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Review article

Sexual selection in *Apis* bees¹

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Abstract – Honey bees in the genus *Apis* share many reproductive features with other social insects, but have also a number of highly derived mating characteristics, such as obligatory polyandry and – in at least two species – males who deposit their ejaculates directly into the spermathecal duct. These characteristics make the honeybees highly interesting and a special model system for studying sexual selection. Furthermore, the numerical sex ratio of *Apis* bees is extremely male biased and males die during their first and only copulation. This review updates our present knowledge of the mating biology of *Apis* bees and places this information into a broader concept of sexual selection. I concentrate on two intensively studied aspects of sexual selection: Sperm competition and cryptic female choice. I present evidence that sperm competition is likely to occur during the egg fertilization process, whereas cryptic female choice is likely to operate shortly after insemination when ejaculates of many males get stored in the spermatheca of the queen.

Apis / honeybee / polyandry / sperm storage / sperm use / sperm competition / cryptic female choice

1. GENERAL INTRODUCTION TO SOCIAL INSECT REPRODUCTION

Sexual selection has been only marginally investigated in social insects (Boomsma and Ratnieks, 1996) but there is no reason to believe that it is not of evolutionary importance compared to non-social insects and vertebrates (Baer, 2003; Birkhead and Moller, 1998; Boomsma et al., 2005; Simmons, 2001). As two recent reviews (Baer, 2003; Boomsma et al., 2005) have pointed out, the idiosyncrasies of social insect mating systems have induced several unusual forces of sexual selection. This makes social insects interesting study organisms not only to challenge present theories of sexual selection, but also to combine two intensively studied fields: sexual selection and kin selection.

1.1. Social insect queens

Social Hymenopterans are characterized by reproductive females (queens) that only mate at

the beginning of their life, typically during one or very few nuptial flights (Baer, 2003; Boomsma et al., 2005; for honeybees see Ruttner, 1956) Afterward, sperm is stored within a specialized organ of the queen (the spermatheca), where it survives and keeps its viability for years or even decades in many ants (Hölldobler and Wilson, 1990; Pamilo, 1991). The absence of re-mating later in life implies that pairs are permanently committed, so that – in contrast to most other insects – mating does not interfere with normal colony life (Boomsma et al., 2005). Advanced insect societies also depend on the continuous production of sterile worker helpers, which originate from inseminated eggs laid by the mother queen. Consequently, social insect queens need to maintain high fertility, especially in long-lived species with large colonies such as army ants with colonies up to 20 million workers (Raignier and van Boven, 1955) or *Atta* leaf-cutting ants, which maintain colony sizes of up to 8 million workers for 20 years

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(Weber, 1972). Queens of the honeybee *Apis mellifera* are less spectacular but can still survive for up to eight years (Bozina, 1961, cited in Winston, 1991) and lay around 200 000 eggs a year (Winston, 1991; Snodgrass, 1984). Consequently, to complete a successful colony cycle a honeybee queen must be able to lay 1.0–1.6 million fertilized eggs throughout her life, all of which need to develop into viable offspring. Colony size is an important fitness variable, not only because larger colonies produce more sexuals, but also because *Apis* bees found new colonies by colony fission (swarming) (Winston, 1991). The success of a swarm depends upon its size, i.e. the number of workers present. Larger swarms are more successful in establishing a new nest site, surviving colony initiation, hibernating and successfully reproducing in the following year (Lee and Winston, 1985; see also Seeley and Visscher, 1985). Consequently, the reproductive success of a colony is very closely associated with the fertility of the mother queen.

1.2. Social insect males

Males and their reproductive agenda have received far less attention in social insects than in other organisms (Baer, 2003; Boomsma et al., 2005). This might have been a consequence of their cryptic life style, as hymenopteran social insect fathers generally die during or shortly after copulating, while their ejaculates survive in the spermatheca of their mates. The physical absence of fathers during most of the queen's life span has made researchers neglect social insect males, so that many subtleties of their shares in paternity have remained ambiguous. However, recent findings indicate that males might be able to substantially influence paternity (Baer, 2003). For example in bumblebees, males mate-guard queens and use chemical compounds of the accessory glands to monopolize females by reducing the females willingness to re-mate (Baer et al., 2000, 2001; Sauter et al., 2001; see also Baer and Schmid-Hempel, 2005). Male mate guarding is also known in ants (Foitzik et al., 2002) as is the transfer of accessory gland compounds during copulation (either as mating plugs or as spermatophores, see Baer, 2003; Boomsma et al., 2005), but little is known about their effects on paternity distributions. Since

hymenopteran social insect males originate from non-fertilized eggs they are haploid, which has several implications. First, male haploidy results in an increase in relatedness among a father's offspring compared to the offspring of a diploid father, because haploid males only contribute a single set of genes to their daughters (Crozier and Pamilo, 1996). Consequently, a haploid father's offspring has a higher relatedness ($r = 0.75$) compared to offspring of a diploid father ($r = 0.5$) (Page and Metcalf, 1982). This increased relatedness is important, because kin selection often depends upon a high degree of genetic relatedness among siblings (Hamilton, 1964). It is therefore a male characteristic (male haploidy) that induces a skew in offspring relatedness, which most likely favored the evolution of social systems in this group of insects. A second interesting effect of male haploidy is that sperm is clonal, so that intra-ejaculatory sperm competition is absent (Baer, 2003). Third, social insect males are sperm limited because spermatogenesis comes to an end during the pupal stage (Zander, 1922; Hölldobler and Bartz, 1985), which implies that social insect males have to adjust their ejaculate size according to the number of expected copulations (Boomsma et al., 2005).

