

**"The evolution of mating systems in
highly eusocial Hymenoptera"**

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“A large number of individuals, by giving a better chance within any given period for the appearance of profitable variations, will compensate for a lesser amount of variability in each individual, and is, I believe, a highly important element of success.”

Charles Darwin (*The Origin of Species*)

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Summary

Genetic diversity within species has demonstrable advantages at various levels of biological organization. Although the genetic diversity found inside colonies of eusocial Hymenoptera (wasps, bees and ants) has proven to confer important benefits, its advantages at the population level have been much less studied. Moreover, the impact of mating system on the genetic structure of wild population is still poorly understood. Evidence of a genetic basis for worker caste polymorphism is still scarce and was previously confined to ant species exhibiting either small or large workers. Showing a high queen mating frequency and an extreme worker polymorphism, with four recognized physical worker castes, the New World army ant *Eciton burchellii* is among the most suitable study models to test the generality of genetic effects on caste differentiation in insect societies. The first manuscript presented here, proved the existence of a genetic component for worker caste determination in *E. burchellii*, showing that high genetic variation combined with a genetic component to worker caste determination, may increase homeostasis in systems with complex division of labour. At the population level, genetic diversity is ultimately determined by the genetically effective population size (N_e), which due to the combination of eusociality, haplodiploidy and complementary sex determination, is severely constrained in eusocial Hymenoptera. Additional limitations on N_e occur in army ants since they have wingless queens and colony fission, both factors causing restricted maternal gene flow and high population viscosity. The second manuscript addresses the impact of dispersal and multiple mating on the structure of *E. burchellii* populations, showing that male dispersal and polyandry seem to enhance gene flow and minimize the deleterious effects associated with small effective population size. Gene flow in wild populations of eusocial Hymenopterans, however, will not only be affected by the colony characteristics exhibited at any given time, but also by the turnover of colonies over time. The western honeybee (*Apis mellifera*) represents an optimal model to study colony turnover since its mating system involves the regular aggregation of thousands of males from many colonies in specific drone congregation areas (DCAs) that virgin queens visit to mate. The third manuscript presented here assesses the temporal changes in the genetic structure of a wild DCA in Africa, finding an extremely high turnover of the queens contributing drones to the DCA. DCAs of African honeybees thus seem to be extremely dynamic systems, which together with colony migrations, boost the effective population size and maintain a high genetic diversity in the population. The second and the third article of this thesis demonstrate that at the population level, genetic diversity also seems to have important implications for the evolution of mating systems in highly eusocial systems. Another factor potentially shaping the genetic diversity of wild Hymenopteran populations is sexual selection. Army ants and honeybees are ideal models to study sexual selection, since they share extremely male biased sex ratios but exhibit different life histories. Army ant males must find a receptive queen and be accepted by its workers in order to mate. In contrast, honeybee drones concentrate at DCAs waiting to mate with virgin queens on flight. Thus, female mate choice is likely to play a more important role in army ants, and male-male competition seems to prevail in honeybees. For instance, while the second manuscript shows that army ant workers choose the males least related to their queen; the fourth article here included demonstrates that honeybee mating flights select the drones with a higher developmental stability, regardless of their genetic background. Hence, whereas female choice in army ants seems to promote outbreeding, male-male competition among honeybee drones is mainly based on environmental cues, and thus not likely to constrain genetic diversity. Addressing the conservation status of European honeybees, the last manuscript here included stands apart from the previous four. The past and present biodiversity of European honeybees are reviewed, coupled with a brief summary of the management and conservation strategies hold by different countries. Beekeeping practices in Europe were found to have a profound influence over the abundance of honeybees. It is therefore concluded that conservation policies directed at promoting responsible beekeeping, while preserving the current honeybee genetic diversity, should be implemented soon to protect this important pollinator.

