Behavioral Ecology
doi:10.1093/beheco/arq016
Advance Access publication 5 March 2010

# Sexual selection in honey bees: colony variation and the importance of size in male mating success

**Margaret J. Couvillon,[a,b] William O.H. Hughes,[a,c] Juan A. Perez-Sato,[a,d] Stephen J. Martin,[a] Gabrielle G.F. Roy,[a] and Francis L.W. Ratnieks[a,e]**

[a]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, S10 2TN, UK, [b]Department of Ecology and Evolutionary Biology, University of Arizona, 1041 E. Lowell St., Tucson, AZ 85721-0088, USA, [c]Institute of Integrative and Comparative Biology, University of Leeds, Leeds, LS2 9JT, UK, [d]Colegio de Postgraduados Campus Cordoba, Km 348 Carretera Federal Cordoba-Veracruz, Congregación Manuel Leon Amatlan de los Reyes, Cordoba, Veracruz, C.P. 94946, Mexico, and [e]Laboratory of Apiculture and Social Insects, Department of Biological and Environmental Science, University of Sussex, Falmer, Brighton BN1 9QG, UK

Sexual selection is a dominant force in the evolution of many animals and can be particularly significant in species that mate in aerial swarms characterized by strong male–male competition. However, such mating biology, typical of many social insects, is also quite challenging to study. Here, we investigate sexual selection in the honey bee that has 2 distinct male morphs (normal sized and small). Males mate only once and females return to their nest after mating, making it possible to measure the lifetime fitness of both sexes. We allowed known numbers of normal-sized males from 6 colonies and small males from another 6 colonies to compete for natural matings with experimental virgin queens. We then determined the mating success of males by genotyping the offspring of these queens. Colonies differed by an order of magnitude in the intrinsic mating success of their males, confirming that the reproductive fitness of honey bee colonies is highly variable. Small males achieved approximately half as many matings as expected given their number of flights and, in addition, had a significantly smaller share of paternity per mating than normal-sized males. Interestingly, the flight activity of small males suggested that they may compensate for their lower competitiveness by flying outside the most competitive mating period in the afternoon. The lower fitness of small males shows that sexual selection is strong in honey bees and contributes to inclusive fitness dynamics that favor worker cooperation within their societies.
*Key words: Apis mellifera*, male competition, male size, paternity, polyandry. *[Behav Ecol 21:520–525 (2010)]*

Competition between males for matings with females is a common and significant feature of the biology of animals (Andersson 1994; Alcock 2005). It can be particularly extreme when the operational sex ratio is male biased (Clutton-Brock and Parker 1992), as is the case in the many insect species in which large numbers of males form swarms within which there is intense competition to mate with females (Thornhill and Alcock 1983). In this competitive arena, females may select males directly precopulation, but more commonly do so indirectly, by mating with males that are faster, more agile, or more persistent fliers. Females, in addition, may select males postcopulation, again directly or indirectly (Eberhard 1996). Female selection precopulation may result in larger males that are more powerful fliers if selection is based on flight speed or persistence, smaller males if selection is based on agility, or both strategies in some species (Neems et al. 1992; Pitnick et al. 2009). Many social insects, specifically ants, termites, and some bees, provide classic examples of such swarm-based mating biology. In some, obligate monogamy combined with a sex ratio close to 1:1 makes sexual selection weak (Boomsma et al. 2005; Boomsma 2007). In other species, though, in which the operational sex ratio is highly male biased or males are capable of remating, sexual selection is likely to be particularly strong (Heinze and Hölldobler 1993; Heinze and Tsuji 1995; Boomsma et al. 2005). However, studying sexual selection in social insects is notoriously difficult, and the evidence is consequently limited. Matings often take place in midair and are stimulated by precise environmental conditions, making controlled matings impossible. Furthermore, females (queens) normally disperse after mating, making it difficult or impossible to quantify the fitness of the partners postmating.

One exception to this is the honey bee (*Apis mellifera*). The act of mating is instantaneously fatal to honey bee males, so their fitness is linked completely to that of a single queen, and queens return to their natal nest after mating. As a result, the subsequent fitness of both partners can be readily determined (Gary 1963; Winston 1995; Koeniger, Koeniger, Gries, and Tingek 2005). Honey bee males (drones) gather in distinct "drone congregation areas," which may contain hundreds or thousands of males from several to hundreds of colonies (Baudry et al. 1998). Honey bee queens join the mating area singly and are then pursued by a dynamic "comet" of males, with the males that reach the front of the comet mating with the queen (Gary 1963; Koeniger, Koeniger, Gries, and Tingek 2005). Unlike most social insects which are monandrous (Hughes et al. 2008), a queen of *A. mellifera* mates with 12

Address correspondence to W.O.H. Hughes. E-mail: w.o.h.hughes@leeds.ac.uk.