Finally, social insect fathers only sire female offspring and they normally (i.e. when workers are completely sterile) only contribute genetically to the next generation through queen offspring. Therefore, fathers are in conflict with their mate as well as with their worker offspring about the preferred sex ratio. Males prefer a high investment into female offspring, whereas the queen prefers an equal sex ratio investment and the workers a 3:1 female biased sex ratio investment (in a monandrous colony) (Trivers and Hare, 1976). Consequently, males are more in agreement over the sex ratio with their worker offspring than they are with their mates (Boomsma and Ratnieks, 1996).

2. THE MATING SYSTEM OF *APIS* BEES

Reproductive issues in *Apis* bees have been intensively studied and several excellent reviews have summarized earlier data, mostly concentrating on the honeybee *A. mellifera*

(e.g. Koeniger and Koeniger, 1991; Page and Metcalf, 1982; Ruttner, 1985; Winston, 1991; see Eickwort and Ginsberg, 1980 for a general review of mating behavior in Apoidea). Rather than repeating information already presented there I here (1) add information from the last decade to update our knowledge on the mating biology of *Apis* bees and (2) broaden the perspective to include other species of the genus *Apis*. Finally, I place the present data on the mating biology of *Apis* bees into a broader conceptual context of sexual selection theory to discuss the possible occurrence of two traits of special evolutionary significance: sperm competition and cryptic female choice.

The mating system of *Apis* has similarities with mating systems of other social insects, especially those of ants (see Boomsma et al., 2005; Hölldobler and Bartz, 1985; see Hölldobler and Wilson, 1990 for a general introduction), but several derived traits have evolved in *Apis* bees, which I will discuss in more detail below. As found in many ants, mating in *Apis* is initiated during mass swarming (Hölldobler and Wilson, 1990; Winston, 1991), which occurs after sexuals (drones and virgin queens) leave their maternal nest and aggregate at specific geographical mating sites (congregation areas), that are re-used by many generations of sexuals (Ruttner and Ruttner, 1972; Winston, 1991). The *Apis* mating system is of the “male aggregation” type, as defined by Boomsma et al. (2005) and Hölldobler and Bartz (1985), since males establish congregation areas before females arrive to mate (Winston, 1991). During these nuptial flights copulations take place on the wing (Koeniger and Koeniger, 1991), but are of very short duration compared to other social insects (see Brown and Baer, 2005). In *A. mellifera*, the entire mating process takes less than 5 seconds (Koeniger and Koeniger, 1991), often no more than 1 or 2 seconds (Koeniger et al., 1979). Females can copulate with other drones in quick succession. Such short copulation durations, together with the “anonymity” of the drones within the mass swarms, is likely to reduce the queen’s ability to express sophisticated mate choice (Boomsma et al., 2005). However, there are indications that matings within a swarm are not completely random, for example because large drones have a reproductive advantage compared to small drones (Berg

et al., 1997). Furthermore, in mating swarms of mixed honey bee races, such as *A. mellifera adansonii* and *A. mellifera ligustica* (Kerr and Bueno, 1970) or *A. mellifera ligustica* and *A. mellifera carnica* (Koeniger et al., 1989a), females are preferentially inseminated by drones of their own race. However, we lack information about the exact mechanisms leading to assortative paternity; for example whether it is females rejecting certain males or males avoiding copulation with a queen of another race. Female mate choice might occur, because a successful copulation depends on queens opening their sting chamber to allow a male to insert his genitalia (Strassmann, 2001; Winston, 1991). Additionally, queens need to contract their bursa to press the endophallus in order to transfer sperm into their lateral oviducts (Koeniger and Koeniger, 1991). The question is, whether and to what degree a queen is able to discriminate among males, given that she is chased by a dense aggregation of more than 100 males called a “drone comet” (Gries and Koeniger, 1996). A male racing behind a queen might in fact have much more information than the queen about his potential mate (or his competitors), based on visual cues or pheromones released by the queen (see Winston, 1991 and references therein). However, with the highly male biased sex ratios in *Apis*, males are not expected to be discriminating given their minimal chance of obtaining even a single opportunity to mate (Boomsma et al., 2005).

The drones of all *Apis* species die during their first and only copulation (Ruttner, 1954), because the eversion of the males endophallus is irreversible and results in the paralysis of the male (Koeniger and Koeniger, 1991). After the ejaculation the male separates from the queen, but leaves a mating sign consisting of several gland secretions and a chitinous plate (Koeniger et al., 1979). As a consequence, males can copulate only once and mating is lethal, so that he commits his entire reproductive effort to a single queen.