Zusammenfassung

Innerartliche genetische Diversität hat auf verschiedenen Ebenen der biologischen Organisation demonstrierbare Vorteile. Obwohl genetische Variation innerhalb der Kolonie bei eusozialen Hautflüglern (Wespen, Bienen und Ameisen) als vorteilhaft bewiesen wurde, sind die Vorteile auf Populationsebene weit weniger untersucht worden. Des Weiteren sind die Auswirkungen des Paarungssystems auf die genetische Struktur wilder Populationen bisher kaum verstanden. Die Hinweise auf eine genetische Basis von Arbeiterinnen-Kasten-Polymorphismen sind spärlich und bisher lediglich auf Ameisenarten mit zwei Größenkategorien von Arbeiterinnen beschränkt. Die Neuwelt-Treiberameise *Eciton burchellii* gehört durch die hohe Paarungsfrequenz der Königin und den mit vier anerkannten physischen Kasten extremen Arbeiterinnen-Polymorphismus zu den am besten geeigneten Modellorganismen, um die Allgemeingültigkeit genetischer Effekte zur Kastendetermination in Insektenstaaten zu studieren. Das erste hier vorgestellte Manuskript belegt das Vorhandensein einer genetischen Komponente der Arbeiterinnen-Kastendetermination in *E. burchellii*, was zeigt, dass hohe genetische Variation in Verbindung mit einer genetischen Komponente der Arbeiterinnen-Kastendetermination das Gleichgewicht in Systemen mit komplexer Arbeitsteilung erhöhen kann. Auf Populationlevel wird genetische Variation ultimat allerdings durch die effektive Populationsgröße (N_e) bestimmt, die bei eusozialen Hymenopteren durch die Kombination von Eusozialität, Haplodiploidie und komplementärer Geschlechtsbestimmung stark eingeschränkt ist. Bei Treiberameisen ist die effektive Populationsgröße durch die Flügellosigkeit der Königinnen und durch Koloniefissionen sogar noch zusätzlich eingeschränkt. Beide Faktoren verursachen einen eingeschränkten maternalen Genfluss sowie eine hohe Populationsviskosität. Das zweite Manuskript befasst sich mit den Auswirkungen von Verbreitung und multipler Verpaarung auf die Struktur von *E. burchellii* Populationen und zeigt, dass sowohl die Verbreitung von Männchen als auch Polyandrie den Genfluss zu erhöhen, und gleichzeitig die mit kleinen effektiven Populationsgrößen zusammenhängenden, schädlichen Effekte zu minimieren scheinen. Jedoch wird der Genfluss in natürlichen Populationen eusozialer Hymenopteren nicht nur durch die Charakteristika der Kolonien zu einer gegebenen Zeit bestimmt, sondern auch durch den zeitlichen Wechsel von Kolonien. Die westliche Honigbiene (*Apis mellifera*) stellt ein ideales Modell zur Untersuchung von Koloniewechseln dar, da ihr Paarungssystem die regelmäßige Aggregation tausender Männchen verschiedener Kolonien an speziellen Drohnensammelplätzen enthält ("drone congregation area", DCA), welche von den unverpaarten Königinnen aufgesucht werden. Das dritte Manuskript behandelt die temporären Veränderungen der genetischen Struktur natürlicher DCAs in Afrika. Es konnte ein extrem hoher Wechsel derjenigen Königinnen gezeigt werden, von denen die Drohnen der DCAs abstammen. Folglich scheinen die DCAs afrikanischer Honigbienen ein extrem dynamisches System zu sein, was zusammen mit Koloniewanderungen die effektive Populationsgröße erhöht und dadurch eine hohe genetische Diversität in der Population erhält. Der zweite und dritte Artikel dieser Arbeit demonstrieren, dass genetische Diversität ebenfalls auf Populationsebene wichtige Auswirkungen auf die Evolution von Paarungssystemen in hoch-eusozialen Insekten hat. Ein weiterer Faktor, der die genetische Diversität natürlicher Hymenopterenpopulationen möglicherweise prägt, ist sexuelle Selektion. Treiberameisen und Honigbienen stellen ideale Modellorganismen dar, um sexuelle Selektion zu untersuchen, da beide ein stark in Richtung der Männchen verschobenes Geschlechtergleichgewicht teilen aber unterschiedliche Lebensentwicklungen aufweisen. Männchen von Treiberameisen müssen, um sich zu verpaaren, eine paarungsbereite Königin finden und von deren Arbeiterinnen akzeptiert werden. Im Gegensatz dazu versammeln sich Honigbienen Drohnen an DCAs und warten darauf, sich mit einer unverpaarten Königin im Flug zu paaren. Folglich spielt wahrscheinlich bei Treiberameisen die weibliche Partnerwahl eine wichtige Rolle, während bei Honigbienen der Wettstreit zwischen den Männchen überwiegt. Während beispielsweise das zweite Manuskript zeigt, dass Treiberameisenarbeiterinnen diejenigen Männchen auswählen, welche am wenigsten mit der Königin verwandt sind, demonstriert der vierte hier vorgestellte Artikel, dass der Hochzeitsflug bei Honigbienen diejenigen Drohnen mit einer höheren Entwicklungsstabilität selektiert und zwar unabhängig ihrer genetischen Herkunft. Während die weibliche

Partnerwahl bei Treiberameisen das Auskreuzen zu fördern scheint, basiert der Konkurrenzkampf zwischen Männchen bei Honigbienen hauptsächlich auf Umwelteinflüssen und beschränkt daher wahrscheinlich die genetische Diversität nicht. Das letzte hier eingefügte Manuskript befasst sich mit dem Stand des Umweltschutzes europäischer Honigbienen und steht somit nicht in Bezug zu den vorigen vier Artikeln. Zusammen mit einer kurzen Zusammenfassung der Management- und Schutzstrategien in verschiedenen Ländern, wird eine Übersicht zur Biodiversität der Europäischen Honigbienen in der Vergangenheit und Gegenwart gegeben. Die Untersuchungen ergaben einen profunden Einfluss der Imkereipraktiken auf die Abundanz von Honigbienen in Europa. Es wird daher der Schluss gezogen, dass Schutzstrategien mit dem Ziel, eine nachhaltige Imkerei zu fördern und gleichzeitig die momentane genetische Diversität der Honigbienen zu erhalten, zeitnah umgesetzt werden sollten, um diesen wichtigen Bestäuber zu schützen.

Keywords: *Apis mellifera*, beekeeping, drone congregation areas, polyandry, *Eciton burchellii*, effective population size, gene flow, honeybee conservation, mating flights, multiple mating, sexual selection, social insects.

General Introduction

The eusocial Hymenoptera

Exhibiting exceptionally high levels of social organization and extremely specialized and complex mating systems, the social Hymenoptera (wasps, bees and ants) constitute an ideal group of insects to test specific predictions of evolutionary theories (Wilson 1971; Hölldobler & Wilson 1990; Thornhill and Alcock 2001). The social Hymenoptera dominate terrestrial ecosystems, are among the main predators of other arthropods, pollinate the majority of flowering plants and are important food sources for many other animals (Wilson 1971). A particular characteristic shared by all Hymenopterans is haplodiploidy. Hymenoptera males are raised from unfertilized eggs laid by diploid females, and hence they only have one half of the genetic material carried by females. In consequence, female daughters of such haploid males share exactly the same paternal genotype, and thus their genetic relatedness is higher than that observed between sisters of diplo-diploid organisms. Following kin selection theory (Hamilton 1964), social organization should be strongly influenced by the genetic relatedness among group members, since it determines the cost/benefits thresholds required to favour the evolution and maintenance of altruistic behaviours (Queller 1993). Haplodiploidy has thus immense implications for the evolution of sociality. Eusociality, an extreme form of altruism, has evolved several times in the Hymenoptera, providing fertile ground to study different kinds of social conflicts in the light of kin selection theory (e.g. Keller *et al.* 1993; Bourke & Franks 1995; Crozier & Pamilo 1996).

