Präferenzen)
3) die Distanz der Pollenverbreitung.

Ich habe die Quantität und Qualität der Pollen auf Bienenkörper (d.h. Pollen die für Bestäubung potenziell verfügbar sind) von zeit- und ortsgleich gesammelten Arbeiterinnen und Drohnen sowohl von *B. terrestris* als auch von *B. lapidarius* verglichen (Kapitel III). Die Drohnen beider Arten trugen erhebliche Mengen Pollen (wenn auch weniger als artgleiche Arbeiterinnen), die mit bekannten Pollenmengen anderer Bestäuber vergleichbar waren. Darüber hinaus konnte über die Pollentypendiversität (d.h. Qualität), eine Blütenstetigkeit bei Drohnen festgestellt werden, die sich nicht signifikant von der der Arbeiterinnen unterschied. Da die Blütenstetigkeit, also der hochselektive aufeinander folgende Blütenbesuch, ein Hauptcharakteristikum von effiziente Bestäubern ist, zeigen diese Ergebnisse deutlich eine hohes, bislang jedoch unbeachtetes, Bestäubungspotenzial von Hummelmännchen. Noch überraschender war der signifikante Unterschied in der Blütenpräferenz zwischen Arbeiterinnen und Drohen bei beiden Hummelarten, der aus der Diversitätsanalyse der Pollen abgeleitet werden konnte. Folglich kann die Bestäubungsleistung von Hummeldrohnen, die der Arbeiterinnen nicht nur quantitativ, sondern auch qualitativ ergänzen. Damit wird die Bestäubungsleistung einer Hummelkolonie von Arbeiterinnen und von Drohnen bestimmt (Kapitel III). Eine Untersuchung zu den kognitiven Fähigkeiten von Drohnen im Bezug auf Blütenmerkmale ist in Bearbeitung, um auch die Mechanismen hinter der Blütenwahl von Drohnen näher zu beleuchten. Dieses Experiment wird potentiell das Verständnis von Drohnen als Pollenvektoren verbessern (Kapitel VII).

Zusätzlich zur Qualität und Quantität der Pollen, die für Bestäubung verfügbar sind, spielt die Räumlichkeit bei der Pollenverbreitung durch Bienen eine wichtige Rolle. Drohnen und Arbeiterinnen unterscheiden sich wesentlich in ihrem Flugverhalten und in ihrer Flugweite.

Der Flug von Hummelarbeiterinnen dient fast ausschließlich der Versorgung der Kolonie, daher sind sie bie der Nahrungssuche lokal gebunden, um eine Rückkehr zum Nest zu gewährleisten (z.B. Cresswell *et al*. 2000; Cresswell & Osborne 2004). Hummeldrohnen fliegen, im Gegesatz dazu, nicht zu ihrer Kolonie zurück, sobald sie diese verlassen haben; ihr Flug ist primär durch die Suche nach Jungköniginnen zur Paarung motiviert.

Unter Verwendung von klassischem Fang-Wiederfang und molekularen Methoden, habe ich die Flugdistanz von Drohnen im Vergleich zur Foragierdistanz von Arbeiterinnen als rahmenbildendes Maß für die Pollenausbreitung, bestimmt. Zuerst habe ich die Foragierdistanz von Arbeiterinnen neu evaluiert, da Schätzungen in der Literatur stark variiren und kontrovers diskutiert sind (zusammengefasst in Goulson & Osborne 2009). Ich habe mich dabei eines geradlinigen und blütengesäumten Transekts bedient, welches das ausschließliche Nahrungsangebot im Untersuchungsgebiet darstellte. Die dabei gefundenen kurzen Foragierdistanzen von *B. terrestris* Arbeiterinnen stehen im Einklang mit den Erkenntnissen anderer Wissenschaftler, die ebenfalls kurze Flugdistanzen für diese und andere Hummelarten gefunden haben (z.B. Darvill *et al.* 2004, Knight *et al.* 2005[a]). Darüber hinaus konnte mit diesem Experiment der starke Zusammenhang von Verteilung und Qualität der Ressourcen und dem Foragierverhalten von Hummelarbeiterinnen deutlich gezeigt werden, was die hohe Plastizität im Verhalten gegenüber diesen Umweltfaktoren unterstreicht.

