"Molecular Ecology of Eusocial Hymenoptera in the Tropics: From Mating Strategy to Population Structure"

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1. Introduction

“…and the army ants - they leave nothing but the bones.”

Tom Waits*, “Earth died screaming”*

Molecular ecology of social insects

Population genetics, the study of allele frequency distribution and change in time and space, provides the fundamental theoretical tools to understand evolutionary and ecological processes in natural poplations. Since Sewall Wright's and Ronald A Fisher's pioneering work (e.g. Fisher 1930, 1941, 1958; Wright 1921a, b, 1931, 1940, 1943, 1946, 1949, 1965), genetic variability in populations is broken down and partitioned into subunits to reflect population sub-structuring, assuming ideal populations with panmictic mating structure and infinite size. These conditions are however rarely met in natural populations. It is the social structure, mating biology and dispersal behaviour, which shape and influence the genetic population structure and ultimately the evolutionary potential of a given species. Thus behavioural ecology complements population genetics, since only if we understand social biology and behaviour we are able to correctly interpret any given genetic pattern found on the population level. With the rise of advance DNA analytical tools, Wright's theories could be tested at an ever increasing detail, and it is the combination of these tools with behavioural ecology and population genetic theory that eventually established the field of molecular ecology (Hadrys *et al.* 1992; Purugganan & Gibson 2003).

Whenever sociality occurs in a given species it is an essential element to population structure. It adds an additional level of complexity. With increasing coherence of the social groups, the society becomes an extra level of selection in addition to the individual (Pamilo *et al.* 1997; Ross 2001). To study the relevance of sociality on natural selection, social insects provide a unique test system in the animal kingdom because they evolved the most extreme form of sociality, with thousands of individuals living together in colonies. They are characterized by reproductive division of labor, where reproduction is monopolized by few, and in many cases even a single individual, the queen. This highly derived eusocial lifestyle and the division of labor has enabled social insects to dominate nearly all terrestrial eco-systems with the only exception being the Polar Regions (Wilson & Hölldobler 2005). One third of the total biomass in the tropical rainforest stems from social hymenoptera. Besides their ecological dominance, the eusocial lifestyle of ants, bees, and wasps result in population genetic structures which deviate fundamentally from the classical population structure of non-social species. The absolute number of individuals is rather meaningless from a population genetic point of view. The number of individuals in a population of social hymenoptera can be literally legion, the population effective size is however ultimately dependent on the number of colonies, reducing overall genetic variability (Pamilo & Crozier 1997; Chapman & Bourke 2001). Thus one might say that the price of eusociality is a permanent genetic bottleneck, through which populations of eusocial hymenoptera have to pass generation after generation. The bottleneck is determined by the number of queens produced and the males they are mated with. The effective population size and thus overall genetic variability is addtionally restricted by male haploidy, which further reduces the genetic effective size compared with a dilplo-diploid population structure.

The eusocial hymenoptera have evolved a wide array of mating systems and different modes of colony foundation (Wilson 1971). The most ancestral type is considered to be the independent colony foundation by a single singly mated queen often combined with balanced sex ratio of the males and queens, a system which is found in many ant species, wasps and the bumblebees. On the other end of the evolutionary scale and thus being highly derived, there are the completely dependent colony foundations of often multiply mated single queens and highly skewed sex ratios, a combination which can be found in some ant species, the stingless bees and the honeybees (Bourke & Franks 1995; Bourke 1999). These different mating and dispersal systems directly feed back to the population structure, facilitating or hindering gene flow, promoting outbreeding or leading to fragmented populations with increased genetic drift and loss off genetic diversity.

Complementary sex determination

While the males in hymenoptera are haploid and females are diploid (Heimpel & de Boer 2008), male haploidy itself is not the causative mechanism for the development of a given individual into a male or female. The underlying mechanism for sex determination in many hymenoptera is the so called complementary sex determination (*csd*) (Whiting 1943; Beye *et al.* 2003). In the *csd* system all individuals which carry only one allele of the single sex determining locus develop into males whereas those that carry two alleles develop into females. As a consequence all haploid individuals are males, since they carry only one allele at any given locus. However, also diploid individuals which happen to be homozygous at the sex determining locus will develop into males. In the case of the eusocial hymenoptera such diploid males are often infertile or unviable, constituting a considerable genetic load, since such males severely reduce the colony fitness. The number and frequency of sex alleles in a given population determines the frequency of diploid drones. At equilibrium, the sex alleles in a population must have equal frequencies due to the strong frequency dependant selection (Crozier & Pamilo 1996). Any rare sex allele will be strongly favoured by selection because it will rarely become homozygous and not produce any diploid drone offspring for any female carrying this rare allele. In contrast frequent alleles will be selected against, because they increase the chances of diploid drone offspring, thus reducing overall fitness of any individual carrying it.

Conservation genetics and the extinction vortex

While the occurrence of diploid drones was already known for decades from beekeeping, the potential role of the *csd* system for conservation genetics was only recently discussed in more detail. Zayed (2004) pointed out that that the csd system and the occurrence of diploid males could further reduce the effective population size of hymenoptera even more than based on haplo/diploidy alone. This might lead to a so called extinction vortex (Zayed & Packer 2005) in which small or declining populations would be trapped and dragged to inevitable extinction, once they crossed a certain minimum threshold of allelic diversity at the *csd* locus. Small or fragmented populations in eusocial hymenoptera are thus expected to be especially prone to extinction since they combine the negative aspects of the the *csd* system with their eusocial lifestyle, which *a priori* reduces effective population size.

Despite these potential problems resulting from the eusocial lifestyle and the *csd* system, eusocial hymenoptera still merrily roam all ecosystems and prosper, indicating that they evolved effective behavioural mechanisms to counteract these evolutionary constraints. Also a simulation study by Hein *et al.* (2009) shows that the diploid male extinction vortex might be easily avoided by adjusting critical life history strategies of a given species. Studying the mating biology of eusocial hymenopteran species in its many facets thus helps understand how sexual selection is shaping these life history adjustments in different species.

Tropical eusocial insects

Due to their overwhelming biodiversity and abundance of plant and animal life, the tropics have been the focus of biologists for many centuries (e.g. Humboldt & Bonpland 1860; Wallace 1891). Within this tremendous diversity of animal life, the eusocial insects can claim a dominant role in all tropical terrestrial ecosystems and their sheer biomass often exceed those of mammals and other animal groups in Tropical rainforests by far (Wilson 1987). But it is not only the biomass but also the species diversity which is highest in tropical regions for eusocial hymenoptera.

This Tropical diversity covers all levels of eusociality from quite basic forms as found in bumblebees or *Polistes* wasps, up to highly derived species in the honeybees, stingless bees and ants. Nevertheless, and in stark contrast to this high biodiversity, our knowledge on tropical species is incipient at best. Yet, the huge biological diversity in tropical regions provides an ideal setting to study the adaptations and evolutionary responses across a broad range of species, allowing a comparative approach between the different phyla of eusocial hymenoptera.