3. WHY STUDY SEXUAL SELECTION IN *APIS*?

Because honeybees are the best-studied social insect species at present, a large body of literature on their mating biology is available,

Table I. Synopsis of available data on reproductive key figures of *Apis* bees. Shown are the typical values reported in the literature, being sperm numbers as found in males and female spermathecae. Colony size is measured in number of workers and the weight of the males in mg fresh weight. Sperm length data are taken from unpublished data by Baer and Koeniger. For the queen mating frequencies, corrected values as recently published by Tarpy et al., 2004 are presented but the original references have also been added in the last column.

Species	# of sperm male	# sperm in spermatheca	Colony size	Male weight ± SD	Sperm length ± s.e.m.	Queen mating frequency	References
<i>A. mellifera</i>	12' 700' 000	4' 730' 000	60 000	211.1±11.8	262.69±3.52	11.6	1, 8
<i>A. andreniformis</i>	130' 000	1' 260' 000		70.8±3.0		10.5	1, 3, 8, 9
<i>A. florea</i>	430' 000	1' 050' 000	6271	77.6±2.6	205.81±0.68	7.9	1, 8, 10, 11
<i>A. cerana</i>	1' 100 000	1' 350 000	20 000	83.4±8.9	267.07±0.74	14.1	1, 6, 7, 8, 11
<i>A. koschnevnikovi</i>	1' 700 000	2' 130 000		105.5±5.6		13.3	1, 6, 8
<i>A. nuluensis</i>	1' 300' 000			107.0±6.7			1, 4
<i>A. nigrocincta</i>						40.3	8
<i>A. dorsata</i>	1' 590 000	3' 940 000	65 000	155.7±8.5	218.69±0.88	44.2	1, 2, 8, 11
<i>A. laboriosa</i>						28.4	5, 8

References:

1: Koeniger and Koeniger, 2000; 2: Koeniger et al., 2005; 3: Koeniger et al., 2000; 4: Koeniger et al., 1996; 5: Takahashi and Nakamura, 2003; 6: Koeniger and Koeniger, 1991; 7: Oldroyd et al., 1998; 8: Tarpy et al., 2004; 9: Oldroyd et al., 1997; 10: Oldroyd et al., 1996; 11: Ruttner, 1988.

especially about the species of economic interest such as the honeybee *Apis mellifera* and its numerous subspecies. The most important reproductive characteristic of *Apis* bees is that obligatory multiple mating of females (polyandry) is present in all species investigated (Tab. I) and females seem to mate with many more males than needed to simply fill the spermatheca. Obligate multiple mating is otherwise rare in social insects and occurs only in *Vespula* wasps, *Atta* and *Acromyrmex* leaf cutting ants, as well as in *Dorylus* and *Eciton* army ants and in *Pogonomyrmex* harvester ants (Denny et al., 2004; Kronauer et al., 2004; Rheindt et al., 2004; Strassmann, 2001). The evolutionary advantages of polyandrous mating systems have received intense attention over recent years, not only in social insects (Boomsma and Ratnieks, 1996; Crozier and Fjerdingstad, 2001; Strassmann, 2001) but in many other taxa (Arnqvist and Nilsson, 2000; Jennions and Petrie, 2000). However, in social insects, researchers have been additionally challenged to explain polyandry in the light of kin selection theory (Boomsma and Ratnieks, 1996). From

the various hypotheses proposed to explain the evolution of polyandry (Arnqvist and Nilsson, 2000; Crozier and Fjerdingstad, 2001; Jennions and Petrie, 2000), only a subset are relevant to social insects (Boomsma and Ratnieks, 1996; Strassmann, 2001), and only some of these have been rigorously tested so far. The presently most promising hypothesis is that increased genetic diversity has (several) beneficial effects for the colony, as it: 1. reduces parasitism (Baer and Schmid-Hempel, 1999, 2001, 2003; Shykoff and Schmid-Hempel, 1991; Tarpy, 2003), 2. reduces sex ratio conflict between the workers and the queen when split sex ratios apply (Ratnieks and Boomsma, 1995), 3. reduces the variation in diploid male production (Tarpy and Page, 2002), 4. may help to buffer colony performance against fluctuating environmental conditions (Crozier and Page, 1985), and 6. may increase the efficiency of worker task performance (Fuchs and Moritz, 1999; Jones et al., 2004). Polyandry is obviously a derived trait in *Apis* but it has several evolutionary consequences, two of which I will discuss in further

detail below: first, multiple queen mating in *Apis* bees might have induced the evolution of sperm competition (Parker, 1970), which occurs when ejaculates of two or more males compete for the opportunity to fertilize a given set of eggs. Secondly, since females mate with several males in quick succession, cryptic female choice can also evolve (Eberhard, 1996). Cryptic female choice occurs when a female influences male reproductive success after two or more males have copulated with her. The discrimination of the female between potential fathers is “cryptic” when the female-induced processes influencing paternity are hidden to the male. In *Apis* bees, any possible manipulation of ejaculates between insemination and storage would thus by definition be cryptic.

