Evolutionary aspects of mating and reproduction in honeybees

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“Success in sexual reproduction is at the heart of the evolutionary process”
Judson 2002

1 Introduction

An intriguing variety of mating systems and parental investment strategies has been evolved within the insect order Hymenoptera (Thornhill & Alcock 1983, Ayasse et al. 2001). Females of many taxa build nests and provide nutrition to their offspring. For instance, females of sphecid wasps (Apoidea: Spheciformes; the close phylogenetic relatives to the bees; Michener 2000) hunt insects or spiders in order to provision their brood; whereas bees (Apoidea: Apiformes; Michener 2000) in general collect nectar, honeydew and pollen for the same purpose. Parental care and sociality have progressively evolved in the Hymenoptera from the solitary state to a pinnacle in the eusocial bees, wasps and ants. Eusociality is characterised by cooperative brood care, overlapping generations and reproductive reproductive division of labour (Wilson 1971).

1.1 Mating and Reproduction in social Hymenoptera

There are two or more female castes in the eusocial Hymenoptera (Wilson 1971). The females of the worker castes usually don’t mate and rarely lay eggs. They are involved in many different tasks such as brood care, foraging or colony defence. The females of the reproductive castes are called either queens (Wilson 1971) or gamergates (=mated workers; Peeters & Crewe 1984, Peeters 1993). The term gyne indicates both potential and actual queens (Michener 1974).

The mating behaviour varies substantially in the social Hymenoptera (Hölldobler & Wilson 1990, Ayasse et al. 2001, Strassmann 2001). The most common form of mating system is monandry (Boomsma & Ratnieks 1996). Multiple mating of females is rather rare (Strassmann 2001). In some genera, however, for example *Vespula* wasps and *Atta* ants (Villesen et al. 1999, Foster et al. 1999), polyandry is the rule rather than the exception. The mating patterns in ants are classified in two major syndromes: the female calling syndrome and the male congregation syndrome (Hölldobler & Bartz 1985). This classification can be applied to many hymenopteran mating systems (Ayasse et al. 2001). Typically, females which “call” males release a sex pheromone near to their emergence site (Ayasse et al. 2001). Males and females in harvester ants of the genus *Pogonomyrmex* mate at specific landmarks (Hölldobler & Bartz 1985). At these sites many males gather (=male congregation syndrome) and the operational sex ratio is strongly male biased (Hölldobler & Bartz 1985). In *Pogonomyrmex* ants males do not fight for matings but engage in scramble competition (Hölldobler & Bartz 1985).

In the close relatives of the honeybees (Apini), there is much variation in mating patterns. The honeybees are one of four tribes (Euglossini, Bombini, Meliponini) of the corbiculate Apidae (Lockhart & Cameron 2001). Gynes of many bumblee bee species (Bombini) mate on routes marked by the males (Ayasse et al. 2001). Males of some species of the solitary orchid bees (Euglossini) establish small mating territories near to tree-trunks. Here, the males show a typical display behaviour and they also copulate in these territories with the females (Dodson 1966, Kimsey 1980, Stern 1991). Males of many stingless bees (Meliponini) aggregate in “swarms” but, in contrast to honeybees, the congregations are located near the nesting sites (Engels & Imperatriz-Fonseca 1990).

Reproduction in social Hymenoptera includes on the one hand individual reproduction of males and females, and on the other hand reproduction of the colony (Ruttner 1985, Koeniger 1986). Mating, fertilisation of eggs, and egg-laying are in principle not different from non-social insects. However, colony reproduction constitutes a special event in the life cycle of social insects. Reproduction of the colony can either occur by colony fission or by the release of new sexuals with subsequent colony foundation of the gynes (Michener 1974, Hölldobler & Wilson 1990). In both kinds of colony reproduction, females have to mate before they can establish a colony of their own. Rare exceptions are species that obligatorily reproduce by thelytokous parthenogenesis (Heinze & Tsuji 1995). An example is the queenless myrmicine ant *Pristomyrmex pungens* (Tsuji 1988).