Eusocial insects exhibit reproductive division of labour, with one or few individuals undertaking reproduction (the queens), cooperative brood care, and the presence of overlapping cohorts of specialized sterile helpers (the workers; Wilson 1971). In some groups, here termed as “highly eusocial”, the workers have undergone an extreme level of specialization, losing their ability to mate and reproduce sexually. An intricate division of labour has evolved in these groups, involving clearly distinct worker castes (Oster & Wilson 1978). Such high levels of social organization might have facilitated the appearance of complex mating systems, such as multiple queens in a single colony (polygyny) or multiply mated queens (polyandry; see Hughes *et al.* 2008a). Both mating systems have profound consequences for social organization since they reduce the intracolony worker-worker relatedness, and hence the benefits gained through inclusive fitness may not exceed the cost associated with the maintenance of the sterile worker behaviour (Hamilton 1964; Queller 1993). Non-kin related adaptive advantages must therefore influence social organization in order to maintain the colony structure in these cases (Korb & Heinze 2004).

Genetic diversity in eusocial Hymenoptera

The occurrence of polygyny and polyandry has been found to be negatively correlated across taxa (Keller & Reeve 1994; Hughes *et al.* 2008b), which suggests they are alternative mechanisms to boost intracolony genetic diversity. Genetic diversity within social insect colonies has proven to confer important benefits, such as increased productivity, broader tolerance to environmental changes, increased resistance to pathogens, and improved task performance through a more efficient division of labour (reviewed by Palmer & Oldroyd 2000 and Crozier & Fjerdingstad 2001). The benefits of a high genetic diversity at the population level, however, have been much

less studied, and the impact of such mating system on the genetic structure of wild population is still poorly understood.

The amount of genetic diversity found in a given population is ultimately determined by its genetically effective size (N_e), which refers to the number of individuals in an idealized population that would experience the same rate of random genetic change over time as the real population under consideration (Wright 1931; Hartl & Clark 2007). In eusocial Hymenoptera, the combination of eusociality, haplodiploidy and complementary sex determination, constrains effective population size to a greater extent than in other insect taxa (Hedrick & Parker 1997; Chapman & Bourke 2001; Packer & Owen 2001). Eusociality is characterized by the occurrence of one or few reproductive females per colony (the queens). Since N_e is mostly limited by the abundance of the rare sex, eusocial Hymenoptera have smaller effective population sizes than solitary species with a larger number of reproductive individuals per unit area (Wilson 1963; Chapman & Bourke 2001). In addition, the haploid condition of males reduces the number of copies of a given allele in the population, and hence the effective population size of haplodiploids is $\frac{3}{4}$ the effective population size of diploid organisms under equal sex ratios (Hedrick & Parker 1997). Finally, the common sex determination system based on the complementary action of specific sex alleles at an autosomal locus (Cook & Crozier 1995), inevitably results in a fraction of inviable or effectively sterile diploid males in the population. The production of diploid males not only limits colony growth, but also biases the effective breeding sex ratio in favour of haploid males, further reducing N_e (Zayed 2004). The combination of these factors makes populations of eusocial Hymenoptera particularly susceptible to losing genetic variability due to genetic drift. In consequence, strong selective pressures are expected to have shaped mating systems in order to avoid the negative effects associated with inbreeding depression (reviewed by Keller & Waller 2002), which in the Hymenoptera include decreased colony foundation success, reduced colony growth and reproduction, and a shorter colony life span (Gerloff & Schmid-Hempel 2005; Haag-Liautard *et al.* 2008).

Gene flow in army ant and honeybee populations

Although rare, polyandry is widespread among eusocial Hymenoptera (Boomsma & Ratnieks 1996; Strassmann 2001; Hughes *et al.* 2008b). The most extreme levels of polyandry are found in honeybees and army ants, where queens usually mate with more than ten males. Both are highly eusocial groups sharing obligatory multiple mating by queens and the same mode of colony reproduction. In contrast to species where the queens found new colonies alone (independent colony foundation), army ants and honeybee queens ground new colonies accompanied by a group of workers (dependent colony foundation). Honeybee queens leave their old nest along with a large proportion of workers to fund a new nest at a different location (Seeley 1985). Pupae containing daughter queens, who will take over reproduction, are left behind in the old nest along with the remaining workers. Dispersal in honeybees is therefore determined by the flight ability of the sexuals and the workers (Peters and Ito 2001). Similarly, army ant colonies reproduce through colony fission, with the old queen taking a large proportion of workers and leaving behind freshly emerged daughter queens. Army ant queens and workers, however, have permanently lost their wings, and thus dispersal is mainly undertaken by the males (Hölldobler and Wilson 1990). Effective dispersal and gene flow is therefore very different in honeybees and army ants, and hence alternative mechanisms are expected to maximize gene flow in each group.

Army ants are a large group of mostly tropical and subterranean ants characterized by the “army ant adaptive syndrome” (Gotwald 1995), namely the combination of group predation and

nomadism. The enhanced group predation efficiency that results from very large colonies reproducing through colony fission, probably selected for the loss of wings in army ant queens, at the expense of higher dispersion ability. In addition, as an inevitable consequence of colony fission, army ants exhibit extremely male biased sex ratios, enhancing the opportunity for random genetic drift and making this species more susceptible to inbreeding (Hartl and Clark 2007). Army ants thus represent an excellent test case for the efficiency of dispersal and mating strategies in enhancing gene flow (Hölldobler and Bartz 1985; Gadagkar 1991).

