Ann. Zool. Fennici 38: 255–265 ISSN 0003-455X
Helsinki 10 September 2001

Dedicated to the memory of William Donald Hamilton

The curious promiscuity of queen honey bees (*Apis mellifera*): evolutionary and behavioral mechanisms

David R. Tarpy[1,2] & Robert E. Page Jr.[1]

[1)] *Entomology Department, University of California, Davis CA 95616, USA*
[2)] *Present address: Department of Neurobiology and Behavior, Seeley G. Mudd Hall, Cornell University, Ithaca NY 14853, USA*

Received 10 January 2001, accepted 1 June 2001

Tarpy, D. R. & Page, R. E. Jr. 2001: The curious promiscuity of queen honey bees (*Apis mellifera*): evolutionary and behavioral mechanisms. — *Ann. Zool. Fennici* 38: 255–265.

Even after considerable effort and debate, it remains unclear why honey bee queens frequently mate with 10 or more males. We address both why polyandry is adaptive to queens and how queens obtain such extreme numbers of mates. We review a manipulative experiment which tested the hypothesis that multiple mating reduces the genetic load caused by the honey bee sex determination system. Our results suggest that multiple mating (i.e., mating more than once) increases a queen's fitness by lowering the probability that she produces a high proportion of inviable, diploid males within her brood. Furthermore, we examined the relationship between a queen's mating behavior and her mating number. We propose that "extreme" polyandry in honey bees (i.e., mating numbers ≥ 10) may be inadvertent consequences of a queen's mating behavior, therefore additional adaptive arguments are not needed to explain why honey bees have some of the highest mating numbers among the social insects.

Introduction

Throughout his career, W. D. Hamilton discussed the impact that multiple mating by social insect queens, or polyandry, has on the genetic structure of colonies (Hamilton 1964, Hamilton 1972, Hamilton 1987). Haplodiploidy in the social Hymenoptera results in asymmetries in genetic relatedness among colony members and creates high coefficients of relatedness between sisters. High relatedness enables altruism to evolve because inclusive fitness effects derived from donating personal fitness to a nestmate are an inverse function of genetic relatedness (i.e., Hamilton's rule). Polyandry, however, lowers the average genetic relatedness among female nestmates, and thus lowers their potential inclusive fitness. To overcome this cost, there are likely to be one or more benefits to multiple mating, the mechanisms of which have gained increased attention in evolutionary biology.

Polyandry may increase female fitness by any number of direct or indirect mechanisms (Table 1; *see also* recent reviews by Arnqvist & Nilsson 2000, Jennions & Petrie 2000). In social insects, direct benefits (e.g., resource defense polyandry or nuptial gifts; Thornhill & Alcock 1983) probably do not affect the fitness of reproductive females because of their life histories (*see* Table 1). Crozier and Page (1985) discussed several hypotheses that are more applicable to polyandry in the social Hymenoptera, including sperm limitation (Cole 1983), sperm competition (Parker 1984), and several possible benefits of increased genetic diversity (Oster & Wilson 1978, Page 1980, Moritz 1985). Several additional theories have since been proposed (*see* Palmer & Oldroyd 2000, Crozier & Fjerdingstad 2001). Hamilton (1987) and Sherman *et al.* (1988) proposed a mechanism that is analogous to the 'Red Queen' hypothesis for the evolution of sex (Hamilton *et al.* 1990), whereby increased genetic diversity reduces the prevalence of disease within a colony. Fuchs and Moritz (1999) proposed that rare 'specialist' tasks are favored only at low frequencies within a colony, so that increased genetic diversity reduces the costs of over- or under-expressing those traits.

Any single hypothesis is insufficient to explain why polyandry is widespread in the social Hymenoptera (Crozier & Fjerdingstad 2001). Comparative studies are helpful to determine trends across taxa (e.g., Keller & Reeve 1994, Boomsma & Ratnieks 1996, Pedersen & Boomsma 1999, Schmid-Hempel & Crozier 1999), but manipulative experiments (e.g., Baer & Schmid-Hempel 1999) are desirable to test individual hypotheses at the species level. Honey bees (*Apis mellifera*) are a model system to investigate polyandry in social insects because mating number can be experimentally controlled using the technique of instrumental insemination (Laidlaw 1977). Moreover, queen bees exhibit some of the highest mating frequencies in the Hymenoptera (Page 1986), mating with upwards of 24 males (Neumann *et al.* 1999). The purpose of this paper is to review a recent set of experiments that investigated polyandry in honey bees. First, we tested the effects of polyandry on queen fitness as a function of the sex determination system. Then we determined if honey bee queens optimize their mating numbers by studying mechanisms of their mating behavior. We argue that it was necessary to take this multilevel approach to understand the evolution of polyandry in honey bees.

Sex determination and multiple mating

Page (1980) presented a hypothesis for the evolution of polyandry in honey bees based on their genic mechanism of sex determination. Many species within the insect order Hymenoptera, including honey bees, have a sex determination system based on a single gene that determines the developmental fate of larvae (Mackensen 1951, Cook & Crozier 1995). There are numerous allelic forms of the sex gene, ranging from six to 17 among different honey bee populations (Woyke 1976, Adams *et al.* 1977). Haploid individuals are hemizygous at the sex locus and are male. Diploid individuals that are heterozygous at the sex locus are female. Diploid individuals that are homozygous, however, are sterile males which, in *Apis mellifera*, are cannibalized by workers soon after hatching from their eggs (Woyke 1963). If a queen and her mate both possess a common sex allele, then half of their diploid offspring will not develop and the viability of their brood will be reduced. This sex determination system, therefore, imposes a genetic load onto a colony and may affect its fitness.