Margaret J. Couvillon and William O.H. Hughes contributed equally to this work.

Received 11 June 2009; revised 11 January 2010; accepted 17 January 2010.

males on average over 1–3 mating flights (Tarpy et al. 2004), storing and using the sperm from these males randomly over the remainder of her life (Franck et al. 1999, 2002). Colonies produce thousands of males but only a few queens, so only very few males are successful in mating with a queen, and male–male competition for matings is thus extreme.

Honey bee males are also a rare (but certainly not unique; Andersson 1994) case in which selection has designed an animal exclusively for mating. Males live in their natal colony where they are protected and provided with food by their sister workers. Males do no work themselves, and their only role is to mate, having evolved large flight muscles and eyes to aid them in obtaining matings. The strength of selection on honey bee males, combined with their individual fitness being traded off by their natal colony against the numbers of males produced and other colony-level traits, makes differences in male competitiveness likely. Indeed, males show considerable variation in the number of spermatozoa they possess (Schluns et al. 2003; Koeniger, Koeniger, Tingek, and Phiancharoen 2005). Their fitness may also potentially differ between colonies (Kraus et al. 2003). A particular source of variation in male mating success is male size. Honey bee males are normally reared in special "drone" cells, which are larger (~6.2 mm diameter) than the cells used for rearing workers (5.2–5.8 mm diameter) (Winston 1995). However, males can also be reared in worker cells. These resulting males are smaller in body size than normal-sized males, with fewer spermatozoa (though proportionally more relative to body size; Schluns et al. 2003), but are otherwise identical and have the same access to nutrition as adults. These "small" males may make up as much as 9% of the males in a drone congregation area (Berg 1991) and are successful at mating and fathering offspring (Berg et al. 1997; Schluns et al. 2003). Precisely how successful they are though is unknown, and quantifying this will reveal the extent of sexual selection on male size.

In addition, the relative success of small males has important implications for understanding the reproductive behavior of honey bee workers. Most males are reared from unfertilized queen-laid eggs (Ratnieks and Keller 1998), but a small proportion of workers lay unfertilized (male) eggs, even in a colony with a queen (Page and Erickson 1988; Ratnieks 1993). Intriguingly, they do this preferentially in drone cells (Page and Erickson 1988; Ratnieks 1993), even though worker cells are far more numerous. We hypothesize that this preference may be due to kin selection. Individual workers have an incentive to lay eggs because a worker is more related to sons (0.5) than brothers (queen's sons, 0.25) (Ratnieks 1988). However, queenright honey bee colonies regulate the total number of males reared, so if workers' sons are reared, this will reduce the number of queen's sons. If the success of small males per unit investment is less than half that of normal-sized males, then a worker will achieve greater inclusive fitness by helping rear an additional normal-sized brother rather than a small male that is her own son. That is, egg-laying workers may only be able to enhance their inclusive fitness if they lay their eggs in drone cells. Thus, the effect of male size on mating success (sexual selection) may have an important effect on reducing intracolony conflict over male production (kin selection) (Boomsma 2007).

Here we examine the mating success of honey bee males using a semi-isolated mating area in which we directly manipulated the numbers and sizes of males present. We test 2 predictions. First, that the colonies differ significantly in the fitness of the males they produce. Second, that the mating success of small males is lower than normal-sized males and, in particular, is insufficient for it to be worthwhile for worker bees to lay eggs in worker cells.

## MATERIALS AND METHODS

### Experimental males

During spring 2005, we reared normal-sized and small males. Normal-sized males were sons of mated queens reared in drone cells ($n = 6$ colonies). Small males were the sons of virgin queens ($n = 6$ colonies) who had been prevented from mating by clipping their wings and had been induced to lay eggs by $CO_2$ treatment. These virgin queens were placed in colonies with 10 full frames of bees, brood, and food that were given frames of worker brood from other colonies to provide a source of workers and were fed syrup to encourage brood production. These colonies were only provided with worker comb to ensure that the queens only laid eggs in worker cells and thus produced only small males. As colonies headed by virgin queens do not reach sufficient size to produce males in drone cells, whereas colonies headed by mated queens will rarely produce significant numbers of males from worker cells, using queens of different type to produce the different sizes of males was unavoidable. However, all queens were in similar condition, and both mated and virgin queens produce healthy offspring.