Tropical eusocial hymenoptera show a large diversity in life history strategies. Their colonies can reach enormous sizes with up to several million individuals like the African army ant *Dorylus molestus* or the Neotropical Leafcutter ant *Atta sexdens* (Hölldobler & Wilson 1990). Even taxonomic groups like the eusocial wasps and bumblebees, where temperate species form relatively small annual colonies, can have considerably larger, perennial colonies in the Tropics (Wilson 1971; Michener 1974). Important behavioural traits which directly influence genetic colony structure, like the number queens in a colony and the number of males they are mated to, show their highest variation in tropical regions (Roubik 1989). In fact most of the polyandrous species can be found in the Tropics, together with the most extreme examples of polyandry: the army ants and honeybees. Also many highly derived symbioses between eusocial hymenoptera and other groups of organisms have evolved there, like the ant-plant symbiosis between *Atzteca* ants and *Cecropia* trees in the Neotropics (Huxley & Cutler 1991).

Tropical eusocial hymenoptera are therefore the study object of the key publications united in this thesis. This focus on the Tropics resulted from more than three years of field work in tropical Mexico and Borneo as well as subtropical South Africa, all major biodiversity hot-spots of the world. This thesis will focus on four major genera: the honeybees, the stingless bees, the bumble bees and army ants.

The Honeybees

The region of origin of the honeybees (genus *Apis*), are the South-East Asian tropical regions, where also the vast majority of *Apis* species occur. Nine species of honeybees have been described so far and only *Apis mellifera*, the western honeybee has a worldwide distribution today, due to its use in apiculture (Ruttner 1988). All honeybee species are highly eusocial and can be considered as a high-end product of the evolution of sociality. Honeybee colonies are large and perennial, reproduce via colony fission and have a single, multiply mated queen. The polyandry of the honeybee queens is outstanding with queens of the giant Asian honeybee *Apis dorsata* mating with up to 100 males on just a few mating flights at the beginning of their lives (Wattanachaiyingcharoen *et al.* 2003). This “curious promiscuity” of the honeybee queens, as Tarpy & Page (2001) put it, has attracted the attention of many researchers and today several mutually non-exclusive hypotheses have been formulated to explain this rather unusual mating behaviour. Most of them are based on the increased within genetic variability resulting from the queens polyandry, which is thought to lead to an increased parasite resistance or increased efficiency of division of labour (Crozier & Fjerdingstad 2001). The problem of all "genetic variance" hypotheses for the evolution of extreme polyandry is that they fail to explain the evolution of polyandry with more than ten matings. This is because the benefit, in terms of increased within colony genetic variability, for each additional mating is rapidly decreasing above ten, while the costs of mating (time, energy, predation risk and diseases) increase constantly for each mating (Kraus & Moritz 2010). An alternative explanation for the evolution of polyandry is the sperm limitation hypothesis (Cole 1983; Kraus *et al.* 2004), which states that queens with large colonies need to ensure mating with a sufficiently high number of males to secure a lifelong capacity of producing fertilized eggs. However the sperm limitation hypothesis is often rejected since the sperm of a single or just several males should be sufficient (Tarpy & Page 2001).

Another feature of mating biology which is shared by all honeybee species is the formation of so called drone congregation areas (DCA), where hundreds of drones gather in mid-air to wait for virgin queens. Also mating itself takes place in mid-air, and arriving queens are immediately followed by a swarm of drones trying to mate with her (Ruttner & Ruttner 1972; Koeniger & Koeniger 2000). This peculiar form of mating behaviour with drones from many colonies of the population gathering at a particular place for mating is thought to have evolved to achieve a highly promiscuous, almost panmictic population structure probably as a mechanism to reduce the risk of inbreeding and the production of diploid drones (Baudry *et al.* 1998).

Figure 1.1 Africanized Honeybees (*A. mellifera*) on brood comb. These highly invasive honeybees have colonized the tropical and subtropical Americas within just a few decades starting from the initial introduction of just 26 hives of African *A. m. scutellata* to Brasil in 1957 (Kerr 1967).

Besides behavioural studies also the phylogeny of honeybees and especially the Western honeybee *A. mellifera* has been a popular research field for decades. Traditionally such studies have been conducted based on morphological characteristics splitting up *A. mellifera* into dozens of subspecies over its large distribution area (Ruttner 1988). With the development of molecular markers, as a complement to the already existing extensive studies on morphological variation, this research field gained an additional boost. Most studies were and are still being conducted based on mtDNA markers, since these are often sufficient to investigate the large scale phylogenetic relationships between the different evolutionary lineages of *A. mellifera* (e.g. Garnery *et al.* 1992; de la Rua et al 1998). However to disentangle more subtle population genetic aspects and to monitor nuclear gene flow, the development and usage of microsatellites became necessary (Franck *et al.* 2001). More recently with the availability of the honeybee genome, also studies implementing a large number of nuclear markers became feasible and large scale studies also employed SNPs to unravel the evolutionary history and worldwide population structure of the honeybee (Whitfield *et al.* 2006).

Microsatellite analyses proved especially useful in the understanding of the so called Africanization of the honeybees in the Americas (Figure 1.1). The Africanization process is a very successful biological invasion, which started in 1957 in Brasil with the introduction of 26 African queens of the subspecies *A. m. scutellata*. The accidental release of theses colonies then triggered hybridisation and spread of the African honeybees over the Americas (Kerr 1967).

The Africanization process is still ongoing and seems only to be stopped by temperate climate conditions. The population genetic processes driving the Africanisation of the honeybees in the Americas is a quite complex, because nearly all European mitotypes get replaced by African ones, whereas on the nuclear level African and European genes seem to be at an equilibrium (Scheider *et al.* 2004).

The Stingless bees

The Stingless bees (Tribe Meliponini) are the largest group of eusocial bees with a worldwide distribution and several hundred species described so far (Rasmussen & Cameron 2010). Their distribution is mainly tropical, but also reaches into southern subtropical regions in Latin America and Africa. As their name implies they have lost the capacity to use their stinging apparatus for defensive behaviour. Nevertheless, they have evolved several other sophisticated defensive mechanisms ranging from fortress-like constructed nests to biting behaviour and secretion of deterrent gland substances. Like the honeybees they are highly eusocial and live in permanent, perennial colonies, composed of up to several thousand individuals and their degree of sociality is comparable to that of the honeybees (Michener 1974; Wille 1983; Roubik 1989).