The expected viability of worker brood within a colony is a binomial function of the effective number of mates by its queen and the number of sex alleles in the population. Let n equal the number of mates by a queen that, for simplicity, are equally frequent in their fertilization of eggs. The proportion of diploid males is determined by the number of a queen's mates that carry a sex allele in common with her, y, which ranges between 0 and n. The probability that a queen produces a given proportion of diploid males depends on the number of differ-

Table 1. A list of some hypotheses explaining the evolution and maintenance of social insect polyandry in general, and their applicability to honey bees in particular.

Hypothesis	Description	Applicable to honey bees	Reference(s)
Direct mechanisms			
1 Convenience	Easier to let multiple males mate than to resist	No, because queens take multiple mating flights	Alcock *et al.* (1977)
2 Sperm replenishment	Females mate multiply to bolster depleted sperm supply	No, because queens only mate once in their lifetimes	Thornhill (1976)
3 Sperm limitation	Need for/storage capacity is greater than what one male can provide	No, because males produce enough sperm to fill a queen's spermatheca (Bolten & Harbo 1982, Page *et al.* 1984)	Cole (1983), Boomsma and Ratnieks (1996), Fjerdingstad and Boomsma (1998)
4 Nutritive sperm	Males contribute beneficial proteins or compounds in the ejaculate	No, because queens lay eggs over a protracted time span	Lamunyon and Eisner (1994)
5 Fertility-certainty	'Bet-hedging' against mating with only an infertile male	No, because sperm transfer is inefficient (reviewed by Oldroyd *et al.* 1998)	Walker (1980), Ridley (1988)
Indirect mechanisms			
6 "Good genes"	Increase probability of mating with a genetically superior male	Yes, but no evidence for mate choice by queen bees (Gary 1963, Koeniger 1990)	Thornhill and Alcock (1983), Zeh (1997)
7 Sperm competition	Form of passive female choice that allows "best" male to win	Yes, but tested and not supported (Laidlaw & Page 1984, Haberl & Tautz 1998)	Parker (1984), Keller and Reeve (1995)
8 Effective population size	Increases the N_e of the population	No, because polygyny is more effective than polyandry to increase N_e (Crozier & Page 1985)	Wilson (1963)
9 Genetic variation			
a Task diversity	Increased fitness due to more diverse worker force	Yes	Oster and Wilson (1978), Robinson and Page (1989)
	More efficient exploitation of foraging environment	Yes	Oldroyd *et al.* (1991), Oldroyd *et al.* (1992), Oldroyd *et al.* (1993)
	Rare tasks	Yes, but only at extreme mating numbers	Fuchs and Moritz (1999)
b Environmental tolerance	Allows for a buffer against environmental changes	Yes	Calderone *et al.* (1989), Page *et al.* (1995)
c Disease tolerance	Reduces the transmission of parasites and pathogens among colony members	Yes	Hamilton (1987), Sherman *et al.* (1988), Schmid-Hempel (1998)
d Genetic benefits	Reduces the optimal sex ratio of the workers to that of the queen	Yes[1]	Moritz (1985), Woyciechowski and Lomnicki (1987), Pamilo (1991), Queller (1993), Ratnieks and Boomsma (1995), Sundström and Ratnieks (1998)
	Reduces the genetic load caused by the sex locus	Yes	Page (1980), Page and Metcalf (1982), Crozier and Page (1985), Ratnieks (1990), Pamilo *et al.* (1994), Crozier and Pamilo (1996)

---

[1] Difficult to distinguish cause from effect; polyandry may have evolved in honey bees for other reasons, and the approximate 1:1 sex ratio (Page & Metcalf 1984) is a consequence of the resultant change in genetic relatedness among female nestmates.