Between 6th and 20th July, we collected adult males soon after eclosion from each male-producing colony and marked them with paint dots to indicate their size (small or normal sized), mother colony, and marking date. Small males were approximately 71% of the size of normal-sized males, weighing 174 ± 11.1 mg fresh and 55 ± 3.9 mg dry versus 240 ± 3 mg fresh and 80 ± 2.5 mg dry (*t*-tests: dry weight $t_{17} = 6.27$, $P < 0.001$ and fresh weight $t_{17} = 5.19$, $P < 0.001$). All experimental males (normal and small) were then fostered into a single queenless colony to standardize their adult environment and to facilitate observations of their flight activity. This colony was relocated to the Barber Booth mating apiary in Edale Valley, Derbyshire, United Kingdom, an area that is semi-isolated from other honey bee populations (Jensen et al. 2005). In total, we marked and introduced 4810 normal-sized males and 763 small males to ensure strong competition. We introduced fewer small males to mimic the natural situation in which small males are less abundant (Berg et al. 1997). Due to differences in the number of males reared, the numbers of introduced males from particular drone-rearing colonies varied. For small males, it was colony E: 41, F: 13, G: 62, H: 59, I: 477, J: 111; for normal males, colony K: 147, L: 1631, M: 637, N: 1412, O: 419, P: 564. For 2 days (2nd and 3rd August) during the mating period, we videoed the entrance of the drone foster colony and subsequently counted the numbers of males of each type (size: small or normal-sized, colony of origin, and cohort: day of introduction) leaving for mating flights. This allowed us to compare our estimates of mating success with the number of males actually flying, thereby controlling for any mortality of drones after marking and before mating flights.

### Experimental queens

We reared 40 virgin queens using standard beekeeping methods and introduced each queen into her own nucleus hive, comprised 5 frames of bees, brood, and honey. These hives were placed at 2 apiaries in the Edale Valley. One apiary (Barber Booth) was where the foster hive containing the experimental males was located. The other (Edale Mill) was 3 km away, well within the mating flight range (Jensen et al. 2005). All males were eliminated from these nucleus hives prior to the experiment. The queens (in nucleus hives) and the male-foster colony were left in place for 2 weeks during August 2005 to allow the queens and males to mate. Observations indicated that both experimental males and queens flew

freely during this period. Twenty-nine of the queens mated successfully and began laying eggs. Approximately 3 weeks later, we collected a sample of worker brood from each of these 29 queens and froze it at −20 °C for later DNA analysis.

### Genetic analysis

Tissue samples were removed from the brood either a leg from pupae or the anterior section from larvae. We detected no difference in successful polymerase chain reaction (PCR)/sequencing between pupae and larvae. DNA extraction was performed using 5% Chelex 100 solution (Bio-Rad, Hercules, CA). Samples were then amplified at microsatellite markers by PCR (Châline et al. 2004) and analyzed on an ABI 3730 capillary sequencer. Allele sizes were scored by comparison with internal size markers and the multilocus genotypes used to infer the genotypes of the mother queens and their multiple mates. The patrilines of the genotyped workers were then determined based on their paternal alleles. We initially screened 17 microsatellite loci and selected a combination of 8 markers (A88, A35, Ap37, A14, A76, A113, Ap14, and A29) that were most informative. First, we determined the genotypes of the mother queens of the male-producing colonies (colonies E–O), which allowed us to know all the available patrilines in the male population we produced, as males arise from unfertilized eggs and therefore only carry maternal genes. For the colonies that produced small males, we determined available patrilines directly by genotyping the mother queens. For the colonies that produced normal males, we genotyped 10 males from each colony and then deduced the genotypes of their mothers. By using the 8 polymorphic loci, we were then able to identify uniquely each male-producing colony and, therefore, the patriline of each offspring worker. We initially analyzed 46 worker offspring for each mated queen. We then analyzed an additional 46 (= 92 in total) offspring for each of the queens that had mated with a small male in order to improve resolution of their paternity shares.