In relation to their high number of species, the stingless bees are much less studied than the honeybee, probably due to their tropical distribution, which makes them less accessible for molecular ecological studies. Concerning their economic importance, stingless bees have been the only source of honey in pre-Columbian times in Latin America and were cultivated to a large extend by many pre-Columbian cultures, like e.g. the Mayans in Central America. In fact one of the four written Mayan books which survived destruction by the Spanish conquistadores and priests is the Madrid Codex, which also includes an extensive description of Mayan meliponiculture with *Melipona beecheii* (Lopez-Maldonado 2010). Stingless bees have been replaced as the prime source for honey by the Western honeybee *Apis mellifera* which has been introduced by the European settlers to the Americas. However, today stingless bees are still kept in Latin America even though on a much smaller scale and mostly for local markets, for keeping up cultural traditions or conservation reasons. More recently stingless bees are also being used and tested as pollinators for commercially grown crops like Rambutan (*Nephelium lappaceum*) (Slaa *et al.* 2006). Even though they lack the spectacular dance language of the honeybees, stingless bees have evolved others means of precise food source communication often involving pheromone marking or piloting (Biesmeijer & Slaa 2004; Nieh 2004). Also their nest architecture (Figure 1.2) is complex and shows a remarkably diversity across species ranging from cluster type nests, which appear rather irregular in their arrangement of brood cells, up to nest with neatly arranged horizontal combs in several layers (Wille 1983; Roubik 2006).

Figure 1.2 *Melipona beecheii* nest The photo shows an opened nest of *M. beecheii*, exposing the horizontal brood combs and honey pots typical for many stingless bee species. The nest itself is constructed inside a hollow tree trunk and here opened for beekeeping purposes.

Stingless bees have queens that are often single mated or only exhibit a low degree of polyandry (up to 6 matings) depending on species and environmental context (Paxton *et al.* 1999a; Palmer *et al.* 2002). New colonies are founded by colony fission by a daughter queen and her sister workers in close proximity to the mother nest, often being supported by the mother colony for some time. Thus the dispersal capacity and maternal gene flow are limited to the distance between mother and daughter colony (in contrast honeybee colonies can have large range migratory swarms). The low mobility results from the permanent physogastry of the stingless bee queens, which renders them unable to fly once they have started egg laying (Wille 1983).

One especially interesting behavioural feature of many stingless bee species is the formation of drone aggregations, where hundreds of drones gather to wait for arriving virgin queens to mate with. In contrast to the honeybees, these drone aggregation are not airborne, but substrate based, often directly outside of conspecific colonies (Engels & Engels 1984). Similar to the honeybees the prime hypothesis for the formation of drone aggregations in stingless bees is once again inbreeding avoidance, since they are also affected by the *csd* system and the production of diploid drones (Paxton 2002; Cameron *et al.* 2004). However empirical studies on this behavioural phenomenon are scarce and the proximate and ultimate mechanisms involved in the formation of drone aggregations are not well studied yet. The publications on stingless bees included in this Habilitation thesis, are among the few studies which address the ultimate causes for this lek-like mating system of stingless bees.

The Bumblebees

In contrast to the two previously described groups of eusocial bees, the bumblebees (Bombini) are primitively eusocial and many aspects of their life history are phylogenetically ancestral to the honeybees and stingless bees (Goulson 2003a). Another difference to their two sister groups is, that the evolutionary origin of bumblebees is thought to be in the temperate zone of the Northern hemisphere, possibly the Mountainous regions of Asia (Williams 1985; Hines 2008), where the majority of species can be found, rather than in the tropics. Hence, tropical bumblebees constitute the exception rather than the rule in the tribe of Bombini and tropical species probably only secondarily evolved adaptations to tropical regions.

So far the majority of the roughly 250 know bumblebee species seem to have an annual lifecycle with independent colony foundation and a single, singly mated queen. The size of the colonies ranges from several hundred individuals (Figure 1.3) down to very small colonies like in *Bombus polaris*, where only one clutch of worker eggs is laid by the queen before the production of sexuals as an adaptation to the short season in arctic regions.

Figure 1.3 *Bombus wilmattae* nest
The photo shows an opened nest of the tropical bumblebee *B. wilmattae*. The brood and honey pots form the center of the nest; the *involucrum* is constructed out of wax and remains of the nest of the former inhabitants (often mice). The nest was excavated following a 1m long entrance tunnel, approx. 70cm under-ground.

In contrast to the honeybees and the stingless bees, the males of the bumblebees do not form lek-like structures but instead establish patrol route in search on young queens (Darwin 1886; Haas 1949). In some species these patrol territories are even defended and rivalling males are being chased off. Due to the annual colony cycle and the small colony size, conflicts between the queen and workers over male production are often well pronounced at the end of the season leading to frequent matricide in some species (Goulson 2003a).

In the wake of the concern for the worldwide pollinator decline and the usage of bumblebees for greenhouse crop pollination, bumblebees received considerable increased attention by researchers, investigating their role in pollination, their population genetics and vulnerability towards extinction and many other aspects of their biology (Colla *et al.* 2006; Velthuis & van Doorn 2006; Potts *et al.* 2010; Whitehorn *et al.* 2011). Several studies indicate that bumblebee species are in decline at least in Europe and North America, presumably due to changes in land use patterns and fragmentation of populations. Even though several bumblebee species seem to be affected by pollinator decline, species like *B. terrestris* are themselves invasive species e.g. in New Zealand or Japan, where they are considered as a threat to native pollinator species (Buttermore 1997; Nagamitsu & Yamagishi 2009; Williams & Osborne 2009). *B. terrestris* is also the bumblebee species which is most commonly used for large scale greenhouse pollination and individuals escaping can either spread diseases and parasites or establish themselves in the surrounding area, or interbreed with local populations, thus altering their genetic structure and composition (Colla *et al.* 2006; Velthuis & van Doorn 2006).

Compared to the growing literature on temperate bumblebee species, the knowledge on tropical species is still minute and molecular studies are virtually non-existent. However, since tropical bumblebees are only secondarily adapted to the tropics, their mating behaviour and colony structure are of special interest. Comparisons with temperate species have the potential to elucidate the adaptive significance of polyandry and genetic colony structure.

The Army ants

When it comes to colony complexity, clearly the ants out-compete the bees as a study system. They can establish colonies orders of magnitude larger and in addition can have much more morphological variance among the females in the colony including multiple worker castes (Hölldobler & Wilson 1990). The fourth group included in this thesis is a group of extremely derived ants, namely the army ants of the Old and New World Tropics. Although army ants represent a completely independent evolutionary branch within the hymenoptera, army ants, as highly specialized nomadic predators, have some key life history features in common with the most advanced eusocial bees - the honeybees. Both genera are characterized by the extreme polyandry of the queens and the completely dependent colony foundation (Denny *et al.* 2004b; Kronauer *et al.* 2004). As such they offer the unique opportunity to analyse and understand the evolutionary processes which have led to these convergent adaptations on the highest level of sociality (Kronauer *et al.* 2007a; Kronauer 2009).