1.2 Males in social Hymenoptera

Sexual dimorphism is common in Hymenoptera (Ayasse et al. 2001) presumably due to different reproductive and parental strategies of males and females. In the social Hymenoptera mainly the females are involved in sustaining the colonies. Males rarely care for the offspring. They generally don’t contribute to tasks as foraging or nest building (Wilson 1971) and hence in comparison to the female castes, males usually receive little attention in studies of social Hymenoptera. Therefore, social hymenopteran males have been termed “The Neglected Sex” (Heinze 2001). Males are fed by their sisters, consume the food storage of the colony, and live in the shelter of the nest. However, males do constitute an important component of the colony fitness (Kraus et al. 2003). They contribute to the inclusive fitness of their sisters (workers and gynes) as well as to the fitness of their mother queen. Moreover, social Hymenoptera males compete for mates with each other just as males of other non-eusocial species do, and therefore form an important independent level of selection. Thus, both natural and sexual selection (Andersson 1994) basically operate on male traits that are related to sexual competition and success at reproduction. The relative lifetime reproductive success of males strongly depends on characteristics of mating behaviour (e.g. performance in mate finding, courtship, and copulation), and on morphological (e.g. shape of reproductive organs) and physiological traits (e.g. production of gametes and accessory secretions).

Mating frequencies of social hymenopteran males have been addressed only in few species. New world harvester ant males (*Pogonomyrmex rugosus*) have been suspected to mate multiple times (Hölldobler & Bartz 1985). Reichardt & Wheeler (1996) reported multiple mating in leaf-cutter ant males (*Acromyrmex versicolor*) and Tasei et al. (1998) in bumble bee males (*Bombus terrestris*). The wingless males in the ant *Cardiocondyla* were also observed to mate more than once. These ergatoid males, which are one of two male morphs, can replenish their sperm supply (Heinze & Hölldobler 1993). In general, however, hymenopteran males stop to produce sperm early in life (Ayasse et al. 2001) and thus have a limited and fixed sperm supply. It is generally assumed that male animals can increase their fitness by mating with many females (Bateman 1948, Thornhill & Alcock 1983). This may also apply in the Hymenoptera. For instance, in *Pogonomyrmex occidentalis* ants young mated queens found new colonies on their own (=haplometrosis) and only very few succeed (Wiernasz et al. 2001). Thus, fitness of *P. occidentalis* males may depend on their mating frequencies (Wiernasz et al. 2001). However, the males have a dilemma, because their mating strategy must consider both the fixed and limited sperm supply, and the fitness of the mated female and the colony she establishes. If females have not enough semen after mating, they may not be able to establish a new colony. The number of copulations with successful inseminations is therefore apparently strongly constrained in several social hymenopteran males. There might be also a trade-off between the number of males produced by a colony and the size of the males (Hölldobler & Wilson 1990). An increased number of males produced by the colony could cause a decrease of the potential fitness of each single male, if the size of the males and the numbers of spermatozoa produced by the males were correlated (Wiernasz et al. 2001).

The mating frequency is particularly limited in males of eusocial stingless bees (Sakagami 1982), honeybees (Koeniger 1986), and leafcutter ants of the genus *Atta* (Hölldobler & Wilson 1990). Multiple mating of males is precluded in these taxa since copulations result in the deaths of the males, the possibly most extreme strategy to deliver semen in a single copulation.

1.3 Reproduction in Honeybees

Western honeybee workers (*Apis mellifera* L.) never mate and their spermathecae are usually rudimentarily developed (Winston 1987). Honeybees are haplo-diploid and males develop regularly from unfertilised eggs due to the sex determining mechanism (Beye et al. 2003). That is, in all subspecies except the Cape honeybee (*A. m. capensis*) workers reproduce by arrhenotokous parthenogenesis (Crozier & Pamilo 1996, but see Mackensen 1953). Workers, however, rarely lay eggs and successful worker reproduction is rather an exception (Visscher 1996, Barron et al. 2001). Workers gain inclusive fitness (Hamilton 1964) through reproduction of their relatives, i.e. primarily by reproduction of their supersister gynes. Supersisters are more closely related with one another (r = 0.75) than with any other relative because supersisters have a haploid father in common. Nevertheless, the reproductive success of their mother queen, their drone father, their halfsister gynes, and their brothers also increases the workers' inclusive fitness. Honeybee workers do play an important role in the process of colony reproduction, since colonies split and thousands of workers make up the swarm that attends the queen (Winston 1987). The workers search a new nesting site, build the new combs and start foraging.