## RESULTS

### Male flight dynamics

A total of 763 small males and 4810 normal-sized males were marked and placed in the foster hive. Small and normal-sized males did not differ in their estimated age at the time of matings (21 days; general linear model: $F_{1,5571} = 0.918$, $P = 0.338$), although this did differ significantly between colonies (varying from 15 to 23 days average age; small drones: $F_{5,757} = 12.8$, $P < 0.0001$; normal-sized drones: $F_{5,4804} = 455$, $P < 0.0001$; note though the extremely high sample sizes giving high power to the tests). A total of 908 flights by small males and 4864 flights by normal-sized males were observed during the 2 observation periods. This proportion, although significantly different due to the large sample sizes ($G_{adj} = 9.4$, $P = 0.002$), is very similar in proportion to the numbers fostered (86.3% and 84.3% large drones marked and flights, respectively). The numbers of flights for each male-producing colony corresponded well with the numbers marked (Pearson's correlation: $r = 0.823$, $N = 12$, $P = 0.001$), although the 2 normal male-producing colonies from which most males were introduced had substantially fewer males flying than would have been expected from the number marked (Figure 1). There was also a positive relationship across colonies between the numbers of males observed flying on the 2nd and 3rd of August (Pearson's correlation: $r = 0.969$, $N = 12$, $P < 0.001$). Normal-sized males showed a clear peak in flight activity around 13:40 to 14:00 (GMT), whereas small male flights were more temporally uniform (Figure 2). Accordingly, there were significantly more small males than expected relative to normal males at early (before 13:20) and late (after 14:20) times of day ($G$ test for heterogeneity: $G_{11} = 9.41$, $P = 0.002$; Figure 2). Within each size of male, the different male-producing colonies and male cohorts were similar in flight times (see Supplementary Figures S1 and S2).

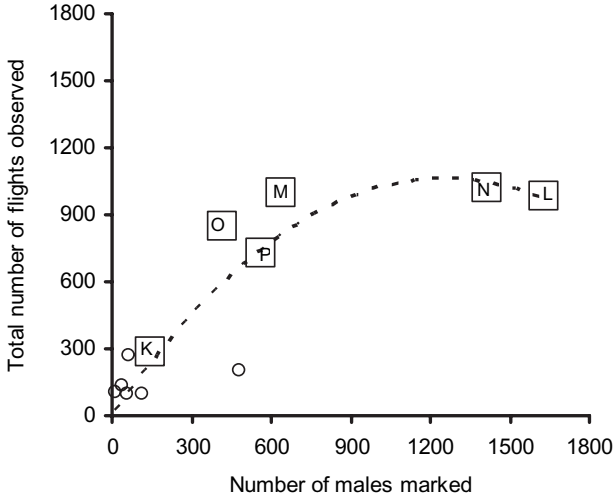


**Figure 1**
The relationship across colonies between the numbers of males marked and the total numbers observed flying during the 2 observation periods. Each of the 6 small male-producing colonies is represented by a circle, and each of the 6 normal-sized male-producing colonies is represented by a square, labeled with the colony identification letter within it because of the differences between colonies for this drone size. The equation of the best-fit curve is $y = -0.0007x^2 + 1.7x$; $r^2 = 0.79$.

### Matings

The 29 experimental queens mated with an average of 13.1 ± 0.59 males (minimum 7 and maximum 19). Of the total of 379

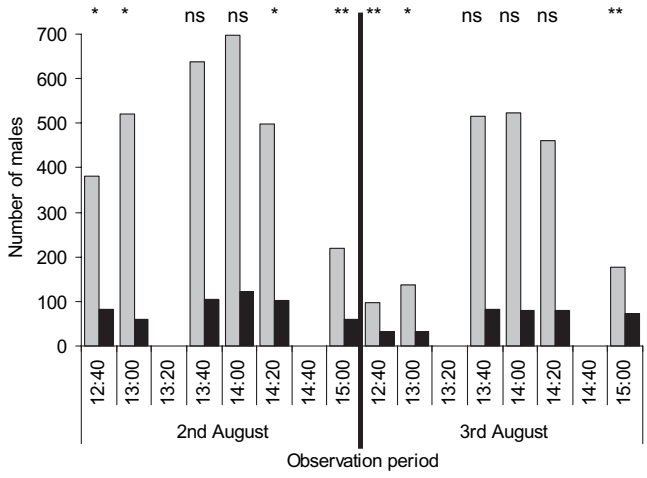


**Figure 2**
The activity patterns of small (black columns) and normal-sized (gray columns) males based on the numbers observed flying during 20-min periods on 2nd and 3rd August. Symbols above columns indicate whether the ratio of small:normal males during a particular 20-min period differed significantly from the ratio recorded overall. This was tested using multiple $G$ tests, with the false discovery rate being controlled using $q$ values (Storey and Tibshirani 2003): ns = $P$ or $q > 0.05$, *$P$ and $q < 0.05$, and **$P$ and $q < 0.01$.

fathers detected, 303 were non–experimental males, 70 were normal-sized experimental males, and 6 were small experimental males (Figure 3). Given the frequency of alleles in the population of non–experimental drones, the probability of a non–experimental drone being misidentified as a normal-sized or small experimental drone was extremely low (0.007 and 0.019, respectively). Four of the queens mated only with non–experimental males (Figure 3) but had a similar mating frequency to the other queens ($t = -0.36$, $P = 0.722$). Three of the 29 queens mated with small males, each to 2 small males (in addition to normal-sized males; Figure 3). The mating frequency of these 3 queens (12.3 ± 2.9) did not differ from that of those who mated with experimental males but only those that were normal-sized males (13.3 ± 0.7; $t_{21} = 0.459$, $P = 0.651$).