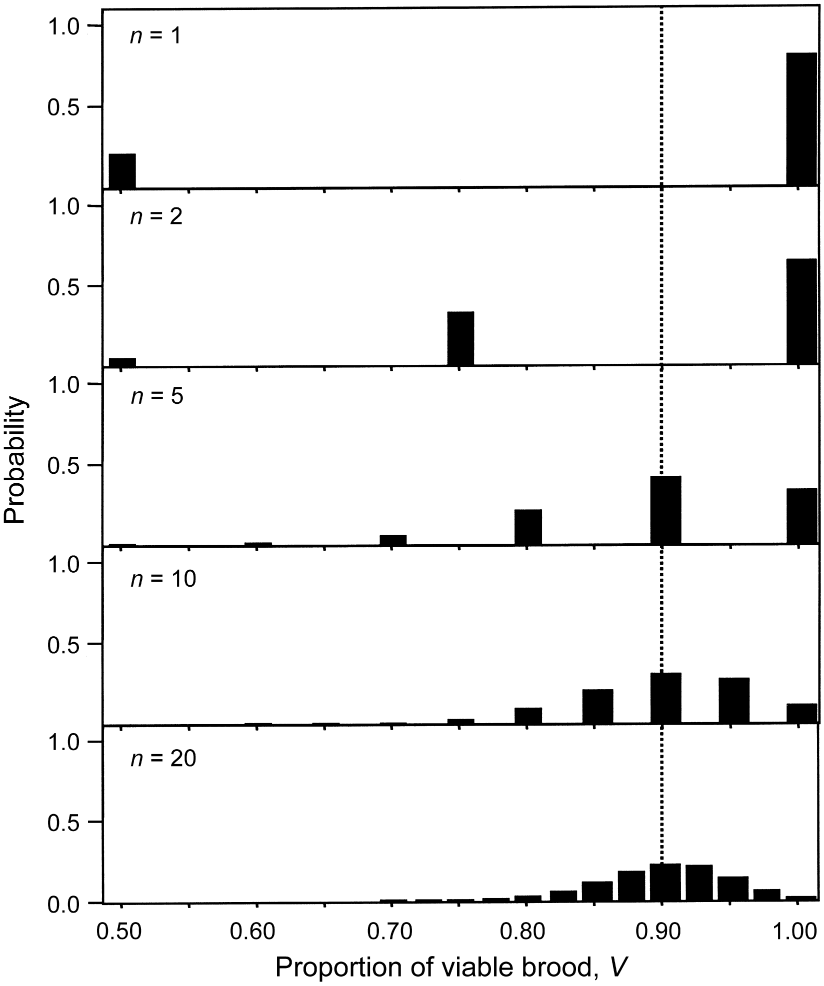

Fig. 1. Brood viability as a function of mating number in a population with ten sex alleles. The probability of producing a given brood viability (*V*) is a binomial function of mating number of the queen (*n*). The expected proportion of viable brood across the population, indicated by the vertical dashed line at *V* = 0.90, is independent of mating number. The variance around the expected viability, however, decreases as mating number increases. Polyandry, therefore, reduces the likelihood that a queen produces a low proportion of viable diploid offspring.

ent sex alleles in the population, k, which are expected to be near equal frequency in the population (Laidlaw *et al.* 1956, Yokoyama & Nei 1979). There is a $2/k$ probability that any given male carries a sex allele identical to one in a queen. Therefore, the probability that y males share a common sex allele with a queen can be determined by the binomial expansion

$$P_{(Y=y)} = \binom{n}{y}\left(\frac{2}{k}\right)^{y}\left(\frac{k-2}{k}\right)^{n-y}, y = 0,1,...,n \quad (1)$$

The proportion of viable diploid brood produced by a queen, therefore, is:

$$V = 1 - \frac{y}{2n} \quad (2)$$

All queens within a population are expected to produce an average brood viability, $\overline{V} = \frac{k-1}{k}$. Although the average brood viability does not change as a function of mating number, the variance in brood viability decreases around the population mean as n increases (Adams *et al.* 1977, Page & Marks 1982). If there are ten equally-frequent sex alleles in a population, the expected viability is 90% for all diploid offspring in the population, and is independent of the mating numbers by queens (Fig. 1; *see also* Page & Marks 1982, Cook & Crozier 1995). When a queen is inseminated by only one male, she has a 0.2 probability of producing 50% viable brood and a 0.8 probability of producing 100% viable brood (note that 0.2 × 0.5 + 0.8 ×

1.0 = 0.9, the population average viability). As *n* increases, the probability decreases that a queen produces either extreme brood viability, and the probability increases that her brood viability is closer to the population average. In other words, polyandry reduces the variance in the proportion of diploid males among queens (colonies) within a population.

It may benefit a queen to mate multiply if the fitness costs of diploid males are disproportionately greater for colonies with low brood viability than they are for colonies with brood viability at or near the population average (Page 1980, Crozier & Page 1985, Page 1986, Pamilo *et al.* 1994, Schmid-Hempel 1994, Cook & Crozier 1995). In other words, according to this model, polyandry is favored if the relationship of colony fitness to brood viability is concave around the average viability of the population. A convex fitness relationship would select against polyandry and favor monandry, and a linear relationship would not favor either mating system. The description of the fitness relationship is, therefore, critical for testing the sex determination hypothesis for the evolution of polyandry.

We tested the sex determination hypothesis (Tarpy 2000) by establishing a population of colonies that varied with respect to their brood viabilities and determined the relationships between brood viability and several measures of colony fitness. To vary brood viability, we instrumentally inseminated super sister queens ($G = 0.75$) each with the mixed sperm of three of their own brothers. This mating scheme controlled the number of common sex alleles that the queens and males carried, enabling us to produce queens that laid viable diploid brood ranging from 50% to 100% while holding "mating" number constant. Note that the genetic effects of other loci (unless they are highly linked to the sex locus) would be random among all the diploid offspring of the population, and thus there is no confounding effect of inbreeding. We placed each queen into her own newly-established colony in the early summer to simulate the foundation of a swarm. We measured brood viability following standard methods (Mackensen 1951) soon after each queen began to lay eggs. Details of our experimental methods may be found in Tarpy (2000).

We evaluated the colonies to determine the effect of brood viability on colony fitness. At three week intervals, we inspected each hive and estimated several variables, including the total number of adult workers, the change in colony biomass, and the total surface areas of the brood nest, honey comb, and pollen stores. We suspended the measurements during the winter, but we inspected each colony periodically to determine if it was still alive. We resumed the evaluations in the spring, and ended the experiment in the mid-summer (*see* Tarpy 2000).