Apart from being polyandrous, the numerical sex ratio of *Apis* bees is extremely male biased. In a congregation area there might be around 25 000 drones (Page, 1986) from several hundred different colonies present (Baudry et al., 1998) so that the numerical sex ratio in the honeybee *A. mellifera* may exceed 20 000:1 (Page and Metcalf, 1984). These highly male biased sex ratios might be a reason for the evolution of suicidal male mating in *A. mellifera* (Page, 1986) because the chance of a male to get a second copulation after having achieved a first one is extremely low (Boomsma et al., 2005). It remains to be seen whether males are able to further influence their chances to achieve a copulation in the absence of male territories, male fighting or any other sophisticated mate choice by females. Finally, *Apis* queens do not found colonies independently but through swarming (Winston, 1991). In *Apis mellifera*, it is the mother queen that leaves the nest before the emergence of virgin queen(s), whereas the newly eclosed queens stay behind. When several virgin queens eclose simultaneously, they fight with each other until only one remains (Pflugfelder and Koeniger, 2003). This combination of traits, lethal queen fighting and reproduction via colony fission is rare in social insects.

In light of the above, the most interesting aspects of the complex reproductive agenda of honeybees are the dynamics of sperm storage and sperm use, and the power of both sexes to influence patterns of paternity. Traits under male control may contribute to sperm competition; traits under female control may result in

cryptic female choice. In the next two sections I will discuss how sperm competition and cryptic female choice might have evolved in *Apis* bees and I develop a hypothesis on how and at which point in time these effects might be expressed in different *Apis* species. As I will also point out, these traits are likely to operate rather differently in *Apis* honebees than in other (non social) organisms.

4. SPERM COMPETITION IN *APIS* BEES

A prerequisite for sperm competition to evolve is that a female mates with more than one male and that there are more sperm than eggs to be fertilized (Boomsma et al., 2005). As I show here both criteria are fulfilled in *Apis*. First of all females are highly polyandrous in *Apis* species with effective paternities ranging from 7.9 in *A. florea* up to 44.2 in *A. dorsata* (Tarpy et al., 2004, see Tab. 1 for references). As in other insects, males of most *Apis* species do not transfer their sperm directly to the spermatheca, but ejaculate them into the female’s sexual tract, the bursa copulatrix (Duvoisin et al., 1999; Koeniger and Koeniger, 1991). Since the total volume of all ejaculates is much larger than the volume of the bursa copulatrix, ejaculates get transferred to the lateral oviducts by contractions of the bursa (see Koeniger and Koeniger, 1991 and references therein). The actual sperm storage process lasts for about 40 hours after mating (Woyke, 1983), during which most of the previously acquired sperm gets lost as it flows back into the bursa copulatrix to be eventually expelled through the vagina (Ruttner and Koeniger, 1971). The rate of sperm migration into the spermatheca is nearly constant during the first 24 hours after copulation, at least for large ejaculates (Woyke, 1983). In *Apis mellifera*, the lateral oviducts of a queen contain about 200 million sperm after a mating flight (see references in Winston, 1991). However, only about 4.7 million of these get stored in the spermatheca, which means that only around 2.5% of the sperm acquired during the nuptial flight(s) gets stored. Extreme sperm dumping is not only known from *A. mellifera* but also for *A. dorsata* (Oldroyd et al., 1996) and must also occur in *A. koschnevnikovi* and *A. cerana*, because also in these species females acquire much more

sperm (approximated by the average ejaculate size \times the queen mating frequency) than is required to fill the spermatheca (Tab. I). This enormous loss of sperm during the sperm storage process has puzzled researchers and some have hypothesized that the specific morphological characteristics of the female's sexual tract are directly responsible for this very inefficient sperm storage process (Kraus et al., 2004; Schlüns et al., 2004; Winston, 1991), so that the extremely high mate numbers of honey bee queens might be "merely a by-product of their variable mating system" (Tapy and Page, 2002). However, given that mating is not completely free of costs (Brown and Baer, 2005) and that an efficient sperm storage process is important for female fitness, this explanation seems incomplete. I will present another not mutually exclusive hypothesis below, which might also explain a significant part of the observed variation in *Apis* queen mating frequency.

Since honeybee queens are polyandrous and store more sperm (4.7 million) than they fertilize eggs (1–1.6 million), sperm competition can occur. To a reasonable approximation, sperm gets completely mixed within the spermatheca, at least when substantial storage time has passed (Franck et al., 1999; Haberl and Tautz, 1998; Laidlaw and Page, 1984; Page, 1986; Page and Metcalf, 1982). Sperm clumping might be expected under special circumstances when workers produce relatedness-induced split sex ratios (Boomsma and Ratnieks, 1996; Trivers and Hare, 1976), but these conditions do not apply in honeybees. Also in non-social insects sperm clumping is rather rare (Simmons, 2001).