Of the males marked, 86.3% were normal sized and 13.7% small, with the representation of small males being very slightly greater than that among those males recorded flying on 2nd (15.2%) and 3rd August (16.5%). However, small males obtained only 7.9% of matings. This was significantly fewer than expected given the number of flights observed ($G$ test: $G_{adj} = 4.07$, $P = 0.044$), although it did not differ significantly from the number expected based on the number of males marked ($G$ test: $G_{adj} = 2.42$, $P = 0.12$). It should be noted though that the relative rarity of small males meant that these analyses involved a statistical effect size of only 0.17 (and thus had low power), even though small males had only half the mating success of normal males per flight.

The colonies producing small males did not differ significantly in the number of matings obtained (analysis of variance [ANOVA]: $F_{5,168} = 0.974$, $P = 0.435$; Figure 4a), although the rarity of matings by small males again means that this analysis has little power. However, the mating success of normal males did vary significantly between mother colonies (ANOVA: $F_{5,174} = 8.06$, $P < 0.0001$). Males from colony M obtained at least one mating with almost all the experimental queens, whereas males from the other colonies mated with very few of the queens (Figure 4a). The differences between colonies in mating success were not due to differences in the age of drones because there was no relationship across colonies between the number of matings obtained and the mean estimated age of drones (Pearson's correlation coefficient, $r = -0.074$, $N = 12$, $P = 0.819$). For most colonies, more male flights were associated with only a small increase in the number of matings obtained (Figure 4b). Two of the colonies (L and N) with the most male flights had substantially more matings relative to their number of flights, whereas the most successful colony (M) obtained more than double the number of matings of even the next most successful colony, in spite of producing approximately the same number of male flights (Figure 4b).

### Paternity share

Small males obtained significantly less paternity share than did normal males ($t$-test: $t_8 = 2.37$, $P = 0.046$; Figure 5). On average, they obtained only 61 ± 15% of the expected paternity share if paternity was equally shared amongst their queen's male partners, with 5 of the 6 small males obtaining a lower share (28–76%) than expected. Overall, the colony of origin did not significantly affect the paternity share of males (ANOVA: $F_{8,67} = 0.922$, $P = 0.504$).

## DISCUSSION

An average of 13.1 matings were detected per queen. This is very close to the overall average observed mating frequency for *A. mellifera* of 12 (Tarpy et al. 2004) and to the estimate of 10.2 obtained previously, using smaller sample sizes, in the same semi-isolated mating area as used in the current study (Jensen et al. 2005). The mating system we were studying, therefore, appears to have been typical of both species and site.

### Colony variation

We found considerable variation in the relative mating success of males from different colonies. This effect was not due to differences between colonies in the age of the males at the time of mating. Importantly, our experiment not only controlled the number of males from different colonies introduced to the mating area but also recorded the numbers flying on 2 days during the mating period. Approximately twice as many males from colonies L and N were introduced into the foster hive as from the other colonies that produced normal males, yet there

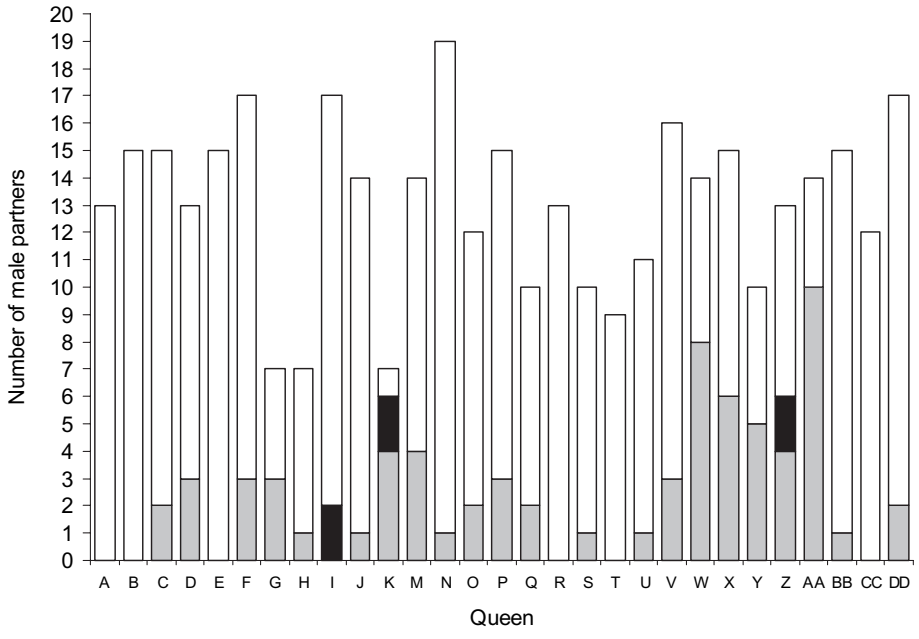


**Figure 3**
The mating success of normal-sized and small males. The number of experimental small males (black), normal-sized males (gray), and non–experimental males (white) with which each queen mated.