There are numerous adaptations due to the different roles of queens and workers. Pollen combs and -corbiculae on the hind legs are only present in workers but not in queens. Likewise wax glands occur only in workers. Many specific adaptations in queen honeybees are clearly associated with mating and egg-laying. Queens store spermatozoa in their spermathecae and can keep them alive for many years (Koeniger 1986). The number of ovarioles is more than a hundred times higher in queens (150-180) than in workers (2-12; Winston 1987). Queens can lay about 200.000 eggs per year (Winston 1992).

Queens are also adapted to fight with their sisters (Pflugfelder & Koeniger 2003). Shortly after emergence from their special cells virgin queens engage in lethal fights, if they meet another queen (Pflugfelder & Koeniger 2003). There is usually only a single egg-laying queen in the colony.

The old queen leaves the nesting site with a prime swarm and a daughter of the old queen inherits the nest. The virgin queen takes one or two short orientation flights (Winston 1987) before she mates on one to several nuptial flights (Roberts 1944, Alber et al. 1955). A considerable number of queens does not return to the colony from their flights indicating a significant mating risk (Ruttner 1980). Matings take place on the wing (Koeniger et al. 1979), on so called drone congregation areas (Ruttner 1985). A honeybee queen usually mates with with more than ten drones (e.g. Estoup et al. 1994 [7-20 matings], Kryger & Moritz 1997 [21-24 matings], Neumann & Moritz 2000 [10-28 matings]). Once queens have started egg-laying, they never remate even if they run out of sperm (Winston 1987). Thus, the sperm number in the spermatheca sets a limit to the fitness of the queen. The sperm utilization pattern of the queen can result in a paternity skew (Moritz 1986) and thus it might have an impact on the fitness of the drones. Due to the extreme degree of polyandry, sperm competition might operate in honeybees (Hunter & Birkhead 2002). Potentially, the rank in the sequence of copulations can affect the male's fitness in polyandrous species (Simmons 2001).

All species of the honeybee genus *Apis* are highly polyandrous (Palmer & Oldroyd 2000). With respect to their mating behaviour, the nine honeybee species show an important difference in the time of day when nuptial flights of drones and queens occur (Koeniger & Koeniger 2000). The different timing of nuptial flights seems to be a reproductive isolation mechanism of sympatric Asian *Apis* species (Koeniger & Koeniger 2000). A strikingly similar situation has been described for sympatric *Pogonomyrmex* species in North America (Hölldobler & Bartz 1985). In addition, there is a little difference in the daily start of nuptial flights between the sexes in the Western honeybee (*Apis mellifera*) with the drones leaving the colony for nuptial flights earlier than queens (Koeniger et al. 1989).

In addition to sympatric separation over time also spatial separation in the same habitat could result in reproductive isolation. The preferred altitude at which drones establish a drone congregation area differentiates two subspecies of the Western honeybee (*A. m. carnica* and *A. m. ligustica*; Koeniger et al. 1989). The sites of drone congregation areas of three sympatric Asian *Apis* species are spatially separated (Koeniger & Koeniger 2000) which might contribute to their reproductive isolation. Reproductive isolation can also arise from the variation in the anatomies of the male reproductive organs (=endophalli) of the different *Apis* species (Koeniger 1991). The varying endophalli seem to be related to different sperm transfer mechanisms (Koeniger 1991).

1.4 Aims and Objectives of the Thesis

This thesis is concerned with traits related to reproduction of individual honeybee drones and queens and with their mating behaviour. Resulting consequences for the colony fitness and thus the fitness of the worker caste are discussed.

The main Objectives of this thesis are as follows:

- to study potential causes of multiple nuptial flights of honeybee queens (e.g. sperm transfer and mating frequency)
- to re-evaluate the relationship between semen production and body size in drone honeybees
- to discuss its potential relevance for the fitness of the individual drone and for the fitness of the colony, respectively
- to study the queen's utilization pattern of spermatozoa from different drones
- to determine the impact of the drone's semen production on his siring success
- to re-evaluate the impact of the drone's rank in the insemination sequence on his siring success
- to study a potential first male advantage in dwarf honeybees and a paternity skew in different *Apis* species
- to study potential different mating frequencies in *Apis* species

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2 Summary

The Western honeybee (*Apis mellifera* L.) has an extremely polyandrous mating system. In general honeybee queens mate with at least ten drones, but even more than forty matings were detected. In contrast, drone honeybees die during copulation and are thus strictly monogamous.