The mating system of the honeybee (*Apis mellifera*), on the other hand, has been regarded as one of the most panmictic in the animal kingdom, with thousands of males aggregating in drone congregation areas (DCAs) that virgin queens visit to mate with tens of partners. DCAs are formed irrespective of the presence of a queen, at open sites delimited by conspicuous geographic landmarks, such as valleys, river shores or forest openings. Moreover, they are stable through time, with some locations known to have served as DCA for decades (Ruttner 1972). Although DCA are known to gather males from most colonies within recruitment range (Baudry *et al.* 1998), the temporal changes in the colonies contributing drones remain unknown. Yet, changes in the DCAs' genetic structure will ultimately determine population gene flow and effective population size.

Study questions

The different life histories of army ants and honeybees constitute two alternative models to study the evolution of mating systems in highly eusocial Hymenopterans with queen multiple mating and male biased sex ratios. Focusing on two species, namely the army ant *Eciton burchellii* and the honeybee *Apis mellifera*, this thesis aims to provide a broad insight into the mating systems of these two groups. The following major questions are addressed in each one of the next five chapters:

- I. Is there a genetic component for worker caste determination in the army ant *Eciton burchellii*?
- II. What is the impact of male dispersal and multiple mating on gene flow in the army ant *Eciton burchellii*?
- III. How dynamic are drone congregation areas of the honeybee *Apis mellifera*?
- IV. Is there male-male competition during the mating flights of *Apis mellifera* drones?
- V. What is the current status of native honeybee populations in Europe and which factors constitute a threat to their conservation?

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I. Worker caste determination in the army ant *Eciton burchellii*

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Abstract

Elaborate division of labour has contributed significantly to the ecological success of social insects. Division of labour is achieved either by behavioural task specialisation 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 intracolony 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 *E. 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.

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

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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 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 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 minimize the deleterious effects associated with small effective population size.

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III. Temporal variation in the genetic structure of a drone congregation area: An insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*)

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Abstract

The mating system of the honeybee (*Apis mellifera*) has been regarded as one of the most panmictic in the animal kingdom, with thousands of males aggregating in drone congregation areas (DCAs) that virgin queens visit to mate with tens of partners. Although males from many colonies gather at such congregations, the temporal changes in the colonies contributing drones remain unknown. Yet, changes in the DCAs' genetic structure will ultimately determine population gene flow and effective population size. By repeatedly sampling drones from an African DCA over a period of three years, we studied the temporal changes in the genetic structure of a wild honeybee population. Using three sets of tightly linked microsatellite markers, we were able to reconstruct individual queen genotypes with a high accuracy, follow them through time and estimate their rate of replacement. The number of queens contributing drones to the DCA varied from 12 to 72 and was correlated with temperature and rainfall. We found that more than 80% of these queens were replaced by mostly unrelated ones in successive eight months sampling intervals, which resulted in a clear temporal genetic differentiation of the DCA. Our results suggest that the frequent long range migration of colonies without nest-site fidelity is the main driver of this high queen turnover. DCAs of African honeybees should thus be regarded as extremely dynamic systems which together with migration boost the effective population size and maintain a high genetic diversity in the population.

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IV. Mating flights select for symmetry in honeybee drones (*Apis mellifera*)

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Abstract

Males of the honeybee (*Apis mellifera*) fly to specific drone congregation areas (DCAs), which virgin queens visit in order to mate. From the thousands of drones that are reared in a single colony only very few succeed in copulating with a queen, and therefore a strong selection is expected to act on adult drones during their mating flights. In consequence, DCAs may serve as a mate selection mechanism, assuring that queens only mate with those individuals having a better flight ability and a higher responsiveness to the queen's visual and chemical cues. We tested this idea relying on fluctuating asymmetry (FA) as a measure of phenotypic quality. By recapturing marked drones at a natural DCA and comparing their FA with a control sample of drones collected at their natal colonies, we were able to detect any selection on wing size and wing FA occurring during the mating flights. Aiming to obtain independent drone samples, sharing the same mother queen and similar rearing conditions, we confirmed drone parentage by genotyping all samples with three sets of linked microsatellite markers. Although we found no evidence for selection on wing size, FA was found to be significantly lower in the drones collected at the DCA than in those collected at the hives. Our results demonstrate that developmental stability can determine the mating ability of honeybee drones. We therefore conclude that selection during the mating flights of drones seems to be an important factor for female mate choice in honeybees.

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V. Biodiversity, conservation and current threats to European honeybees

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Abstract

Europe harbours several endemic honeybee (*Apis mellifera*) subspecies. Yet the distribution of these subspecies is nowadays also much influenced by beekeeping activities. Large scale migratory beekeeping and trade in queens, coupled with the promiscuous mating system of honeybees, have exposed native European honeybees to increasing introgressive hybridization with managed non-native subspecies, which may lead to the loss of valuable combinations of traits shaped by natural selection. Other threats to European honeybees are factors that have caused a progressive decline in *A. mellifera* throughout the world in recent years, leading to large economic losses and jeopardizing ecosystem functioning. We review the biodiversity of European honeybees and summarize the management and conservation strategies employed by different countries. A comprehensive picture of the beekeeping industry in Europe is also provided. Finally we evaluate the potential threats affecting the biodiversity of European honeybee populations and provide some perspectives for future research.