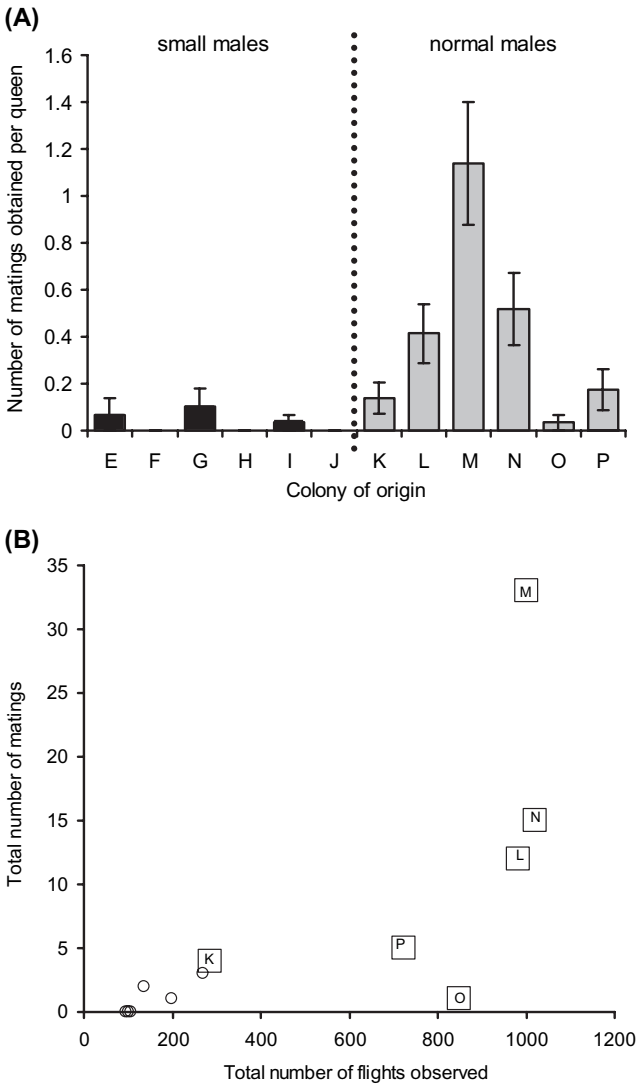


**Figure 4**
The colony variation in mating success. (a) The mean ± standard error number of matings obtained per queen by each of the experimental male-producing colonies, 6 of which produced small males (E–J) and 6 of which produced normal-sized males (K–P). (b) The relationship across colonies between the total number of matings obtained with the total number of male flights observed on 2nd and 3rd August. Each of the 6 small male-producing colonies is represented by a circle, and each of the 6 normal-sized male-producing colonies by a square, labeled with the colony identification letter within it because of the differences between colonies for this drone size.

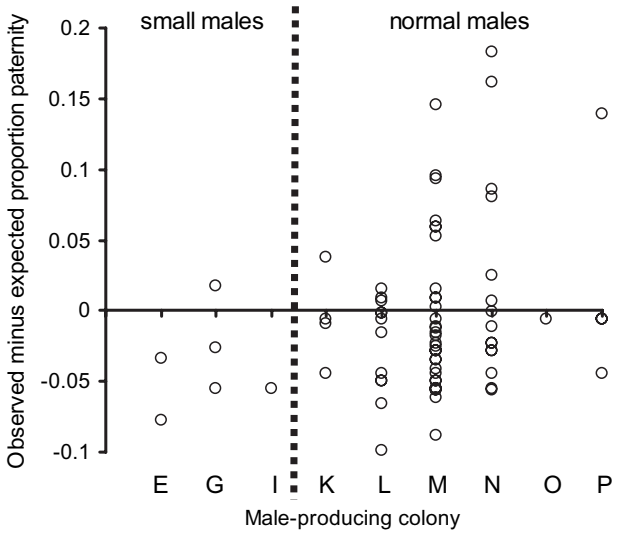


**Figure 5**
The paternity share of males from the 3 small male-producing colonies (E, G, and I) and 6 normal male-producing colonies (K–P), which obtained matings with the experimental queens (no matings were obtained by males from the other 3 small male-producing colonies). Each circle represents a single male and shows its observed proportional paternity share of its mate's offspring minus its expected share based on the mating frequency of the queen and assuming that paternity was shared equally between males.

was very little difference between the colonies in the number of male flights observed. This demonstrates the importance of quantifying the numbers of drones actually flying when assessing colony fitness rather than simply basing relative success on the number believed to be present in the colony.