Queens often take multiple nuptial flights. The cost of multiple nuptial flights was studied in relation to potential benefits. The mating frequency of naturally mated queens was analysed using DNA fingerprinting. Queens that were restricted to one nuptial flight, but wanted to take an additional flight, had significantly fewer matings (7.6±1.9) than queens which started oviposition after a single nuptial flight (14.5±1.2). Moreover, the sperm number stored in the spermatheca significantly increased with the number of matings. Presumably, queens adjust their nuptial flight frequency according to the mating success of the previous nuptial flights. The number of copulations seems to serve as a signal for the initiation of oviposition. Furthermore, the findings suggest that a certain number of matings is required to fill the spermatheca to its storage capacity. This is in accord with the sperm limitation hypothesis for the evolution of extreme polyandry.

The average sperm number per copulation or nuptial flight that a queen receives depends on the sperm numbers produced by the drones. Yet, drones might differ in sperm numbers for several reasons. Here, the effect of drone honeybee's body size on semen production was evaluated. In the same colonies, drones were either reared in drone cells (large drones) or in worker cells (small drones). Wing lengths (size indicator) and sperm numbers of small and large drones were compared. Small drones (~13% reduced wing size) produce significantly fewer spermatozoa (7.5 ± 0.5 million) than normally sized drones (11.9 ± 1.0 million spermatozoa). There is a significant positive correlation between sperm number and wing size within the small drones and in both groups combined. In the large group alone no correlation was found. The rearing investment per spermatozoon is lower for small than for normally sized drones because small drones produce more spermatozoa in relation to their body weight. Since colonies usually produce large drones, the enhanced investment must be outweighed by a mating advantage of large drones.

The varying sperm numbers of drones can have an impact on their individual fitness. But in addition, the pattern of sperm utilization by the queen affects the drones fitness. Thus, the consequences of sperm utilization for the fitness of the queen's mates were studied using DNA-fingerprinting. Eight queens were instrumentally inseminated with semen of six or seven drones. Every drone contributed either 0.5 µl or 1.0 µl semen respectively. The impact of the insemination sequence and the amount of semen on the sperm utilization were analysed. The data show no significant effect of the insemination sequence but a strong impact of the semen volume of a drone on the frequency of his worker offspring in the colony. This effect was not linear and the patriline frequencies of the drones contributing larger semen volumes are disproportionately enhanced. If these observations are also valid for natural matings, drone honeybees should maximise the number of sperm but not apply specific mating tactics to be first or last male in a mating sequence.

In spite of a lacking first or last male advantage in *A. mellifera*, the reproductive success of the siring drones is usually strongly biased as inferred from analysing colonies with naturally mated queens. For the dwarf honeybees (*A. andreniformis* and *A. florea*) a first male advantage was hypothesized due to the peculiar anatomy of their male genitalia which allows for direct injection of semen into the spermaduct. In order to survey the differences and similarities in paternity skew among species in the genus *Apis,* data from the literature were reanalysed by using a sample size calibration method. As a result the paternity skew among seven honeybee species differed significantly, particularly due to the rare patrilines. The sorting algorithm, i.e. the ranking of the patrilines had, however, a considerable effect on the patternity skew pattern. The more frequently occurring patrilines appear to be similarly distributed in all tested species, suggesting one common sperm transfer mechanism for all honeybees. As a consequence, the proposed first male advantage in the dwarf honeybees is not supported by empirical data.

3 Zusammenfassung

Die Westliche Honigbiene (*Apis mellifera* L.) weist ein extrem polyandrisches Paarungssystem auf. Im Allgemeinen paaren sich Honigbienenköniginnen mit mindestens zehn Drohnen, aber es wurden sogar mehr als vierzig Paarungen nachgewiesen. Im Gegensatz dazu sterben Drohnen während der Paarung und sind deshalb strikt monogam.