Given the mixing of ejaculates, sperm competition could occur at two different stages: during the sperm storage process and during sperm use, i.e. when eggs get fertilized. Sperm competition during the sperm storage process would occur if ejaculates of males would compete for access to the spermatheca. However, several recent findings indicate that sperm competition during sperm storage is of limited significance at best. First, male ejaculates remain moderately clumped within the lateral oviducts (Franck et al., 2002; Winston, 1991), which implies that sperm from different ejaculates do not compete for access to the spermatheca simultaneously. We still lack detailed

molecular quantification of the degree of clumping/mixing of ejaculates within the lateral oviducts, but if confirmed it would mean that free competition between sperm is seriously constrained. Second, in artificially inseminated queens of *A. mellifera* all paternal lineages consistently obtain at least some paternity in the worker offspring (see e.g. Laidlaw and Page, 1984; Schlüns et al., 2004). Given that artificial insemination is likely to further decrease sperm clumping before storage, one would expect that certain males would outcompete others if sperm competition was significant. Third, the classical last male precedence, as found in many (non social) insects (Simmons, 2001) is not present in *A. mellifera* after natural mating flights (Franck et al., 2002) and is only weakly present after artificial insemination (Moritz, 1986). It is also worth mentioning that female mating frequencies have been estimated based on realized paternity distributions among worker offspring, so the figures presented in Table I refer to males that successfully contributed to worker offspring. This indicates that females do not simply copulate with a large number of males, but that sperm of most if not all of them actually gets stored and is successfully used for fertilization. Fourth, paternities are generally not highly biased towards one or very few males (Franck et al., 2002; Laidlaw and Page, 1984; Schlüns et al., 2004, 2005) as would be expected when sperm competition is a major force. Fifth, the evolution of "harmful" competitive traits such as sperm removal, spermicide, sperm flushing or sperm incapitation is not expected to evolve in *Apis* bees or any other social Hymenoptera with long-lived colonies. This is because such traits are likely to harm somatic colony growth (worker production) during the relatively long time lag between insemination and reproduction (Baer, 2003; Boomsma et al., 2005). Sixth, the short mating duration makes it rather unlikely that males can achieve anything else than an ejaculation. In summary, *Apis* males seem to have hardly any chance to manipulate the ejaculates of their competitors, because they lack the time to do so and queens store previous ejaculates out of reach of subsequently copulating males and do not start the sperm storage process until after the last mating.

Because sperm becomes completely mixed after being stored (Franck et al., 1999; Haberl

and Tautz, 1998; Laidlaw and Page, 1984; Page, 1986; Page and Metcalf, 1982), sperm competition is more likely to occur during fertilization (Harbo, 1990) when spermatozoa from different males are likely to encounter the same egg simultaneously. The necessary prerequisite that queens use more than one sperm per fertilization is met, although counts differ between 4–12 (Ruttner, 1975; Yu and Omholt, 1999), 25 (Harbo, 1979) and 10–100 (Adam, 1912; Bresslau, 1905). Sperm competition at the egg fertilization stage might be in the interest of the queen to ensure that the most viable sperm fertilizes each egg. Furthermore, *Apis* sperm is very long (Tab. I) compared to other (monandrous) social insects such as bumblebees (166–169 μm , Baer et al., 2003), fire ants (60 μm , Lino Neto and Dolder, 2002) or the non-leafcutter fungus growing ants (Baer, unpublished data). This length would seem puzzling when sperm is stored as a fraction of an entire ejaculate (see also below), as more sperm could be stored in the same fraction when sperm were shorter. Longer sperm would thus only be advantageous when competing with unrelated sperm and when longer sperm would move faster. In *Apis*, these conditions seem much more likely to apply when individual sperm compete for fertilizing an egg (i.e. during competition for reaching the micropyle) than when masses of clonal sperm compete for access to the spermatheca. There is indeed evidence that longer sperm is associated with sperm competition in a number of comparative studies (Briskie and Montgomerie, 1992; Gage, 1994; Gomendio and Roldan, 1991; Morrow and Gage, 2000), although not in some others (Hosken, 1997; Stockley, 1997). The hypothesis that sperm competition in *Apis* is more likely during egg fertilization than during sperm storage implies that sperm storage success is a function of ejaculate size, but that fertilization success is a function of individual sperm quality.

5. CRYPTIC FEMALE CHOICE IN *APIS* BEES

The other factor that might have considerable influence on paternity patterns in *Apis* bees is cryptic female choice. Similar to sperm competition, cryptic female choice could potentially occur during the sperm storage process