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(Invited paper for the *Apidologie* special issue on bee conservation)

General Discussion

The colony level perspective

Genetic diversity within species has verifiable advantages at various levels of biological organization (Hartl & Clark 2007; Nonacs & Kapheim 2007). Within social insect colonies, genetic diversity has proven to confer important benefits, such as an enhanced productivity and a broader tolerance to environmental changes (Crozier & Page 1985; Page *et al.* 1995; Fjerdingstad 2002; Jones *et al.* 2004; Schwander *et al.* 2005; Mattila & Seeley 2007), increased resistance to pathogens (Brown & Schmid-Hempel 2003; Tarpay 2003; Hughes & Boomsma 2004 2006; Tarpay & Seeley 2006; Seeley & Tarpay 2007), and improved task performance through a more efficient division of labour (Fuchs & Moritz 1998, Hughes *et al.* 2003; Julian & Fewell 2004).

The existence of a genetic component for worker task-specialization is now well documented in honeybees (Robinson & Page 1988; Frumhoff & Baker 1988; Moritz & Hillesheim 1989; Oldroyd *et al.* 1992; Robinson *et al.* 1994; Page *et al.* 1995; Page & Erber 2002), but less so among the ants (Julian & Fewell 2004; Rosset *et al.* 2005). Although worker polymorphism and intra-colonial genetic diversity appear to be positively correlated across ant taxa (Fjerdingstad & Crozier 2006), evidence of a genetic basis for worker caste polymorphism is still scarce and was previously confined to ant species from two subfamilies, exhibiting either small or large workers (Fraser *et al.* 2000; Hughes *et al.* 2003; Rheindt, *et al.* 2005). Showing a high queen mating frequency and an extreme worker polymorphism, with four recognized physical worker castes (Franks 1985), the New World army ant *Eciton burchellii* is among the most suitable study models to test for the generality of genetic effects on caste differentiation in insect societies. The first manuscript here presented, proved the existence of a genetic component for worker caste determination in *E. burchellii*, showing that high genetic variation combined with a genetic component for worker caste determination, may increase homeostasis in systems with complex division of labour (Fjerdingstad & Crozier 2006; Oldroyd & Fewell 2007).

This first manuscript supports the notion that genetic diversity is associated to highly eusocial systems, exhibiting an extreme worker caste differentiation. However, a recent study showed that polyandry was not the ancestral state of eusocial insects, suggesting that it only appeared after the workers had lost the ability to reproduce sexually (Hughes *et al.* 2008). Hence, genetic diversity does not seem to be the cause of the high levels of worker specialization observed in some species (Oster & Wilson 1978). By providing the colony members with a broad range of response thresholds to different environmental stimuli, intracolony genetic diversity may nevertheless increase colony homeostasis and thus help to maintain a complex social organization (Oldroyd & Fewell 2007).

The population level perspective

At the population level, genetic diversity is ultimately determined by the genetically effective population size (N_e), which due to the combination of eusociality, haplodiploidy and complementary sex determination is severely constrained in eusocial Hymenoptera (Hedrick & Parker 1997; Chapman & Bourke 2001; Packer & Owen

2001). Army ants are particularly exposed to genetic drift since their queens are wingless and colony fission is the usual way of reproduction, both factors causing restricted maternal gene flow and high population viscosity (Gotwald 1995; Berghoff *et al.* 2008). Winged army ant males thus gain a particular significance to ensure dispersal, facilitate gene flow and avoid inbreeding (Keller & Passera 1993; Nunney 1993; Crozier & Pamilo 1996; Haag-Liautard *et al.* 2008). The second manuscript addresses the impact of dispersal and multiple mating on gene flow in *E. burchellii*, showing that male dispersal and polyandry seem to enhance gene flow and minimize the deleterious effects associated with a small effective population size.

Gene flow in wild populations of eusocial Hymenopterans, however, will not only be affected by the colony characteristics exhibited at any given time, but also by the turnover of colonies over time. Colony turnover is very difficult to quantify in natural populations, because it requires identifying and following particular colonies in the wild. The western honeybee (*Apis mellifera*) represents an optimal model to study colony turnover since its mating system involves the aggregation of thousands of males from many colonies in specific drone congregation areas (DCAs) that virgin queens visit to mate (Winston 1987; Baudry *et al.* 1998). Furthermore, the recent availability of the honeybee genome (The Honeybee Genome Sequencing Consortium 2006) has made possible the development of new molecular DNA tools that allow the very accurate reconstruction of queen genotypes from a random sample of honeybee drones (Moritz *et al.* 2007; Shaibi *et al.* 2008). The third manuscript presented here assesses the temporal changes in the genetic structure of a wild honeybee population. The fact that more than 80% of the queens contributing drones to a particular DCA were found to be replaced by mostly unrelated ones in successive eight months sampling intervals, shows that DCAs of African honeybees are extremely dynamic systems, which together with colony migrations, boost the effective population size and maintain a high genetic diversity in the population (Estoup *et al.* 1995).

The second and third paper of this thesis demonstrate that at a higher, population level, genetic diversity also seems to have important implications for the evolution of mating systems in highly eusocial systems. Group-level adaptations can be easily over-run by individual or colony level benefits, and have therefore remained as alternative or second-order explanations (Bourke & Franks 1995; Crozier & Pamilo 1996). The mating systems of eusocial Hymenoptera, however, probably evolved in response to multiple selection pressures, and therefore a broader, multi-level selection perspective, is likely to yield new interesting insights (Korb & Heinz 2004). For instance, these two papers show that in addition to all the colony-level benefits mentioned above, high genetic diversity is also important at the population level in order to counterbalance small effective population sizes. The mating systems of these two highly eusocial Hymenopterans can therefore be regarded as alternative approaches to maximize gene flow and avoid inbreeding.