Army ants are often referred to as the ultimate social predators and their colonies are indeed the top predators of arthropods and other small animals in the Tropics (Schneirla 1971; Gotwald 1995). The term army ant in its broader sense refers to all ant species showing the so called army ant adaptive syndrome, which is a combination of evolutionary interrelated traits: nomadism of the whole colony, obligatory group predation and highly specialized queens (Gotwald 1995; Kronauer 2009). In its narrow sense it refers to the “true” army ants of a monophyletic clade compromising the subfamilies of the Ecitoninae, Dorylninae and Aenictinae (Brady 2003; Kronauer *et al.* 2007b). The colonies of these ants do not have a permanent nest site and are regularly on the move. In case of the New World army ants of the genus *Eciton* these migrations follow a regular pattern and the temporal nest are constructed out of the ants themselves. The ants form so called bivouacs out of their bodies, with the brood and queen at the centre (Figure 1.4).

The permanently wingless queens of the army ants never leave their colonies and also mate there with males from other colonies. These males are excellent flyers and must be capable of finding conspecific nest and be granted access to these colonies by the foreign workers. Army ant colonies are monogynous, reproduce via colony fission and their queens are highly polyandric, mating with 10 to 20 males (Denny et al. 2004b). The only known exception from this pattern is the polygynous species *Neivamyrmex carolinensis*, where queens have an effective mating frequency of less than 1.5 (Kronauer et al. 2007c). Thus the army ants have evolved a mating system with extreme polyandry of the queens and large monogynous colony which can only propagate via colony fission (convergent to the honeybees). Also similar to honeybees there is a distinctive division of labour among the workers of an army ant colony.

Figure 1.4 *Eciton burchelli* bivouac The photo shows a close up of a bivouac of the Neotropical army ant *E. burchelli*. As it can be seen the workers of various castes form the bivouac with their bodies, clinging to each other and attaching it to a log.

However, unlike in the honeybees this division of labour is not a temporal one and division of labour is manifested in physical worker castes. These castes range from minute “minor” workers participating in brood care up to the large “soldier” caste (Figure 1.5) which mainly is for the purpose of defence of the colonies and the foraging trails (Schneirla 1971; Gotwald 1995). The division of labour in physical castes and the highly complex genetic colony structure resulting from the high queen mating frequency are the topic of one publication included in this thesis.

Figure 1.5 *E. burchelli* major worker The photo shows the impressive mandibles of a major worker (soldier) of *E. burchelli*, demonstrating the phenotypic plasticity within a colony of army ants where workers differ considerably in size and body proportions.

Based on the limited dispersal ability of the queens, army ant population are expected to be subject to strong population fragmentation and isolation. In combination with the above described general restrictions in population size in social hymenoptera, this raises the question which behavioural mechanism army ants have evolved (if any) to counteract this inevitable fragmentation. The males of the army ant are in fact the prime candidates for counteracting this population fragmentation, since they are the only individuals in army ants which potentially could cover larger distances. A study by Berghoff *et al.* (2008) indicate that mitochondrial gene flow by the queen is indeed easily hindered by obstacles like rivers wheras the nuclear gene flow was unhindered over short geographical distances and driven by the males. Two additional chapters in this thesis deal with the topic of the flight capacity of males in Army ants and large scale population structure in an African Army ant.

Aims and scope of the Habilitation Thesis

The fourteen chapters united in this cumulative thesis are centred on the molecular ecology of mostly tropical species of eusocial hymenoptera and is sub-divided in three sections. The studies the chapters are based upon explore the causes and consequences of mating systems from the behavioural level to colony and population genetic structure. Section one deals with multiple mating of females and its consequences for colony genetic structure, Section two covers the male mating strategies and their impact on population subdivision. The third section aims at analysing colony based gene flow and overall population genetic structure.

The first section “Polyandrous queens and colony organization” unites four chapters starting with two chapters on the tropical Bumblebee *B. wilmattae* (Huth-Schwarz *et al.* 2011 a, b). They describe for the first time the mating frequency of a tropical bumblebee species with molecular tools and explore the consequences of the mating biology of the queens for intra-colonial queen-worker conflict. The third and the fourth chapter of the first section are on the Western honeybee *A. mellifera* and the Neotropical Army ant *E. burchellii*, with both species showing an extreme degree of polyandry. These two chapters study the impact of extreme polyandry on one of the most important aspects of colony organisation and structure: the division of labour among the workers (Jaffé *et al.* 2007; Kraus *et al.* 2010).

The second section “Male mating strategies and gene flow” includes six chapters all exploring male mating strategy, its possible ultimate and proximate causes, as well as its consequences for population genetic structure and connectivity. The first three chapters presented are on the Neotropical stingless bee *Scaptotrigona mexicana* and cover both behavioural and population genetic aspects of the formation of the male aggregations in this species (Kraus *et al.* 2008; Lopez & Kraus 2009; Müller *et al.* 2012). The fourth chapter is also on male aggregations, but this time on the drone congregation areas of the Giant Asian honeybee *A. dorsata*, where the temporal genetic composition of one such congregation and its implications for drone behaviour are explored (Kraus *et al.* 2005a). The fifth chapter again has the Neotropical Army ant *E. burchellii* as study organisms and investigates the role of the males of this species as carriers of nuclear gene flow (Jaffé *et al.* 2009). The sixth and last chapter of the second section gives an estimate of the flight distances of male bumblebees in the temperate species *B. terrestris* and their impact on population sub-structuring (Kraus *et al.* 2009).

The third section “Colony density and population structure” unites chapters on population genetics of eusocial hymenoptera without an explicit focus on either sex. The first two chapters focus on Africanized honeybees in Mexico and describe in detail the genetic aspects of the Africanization process in Veracruz (Mexico) and explore colony densities and population genetic aspects with respect to the presence or absence of beekeeping in Chiapas and Yucatan (Kraus *et al.* 2007; Moritz *et al.* 2012). The third chapter for the first time shows that the bumblebee *B. terrestris,* which is bred and used for greenhouse pollination on a large scale indeed spreads its gene from greenhouse populations into feral populations, indicating a potential hazard for biological conservation (Kraus *et al.* 2010). The fourth and last chapter provides a large scale population genetic study of the African Army ant *Dorylus fulvus* and disentangles the contribution of both the queens and males to gene flow among even distant populations (Barth *et al.* 2012).

2. Polyandrous queens and colony organization

2.1 Mating frequency and genetic colony structure of the Neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

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Contribution FB Kraus: study design/supervisor, sampling, manuscript preparation

Abstract

So far, nearly all studies concerning the mating frequency of bumblebees have been conducted with temperate species, showing that single mating seems to be the predominant pattern in bumblebees. Studies involving tropical species, however, are still scarce. Here, we determined the mating frequency of queens of the tropical bumblebee species, *Bombus wilmattae* by using microsatellite genotyping based on a sample of nine colonies from Chiapas, Southern Mexico. A total of 204 workers were genotyped with microsatellite markers to infer the queen genotype and the number of males with which each queen had mated. Two of the nine queens were doubly mated and seven singly mated. In the colonies with the double-mated queens, the distribution of the patrilines was not even, resulting in effective mating frequencies of 1.34 and 1.70, respectively, and an average relatedness of g = 0.58 ± 0.06.