All of the internal colony variables were highly correlated with each other. The most significant correlations — both statistically and biologically — were those with adult worker population, since a colony's phenotype is a reflection of the tasks performed by its workers. Indeed, a colony's population affects its fitness in two ways. First, a larger colony is able to collect more nectar and store more honey during the active foraging season, thereby increasing the food reserves that are necessary for it to survive the winter (Seeley 1985). Second, a larger colony is more likely to issue one or more swarms after its rapid population increase in the spring (Lee & Winston 1987). Worker population, therefore, is an important indicator of colony fitness and is arguably the best variable to distinguish non-linear effects of brood viability. We observed a significant concave relationship between brood viability and worker population at the end of the first active season, approximately 18 weeks after the colonies were established ($r^2 = 0.29$, $p < 0.05$).

A colony's brood viability also significantly influenced the probability that it survived the winter, a direct component of fitness. All colonies survived the winter that were above the median brood viability of 72%. In contrast, only 37.5% of the colonies below the median brood viability survived the winter (Fig. 2). Polyandry decreases the probability that a queen produces brood with a viability below 72% (*see* Fig. 1), thereby demonstrating the direct fitness advantage of polyandry.

It is critical to distinguish among the genetic

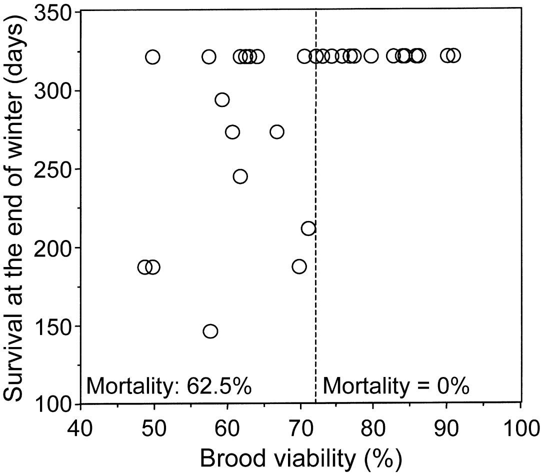

Fig. 2. Winter survival of colonies as a function of their brood viability (from Tarpy 2000). Colonies with brood viabilities in the lower half of the experimental population (left of the vertical dashed line) were much more likely to die during the winter than did colonies with brood viabilities in the upper half.

diversity hypotheses because, given the proper conditions, they all predict that increased genetic diversity can increase colony fitness (Crozier & Fjerdingstad 2001). Our experimental design (*see* Tarpy 2000) provided an independent test of the sex locus hypothesis. Each experimental queen was inseminated with three males derived from their own mother. Thus the total genetic diversity did not correlate with brood viability among the colonies. These results, therefore, cannot be explained by other potential benefits of increased genetic diversity.

There are several genetic and ecological factors that impact how the sex locus influences the mating systems of social insects. First, the time at which diploid drone larvae are removed, which affects the efficiency of cell use in the brood chamber, has been suggested to be a selective factor for mating number (Ratnieks 1990). Second, polyandry is favored if selection occurs during the asymptotic phase of a colony's growth cycle, as it would for a swarming species such as honey bees (Page 1986). On the other hand, monandry is favored if selection acts during the exponential phase, as it would for solitary-founding species such as *Bombus terrestris* and *Solenopsis invicta* (Pamilo *et al.* 1994). Third, greater allelic variation at the sex locus reduces the probability that a queen produces 50% viable brood (*see* Eq. 1) and lowers the impact of the genetic load. Even though novel sex alleles spread within a population as a result of overdominant selection, the effective population size limits their total number at the selection-drift equilibrium (Cook & Crozier 1995). *Solenopsis invicta*, like *Apis mellifera*, have an estimated 15 sex alleles (Ross & Fletcher 1986), but *Bombus terrestris* has at least 24 sex alleles (Duchateau *et al.* 1994), presumably because they have a higher effective population size. The sex locus may therefore not only explain polyandry in *Apis*, but also the predominantly monandrous mating systems of *Bombus* and *Solenopsis*. As Crozier and Pamilo (1996) point out, however, these few examples do not imply that the sex locus accounts for all social insect mating systems, but they do demonstrate the potential impact that the sex determination system has on the biology of social insects.

Mating behavior and extreme polyandry

It may be advantageous for a honey bee queen to mate multiply and ensure that she does not produce a brood of low viability, but such a benefit is subject to diminishing returns. The costs of mating — such as the risks of predation and physical injury — will always favor a lower mating number (Crozier & Fjerdingstad 2001) and are likely to outweigh the asymptotic benefit of polyandry after only a few matings. It has been argued, therefore, that the sex locus mechanism cannot explain polyandry above 6–10 matings (Palmer & Oldroyd 2000), and that additional or alternative adaptations are responsible for "extreme" polyandry in honey bees (e.g., Queller 1993, Fuchs & Moritz 1999).

Queens of *Apis mellifera*, however, have effectively mated with fewer than five males in 23.3% of reported cases, and fewer than ten males in 47.8% of reported cases (D. Tarpy unpubl.; effective mating number takes into account unequal paternity among the males (Pamilo 1993), and is the mating number by which benefits are accrued at the colony level). It appears, therefore, that only a slim majority of queens mate with a number of males that exceed the level considered "extreme". The question becomes why some queens mate with an extreme number of males while others do not. To address this issue, it is necessary to understand the mating behavior of queens and the mechanisms by which they assess their mating status.