Interestingly, army ants and honeybees have evolved different mechanisms in response to different selective pressures. Because of the lack of winged queens and workers, army ant dispersal is far more restricted than that of honeybees. In consequence, winged army ant males are responsible for most dispersal. In the second paper we show that army ant males are capable of undertaking long mating flights. In addition, by mating with many males, army ant queens sample genes from males of many different colonies, thus promoting panmixis and reducing the strength of genetic drift. On the contrary, honeybee queens and workers are capable of undertaking long-

distance migratory swarms and hence the males do not play such an important role in dispersal as in army ants (Hepburn & Radloff 1998). Honeybee males, for instance, join the nearest DCA to mate (Koeniger *et al.* 2005a), probably aiming at minimizing their energy expenditure during the mating flights. Nevertheless, honeybees have been regarded as one of the most panmictic organisms in the animal kingdom (Baudry *et al.* 1998), because such DCAs can comprise males from most colonies within recruitment area. The third paper here presented, quantified the temporal changes in the genetic composition of a wild DCA, showing that such drone-contributing colonies are extremely mobile, causing a significant temporal genetic differentiation of African DCAs. Hence, in contrast to army ants, dispersal in honeybees is mainly undertaken by migratory colonies.

Sexual selection

The selection processes occurring during the location of mates, the copula and the fertilization, can act either among members of one sex (intra-sexual selection) or among the members of both sexes (inter-sexual selection). Sexual selection primarily operates on the sex with the greatest variance in reproductive success. As this is usually the male sex, intra-sexual selection is normally referred to as male-male competition, and intra-sexual selection as female choice (Andersson 1994). Both kinds of selection result in the greater reproductive success of the selected males, at the expense of a reduction in the genotypic and phenotypic variance found in the initial male population (Andersson 1994). It is therefore a challenge to understand how genetic diversity is maintained under different kinds of sexual selection (Thornhill & Alcock 2001; Andersson & Simmons 2006).

The strength of sexual selection increases with the variance in the reproductive success of the males, i.e. when the operational sex ratios are more male biased. Along with stingless bees, army ants and honeybees have the most extreme male biased sex ratios among the social Hymenoptera (Boomsma *et al.* 2005). Army ants and honeybees are therefore particularly suitable models to compare sexual selection mechanisms in species with different life histories. Before gaining access to a queen, *E. burchellii* males must cross the potentially deadly worker force, ultimately “choosing” the next fathers of their colony (Schneirla 1971). Franks and Hölldobler (1987) pointed out that if workers are to maximize their own inclusive fitness, they should choose the males that offer the greatest fertility, presumably the more robust ones exhibiting the most queen-like parafumarie. Such a direct mate choice over many generations would facilitate the development of a greater worker preference and more pronounced male traits, resulting in a Fisherian runaway selection. This circular process of positive feedback between female preference and male traits usually cause a significant reduction in genetic diversity (Andersson 1994), which would be particularly harmful for species with small effective population sizes, such as army ants. In the second manuscript, *E. burchellii* queens were found to be less related than average to their mates. Moreover, the same result was found in three other *E. burchellii* populations in Panama (Berghoff *et al.* 2008), which provides strong evidence for a relatedness-based worker mate choice. Army ant workers thus seem to choose the queen’s mates based on their relatedness to the queen, preferring the least related males among those having accomplished a successful mating flight. This mate choice mechanism might have

evolved to reduce sib-matings and the deleterious effects associated with inbreeding or genetic incompatibility (Cook & Crozier 1995; Ayasse *et al.* 2001; Oppelt *et al.* 2008).

In contrast to army ants, honeybee males do not experience such a worker-driven selection, because they never enter the colony of their future mate. Moreover, honeybee copulas are extremely fast and occur in mid-air, making any direct pre-copulatory mate selection by the queens unlikely (Baer 2005). On the other hand, male-male competition is probably very strong in honeybees. From the thousands of drones that are reared in a single colony, only very few succeed in copulating with a queen, and hence a strong selection is expected to act on adult drones during their mating flights (Koeniger *et al.* 2005b). The formation of DCAs may thus serve as an indirect mate choice mechanism, assuring that queens only mate with those individuals having a better flight ability and a higher responsiveness to the queen's visual and chemical cues. This idea was tested in the fourth manuscript, which shows that developmental stability, assessed through fluctuating asymmetry (Møller and Swaddle 1997; Polak 2003), seems to determine the mating ability of honeybee drones. Moreover, such developmental stability was found to be independent of genetic factors, being the rearing environment (or the interaction between genotype and the rearing environment) the main source of phenotypic variance. Drone mating flights thus seem to select the drones with a higher developmental stability (which are also likely to have more and higher quality sperms), without causing an important reduction in genetic diversity. Taken together, these results highlight once more the importance of dispersal and gene flow as key factors shaping the evolution of mating systems in highly eusocial Hymenoptera (Boomsma *et al.* 2005).

Beekeeping and the conservation of native honeybees in Europe

Apis mellifera is arguably the world's most important beneficial insect, being of great value to man and nature. They produce honey, pollinate crops worth billions of Euros per year, and provide full and part time employment to beekeepers throughout the globe (Southwick & Southwick 1992; Kevan & Phillips 2001; Moritz *et al.* 2005). The honeybee is also a vital member of many terrestrial ecosystems, pollinating a broad spectrum of wild flora. Moreover, because they hibernate as colonies with large numbers of workers they are essential for ensuring early spring pollination, when other pollinators are absent (Aizen & Feinsinger 1994; Dick 2001).