2.2 Workers dominate male production in the Neotropical bumblebee *Bombus wilmattae* (Hymenoptera: Apidae)

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Contribution FB Kraus: study design/supervisor, sampling, manuscript preparation

Abstract

Background: Cooperation and conflict in social insects are closely linked to the genetic structure of the colony. Kin selection theory predicts conflict over the production of males between the workers and the queen and between the workers themselves, depending on intra-colonial relatedness but also on other factors like colony efficiency, sex ratios, cost of worker reproduction and worker dominance behaviour. In most bumblebee (*Bombus*) species the queen wins this conflict and often dominates male production. However, most studies in bumblebees have been conducted with only a few selected, mostly single mated species from temperate climate regions. Here we study the genetic colony composition of the facultative polyandrous Neotropical bumblebee *Bombus wilmattae*, to assess the outcome of the queen-worker conflict over male production and to detect potential worker policing.

Results: A total of 120 males from five colonies were genotyped with up to nine microsatellite markers to infer their parentage. Four of the five colonies were queen right at point of time of male sampling, while one had an uncertain queen status. The workers clearly dominated production of males with an average of 84.9% ± 14.3% of males being worker sons. In the two doubly mated colonies 62.5% and 96.7% of the male offspring originated from workers and both patrilines participated in male production. Inferring the mother genotypes from the male offspring, between four to eight workers participated in the production of males.

Conclusions: In this study we show that the workers clearly win the queen-worker conflict over male production in *B. wilmattae*, which sets them apart from the temperate bumblebee species studied so far. Workers clearly dominated male production in the singly as well the doubly mated colonies, with up to eight workers producing male offspring in a single colony. Moreover no monopolization of reproduction by single workers occurred.

2.3 Shift work has a genetic basis in honeybee pollen foragers (*Apis mellifera* L.)

(Published in: *Behavior Genetics (2010) 41:323-328*)

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Contribution FB Kraus: study design/supervision, data analysis, manuscript writing

Abstract

Division of labour is a fundamental property of any social system. The specialization of different individuals in different tasks increases the overall work performance and efficiency. Specialization is thought to be the very foundation of the success of human societies but also in complex colonies of social insects. In human societies an advanced form of division of labour, especially since the industrialisation, is shift work, where individuals perform the same task but in subsequent cohorts in time. Although social insects can measure and are aware of time, shift work has not been documented in colonies of social insects so far. We observed foragers of two honeybee (*Apis mellifera*) colonies (approx. 140 workers each) and genotyped them with microsatellite DNA markers. We determined paternity and assigned them to the various subfamilies in the colony to test whether there is genetic variance for shift work in foraging honeybees. We could show that the patriline identity of the foragers had a significant effect on foraging either in the morning or evening. Individual foragers differed in their preference for the “early” or “late” shift, and shift work indeed existed in the colony.

2.4 Worker caste determination in the army ant *Eciton burchellii*

(Published in: *Biology Letters (2007) 3: 513-516*)

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Contribution FB Kraus: study design/supervisor, sampling, manuscript preparation

Abstract

Elaborate division of labour has contributed significantly to the ecological success of social insects. Division of labour is achieved either by behavioural task specialization or by morphological specialization of colony members. In physical caste systems, the diet and rearing environment of developing larvae is known to determine the phenotype of adult individuals, but recent studies have shown that genetic components also contribute to the determination of worker caste. One of the most extreme cases of worker caste differentiation occurs in the army ant genus *Eciton*, where queens mate with many males and colonies are therefore composed of numerous full-sister subfamilies. This high intracolonial genetic diversity, in combination with the extreme caste polymorphism, provides an excellent test system for studying the extent to which caste determination is genetically controlled. Here we show that genetic effects contribute significantly to worker caste fate in *Eciton burchellii*. We conclude that the combination of polyandry and genetic variation for caste determination may have facilitated the evolution of worker caste diversity in some lineages of social insects.

3. Male mating strategies and gene flow

3.1 *Cherchez la femme*? - Site choice of drone congregations in the stingless bee *Scaptotrigona mexicana*

(Published in: *Animal Behaviour (2009) 77: 1247-1252*)

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Contribution FB Kraus: study design/supervisor, data analysis, manuscript writing

Abstract

Drone congregations are a widespread phenomenon among stingless bee species. Such congregations can consist of more than 1000 individuals and persist for several days or even weeks. Because congregations often form directly outside of conspecific colonies, it has been hypothesized that the presence of a virgin queen inside the colony triggers the aggregation of the drones. Here we use drone congregations of the Neotropical stingless bee *Scaptotrigona mexicana* to test this hypothesis. We performed behavioral experiments where the drones of a given congregation could decide between a previously chosen colony and a new unknown one as site for the formation of a congregation. Our results show that drone congregations are not associated with a particular colony (and its content) but rather with specific sites at the test location. Thus the content of a colony (e.g. the presence of a virgin queen) is unlikely to be the trigger for the formation of a congregation. Further we could show that pheromonal markings are used in the short distance orientation of drones towards a given site. Choice experiments of individual drones in the laboratory showed that drones are attracted to groups of other drones while they do not react to groups of workers. These results imply that once a given site was chosen by some drones they will attract more drones via positive feedback from the visual presence and odors of increasing numbers of drones.

3.2 Genetic structure of drone congregations of the stingless bee *Scaptotrigona mexicana*

(Published in: *Insctes Sociaux (2008) 55: 22-27*)

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Contribution FB Kraus: study design/supervisor, sampling, data analysis, manuscript writing

Abstract

Drones of stingless bee species often form distinctive congregations of up to several hundred individuals which can persist over considerable periods of time. Here we analyse the genetic structure of three drone congregations of the Neotropical stingless bee *Scaptotrigona mexicana* employing eight microsatellite markers. Two congregations were close to each other (50m), the third one was located more than 10km away from them. This spatial pattern was also reflected on the genetic level: the two close congregations did not show any population sub-structuring, whereas the more distant congregation showed a significant population differentiation to both of them. Population subdifferentiation was however low with F_{st} values (F_{st} = 0.020 and 0.014) between the distant congregations, suggesting gene flow over larger distances mediated by the drones of *S. mexicana*. Based on the genotypic data we also estimated the number of colonies contributing drones to the congregations.The two joint congregations consisted of drones originating from 39.6 colonies, while the third congregation was composed of drones from 21.8 colonies, thus proving that congregations of *S. mexicana* are constituted of unrelated drones of multi-colonial origin.