Königinnen unternehmen häufig mehrere Hochzeitsflüge. Die Kosten von mehrfachen Hochzeitsflügen wurden in Beziehung zu möglichen Vorteilen untersucht. Die Paarungshäufigkeiten von natürlich gepaarten Königinnen wurden mittels DNA-Fingerprinting analysiert. Königinnen, die auf einen Hochzeitsflug eingeschränkt wurden, die aber zusätzliche Flüge unternehmen wollten, wiesen signifikant weniger Paarungen auf (7,6±1,9) als Königinnen, die nach nur einem Hochzeitsflug mit der Eiablage begannen (14,5±1,2). Außerdem erhöhte sich die Anzahl der in den Spermatheken gespeicherten Spermien signifikant mit der Anzahl der Paarungen. Vermutlich passen Königinnen die Häufigkeit ihrer Hochzeitsflüge dem Paarungserfolg der vorangegangenen Hochzeitsflügen an. Die Anzahl der Kopulationen scheint als ein Signal für die Einleitung der Eiablage zu dienen. Desweiteren lassen die Ergebnisse vermuten, dass eine bestimmte Zahl an Paarungen nötig ist, um die Spermatheka bis zu ihrer Kapazitätsgrenze zu füllen. Dies steht im Einklang mit der Spermienlimitierungshypothese für die Evolution der extremen Polyandrie.

Die durchschnittliche Spermienzahl, die eine Königin je Kopulation oder Hochzeitsflug erhält, hängt von der Anzahl der Spermien ab, die von Drohnen produziert wird. Doch Drohnen könnten sich in ihren Spermienzahlen aus verschiedenen Gründen unterscheiden. Hier wurde der Effekt der Körpergröße von Honigbienendrohnen auf die Spermienproduktion untersucht. In denselben Kolonien wurden Drohnen entweder in Drohnenzellen (große Drohnen) oder in Arbeiterinnenzellen (kleine Drohnen) aufgezogen. Die Flügellängen (als Größenmaß) und die Spermienzahlen von kleinen und großen Drohnen wurden verglichen. Kleine Drohnen (~13% reduzierte Flügelgröße) produzieren signifikant weniger Spermien (7,5 ± 0,5 Millionen) als normal große Drohnen (11,9 ± 1,0 Millionen Spermien). Es gibt eine signifikante, positive Korrelation zwischen den Spermienzahlen und den Flügelgrößen innerhalb der kleinen Drohnen und auch, wenn beide Gruppen zusammengefasst werden. In der großen Gruppe allein wurde keine Korrelation gefunden. Die Aufzuchtsinvestition pro Spermium ist für die kleinen Drohnen niedriger als für die normal großen Drohnen, weil kleine Drohnen mehr Spermien im Verhältnis zu ihrem Körpergewicht produzieren. Da Kolonien gewöhnlich große Drohnen produzieren, muss die erhöhte Investition durch einen Paarungsvorteil der große Drohnen aufgewogen werden.

Die variierenden Spermienzahlen der Drohnen können einen Einfluss auf ihre individuelle Fitness haben, aber zusätzlich beeinflusst das Muster der Spermiennutzung der Königin die Fitness der Drohnen. Deshalb wurden mittels DNA-Fingerprinting die Konsequenzen der Spermiennutzung für die Fitness der Paarungspartner der Königin untersucht. Acht Königinnen wurden instrumentell mit Sperma von sechs oder sieben Drohnen besamt. Jeder Drohn trug entweder 0,5 µl oder 1,0 µl Sperma bei. Der Einfluss der Besamungsreihenfolge als auch der Einfluss der Spermamenge auf die Spermiennutzung wurde analysiert. Die Daten zeigen keinen signifikanten Effekt der Besamungsreihenfolge dafür aber einen starken Effekt des Spermavolumens eines Drohns auf die Häufigkeit seiner Arbeiterinnennachkommen in der Kolonie. Dieser Effekt war nicht linear und die Patrilinienhäufigkeiten der Drohnen, die größere Spermavolumina beitrugen, war überproportional erhöht. Wenn diese Beobachtungen auch für natürliche Paarungen gelten, sollten Honigbienendrohnen die Spermienzahlen maximieren. Sie sollten aber keine besonderen Paarungstaktiken anwenden, um das erste oder das letzte Männchen in der Paarungsreihenfolge zu sein.