Like queens of many social Hymenoptera, a queen honey bee mates during a brief period soon after she becomes an adult and stores the sperm that she uses throughout her egg-laying life. A queen mates outside her nest at a 'drone congregation area' where she copulates with many males in rapid succession before she returns to her colony. A queen may take as many as three mating flights (Ruttner 1956), storing a small proportion of each male's sperm in her spermatheca (Page 1986). A queen never mates again after she begins to oviposit.

A queen's mating number is a consequence of her mating behavior. Queens are expected to optimize their mating numbers based on the relative costs and benefits of multiple mating. We investigated how the duration and number of mating flights by a queen affect the number of times she mates (Tarpy & Page 2000). If there is an extreme optimal number of mates for queens, then they would be expected to adjust their mating behavior on the basis on the number of times they have mated. In other words, we asked the question "can honey bee queens 'count' their mates?"

We monitored 30 queens during their nuptial flights (*see* Tarpy 2000, Tarpy & Page 2000 for detailed methods). With minimal disturbance to the queen and colony, we estimated the duration of a queen's nuptial flight by recording her exit and re-entry times. If queens actively optimize the number of times they mate, then there should be little variation in mating numbers among queens independent of flight time. If queens do not adjust their flight times to influence mating number, then there should be a weak correlation, if any, between flight duration and mating number, with significant variance among queens for both measures.

If a queen attempted a second mating flight after her first mating flight, then we prevented her from exiting the colony by blocking the entrance with queen excluder material. A queen that did not attempt a second flight began to lay eggs within a few days. Thus we recorded two groups of queens that had an equal number of mating opportunities (i.e., one mating flight) but differed in their motivation to continue mating: (1) those that started to lay eggs after their mating flight and (2) those that attempted, unsuccessfully, to take second mating flights. If queens have been selected to optimize their mating number, then queens that attempted second flights presumably had a lower mating success on their first flights. We predicted, therefore, that queens in group 1 had mated more times than queens in group 2.

We sampled adult workers from each colony to obtain the progeny of each queen. We determined the genotypes of each worker at five loci: the allozyme malate dehydrogenase (Nielsen *et al.* 1994), two RAPD-PCR markers (Lewis & Snow 1992), and the microsatellites A76 and A107 (Estoup *et al.* 1994). Based on these markers, a standard paternity analysis of the workers (e.g., Haberl & Tautz 1999) yielded an estimate of the effective mating number of each queen (Pamilo 1993). We correlated the estimated mating numbers of the queens to their mating behavior to determine how selection for extreme polyandry has influenced a queen's behavior on her nuptial flight.

Queens flew for an average of 24.2 ± 9.21 minutes on their mating flights, which is consistent with previous findings (Roberts 1944, Ruttner 1956). On those flights, queens effectively mated with 4.6 ± 3.47 males (mean ± SD). There was no significant correlation between flight time and mating number ($t_{25} = -0.27$, $p = 0.79$, $r^2 = 0.003$). Only 26.7% of the queens attempted a second mating flight. We compared the effective mating numbers between the two groups of queens (group 1: $n = 22$, 4.7 ± 3.50; group 2: $n = 8$, 4.3 ± 3.60) and found no significant difference between them ($t_{28} = 0.28$, $p = 0.78$). Thus we found no evidence that queens adjust their mating behavior in response to the number of males with which they mate.

The observed variation in effective insemination numbers on a single flight (0 to 8.67) is not expected if queens have been selected to optimize their mating numbers. Low worker samples (Pamilo 1993) or sperm clumping (Franck

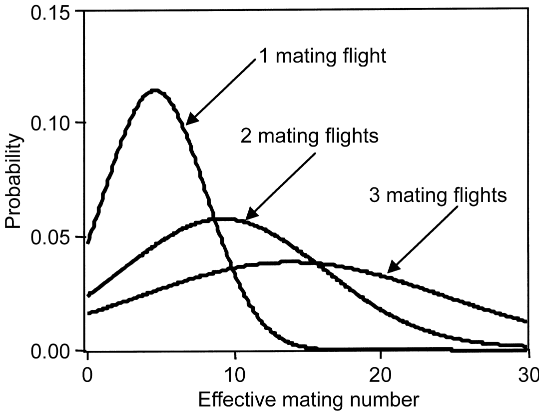

Fig. 3. The effect of the honey bee mating system on effective mating numbers of queens. The results of Tarpy and Page (2000) indicate a high variance in mating number for queens on a single mating flight (4.6 ± 3.47 males). Queens may take up to three mating flights before they begin to oviposit. Assuming that mating number is additive with each successive flight, the inability of queens to assess their number of mates produces extremely large variation in their final mating numbers. Extreme polyandry may occur in some queens by chance rather than by design.

et al. 1999) may partially account for this variability, but it is likely that queens cannot directly assess (count) the number of times they mate (Tarpy & Page 2000). The high variation in mating numbers on a single flight, coupled with the inability of queens to assess their mating numbers and subsequent mating flights, may result in extreme final mating numbers (Fig. 3). Extreme polyandry in *Apis*, therefore, may not require an additional benefit beyond reducing the probability of producing a large proportion of diploid males because differences in the mating behavior among queens may inadvertently result in extreme mating numbers. This argument presumes that the fitness costs of mating are relatively low, which is a matter that remains to be fully resolved (Ratnieks 1990, Tarpy & Page 2000; c.f. Moritz 1985, Palmer & Oldroyd 2000). Future research must determine the relative costs associated with mating, the biological cue that prompts a queen to take more than one mating flight, and the factors that contribute to the variation in mating number on a given mating flight.