and/or during sperm use. There is good evidence from non-social insects that females indeed have considerable influence on these processes (Eberhard, 1996; Simmons, 2001). Whereas sperm competition in *Apis* bees is more likely to occur during sperm use (i.e. during egg fertilization), I here argue that cryptic female choice is more likely to occur during the sperm storage process. First of all the queen can actively close the bursa copulatrix with a valve-fold, known as *vavulva vaginalis* so that entire ejaculates could potentially be rejected during ejaculation. As mentioned earlier, the queen mates during her nuptial flight with several males in quick succession acquiring large amounts of spermatozoa, and the subsequent transfer to the lateral oviducts is influenced by muscular contractions of the queen (Eberhard, 1996; Koeniger and Koeniger, 1991 and references therein). This process offers another possibility for the queen to reject entire or partial ejaculates because she might decide to eject sperm rather than transferring it to her lateral oviducts. *Apis* queens also have the possibility to pump sperm backwards via muscular contractions which are normally used to move sperm towards the opening of the spermathecal duct during the sperm storage process (Snodgrass, 1984). This idea would also shed light on possible additional functions of the mating sign, which is very complex structure in all *Apis* bees (Ruttner, 1988). The mating sign is obviously not preventing additional copulations by subsequent males, and was hypothesized to mostly serve as a plug to prevent sperm backflow (Woyciechowski et al., 1994) or even as a promoter of additional copulations (Koeniger, 1990). Here I add the hypothesis that the mating sign might actually be the result of a sexually selected arms race between the queen and the males, with males evolving mating signs in order to guarantee successful sperm transfer to the lateral oviducts (but not preventing further copulations) and females evolving counter adaptations to be able to perform cryptic female choice. Since the mating sign consists of a sclerotized part as well as of mucus secretions, males might use mechanical as well as chemical stimuli to promote the transfer of their sperm to the lateral oviducts. More detailed studies are needed about the functional morphology of the mating sign or biochemical investigations of the mucus secretion to shed further light on this issue.

Unfortunately, copulating honey bee queens are difficult to observe during nuptial flights so we do not know whether queens actually reject sperm at this stage. The migration of spermatozoa into the spermatheca occurs not until the queen has returned to the hive (Woyke, 1983). Consequently, any manipulation of these temporarily stored ejaculates in the bursa copulatrix and the lateral oviducts would per definition be “cryptic female choice”, because sperm storage occurs after decoupling of the males and the queen and after the nuptial flight when the drones are no longer present.

Sperm storage in *A. mellifera* is a combination of sperm actively swimming up the spermathecal duct as well as muscular movements of the female (Ruttner and Koeniger, 1971). The fact that sperm is ultimately forced to actively swim for successful storage might be in the queen’s interest to avoid the storage of dead or non-viable spermatozoa. Indeed damaged sperm is not stored in the spermatheca (Ruttner and Koeniger, 1971). The queen controls the migration of sperm into the spermatheca by abdominal contractions pushing temporarily stored sperm out of the lateral oviducts into the bursa copulatrix as well as by muscles attached to the oviduct, also known as Bresslau’s sperm pump (Bresslau, 1905). If the female controls the flow back of sperm as well as the uptake of sperm into the spermatheca, she might also use this ability to manipulate the storage process of different ejaculates. As a consequence of the clumping of temporarily stored ejaculates in the lateral oviducts (Franck et al., 2002; Winston, 1991), sperm from different males might also appear as one clump after another at the opening of the spermathecal duct. The queen therefore has two different ways to control sperm influx of specific males into the spermatheca: differential backflow of (fragments of) ejaculates and the time span that (fragments of) ejaculates are allowed to be positioned before the opening of the spermathecal duct. If (fragments of) ejaculates are stored one after another, some sperm clumping is expected to occur in the early phase of sperm storage, which has indeed been found, both directly (Franck et al., 2002) and indirectly by showing that the variance in patriline distributions decreases over time (Franck et al., 1999); i.e., as sperm becomes more mixed, patrilines

vary less in their contributions to worker offspring.

It is reasonable to assume that a honeybee queen has the necessary mechanisms to estimate the amount (or what is left) of sperm she has in her oviducts or even the degree of filling of the spermatheca. This assumption seems reasonable, because in the absence of re-mating later in life, queens depend on maximal sperm storage so that neuronal mechanisms to control this process would provide an immediate selective advantage. Queens perform additional mating flights in cases of insufficient spermatheca filling (Woyke, 1964), sometimes even on the same day (Snodgrass, 1984). The question then arises how well queens can manipulate ejaculates, given that they only have restricted information about their mating partners (see above). After discarding unwanted ejaculates, queens might in fact not favor any specific male but try to equalize the contributions from their mates, independently of how much sperm each male contributed. The reason to perform such “genotype scrambling” is that this results in a general increase in genetic diversity among worker offspring (see also Boomsma and Ratnieks, 1996). As mentioned earlier this has repeatedly been shown to benefit colony performance in various different ways.

Further support for the “genotype scrambling” hypothesis comes from studies of patriline distributions in honeybee colonies. As Schlüns et al. (2005) show, patrilines are relatively evenly distributed in seven honeybee species investigated, at least for the most frequent patrilines, and no patriline is able to monopolize paternity in the ways expected from last or first male precedence. This finding is not necessarily expected, because males vary greatly in ejaculate size, (see Koeniger et al., 2005 and references therein): in *Apis mellifera* between 2 and 12 million and in *Apis dorsata* between 1.2 and 2.4 million. The fact that patrilines are rather evenly distributed in honeybee worker offspring thus supports the idea that females might indeed try to equalize male contributions during the sperm storage process. Experiments where queens were serially inseminated with sperm from different males also support active equalizing of sperm contributions by queens, as the order of injection had no or only a weak effect upon the success of ejaculates in siring worker offspring (Laidlaw

and Page, 1984; Schlüns et al., 2004; Moritz, 1986). Thus, the sperm of the first male inseminated was not more successful during storage than the last one, even though the storage constraint was smaller for the first ejaculate compared to later ones.