3.3 Outbreeding and lack of temporal genetic structure in a drone congregation of the Neotropical stingless bee *Scaptotrigona mexicana*

(Published in: *Ecology & Evolution (2012) in print*)

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Contribution FB Kraus: study design/supervisor, sampling, manuscript preparation

Abstract

Drone aggregations are a widespread phenomenon in many stingless bee species (Meliponini), but the ultimate and proximate causes for their formation are still not well understood. One adaptive explanation for this phenomenon is the avoidance of inbreeding, which is especially detrimental for stingless bees due to the combined effects of the complementary sex-determining system and the small effective population size caused by eusociality and monandry. We analyzed the temporal genetic dynamics of a drone aggregation of the stingless bee *Scaptotrigona mexicana* with microsatellite markers over a time window of four weeks. We estimated the drones of the aggregation to originate from a total of 55 colonies using sibship reconstruction. There was no detectable temporal genetic differentiation or sub structuring in the aggregation. Most important, we could exclude all colonies in close proximity of the aggregation as origin of the drones in the aggregation, implicating that they originate from more distant colonies. We conclude that the diverse genetic composition and the distant origin of the drones of the *S. mexicana* drone congregation provides an effective mechanism to avoid mating among close relatives.

3.4 Temporal genetic structure of a drone congregation area of the giant Asian honeybee (*Apis dorsata*)

(Published in: *Naturwissenschaften (2005) 92: 578-581*).

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Contribution FB Kraus: study design, data analysis, manuscript writing

Abstract

The giant Asian honeybee (*Apis dorsata*), like all other members of the genus *Apis*, has a complex mating system in which the queens and males (drones) mate at spatially defined drone congregation areas (DCAs). Here, we studied the temporal genetic structure of a DCA of *A. dorsata* over an 8-day time window by the genotyping of sampled drones with microsatellite markers. Analysis of the genotypic data revealed a significant genetic differentiation between 3 sampling days and indicated that the DCA was used by at least two subpopulations at all days in varying proportions. The estimation of the number of colonies which used the DCA ranged between 20 and 40 colonies per subpopulation, depending on the estimation procedure and population. The overall effective population size was estimated as high as Ne = 140. The DCA seems to counteract known tendencies of *A. dorsata* for inbreeding within colony aggregations by facilitating gene flow among subpopulations and increasing the effective population size.

3.5 Gene flow is maintained by polyandry and male dispersal in the army ant *Eciton burchellii*

(Published in: *Population Ecology (2009) 5: 227-236*)

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Contribution FB Kraus: study design/supervisor, sampling, manuscript preparation

Abstract

The combination of haplodiploidy, complementary sex determination and eusociality constrains the effective population size (N_e) of social Hymenoptera far more than in any other insect group. Additional limitations on N_e occur in army ants since they have wingless queens and colony fission, both of which are factors causing restricted maternal gene flow and high population viscosity. Therefore, winged army ant males gain a particular significance to ensure dispersal, facilitate gene flow and avoid inbreeding. Based on population genetic analyses with microsatellite markers, we studied a population of the Neotropical army ant *Eciton burchellii*, finding a high level of heterozygosity, weak population differentiation and no evidence for inbreeding. Moreover, by using sibship reconstruction analyses, we quantified the actual number of male contributing colonies represented in a queen's mate sample, demonstrating that, through extreme multiple mating, the queens are able to sample the genes of males from up to ten different colonies, usually located within an approximate radius of 1km. We finally correlated the individual mating success of each male contributing colony with the relative siring success of individual males and found a significant colony-dependent male fitness component. Our results imply that the dispersal and mating system of these army ants seem to enhance gene flow and minimise the deleterious effects associated with small effective population size.

3.6 Male flight distance and population substructure in the bumblebee *Bombus terrestris*

(Published in: *Journal of Animal Ecology (2009) 78: 247-252*)

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Contribution FB Kraus: study design, sampling, data analysis, manuscript writing

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 subdifferentiation 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 2.6km to 9.9km, much further than worker flight ranges. Bumblebee-mediated pollen flow will therefore be much further than expected based on the foraging range of workers alone if males also contribute to pollination.

4. Colony density and population structure

4.1 Asymetric introgression of African genes in honeybee populations (*Apis mellifera* L.) in Central Mexico

(Published in: *Heredity (2007) 99: 233-240*)

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Contribution FB Kraus: study design, data analysis, manuscript writing

Abstract

The Africanization of the honeybee (*Apis mellifera*) in South America is one of the most spectacular examples of biological invasions. In this study, we analyzed the Africanization process in Central Mexico along an altitudinal transect from 72 to 2800m, using both mitochondrial and nuclear DNA markers. The mitochondrial analysis revealed that the two high-altitude populations had a significantly greater percentage of African mitotypes (95%) than the three lowland populations (67%), indicating successful spreading of Africanized swarms to these altitudes. All populations (highland and lowland) had a similar overall proportion of African alleles at nuclear loci (58%). Thus, all populations showed an asymmetric introgression of African nuclear and mtDNA. Colonies with African mitotypes had, on average, significantly more African nuclear alleles (60%) than those with European mitotypes (51%). Furthermore, the three lowland populations showed clear signs of linkage disequilibrium, while the two high-altitude populations did not, indicating recent genetic introgression events into the lowland populations.

4.2 Honeybee colony density in areas with managed and feral honeybee populations in Southern Mexico

(Submitted to *Apidologie*)

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Contribution FB Kraus: study design, data analysis, manuscript preparation, correspondance

Abstract

The density of feral and managed honeybee populations (*Apis mellifera*) was determined for various sampling locations in Chiapas and Yucatan (Mexico) to asses the impact of apiculture on populations in the wild. We used a paired sampling approach determining colony densities in similar habitats and landscapes but with different intensity of beekeeping. Sampling sites included nature reserves, mango, and shaded coffee plantations. The agricultural sites were all set in high diversity landscapes with plenty of surrounding secondary forest. Colony density was determined by genotyping drones caught on drone congregation areas and assigning the drone genotypes to mother queens each heading a colony. We use three sets of linked markers each to achieve sufficient resolution for a precise colony assignment. The estimated colony densities ranged from 13.6 col/km² to 21.6 col/km². We found no significant difference in colony densities between the sites with and without beekeeping suggesting the managed honeybee populations do not substantially add to the overall number of honeybee colonies supported in the wild. This indicates that restrictions on apicultural activities to prevent any potential conservation conflict with native pollinators might not be useful, since honeybee colonies are very abundant in many different landscapes in Southern Mexico independent of apiculture.

4.3 Greenhouse Bumblebees (*Bombus terrestris*) spread their genes into the wild

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Contribution FB Kraus: study design, data analysis, manuscript writing

Abstract

Bumblebees (*Bombus* spp.) are commonly used for greenhouse pollination of tomatoes and other crop plants. The colonies used for this purpose are provided by commercial bumblebee breeders, which by now operate at a professional company level. As a result of this practice commercially bred bumblebee colonies are transported and used over large distances and national borders, introducing subspecies into non-endemic regions. The question whether and to what extends gene flow between such managed greenhouse and wild bumblebee populations exists, so far has not been addressed. Here we used samples from three greenhouses in Poland and the surrounding populations to address this question. Using microsatellite DNA data we found strong genetic introgression from the sampled greenhouse populations into the adjacent populations. Depending on the analysed population the number of individuals assigned to the greenhouse populations ranged from 0.08 up to 0.47. We also found that more distant populations were much less affected by genetic introgression from the greenhouses.