Trotz des Fehlens eines Vorteils für das erste oder das letzte Männchen bei *A. mellifera* ist der Fortpflanzungserfolg der nachkommenzeugenden Drohnen, wie man von der Analyse von Kolonien mit natürlich gepaarten Königinnen weiß, gewöhnlich sehr ungleich. Für die Zwerghonigbienen (*A. andreniformis* und *A. florea*) wurde die Hypothese eines Vorteils der ersten Männchen aufgrund der eigentümlichen Anatomie ihrer Genitalien vorgeschlagen. Diese Anatomie ermöglicht eine direkte Injektion des Spermas in den Spermaduct. Um die Unterschiede und Ähnlichkeiten im „paternity skew” zwischen Arten der Gattung *Apis* zu überprüfen, wurden Daten aus der Literatur mittels einer Kalibrierungsmethode für unterschiedliche Stichprobengrößen reanalysiert. Im Ergebnis unterschieden sich die sieben Arten im „paternity skew“ signifikant, vor allem aufgrund der seltenen Patrilinien. Der Sortierungsalgorithmus, d.h. die Rangfolge der Patrilinien hatte jedoch einen beträchtlichen Effekt auf das Muster der „paternity skews“. Die häufiger auftretenden Patrilinien scheinen bei allen untersuchten Arten ähnlich verteilt zu sein. Dies lässt auf einen allgemeinen Spermienübertragungsmechanismus für alle Honigbienen schließen. Folglich wird der vorgeschlagene Vorteil der ersten Männchen der Zwerghonigbienen nicht durch die empirischen Daten unterstützt.

4 Multiple nuptial flights, sperm transfer and the evolution of extreme polyandry in honeybee queens

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running headline: SCHLÜNS ET AL: HONEYBEE MATING BEHAVIOUR

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4.1 Abstract

The honeybee, *Apis mellifera*, has an extremely polyandrous mating system, which often involves multiple nuptial flights by the queens. Here we study the cost of multiple nuptial flights in relation to potential benefits in order to understand the evolution of extreme polyandry. We analysed the paternity of worker offspring of naturally mated queens with eight DNA microsatellite loci. Queens that were restricted to one nuptial flight, but wanted to take an additional flight, had significantly fewer matings (7.6±1.9) than queens which started oviposition after a single nuptial flight (14.5±1.2). Furthermore, the sperm number stored in the spermatheca significantly increased with the number of matings. We suggest that queens adjust their nuptial flight frequency according to the mating success of the previous nuptial flights. The number of copulations seems to serve as a signal for the initiation of oviposition. In the light of these findings, we reconsider and discuss the significance of the sperm limitation hypothesis for the evolution of extreme polyandry in *A. mellifera*.

5 Sperm numbers in drone honeybees (*Apis mellifera*) depend on body size

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5.1 Abstract

The effect of drone honeybee's body size on semen production was evaluated. In the same colonies, drones were either reared in drone cells (large drones) or in worker cells (small drones). Wing lengths (size indicator) and sperm numbers of small and large drones were compared. Small drones (~13% reduced wing size) produce significantly fewer spermatozoa (7.5 ± 0.5 million) than normally sized drones (11.9 ± 1.0 million spermatozoa). There is a significant positive correlation between sperm number and wing size within the small drones and in both groups combined. In the large group alone no correlation was found. The rearing investment per spermatozoon is lower for small than for normally sized drones because small drones produce more spermatozoa in relation to their body weight. Since colonies usually produce large drones, the enhanced investment must be outweighed by a mating advantage of large drones.

Apis mellifera **/ spermatozoa / male size / small drone / large drone / reproductive success**

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6 Sperm utilization pattern in the honeybee (*Apis mellifera* L.)

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6.1 Abstract

Queen honeybees (*Apis mellifera* L.) mate with a large number of drones on their nuptial flights. Not all drones contribute equally to the queen's offspring and the queen's utilization pattern of spermatozoa from different drones has an important impact on the genetic composition of the colony. Here we study the consequences of sperm utilization for the fitness of the queen's mates with microsatellite DNA-fingerprinting. Eight queens were instrumentally inseminated with semen of six or seven drones. Every drone contributed either 0.5 µl or 1.0 µl semen respectively and we analysed both the impact of the insemination sequence and the amount of semen on the sperm utilization. Our data show no significant effect of the insemination sequence but a strong impact of the semen volume of a drone on the frequency of his worker offspring in the colony. This effect was not linear and the patriline frequencies of the drones contributing larger semen volumes are disproportionately enhanced. If these observations are also valid for natural matings, drone honeybees should maximise the number of sperm but not apply specific mating tactics to be first or last male in a mating sequence.