The across-colony relationship between number of flights observed and number of matings obtained is particularly intriguing. The expected relationship would be linear, with more male flights resulting in proportionally more matings. However, for most colonies, more male flights resulted in only slightly more matings. The vast majority of matings were instead obtained by the 3 colonies with the greatest number of flights, L, N, and M. The number of matings obtained relative to male flights was particularly disproportionate for the last of these, which obtained more than double the matings of the next most successful colonies. The differences in mating success were not due to the numbers of males flying (which did not differ between the 3 most successful colonies), the colony of residence (all the males having been fostered in the same colony), the age of the males, or the times of flight. It therefore appears that the males produced by some colonies are better able to obtain matings than those from other colonies and that this intercolony variation is quite large. This is the most conclusive evidence to date of colony variation in the fitness of honey bee males under natural conditions and has important implications for honey bee breeding programs.

### Does size matter?

We found clear evidence that small honey bee males were less successful at mating than normal-sized males. Small males, introduced at a frequency similar to that under natural conditions (Berg 1991), obtained approximately half the number of matings expected, given both the numbers of males marked and the numbers observed flying. In addition, small males that did mate obtained only 61% of the paternity share obtained by normal-sized males, very close to what is expected given that they have only 63% of the spermatozoa of a normal-sized male (Schluns et al. 2003). In some other insects, small males can be more successful at mating in swarms due to their greater agility (Neems et al. 1992), but this therefore does not appear to be the case in honey bees. Small males were also observed to fly at a more constant rate in the afternoon observation period than normal males, who had a distinct peak in flight activity. The lower mating success of small males could therefore be because they flew at times of day when fewer queens were available or because they are less successful in male–male competition. The latter seems more likely. The large size of normal males is presumably under strong selective pressure because it affords greater mating success, and it seems probable that small males may be weaker fliers or less

able to detect queens (Thornhill and Alcock 1983). Perhaps, therefore, the observed tendency of small males to fly earlier and later in the day may be an adaptation to exploit times when normal-sized male activity is lower and competition less. Intriguingly, all 3 of the queens that mated with small males did so twice. Possibly, these queens selected their mates differently to other queens, but more probably they flew earlier or later in the day, when relatively more small males were in the mating area. This conforms to our anecdotal observations: Although the majority of queens confined their mating flights to a narrow time window, a few queens flew earlier and later in the afternoon. Such a change in small male behavior to exploit times of day with lower competition would mirror the pattern previously noted across the mating season (Berg et al. 1997), and seen in other animals (Alcock 2005).

In terms of inclusive fitness, workers could benefit from laying eggs in worker cells if the fitness of their resulting male offspring per unit of investment is at least half (a proportion determined by the relative amount of shared genes between sons vs. brothers) that of their brothers reared in drone cells. Our results, however, suggest that the fitness of small males is less than this. Small males obtained approximately half the number of matings of normal-sized males, and those small males that did mate also obtained only 61% of the paternity of normal-sized males, which matches well with data showing that they have only 63% as many spermatozoa (Schluns et al. 2003). Small males were 70% as big as normal-sized males, so their relative fitness per unit of investment was 43% of normal-sized males, below the 50% threshold at which a worker benefits more from the colony investing in a normal-sized brother than a small son. The relatively low fitness of small males helps explain why workers, when they lay eggs, do so specifically in drone cells rather than in the more numerous workers cells (Ratnieks 1993). By reducing the opportunities for personal reproduction to enhance inclusive fitness, sexual selection on male size therefore further biases the inclusive fitness dynamics within honey bee colonies toward worker cooperation.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/.

## FUNDING

Natural Environment Research Council (NE/C514115/1); National Science Foundation (PhD scholarship to M.J.C.); and Sixth European Community Framework Programme (Marie Curie Outgoing International Fellowship to W.O.H.H.).

We are grateful to John Neild and Richard Grimes for providing the Barber Booth and Edale Mill apiaries, respectively, to Prof. Terry Burke for providing facilities for the genetic analysis in the Sheffield Molecular Genetics Facility, and to 2 anonymous referees for their constructive comments.

## REFERENCES

Alcock J. 2005. Animal behaviour: an evolutionary approach. Sunderland (MA): Sinauer.