Cryptic female choice could also occur during sperm use, i.e. when eggs get fertilized, which was defined as sperm selection (Simmons, 2001) and has been postulated in a number of insect species. However, given that honeybee queens store ejaculates in a single spermatheca where they become completely mixed, it is difficult to imagine a mechanism that would give the female the ability to select sperm from specific males. Also, given that sperm storage is constrained by the volume of the spermatheca, females are expected to select against undesired sperm before storage. Consequently, cryptic female choice via sperm selection seems unlikely to occur during sperm use, but given the little information available, it is difficult rule it out completely and more studies are needed.

Given the scenario presented here, cryptic female choice would act rather differently in *Apis* bees compared to other organisms, where females typically prefer certain types of males to others (Eberhard and Cordero, 1995). Queens would use the congregation areas to quickly collect ejaculates of different males in their oviducts and process them later in their own interest to ensure that (1) they transfer enough sperm to completely fill the spermatheca (based on the amount of sperm stored in the lateral oviducts) and (2) in case of an excess of sperm or the possibility of more nuptial flights, queens mate and store sub-samples of sperm from as many male genotypes as possible. Consequently, the “genotype scrambling” hypothesis can in fact explain the excessive sperm intake and sperm dumping of *Apis* queens. One important reason for genotype collecting is that the queen has insufficient information about her mates and insufficient time to discriminate among them, for example to detect males with specific qualities such as their sex locus alleles or disease resistance.

At least two species of *Apis* bees, *A. florea* and *A. andreniformis*, differ from this general mating pattern in that males ejaculate their sperm directly into the spermathecal duct from where it is rapidly transferred to the spermatheca

(Koeniger and Koeniger, 1991; Koeniger et al., 1989b). In both species, returning queens have sperm in their spermathecae but not in their lateral oviducts, as is the default pattern in the other *Apis* bees. Consequently, ejaculates are transferred to the spermatheca during or shortly after copulation. Such a change in mating biology has important consequences because it implies that the females might have lost the possibilities to perform cryptic female choice during the sperm storage process in the ways as described above. Several characteristics of the mating biology of these two species indicate that such a loss of female influence might indeed be the case. First of all, if extensive sperm dumping is a result of cryptic female choice it should be absent in *A. florea* and *A. andreniformis*. Indeed, males seem to be able to get large proportions of their ejaculates stored in the spermatheca: 66% in *A. andreniformis* and 44% in *A. florea* (Koeniger et al., 1989b; Koeniger et al., 2000; Koeniger and Koeniger, 2000). The same conclusion can be drawn after looking at the typical ejaculate sizes of males (Tab. I) and comparing them with female mating frequencies and the number of sperm stored in the spermatheca. An *A. andreniformis* queen mating 11 times receives about 1.43 million sperm, which is very close to the 1.26 million actually found in the spermatheca. In *A. florea*, male storage success seems lower (about 30%, given the 3.44 million sperm transferred during copulations and the typical storage of 1.05 million sperm in the spermatheca) but this figure is still high compared to the other *Apis* species. Males appear to have gained control over the sperm storage process by avoiding the typical temporary storage of their ejaculates in the queen’s lateral oviducts. If females are unable to reject entire ejaculates or subsamples of them, the queen mating frequency is expected to be lower than in species where queens can store sperm from as many males as they want. Indeed, *A. florea* and *A. andreniformis* have the lowest mating frequencies of all *Apis* bees investigated (Tab. I). Polyandry in these species may still have been maintained because males might also benefit from genetic diversity among worker offspring or because they do not have enough sperm to fully inseminate a female, as suggested by the sperm limitation hypothesis (Kraus et al., 2004). There is some support for the latter idea

because males indeed possess less sperm than queens typically store after mating (Tab. I) but as Eberhard (1996) pointed out, there might be other reasons for the evolution of undersized ejaculates. Furthermore, if the number of sperm that a male possesses defines his mating success, males might reallocate their resources to invest in sperm numbers and reduce investment in accessory glands, which are used in many insects to manipulate male postcopulatory mating success (Baer, 2003; Boomsma et al., 2005; Simmons, 2001). The accessory glands (mucus glands) of *A. florea* and *A. andreniformis* are indeed only rudimentary (Koeniger and Koeniger, 1991). This evolutionary trend is very similar to another group of polyandrous social insects. In *Atta* leaf cutting ants, males have also evolved a mechanism of a more direct sperm transfer to the spermatheca (Baer and Boomsma, unpublished data) and males in these species also have reduced investments in accessory glands (Baer and Boomsma, 2004). As it seems, sexual selection may have identified alternative traits to work on in *A. florea* and *A. andreniformis*. Males of these species are the most able flyers of all the *Apis* males investigated so far (Radloff et al., 2003), which might suggest that females came to challenge them for their general stamina and condition after they had lost the possibilities for cryptic female choice (Boomsma et al., 2005). Consequently, the idiosyncrasies of the mating system of *A. florea* and *A. andreniformis* with the hypothesized reduction of female manipulations of paternity and their higher performance and efficiency of males make them interesting species for comparative sexual selection studies with the other honeybees.