7 Paternity skew in seven species of honeybees (Hymenoptera: Apidae: Apis L.)

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7.1 Abstract

Honeybees (*Apis)* show an extremely polyandrous mating system. In general honeybee queens mate with at least ten drones, but even more than fifty matings were detected in two species (*A. dorsata* and *A. nigrocincta*). The reproductive success of the siring drones is usually strongly biased giving rise to speculations of first or last mate advantage. Especially for the dwarf honeybees (*A. andreniformis* and *A. florea*) a first male advantage was hypothesized due to the peculiar anatomy of their male genitalia which allows for direct injection of semen into the spermaduct. In order to survey the differences and similarities in paternity skew among species in the genus *Apis,* we reanalyzed data from the literature by using a sample size calibration method. As a result the paternity skew among seven honeybee species differed significantly, particularly due to the rare patrilines. The sorting algorithm, i.e. the ranking of the patrilines had, however, a considerable effect on the patternity skew pattern. The more frequent occurring patrilines appear to be similarly distributed in all tested species, suggesting one common sperm transfer mechanism for all honeybees. As a consequence neither the proposed first male advantage in the dwarf honeybees nor the last male advantage in the Western honeybee (*A. mellifera*) are not supported by empirical data.

Keywords: Polyandry, patrilines, paternity skew, microsatellite DNA, Apis, honeybee

8 Appendix

Declaration on the contributions to the manuscripts/papers on which this thesis is based

Multiple nuptial flights, sperm transfer and the evolution of extreme polyandry in honeybee queens
Schlüns H, Moritz RFA, Neumann P, Kryger P, Koeniger G
in *Animal Behaviour* in review

> The manuscript was written by myself. The behavioural experiments (interfering with the queens' nuptial flights) and the counts of the sperm numbers were carried out by P. Kryger. I have genotyped the queens and their worker offspring using DNA microsatellites in Halle and I have perfomed the statistics. The mating frequency estimates and the counts of the sperm numbers of the queens from Baltrum were determined by P. Neumann and G. Koeniger.

Sperm numbers in drone honeybees (*Apis mellifera* L.) depend on body size
Schlüns H, Schlüns EA, van Praagh J, Moritz RFA (2003)
Apidologie 34: 577-584

> The manuscript was written by myself, and I had the idea of reevaluating the sperm numbers in drone honeybees. The drones were bred by J. van Praagh in Celle and on the island of Neuwerk, respectively. I have taken the drone samples on Neuwerk, and I have measured the wing lengths. Dissections of the drones and sperm number counts were carried out by myself together with E.A. Schlüns in Halle.

Sperm utilization pattern in the honeybee (*Apis mellifera* L.)
Schlüns H, Koeniger G, Koeniger N, Moritz RFA
in *Behavioral Ecology and Sociobiology* in review

> The manuscript was written by myself and I performed the statistics. I had the idea of determining the impact of the drone's semen volume on his patriline frequency. The queens and drones were bred by the Bieneninstitut in Oberursel (N. Koeniger & G. Koeniger). I have established the instrumental insemination with precise measurement of the semen volume together with B. Springer (Oberursel) who also inseminated the queens. After breeding the worker offspring in small nucs, I have genotyped the drones and their worker offspring using DNA microsatellites in Halle.

Paternity skew in seven species of honeybees (Hymenoptera: Apidae: *Apis* L.)
Schlüns H, Moritz RFA, Lattorff HMG, Koeniger G
to be submitted

> The manuscript was written by myself. I had the idea of using the re-sampling method in order to compare mating frequency estimates with different sample sizes. I have implemented the bootstrapping computer program and I have performed the statistics. I had very stimulating and fruitful discussions on sperm utilization and reproductive skew with H.M.G. Lattorff, R.F.A. Moritz, and G. Koeniger.

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I thank Dr. Job van Praagh (Niedersächsisches Landesinstitut für Bienenkunde) for his kind collaboration in the study of small and normally sized drones. I also thank Dr. Stefan Berg (Bieneninstitut Kirchhain) and Prof. Dr. Gard W. Otis (University of Guelph, Canada) who provided essential information on small drones.

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Many professional beekeepers (Beate Springer, Matthias Uhlmann, Hansgeorg Schell, Günter Köhler, Anna Lisa Petzold) shared their knowledge on keeping bees with me. Thanks a lot to all of them.

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Curriculum vitae

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Erklärung

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

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

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

Halle (Saale), den 16. Dezember 2003

Helge Schlüns