Considering the importance of genetic diversity for honeybee populations, any effort aiming at the conservation of this key pollinator should preserve its current biodiversity. In many regions of Africa, where beekeeping is poorly developed, truly wild and undisturbed honeybee populations can still be found (Moritz *et al.* 2005). In contrast, the current distribution and zones of natural hybridization of the ten morphologically and genetically distinct European *A. mellifera* subspecies have been strongly influenced by beekeeping activities over the course of history (Ruttner 1988). The promiscuous mating system of honeybees, coupled with large scale migratory beekeeping and queen trade, has exposed native European honeybees to increasing introgressive hybridization with managed non-native subspecies (Jensen *et al.* 2005; Dall'Olio *et al.* 2007). Such hybridization modifies the genetic pool of local honeybee populations, leading to the loss of their genetic identity. This is alarming since native subspecies constitute important reservoirs of local adaptations, ultimately determining the survival of honeybees in natural habitats (Randi 2008).

Addressing the conservation status of European honeybees, the last manuscript stands apart from the previous four. The past and present biodiversity of European honeybees are reviewed in the fifth manuscript of this thesis, coupled with a brief summary of the management and conservation strategies held by different countries. By surveying the apicultural practices of 33 European countries, a comprehensive picture of the magnitude and nature of the beekeeping industry in the continent is provided. The density of managed hives across all countries was found to be positively correlated to the mean number of hives kept by local beekeepers, showing that the size of beekeeping operations can profoundly influence the local abundance of beehives. Pollution, land use, and harmful beekeeping practices were found among the main factors currently threatening honeybee populations (van Engelsdorp *et al.* 2007; Potts *et al.* 2008). It is therefore concluded, that conservation policies directed at promoting responsible beekeeping while preserving the current honeybee genetic diversity, should be implemented soon to avoid the extinction of valuable native subspecies.

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Appendix

Declaration on the Author Contributions

- I. Jaffé R, Kronauer DJC, Kraus FB, Boomsma JJ, Moritz RFA (2007) Worker caste determination in the army ant *Eciton burchellii*. *Biology Letters* 3(5): 513-516.

I genotyped the samples, merged our data with that from our Copenhagen partners, performed the analyses and wrote the paper. D. Kronauer collected and genotyped the Venezuelan samples and participated in the analyses and the writing of the paper. B. Kraus collected the Mexican samples and participated in the analyses and the writing of the paper. J. Boomsma and R. Moritz designed and supervised the project, and provided helpful discussions.

- II. Jaffé R, Moritz RFA, Kraus FB (2009) Gene flow is maintained by polyandry and male dispersal in the army ant *Eciton burchellii*. *Population Ecology*. Online First, DOI: 10.1007/s10144-008-0133-1.

I participated in the design of the project, genotyped the samples, performed the analyses and wrote the paper. B. Kraus collected the samples and participated in the analyses and the writing of the paper. R. Moritz participated in the design of the project, supervised the work, and provided helpful discussions.

- III. Jaffé R, Dietemann V, Crewe RM, Moritz RFA (2009) Temporal variation in the genetic structure of a drone congregation area: An insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). *Molecular Ecology*, *in press*.

I participated in the design of the project, collected and genotyped the samples, performed the analyses and wrote the paper. V. Dietemann collected the samples and participated in the analyses and the writing of the paper. R. Crewe facilitated the field work in South Africa and provided helpful discussions. R. Moritz participated in the design of the project, supervised the work, and provided helpful discussions.

- IV. Jaffé R, Moritz RFA (in review) Mating flights select for symmetry in honeybee drones (*Apis mellifera*). *Naturwissenschaften*.

I designed the project, collected, genotyped and measured the samples, performed the analyses and wrote the paper. R. Moritz supervised the work, and provided helpful discussions.

- V. De la Rúa P, Jaffé R, Dall 'Olio R, Muñoz I and Serrano J (2009) Diversity, conservation and current threats of European honeybees. *Apidologie*, *in press*.

I surveyed the apicultural practices of 33 European countries, wrote the section "Introgression in Honeybee Populations", most of "Management and Conservation" and part of "Future Research", and helped with the edition of the paper. R. Dall 'Olio wrote the section "Current Threats" and helped with the edition of the paper. I. Muñoz reviewed the literature on honeybee molecular diversity, wrote part of the section "Overview of the Evolutionary Branches and Molecular Lineages of *Apis mellifera*" and helped with the edition of the paper. P. De la Rúa designed and organized the project, wrote the remaining sections of the manuscript as well as the boxes, and edited the paper. J. Serrano supervised the whole work and wrote the conclusions of the manuscript.

Curriculum Vitae

Personal information

Birth	October 13 th 1980, Caracas, Venezuela.
Nationality	Venezuelan.
Languages	Spanish as a mother tongue, fluent English (TOEFL score 283/300) and conversation level in French, German and Portuguese.
Current Affiliation	Molecular Ecology Research Group, Institute of Biology, Martin Luther University Halle-Wittenberg. Hoher Weg 4, # 125, 06120 Halle (Saale), Germany.
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Higher education

2006-2009	PhD at the Martin Luther University Halle-Wittenberg, Germany. Dissertation thesis concerned with "The evolution of mating systems in highly eusocial Hymenoptera", supervised by Prof. Dr. Robin F.A. Moritz.
2005-2006	MSc in Molecular Ecology at the Martin Luther University Halle-Wittenberg, Germany. Dissertation thesis concerned with "Genetic basis for worker caste determination in the New World army ant <i>Eciton burchellii</i> ", supervised by Prof. Dr. Robin F.A. Moritz.
1998-2004	Licentiate in Biology at the Simón Bolívar University, Caracas, Venezuela. Dissertation thesis concerned with "Growth rate, locomotive performance and behavior of juvenile Amazon Turtles (<i>Podocnemis expansa</i>)", supervised by Dr. Guillermo Barreto.