Conclusions

We demonstrate a significant fitness effect of the sex determination system of honey bees. As a consequence, queens that mate more times are expected to reduce the probability of having lower brood viability and, on average, survive better and produce more populous colonies. This does not exclude the existence of other potential benefits derived from increasing the genotypic diversity within a colony. Indeed, we maintain a pluralistic view of how polyandry may benefit a colony. It is necessary, however, to perform controlled, manipulative experiments that independently test those other genetic diversity hypotheses — such as the parasite and pathogen hypothesis (Sherman *et al.* 1988) — to determine their relative fitness consequences. Nonetheless, we believe that it is not necessary to evoke a complex argument to explain why extreme polyandry evolved in honey bees. Given the manner in which matings are acquired, we feel the unusually high levels of polyandry reported for honey bees is best explained as an inadvertent consequence of mating behavior rather than an adaptive optimal solution to a need for even more genotypic diversity.

Acknowledgements

We thank Kim Fondrk, Tanya Pankiw, Jürgen Gadau, Helen Chan, Meredith Humphries, and David Nielsen for their help in the field and in the laboratory. Special thanks to Francis Ratnieks, Phil Starks, and an anonymous reviewer for their helpful input. Research was funded by grants from the Almond Board of California, the Jastro-Sheilds foundation, and the Animal Behavior Society. The paper was written while funded from USDA grant 2001-35302-09905 awarded to DRT.

References

Adams, J., Rothman, E. D., Kerr, W. E. & Paulino, Z. L. 1977: Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. — *Genetics* 86: 583–596.

Alcock, J., Eickwort, G. C. & Eckwort, K. R. 1977: The reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. — *Behav. Ecol. Sociobiol.* 2: 385–396.

Arnqvist, G. & Nilsson, T. 2000: The evolution of polyandry: multiple mating and female fitness in insects. — *Anim. Behav.* 60: 145–164.

Baer, B. & Schmid-Hempel, P. 1999: Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. — *Nature* 397: 151–154.

Bolten, A. B. & Harbo, J. R. 1982: Numbers of spermatozoa in the spermatheca of the queen honeybee after multiple inseminations with small volumes of semen. — *J. Apicult. Res.* 21: 7–10.

Boomsma, J. J. & Ratnieks, F. L. 1996: Paternity in eusocial Hymenoptera. — *Phil. Trans. Roy. Soc. Lond.,* B 351: 947–975.

Calderone, N. W., Robinson, G. E. & Page, R. E. Jr. 1989: Genetic structure and division of labor in honeybee societies. — *Experientia* (Basel) 45: 765–767.

Cole, B. J. 1983: Multiple mating and the evolution of social behavior in the Hymenoptera. — *Behav. Ecol. Sociobiol.* 12: 191–201.

Cook, J. M. & Crozier, R. H. 1995: Sex determination and population biology in the Hymenoptera. — *Trends Ecol. Evol.* 10: 281–286.

Crozier, R. H. & Page, R. E. Jr. 1985: On being the right size: male contributions and multiple mating in social Hymenoptera. — *Behav. Ecol. Sociobiol.* 18: 105–115.

Crozier, R. H. & Pamilo, P. 1996: *Evolution of social insect colonies: sex allocation and kin selection.* — Oxford University Press, New York. 306 pp.

Duchateau, M. J., Hoshiba, H. & Velthuis, H. H. W. 1994: Diploid males in the bumble bee *Bombus terrestris*. — *Entomol. Exp. Appl.* 71: 263–269.

Estoup, A., Solignac, M. & Cornuet, J. 1994: Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. — *Proc. Roy. Soc. Lond.* B 258: 1–7.

Fjerdingstad, E. J. & Boomsma, J. J. 1998: Multiple mating increases the sperm stores of *Atta colombica* leafcutter ant queens. — *Behav. Ecol. Sociobiol.* 42: 257–261.

Franck, P., Coussy, H., Le, C. Y., Solignac, M., Garnery, L. & Cornuet, J. M. 1999: Microsatellite analysis of sperm admixture in honeybee. — *Ins. Molec. Biol.* 8: 419–421.

Fuchs, S. & Moritz, R. F. A. 1999: Evolution of extreme polyandry in the honeybee *Apis mellifera* L. — *Behav. Ecol. Sociobiol.* 45: 269–275.

Gary, N. E. 1963: Observations of mating behaviour in the honeybee. — *J. Apicult. Res.* 2: 3–13.

Haberl, M. & Tautz, D. 1998: Sperm usage in honey bees. — *Behav. Ecol. Sociobiol.* 42: 247–255.

Haberl, M. & Tautz, D. 1999: Paternity and maternity frequencies in *Apis mellifera sicula*. — *In. Soc.* 46: 137–145.

Hamilton, W. D. 1964: The genetical evolution of social behaviour. I and II. — *J. Theor. Biol.* 7: 1–52.

Hamilton, W. D. 1972: Altruism and related phenomena, mainly in social insects. — *Ann. Rev. Syst. Ecol.* 3: 193–232.