Andersson M. 1994. Sexual selection. Princeton (NJ): Princeton University Press.

Baudry E, Solignac M, Garnery L, Gries M, Cornuet JM, Koeniger N. 1998. Relatedness among honeybees (*Apis mellifera*) of a drone congregation. Proc R Soc Lond B Biol Sci. 265:2009–2014.

Berg S. 1991. Investigation of the rates of large and small drones at a drone congregation area. Apidologie. 22:437–438.

Berg S, Koeniger N, Koeniger G, Fuchs S. 1997. Body size and reproductive success of drones (*Apis mellifera* L). Apidologie. 28:449–460.

Boomsma JJ. 2007. Kin selection versus sexual selection: why the ends do not meet. Curr Biol. 17:R673–R683.

Boomsma JJ, Baer B, Heinze J. 2005. The evolution of male traits in social insects. Annu Rev Entomol. 50:395–420.

Châline N, Ratnieks FLW, Raine NE, Badcock NS, Burke T. 2004. Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips. Apidologie. 35:311–318.

Clutton-Brock TH, Parker GA. 1992. Potential reproductive rates and the operation of sexual selection. Q Rev Biol. 67:437–456.

Eberhard WG. 1996. Female control: sexual selection by cryptic female choice. Princeton (NJ): Princeton University Press.

Franck P, Coussy H, Le Conte Y, Solignac R, Garnery L, Cornuet JM. 1999. Microsatellite analysis of sperm admixture in honeybee. Insect Mol Biol. 8:419–421.

Franck P, Solignac M, Vautrin D, Cornuet JM, Koeniger G, Koeniger N. 2002. Sperm competition and last-male precedence in the honeybee. Anim Behav. 64:503–509.

Gary NE. 1963. Observations of mating behaviour in the honeybee. J Apic Res. 2:3–13.

Heinze J, Hölldobler B. 1993. Fighting for a harem of queens—physiology of reproduction in *Cardiocondyla* male ants. Proc Natl Acad Sci U S A. 90:8412–8414.

Heinze J, Tsuji K. 1995. Ant reproductive strategies. Res Popul Ecol. 37:135–149.

Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW. 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. Science. 320:1213–1216.

Jensen AB, Palmer KA, Châline N, Raine NE, Tofilski A, Martin SJ, Pedersen BV, Boomsma JJ, Ratnieks FLW. 2005. Quantifying honey bee mating range and isolation in semi-isolated valleys by DNA microsatellite paternity analysis. Conserv Genet. 6:527–537.

Koeniger G, Koeniger N, Tingek S, Phiancharoen M. 2005. Variance in spermatozoa number among *Apis dorsata* drones and among *Apis mellifera* drones. Apidologie. 36:279–284.

Koeniger N, Koeniger G, Gries M, Tingek S. 2005. Drone competition at drone congregation areas in four *Apis* species. Apidologie. 36: 211–221.

Kraus FB, Neumann P, Scharpenberg H, Van Praagh J, Moritz RFA. 2003. Male fitness of honeybee colonies (*Apis mellifera* L.). J Evol Biol. 16:914–920.

Neems RM, Lazarus J, McLachlan AJ. 1992. Swarming behavior in male chironomid midges: a cost-benefit analysis. Behav Ecol. 3: 285–290.

Page RE, Erickson EH. 1988. Reproduction by worker honey bees (*Apis mellifera* L.). Behav Ecol Sociobiol. 23:117–126.

Pitnick S, Henn KRH, Maheux SD, Higginson DM, Hurtado-Gonzales JL, Manier MK, Berben KS, Guptill C, Uy JAC. 2009. Size-dependent alternative male mating tactics in the yellow dung fly, *Scathophaga stercoraria*. Proc R Soc Lond B Biol Sci. 276:3229–3237.

Ratnieks FLW. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. Am Nat. 132:217–236.

Ratnieks FLW. 1993. Egg-laying, egg-removal, and ovary development by workers in queenright honey-bee colonies. Behav Ecol Sociobiol. 32:191–198.

Ratnieks FLW, Keller L. 1998. Queen control of egg fertilization in the honey bee. Behav Ecol Sociobiol. 44:57–61.

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

Storey JD, Tibshirani R. 2003. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 100:9440–9445.

Tarpy DR, Nielsen R, Nielsen DI. 2004. A scientific note on the revised estimates of effective paternity frequency in *Apis*. Insectes Soc. 51: 203–204.

Thornhill R, Alcock J. 1983. The evolution of insect mating systems. Cambridge (MA): Harvard University Press.

Winston ML. 1995. The biology of the honey bee. London: Harvard University Press.