4.4 Male-biased dispersal counteracts limited female gene flow in a subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*

(Submitted to: *Molecular Ecology*)

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Contribution FB Kraus: study design/supervisor, manuscript preparation

Abstract

Sex-biased dispersal is a widespread phenomenon in the animal kingdom, which strongly influences gene flow and population structure. Particularly army ants, important key-stone predators in tropical ecosystems, are prone to population fragmentation and isolation due to their extraordinary mating system with permanently wingless queens and therefore male dependent dispersal and gene flow. Here we report on sex-biased dispersal and the genetic population structure of an African subterranean army ant, *Dorylus* (*Typhlopone*) *fulvus*. Colony and queen dispersal is confined underground in this species, and only the male sex disperses in mating flights. Using maternally inherited mitochondrial markers (mtDNA) and bi-parentally inherited nuclear microsatellite markers we found strong geographical structuring of mtDNA haplotypes, whereas the nuclear genetic population structure was less pronounced, with no indications of inbreeding within populations. Strong mtDNA ($\Phi_{ST} = 0.85$), but far lower nuclear ($F_{ST} = 0.23$) genetic differentiation translated to a more than an order of magnitude larger male migration rate compared to that of queens, reflecting the low motility of queens but a large scale promiscuous gene flow by males. Thus, the well flying *D. fulvus* males appear to counteract limited queen dispersal at the nuclear but not at the mitochondrial level. With this study we aim to achieve a better understanding of how sex specific dispersal patterns and mating systems affect the population structure and phylogeography of species.

5. Summary and Conclusions

E pluribus unum

The 14 chapters united in this thesis focus on seven, mainly tropical species, from four taxonomic groups of the eusocial hymenoptera: the honeybees, the stingless bees, the bumblebees, and the army ants. By applying molecular ecological tools, these chapters explore the causes and consequences of mating strategies for both sexes and their impact on colony and population genetic structure. This cross taxa perspective takes a bottom up approach to detect consistent patterns in the different groups of eusocial hymenoptera to allow for a more meaningful and general inferences of the adaptive significance of mating strategies and colony organization for natural selection and the population structure.

Polyandrous queens and colony organization

The first section provides the female perspective of mating biology in eusocial hymenoptera and its implications for colony genetic structure and organization. The first two chapters (Huth-Schwarz et al 2011a, b) deal with the tropical bumblebee *B. wilmattae.* For the first time reliable data on the mating frequency of a tropical bumblebee is provided, showing that *B. willmattae* queens are facultatively polyandrous, albeit at a low level and with a maximum of two matings. While this is not unusual for Bumblebees, *B. wilmattae* strongly deviates from temperate species in terms of worker reproduction with an average of 84.9 % of all males being offspring of workers. Thus the workers dominate and clearly win the queen-worker conflict in this tropical Bumblebee. Given that Bumblebees only secondarily adapted to the tropics, it remains to be answered whether this high level of worker reproduction is a general pattern and adaptation of tropical bumblebees or rather a particularity of *B. wilmattae* (Jenny 1974; El-Niweiri & Moritz 2011).

The two following chapters (Kraus et al 2011, Jaffé et al 2007) switch from the more primitively eusocial bumblebees to two highly derived eusocial hymenopteran species: the honeybee *A. mellifera* and the army ant *Eciton burchellii*. The queens of these two species are highly polyandrous and thus their colonies are genetically complex entities, with workers originating from dozens of subfamilies. In both chapters we could show that this high genetic variability is directly linked to colony organization in form of division of labor between the workers. In case of the honeybee this division of labor is a temporal one, with foragers of different subfamilies having different preferences for flight times, thus engaging in shift work and a temporal division of labor. In the Army ant *E. burchellii* division of labor is manifested in morphological castes, where workers can be minors, medias or majors. The siring father and hence the subfamily identity of a worker determines the probability of a given individual to develop into one of the several castes. So both honeybees and army ants, despite their phylogenetic distance, congruently provide evidence that within-colony genetic variability is tightly linked with the organization of task assignment in the colony. It becomes very clear that the combination of polyandry and genetic variation for caste determination is a major force in driving colony organization in highly eusocial hymenoptera in general.

Generally, as shown by the four chapters in this first part, the mating behavior of the queens has a strong impact on colony level selection, with the degree of polyandry (or monandry respectively) directly influencing both within-colony genetic variability and colony fitness. In the more primitively eusocial bumblebees the number of matings influences the inter-individual conflict potential over reproduction. Here the reproductive division of labor itself is not as strict as in more derived species, as shown by the queen worker conflict over male production. In highly derived eusocial species, polyandry is associated with further colony level traits, where the increased genetic variability enhances colony efficiency in form of improved division of labor either in physical castes or shift work, as shown by the chapters on army ants and honeybees.

Male mating strategies and gene flow

In contrast to female mating strategies the males have long been the neglected gender in research on eusocial hymenoptera (Koeniger 2005). The second section of this thesis compromises six chapters on male mating strategies of four species from four different taxa, as an effort to put a certain emphasis on the male perspective, which is of course equally important than female mating strategies.

The first three chapters explore the phenomena of male aggregations in the stingless bee *Scaptotrigona mexicana* (Kraus *et al*. 2008, López & Kraus 2009, Müller *et al*. 2012). Males of this species establish daily aggregations next to the flight entrance of specific coloniesduring mati8ng season. Surprisingly, *S. mexicana* male aggregations are not associated with a particular conspecific colony they are positioned upon, but rather with specific locations. Also the males are attracted to the aggregations by the scent of other males, triggering a snow ball effect once an aggregation starts to cluster. Furthermore, the genetic analysis of aggregations showed that the males participating in it come from dozens of colonies, but not a single male originated from colonies in the immediate vicinity of the aggregation. Also no temporal or spatial sub-structuring was found among the analyzed aggregations on subsequent days. Taken together, these results indicate that male aggregations in this stingless bee are very effective in avoiding any inbreeding tendencies and promote gene flow via the males counteracting the limited mobility of the females and their colonies. The fourth chapter deals with a quite similar phenomenon the drone congregations of the Giant Asian honeybee *A. dorsata (*Kraus *et al.* 2005a). Here the drones do not aggregate in front of a colony but fly to drone congregation areas at the level of the high canopy in the rainforest. In our study a single drone congregation was visited by drones of a high number of colonies from two distinct sub populations in varying proportions over several sampling days. However also here, like in *S. mexicana*, the very existence of such congregations is curcial for the promotion of gene flow among subpopulations.

Also in the fifth chapter, which deals with the role of male *E. burchellii* army ants for gene flow (Jaffé *et al*. 2009), we could show that the males are the prime agents for connectivity and gene flow among populations. In case of army ants this is probably even more important, since the colonies and queens are restricted to “walking distance” without the possibility to cross even medium sized creeks or water bodies.