Hamilton, W. D. 1987: Kinship, recognition, disease, and intelligence: constraints of social evolution. — In: Ito, Y., Brown, J. L. & Kikkawa, J. (eds.), *Animal societies: theory and facts*: 81–102. Japanese Scientific Society Press.

Hamilton, W. D., Axelrod, R. & Tanese, R. 1990: Sexual reproduction as an adaptation to resist parasites (A review). — *Proc. Nat. Acad. Sci.* 87: 3566–3573.

Jennions, M. D. & Petrie, M. 2000: Why do females mate multiply? A review of the genetic benefits. — *Biol. Rev.* 75: 21–64.

Keller, L. & Reeve, H. K. 1994: Genetic variability, queen number, and polyandry in social Hymenoptera. — *Evolution* 48: 694–704.

Keller, L. & Reeve, H. K. 1995: Why do females mate with multiple males? The sexually selected sperm hypothesis. — *Adv. Stud. Behav.* 24: 291–315.

Koeniger, G. 1990: The role of the mating sign in honey bees, *Apis mellifera* L.: does it hinder or promote multiple mating? — *Anim. Behav.* 39: 444–449.

Laidlaw, H. H. Jr. 1977: *Instrumental insemination of honey bee queens.* — Dandant, Hamilton, IL. 160 pp.

Laidlaw, H. H. Jr., Gomes, F. P. & Kerr, W. E. 1956: Estimations of the number of lethal alleles in a panmictic population of *Apis mellifera*. — *Genetics* 41: 179–188.

Laidlaw, H. H. Jr. & Page, R. E. Jr. 1984: Polyandry in honey bees (*Apis mellifera* L.): sperm utilization and intracolony genetic relationships. — *Genetics* 108: 985–997.

Lamunyon, C. W. & Eisner, T. 1994: Spermatophore size as determinant of paternity in an arctiid moth (*Utetheisa ornatrix*). — *Proc. Nat. Acad. Sci. USA* 91: 7081–7084.

Lee, P. C. & Winston, M. L. 1987: Effects of reproductive timing and colony size on the survival, offspring colony size and drone production in the honey bee (*Apis mellifera*). — *Ecol. Entomol.* 12: 187–195.

Lewis, P. O. & Snow, A. A. 1992: Deterministic paternity exclusion using RAPD markers. — *Molec. Ecol.* 2: 275–283.

Mackensen, O. 1951: Viability and sex determination in the honey bee (*Apis mellifera* L.). — *Genetics* 36: 500–509.

Moritz, R. F. A. 1985: The effects of multiple mating on the worker-queen conflict in *Apis mellifera* L. — *Behav. Ecol. Sociobiol.* 16: 375–377.

Neumann, P., Moritz, R. F. A. & Mautz, D. 1999: Using DNA microsatellites for maternity testing in honeybees (*Apis mellifera* L.). — *Apidologie* 30: 505–512.

Nielsen, D., Page, R. E. J. & Crosland, M. W. J. 1994: Clinal variation and selection of MDH allozymes in honey bee populations. — *Experientia* 50: 867–871.

Oldroyd, B. P., Rinderer, T. E., Buco, S. M. & Beaman,

L. D. 1993: Genetic variance in honey bees for preferred foraging distance. — *Anim. Behav.* 45: 323–332.

Oldroyd, B. P., Clifton, M. J., Parker, K., Wongsiri, S., Rinderer, T. E. & Crozier, R. H. 1998: Evolution of mating behavior in the genus Apis and an estimate of mating frequency in *Apis cerana* (Hymenoptera: Apidae). — *Ann. Entomol. Soc. Am.* 91: 700–709.

Oldroyd, B. P., Rinderer, T. E. & Buco, S. M. 1991: Intracolonial variance in honey bee foraging behavior: the effects of sucrose concentration. — *J. Apicult. Res.* 30: 137–145.

Oldroyd, B. P., Rinderer, T. E., Harbo, J. R. & Buco, S. M. 1992: Effects of intracolonial genetic diversity on honey bee (Hymenoptera: Apidae) colony performance. — *Ann. Entomol. Soc. Am.* 85: 335–343.

Oster, G. F. & Wilson, E. O. 1978: *Caste and Ecology in the Social Insects.* — Princeton University Press, Princeton, N.J.

Page, R. E. Jr. 1986: Sperm utilization in social insects. — *Ann. Rev. Entomol.* 31: 297–320.

Page, R. E. Jr., Kimsey, R. B. & Laidlaw, H. H. Jr. 1984: Migration and dispersal of spermatozoa in spermathecae of queen honeybees (*Apis mellifera* L.). — *Experientia* 40: 182–184.

Page, R. E. Jr. & Marks, R. W. 1982: The population genetics of sex determinations in honey bees: random mating in closed populations. — *Heredity* 48: 263–270.

Page, R. E. Jr. & Metcalf, R. A. 1982: Multiple mating, sperm utilization, and social evolution. — *Am. Nat.* 119: 263–281.

Page, R. E. Jr. & Metcalf, R. A. 1984: A population investment sex ratio for the honey bee (*Apis mellifera* L.). — *Am. Nat.* 124: 680–702.