Courses and internships

2007	Participant of the R-programming courses "First Steps in R" and "Data Analysis in R" offered by the Center for Statistical Consulting from the University of Bielefeld, Germany.
2006	Participant of the "Population Genetics Computer Lab" offered by the Evolutionary Biology Research Group from the University of Munich, Germany.
2004	Selected participant of the "Tropical Ecology and Conservation" course, taught by the Organization for Tropical Studies, Costa Rica.
2000	Exchange student at the São Paulo State University, Rio Claro, Brazil.

Work experience

- 2006-2009 Research associate in the BEESHOP European network. Martin Luther University Halle-Wittenberg, Germany.
- 2006-2009 Lecture assistant in honeybee biology and population genetics. Martin Luther University Halle-Wittenberg, Germany.
- 2000-2004 Technical assistant in diverse projects concerned with biological pest control, ecophysiology of nectarivorous birds, maintenance of cell lines and infectivity essays and biodiversity and abundance of marine invertebrates. Simón Bolívar University, Caracas, Venezuela.
- 1999-2004 Field assistant in diverse projects concerned with dispersion behavior in the capybara (*Hydrochaerus hydrochaeris*), ecophysiology of juvenile Amazon Turtles (*Podocnemis expansa*), biogeography of the Tegu lizard (*Tupinambis teguixin*), and plant-ant relationship in the amazon forest canopy. Simón Bolívar University, Caracas, Venezuela.
- 2001-2002 Lecture assistant in general zoology. Simón Bolívar University, Caracas, Venezuela.

Awards

- 2009 PhD dissertation graded *Magna Cum Laude*.
- 2009 Article awarded with the "Open Choice" option on the 51-2 issue of *Population Ecology*.
- 2005 Awarded with a scholarship from the DAAD-FUNDAYACUCHO (Germany-Venezuela) to cover the full costs of my MSc. at the Martin Luther University, Germany.
- 2004 Awarded with a scholarship from the Organization for Tropical Studies to cover part of the costs of the "Tropical Ecology and Conservation" course, Costa Rica.

Peer-reviewing experience

Referee of several papers submitted to the following journals: *Behavioral Ecology and Sociobiology*, *Journal of Apicultural Research*, *Journal of Arachnology* and *Naturwissenschaften*.

Publication List

Peer-reviewed articles

- Yañez O, **Jaffé R**, Jarosch A, Fries I, Moritz RFA, Paxton RJ, De Miranda JR (*in preparation*) Impact of vector mediation over the vertical transmission of a honeybee (*Apis mellifera*) virus.
- Jaffé R**, Moritz RFA (*submitted*) Mating flights select for symmetry in honeybee drones (*Apis mellifera*).
- Jaffé R**, Dietemann V, Allsopp MH, Costa C, Crewe RM, Dall'Olio R, De la Rúa P, El-Niweiri MAA, Fries I, Kezic N, Meusel MS, Paxton RJ, Shaibi T, Stolle E and Moritz RFA (2009) Filling the gap in pollinator decline censuses: Measuring the density of honeybee (*Apis mellifera*) colonies across their natural range. *Conservation Biology*, in press.
- De la Rúa P, **Jaffé R**, Dall 'Olio R, Muñoz I and Serrano J (2009) Biodiversity, conservation and current threats to European honeybees. *Apidologie* special issue on bee conservation, *in press*: DOI: 10.1051/apido/2009027.
- Jaffé R**, Dietemann V, Crewe RM, Moritz RFA (2009) Temporal variation in the genetic structure of a drone congregation area: An insight into the population dynamics of wild African honeybees (*Apis mellifera scutellata*). *Molecular Ecology*, 18 (7): 1511-1522.
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- Jaffé R**, Peñaloza C, Barreto G (2008) Monitoring an endangered fresh water turtle management program: Effects of nest relocation on growth and locomotive performance of the Giant South American Turtle (*Podocnemis expansa*: Podocnemididae). *Chelonian Conservation and Biology*, 7 (2): 213-222.
- Barrantes G, Sánchez C, Hilje B, **Jaffé R** (2008) Male song variation of Green Violetear (*Colibri thalassinus*) in the Talamanca Mountain Range, Costa Rica. *The Wilson Journal of Ornithology*, 120 (3): 519-524.
- Gianoli E, Sendoya S, Vargas F, Mejía P, **Jaffé R**, Rodríguez M, Gutiérrez A (2008) Patterns of *Azteca* ants' defence of *Cecropia* trees in a tropical rainforest: support for optimal defence theory. *Ecological Research*, 23 (5): 905-908.
- Jaffé R**, Kronauer DJC, Kraus FB, Boomsma JJ, Moritz RFA (2007) Worker caste determination in the army ant *Eciton burchellii*. *Biology Letters* 3(5): 513-516.
- Jaffé K, Horchler P, Verhaagh M, Gomez C, Sievert R, **Jaffé R**, Morawetz W (2007) Comparing the ant fauna in a tropical and a temperate forest canopy. *Ecotropicos*, 20(2): 74-81.
- Jaffé R**, Eberhard W, De Angelo C, Eusse D, Gutierrez A, Quijas S, Rodríguez A, Rodríguez M (2006) Caution webs in the way! Possible functions of silk stabilimenta in *Gasteracantha cancriformis* (Araneae: Araneidae). *Journal of Arachnology*, 34(2): 448-455.

Book chapters and popular articles

- Jaffé R**, Moritz RFA (2009) Beekeeping and the conservation of native honeybees in Europe. In: Atlas of Biodiversity Risks: From Europe to the Globe and from Stories to Maps. ALARM Project.
- Jaffé R**, Wolf S, Moritz RFA (2008) Bees in Europe and Sustainable Honey Production (BEESHOP): A European Research Network. *Bee Improvement and Conservation Magazine*, 1.

Erklärung

Halle (Saale), den 12 Februar 2009

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

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

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

Rodolfo Jaffé