The last chapter of the second section switches from the highly eusocial taxa back to the more primitively eusocial Bumblebees (Kraus *et al.* 2009), where both males and females are produced at the end of the colony cycle, and both sexes in principle have equal chances to migrate away from their mother colonies. Here we analyzed a population of the bumblebee *B. terrestris* and could show that the males have a larger flight radius than the females indicating that also in this species males cover lager distances after leaving the nest than the queens do.

As outlined before, eusocial hymnoptera are particularly affected by various genetic and life history factors which potentially restrict their effective population sizes and gene flow. These include their haplo/diploidy, the genetic load resulting from complementary sex determination and in general their eusocial life style which inevitably restricts the number of reproductive females to the number of colonies. However, all studies in this section show that the analyzed species and taxa have evolved mating strategies to efficiently counteract inbreeding. In all studied cases it is the male sex which is adapted to be the prime carrier for long distance gene flow.

Colony density and population structure

While the two previous sections focused on one of the two sexes each, the third and last section unites four chapters on the more general colony level and population perspective, thus including the impact of both sexes. The first two chapters of this part both deal with the Africanized honeybee in Central America. In the first chapter (Kraus *et al.* 2007), we analyzed the Africanization process in Central Mexico, along an altitudinal transect from 72 to 2800 m, using both mitochondrial and nuclear DNA markers. The two high-altitude populations had a significantly greater percentage of African mitotypes (95%) than the three lowland populations (67%), indicating successful spreading of Africanized swarms to these altitudes. Moreover, all populations had a similar overall proportion of African alleles at nuclear loci, thus showing an asymmetric introgression of African nuclear and mtDNA. The second chapter on Africanized honeybees (Moritz *et al.* submitted) addresses the influence of beekeeping on the density of honeybee colonies using three sets of linked microsatellite markers. We found no significant difference in colony densities between the sites with and without beekeeping suggesting the managed honeybee populations do not substantially add to the overall number of honeybee colonies supported in the wild. Both studies show that the honeybee *A. mellifera,* as an invasive species in America, has successfully established itself firmly in most terrestrial ecosystems in Central America and restrictions on beekeeping (for conservation of native pollinators) are unlikely to significantly reduce overall high colony densities in the feral population.

The third chapter (Kraus *et al.* 2010) also deals with a potentially invasive species, the bumblebee *B. terrestris*. Here we describe for the first time strong genetic introgression from the sampled greenhouse populations into the adjacent wild populations. Depending on the analyzed population, the number of individuals assigned to the greenhouse populations ranged from 8% to 47 %. This implies that greenhouses bumblebees are capable of changing the genetic structure of conspecific endemic subspecies. Since the consequences of this genetic spillover are hard to predict and potentially have negative effects, it seems advisable to handle and manage bumblebee imports with caution also in regions where *B. terrestris* is endemic.

The last chapter in this part is a phylo-geographic study on the African subterranean army ant *Dorylus fulvus* applying both mitochondrial and nuclear markers (Barth *et al.* submitted). The tested *D. fulvus* populations show a strong bio-geographical structure concerning their mitochondrial genetic variation, whereas the nuclear genetic population structure is less clearly pronounced. Moreover, the relative intensity of male gene flow is an order of magnitude higher than that of the females. Our results indicate low motility of queens but a large scale promiscuous gene flow by males. Thus, the well flying *D. fulvus* males appear to efficiently counteract the extremely limited queen dispersal at the nuclear but not at the mitochondrial level.

The last section thus nicely shows that despite all potential population-level restrictions resulting from eusociality, haplo/diploidy and *csd* system, many eusocial hymenoptera are very effective in seizing new habitats and in the maintenance of long distance gene flow. Sociality and the colony based life history does not exclude them from becoming invasive species, which is not only impressively documented by the astounding spread of the Africanized honeybees over the Americas in just a few decades but also by other invasive wasp, ant, and bee species all over the globe

Outlook

The publications united in this thesis comprise results from many years of field work in the Tropics of Latin America, Africa and Asia and the subsequent laboratory work applying molecular ecology tools. Even though the majority of species in this thesis are not model organisms, and the molecular microsatellite markers were often obtained by cross species amplification, the knowledge gain on the biology of these species has already been remarkable. The insights on the mating biology, genetic colony and population structure would have been hard, if not impossible to achieve without molecular markers. With the current advances in next generation sequencing technology and bioinformatics, new approaches like population genomic studies will be feasible also for non-model organisms in the near future. The rise of these new sequencing techniques will clearly further accelerate the knowledge gain on the biology of eusocial hymenoptera, which will in fact grow over-proportional compared to the advances in molecular ecology over the last 20 years. Still, the classical studies as presented here will be the very and essential foundation for the next step in research. Irrespective of novel techniques and methods used, it is equally clear that research on eusocial insects will remain one of the most challenging and exciting fields of research in modern biology. We are still far from understanding how a colony of social insects functions, yet this may be essential when we address the more ultimate question of why it functions in the first place. Equally important will also be what we can learn from social insect colonies for the understanding of our own societies. As Karl von Frisch stated: “The bee's life is like a magic well: the more you draw from it, the more it fills with water” (von Frisch 1950). This is true for research on social insects in general and the well is already deep indeed with floods to expect.

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7. Appendix

Curriculum Vitae

Personal Data

Name:	Frank Bernhard Kraus
Nationality:	German
Year of Birth:	1971
Place of Birth:	Mannheim

Education

PhD:	2003 (*magna cum laude*) Thesis: "Consequences of Mating Behaviour on the Population Ecology of Honeybees (*Apis mellifera* L.)"
Postgraduate Studies:	2000-2003 MLU Halle – Wittenberg, Germany
Diplom (Biologie):	2000, Thesis: "Population Ecology and Genetics of the Keeled Skimmer (*Orthetrum coerulescens*)"
Study of Biology:	1994-2000 J.W. Goethe-University Frankfurt (Main), Germany

Professional Career

Since 2008:	Postdoctoral Researcher, Institut für Biologie, Molekulare Ökologie, MLU Halle, Halle (Saale), Germany. BMBF-Project Fugapis and EU-Project BeeDoc
2005-2007	Researcher (*Investigador associado*) at ECOSUR (El Colegio de la Fronterea Sur), Department of Tropical Entomology in Tapachula, Chiapas, Mexico
2004	Postdoctoral Researcher, Institut für Biologie, Molekulare Ökologie, MLU Halle, Halle (Saale), Germany. EU - Project ALARM
2000-2003:	Research Associate, Institut für Biologie, Molekulare Ökologie, MLU Halle, Halle (Saale), Germany. EU-Project BABE

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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass diese Habilitationsschrift selbständig und ohne fremde Hilfe verfasst wurde. Andere als die angegebenen Quellen und Hilfsmittel wurden nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche kenntlich gemacht.

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