Page, R. E. Jr., Robinson, G. E., Fondrk, M. K. & Nasr, M. E. 1995: Effects of worker genotypic diversity on honey bee colony development and behavior (*Apis mellifera* L.). — *Behav. Ecol. Sociobiol.* 36: 387–396.

Page, R. E. Jr. 1980: The evolution of multiple mating behavior by honey bee queens (*Apis mellifera*). — *Genetics* 96: 263–273.

Palmer, K. A. & Oldroyd, B. P. 2000: Evolution of multiple mating in the genus *Apis*. — *Apidologie* 31: 235–248.

Pamilo, P. 1991: Evolution of colony characteristics in social insects. II. Number of reproductive individuals. — *Am. Nat.* 138: 412–433.

Pamilo, P. 1993: Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. — *Heredity* 70: 472–480.

Pamilo, P., Sundström, L., Fortelius, W. & Rosengren, R. 1994: Diploid males and colony-level selection in *Formica* ants. — *Ethol. Ecol. Evol.* 6: 221–235.

Parker, G. A. 1984: Sperm competition and the evolution of animal mating strategies. — In: Smith, R. L. (ed.), *Sperm competition and the evolution of animal mating systems*: 1–60. Academic Press.

Pedersen, J. S. & Boomsma, J. J. 1999: Positive association of queen number and queen-mating frequency in *Myrmica* ants: A challenge to the genetic-variability hypotheses. — *Behav. Ecol. Sociobiol.* 45: 185–193.

Queller, D. C. 1993: Worker control of sex ratios and selection for extreme multiple mating by queens. — *Am. Nat.* 142: 346–351.

Ratnieks, F. L. W. 1990: The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. — *Behav. Ecol. Sociobiol.* 26: 343–348.

Ratnieks, F. L. W. & Boomsma, J. J. 1995: Facultative sex allocation by workers and the evolution of polyandry by queens in social Hymenoptera. — *Am. Nat.* 145: 969–993.

Ridley, M. 1988: Mating frequency and fecundity in insects. — *Biol. Rev. Camb. Phil. Soc.* 63: 509–550.

Roberts, W. C. 1944: Multiple mating of queen bees proved by progeny and flight tests. — *Glean. Bee Cult.* 72: 255–260.

Robinson, G. E. & Page, R. E. Jr. 1989: Genetic basis for division of labor in insect societies. — In: Breed, M. D. & Page, R. E. Jr. (eds.), *The genetics of social evolution*: 61–80. Westview Press.

Ross, K. G. & Fletcher, D. J. C. 1986: Diploid male production: a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae). — *Behav. Ecol. Sociobiol.* 19: 283–291.

Ruttner, F. 1956: The mating of the honeybee. — *Bee World* 37: 3–15.

Schmid-Hempel, P. 1994: Infection and colony variability in social insects. — *Phil. Trans. Roy. Soc. Lond.* B 346: 313–321.

Schmid-Hempel, P. 1998: *Parasites in social insects.* — Princeton Unversity Press, Princeton, N.J. 409 pp.

Schmid-Hempel, P. & Crozier, R. H. 1999: Polyandry versus polygyny versus parasites. — *Phil. Trans. Roy. Soc. Lond.* B 354: 507–515.

Seeley, T. D. 1985: *Honeybee ecology: a study of adaptation in social life.* — Princeton University Press, Princeton. 201 pp.

Sherman, P. W., Seeley, T. D. & Reeve, H. K. 1988: Parasites, pathogens, and polyandry in social Hymenoptera. — *Am. Nat.* 131: 602–610.

Sundström, L. & Ratnieks, F. L. W. 1998: Sex ratio conflicts, mating frequency, and queen fitness in the ant *Formica truncorum*. — *Behav. Ecol.* 9: 116–121.

Tarpy, D. R. 2000: Behavioral and evolutionary mechanisms of polyandry in honey bees (*Apis mellifera*). Ph.D. Dissertation. University of California, Davis.

Tarpy, D. R. & Page, R. E. Jr. 2000: No behavioral control over mating frequency in queen honey bees (*Apis mellifera* L.): implications for the evolution of extreme polyandry. — *Am. Nat.* 155: 820–827.

Thornhill, R. 1976: Sexual selection and parental investment in insects. — *Am. Nat.* 110: 152–163.

Thornhill, R. & Alcock, J. 1983: *The evolution of insect* *mating systems*. — Harvard University Press, Cambridge. 471 pp.

Walker, W. F. 1980: Sperm utilization strategies in nonsocial insects. — *Am. Nat.* 115: 780–799.

Wilson, E. O. 1963: Social modifications related to rareness in ant species. — *Evolution* 17: 249–253.

Woyciechowski, M. & Lomnicki, A. 1987: Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. — *J. Theor. Biol.* 128: 317–328.

Woyke, J. 1963: What happens to diploid drone larvae in a honeybee colony. — *J. Apicult. Res.* 2: 73–75.

Woyke, J. 1976: Population genetic studies on sex alleles in the honeybee using the example of the Kangaroo island bee sanctuary. — *J. Apicult. Res.* 15: 105–123.

Yokoyama, S. & Nei, M. 1979: Population dynamics of sex-determining alleles in honey bees and self-incompatibility alleles in plants. — *Genetics* 91: 609–626.

Zeh, J. A. 1997: Polyandry and enhanced reproductive success in the harlequin-beetle-riding pseudoscorpion. — *Behav. Ecol. Sociobiol.* 40: 111–118.