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Résumé – Sélection sexuelle chez les abeilles du genre *Apis*. Le comportement reproducteur des abeilles mellifères (*Apis* spp.) ressemble beaucoup à celui des autres insectes sociaux, bien qu'une série de caractéristiques spécifiques, que l'on ne retrouve que très rarement chez les insectes sociaux, se soit développée au cours de l'évolution. Par exemple, chez toutes les espèces d'abeilles mellifères les reines s'accouplent avec plusieurs mâles (polyandres). En outre les mâles d'au moins deux espèces sont capables d'éjaculer leur sperme directement dans le canal de la spermatheque. Enfin les abeilles mellifères produisent des mâles en très grand nombre qui, conséquence de ce sex-ratio défavorable aux mâles, ne peuvent s'accoupler qu'une seule fois et meurent au cours de l'accouplement. Ces caractéristiques font des abeilles mellifères un modèle très intéressant de biologie de l'évolution pour étudier la présence et les conséquences de la sélection sexuelle sur la biologie de la reproduction de ces espèces.

Dans cette synthèse je résume notre connaissance actuelle concernant la biologie de la reproduction chez les abeilles mellifères et replace ces données dans le cadre théorique de la sélection sexuelle. Je développe plus particulièrement deux aspects qui ont fait récemment l'objet d'études intenses chez d'autres organismes : la compétition spermatique et le choix mystérieux de la femelle. Il y a concurrence pour le sperme lorsque les spermés éjaculés par plusieurs mâles concourent simultanément à la fertilisation des œufs. Le choix mystérieux de la femelle existe lorsque les femelles manipulent dans leur propre intérêt les spermés éjaculés des mâles et exercent ainsi une influence sur la paternité. La sélection par la femelle des spermés éjaculés se passe de façon mystérieuse, à savoir qu'elle n'est pas visible pour les mâles.

Je montre ici que notre connaissance actuelle de la biologie de la reproduction des abeilles mellifères permet de conclure que tant la compétition spermatique que le choix mystérieux de la femelle pourraient être présents chez ces espèces. Les données montrent en outre que la concurrence spermatique s'exerce pendant le processus de fertilisation des œufs, alors que le choix énigmatique de la femelle a lieu principalement au cours de la copulation et du stockage du sperme.

***Apis* / polyandrie / stockage du sperme / fertilisation / compétition spermatique / choix mystérieux de la femelle**

Zusammenfassung – Sexuelle Selektion bei *Apis* Bienen. Das Reproduktionsverhalten der Honigbienen ist dem anderer sozialer Insekten sehr ähnlich, obwohl sich im Laufe der Evolution eine Reihe sehr spezifischer Eigenschaften entwickelt haben, die sonst nur selten bei sozialen Insekten zu finden sind. So sind beispielsweise alle bisher untersuchten Honigbienenarten polyandrisch, das heisst, die Königinnen verpaaren sich generell mit mehreren

Männchen. Zudem sind die Männchen in mindestens zwei Arten in der Lage, ihre Ejakulate direkt in den Spermathekengang zu übertragen. Schliesslich produzieren Honigbienen einen extremen numerischen Überschuss an Männchen, die, wohl als Folge dieser männchenlastigen Sex-Ratio, sich nur ein einziges Mal verpaaren können und während der Kopulation sterben. Diese Eigenschaften der Reproduktionsbiologie machen Honigbienen zu einem sehr interessanten Modellsystem der Evolutionsbiologie, um die Präsenz und die Konsequenzen von sexueller Selektion auf die Reproduktionsbiologie dieser Arten zu untersuchen.

In dieser Review fasse ich unser heutiges Wissen über die Reproduktionsbiologie der Honigbienen zusammen und setze diese Daten in einen theoretischen Rahmen der sexuellen Selektion. In meinen Ausführungen konzentriere ich mich auf zwei Aspekte, die in jüngster Vergangenheit intensiv in anderen Organismen untersucht wurden: Spermienkonkurrenz und kryptische Weibchenwahl. Spermienkonkurrenz entsteht, wenn Ejakulate mehrerer Männchen gleichzeitig um die Befruchtung von Eiern konkurrieren. Kryptische Weibchenwahl besteht, wenn Weibchen die Ejakulate von Männchen in ihrem eigenen Interessen manipulieren und so Einfluss auf die Vaterschaft nehmen. Die Selektion der Ejakulate durch das Weibchen geschieht kryptisch, das heisst sie ist nicht ersichtlich für die Männchen.

Hier zeige ich, dass unser gegenwärtiges Wissen über die Reproduktionsbiologie der Honigbienen den Schluss zulässt, dass sowohl Spermienkonkurrenz als auch kryptische Weibchenwahl in Honigbienen vorhanden sein könnte. Allerdings zeigen die Daten auch, dass Spermienkonkurrenz in Honigbienen wohl auf die Befruchtung der Eier beschränkt ist, während kryptische Weibchenwahl hauptsächlich während der Kopulation und der Spermien-speicherung vorhanden ist.

Honigbiene / Polyandrie / Spermien-speicherung / Befruchtung / Spermienkonkurrenz / kryptische Weibchenwahl

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