Das spezifische Flugverhalten von Drohen (z.B. Patrollieren), die Unabhängigkeit von der Kolonie und die Selbstversorgung mit Energie an Blüten machen eine direkte Abschätzung der (Paarungs-) Flugdistanz mittels Fang-Wiederfang entlang eines Transekts unmöglich. Ich habe daher stattdessen molekulare Methoden genutzt, um die Flugdistanz von Hummelmännchen abzuschätzen. Da drohnenproduzierende Kolonien gewöhnlich groß sind, haben sie viele Arbeiterinnen, die im Umkreis der Kolonie foragieren. Man kann daher annehmen, dass Kolonien von denen ausschließlich Drohnen gefunden werden, weiter vom Fangort der Drohnen entfernt sind als die Foragierdistanz der Arbeiterinnen (vorausgesetzt der Schätzfehler durch kleine Stichprobengröße ist gering). Die Flugdistanz der Drohnen kann demnach indirekt aus der Koloniedichte von Arbeiterinnen- und Drohnen-beitragenden Kolonien und der Gesamtanzahl von Drohnen-beitragenden Kolonien abgeschätzt werden. Dieser Ansatz setzt die Verwendung von verlässlichen molekularen Markern voraus, die durch ausreichende Variabilitat eine genau Zuordnung von Drohnen und Arbeiterinnen zu ihren Ursprungskolonien zulassen.

Die Zuordnung von gesammelten Individuen zu ihren mutmaßlichen Mutterkolonien durch hochvariable Mikrosatelliten-DNA hat sich in den vergangenen zehn Jahren zu einer Routinemethode entwickelt. Unter Verwendung einer Vielzahl von Mikrosatellitenmarkern und populationsgenetischen Analysen, konnte ich erstmals zeigen, dass die Flugdistanz von Erdhummeldrohnen (*B. terrestris*) die Foragierdistanz von Arbeiterinnen signifikant übersteigt (Kapitel V). In einem detailierteren Experiment über die zeitliche Dynamik von Drohnenpopulationen bestätigten sich ausgedehnte Flugweiten von Drohnen von *B. terrestris* und *B. lapidarius*. Weiterhin lassen meine Ergebnisse auf große und zeitlich nur schwach strukturierte Drohnenpopulationen schließen. Folglich kann das Verbreitungsvermögen von Drohnen von *B. terrestris* und *B. lapidarius* als hoch angesehen werden, obwohl eine wissenschaftliche Untersuchung der tatsächlichen räumlichen und zeitlichen Dimension der Patrollierwege von individuellen Drohnen noch aussteht.

Fazit

Die vorliegende Arbeit liefert eine erste Bewertung des Bestäubungspotenzials von Hummeldrohnen unter natürlichen Bedingungen. Unvermeidlich muss das hier gezeichnete Bild von den Eigenschaften und der Bedeutung von Drohnen als Bestäuber sehr allgemein und grob bleiben. Ungeachtet dessen zeigen meine Ergebnisse ein eindruckvoll hohes Bestäubungspotenzial von Hummeldrohnen (*B. terrestris* und *B. lapidarius*), welches die Bestäubungsleistung von Arbeiterinnen quantitativ, qualitativ und räumlich ergänzen kann. Daher eröffnet die Bestäubungsbiologie von Hummeldrohnen ein weites Forschungsfeld, welches nicht nur für die Bestäubung unter natürlichen Bedingungen, sondern auch ökonomisch, d.h. landwirtschaftlich, relevant sein kann. Hier erscheint eine zukünftige Vertiefung der Forschung an der Bestäubungsleistung von Drohnen im ökologischen und ökonomischen Kontext lohnenswert.

Chapter X – References

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Appendix

1. ***Danksagung***
2. ***Curriculum Vitae***
3. ***Eidesstattliche Erklärung***

Danksagung

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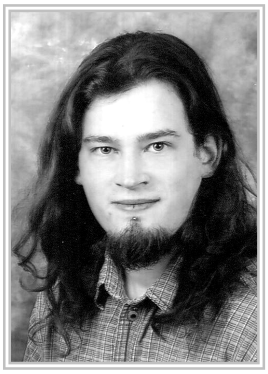

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1987-1992	Elementary school “Curt Wach“ Bockendorf
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2. **WOLF S** & MORITZ RFA (2008): Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie* 39: 419-427.

3. KRAUS FB, **WOLF S**, MORITZ RFA (2009): Male flight distance and population substructure in the bumblebee *Bombus terrestris*. *Journal of Animal Ecology* 78 (1): 247-253.

4. **WOLF S**, ROHDE M & MORITZ RFA (2009): The reliability of morphological traits in the differentiation of *Bombus terrestris* and *B. lucorum* (Hymenoptera: Apidae). *Apidologie* DOI: 10.1051/apido/2009048.

5. **WOLF S** & MORITZ RFA (in review): The pollination potential of bumblebee (*Bombus spec*.) drones (Hymenoptera: Apidae). *Oecologia*

6. **WOLF S**, TOEV T, MORITZ RLV & MORITZ RFA (submitted): Temporal dynamics of the male effective population size in bumblebees (Hymenoptera: Apidae). *Molecular Ecology*

pdfs available at http://www.mol-ecol.uni-halle.de/current_publications/

Stephan Wolf Halle, 04.09.2009

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die vorliegende Arbeit selbständig verfaßt und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben.
Ich erkläre weiterhin, dass ich mich noch nicht um den Doktorgrad beworben habe und diese Arbeit weder der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Halle, den 04.09.2009

Stephan Wolf