



Bumblebee sex ratios: why do bumblebees produce so many males?

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Sex investment ratios in populations of bumblebees are male biased, which contradicts theoretical predictions. Male-biased investment ratios in eusocial Hymenoptera are assumed to be non-stable for both the queen and her workers. In this paper, we show that male-biased sex allocation does not necessarily decrease fitness in the bumblebee *Bombus terrestris*.

A male-biased investment ratio can be the result of an optimal allocation of resources when resources are scarce if (i) there is a large cost difference between male and female production, (ii) there is uncertainty about the amount of resources a colony can invest, and (iii) only a proportion of the investment made in an individual can be reused. This resource allocation then leads to split sex ratios depending on the amount of resources available to a bumblebee colony: colonies under low resource conditions will show a male-biased investment ratio, whereas colonies under high resource conditions allocate more resources towards females. However, the extent to which bumblebee populations show a male-biased sex allocation cannot be explained by cost differences between male and female production alone.

In a recent paper, A. F. G. Bourke argued that male-biased investment ratios in bumblebee populations are a by-product of the occurrence of protandry (males emerge before females). Here we will extend Bourke's argument and show that within a protandrous population, both protandrous and protogynous (females emerge before males) colonies exist. The existence of protandrous and protogynous colonies results in split sex ratios in time, because protogynous colonies rely on males produced by protandrous colonies (partial protandry).

Keywords: investment ratios; split sex ratios; reproductive strategies

1. INTRODUCTION

Bumblebees seem to be an exception to theoretical predictions on stable population sex investment ratios, as these insects show a male-biased instead of female-biased population sex allocation (Owen *et al.* 1980; Owen & Plowright 1982; Fisher 1987, 1992; Duchateau & Velthuis 1988; Röseler & van Honk 1990; Müller & Schmid-Hempel 1992). A female-biased sex investment ratio in social Hymenoptera was predicted by Trivers & Hare in 1976. By combining Hamilton's (1964*a,b*) kin-selection theory with Fisher's (1930) sex-ratio theory, they explained the evolution and maintenance of sociality in haplodiploid Hymenoptera. Because of haplodiploidy (the production of males from unfertilized, haploid eggs), a female shares, on average, 75% of her genes with her sister but only 25% with her brother. This relatedness asymmetry has far reaching evolutionary consequences in that a female may be more likely to help her mother raising sisters and brothers than producing offspring herself provided she can bias sex allocation towards females. In the most simple case, in which a colony has one single-mated queen and workers are sterile, the workers will try to bias the investment ratio towards a 3:1 investment (female:male). Because the mother-queen is equally related to her sons

and daughters, she prefers equal investment in both sexes. Thus, the queen and her workers are in conflict.

Male-biased sex allocation does not only seem to lower the fitness of the workers (who prefer a female-biased allocation ratio) but also the queen's fitness (who prefers equal investment), and therefore it begs for an explanation. In bumblebees, we do not only find male-biased investment at the population level (see Bourke (1997) for an extensive review), but we also find variation in sex allocation between colonies, with some colonies specializing on female production and some specializing on male production, as will be shown for *Bombus terrestris* in this paper. Specialization on the production of one sex leads to a so-called split sex ratio (Grafen 1986). Boomsma & Grafen (1990, 1991) predicted split sex ratios between colonies of eusocial insects when the relatedness asymmetry between the workers and sexual offspring differs: workers within a colony will specialize on producing the sex to which they are most related. Differences in relatedness asymmetry can be caused by different mating frequencies of the queens or differences in queen number. However, differences in relatedness asymmetry cannot account for split sex ratios in *B. terrestris*, as queens of this species are singly mated (Estoup *et al.* 1995) and colonies are monogyne. Therefore, there must be an alternative explanation for the occurrence of sex-ratio specialization in this bumblebee.

According to Grafen (1986) and Boomsma (1993), split sex ratios are expected between colonies if the production

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of males and females require different resources and if colonies differ in resource availability. Therefore, colonies with low resources will bias the sex ratio towards the cheaper sex. In this paper, we will determine the exact male-to-female cost ratio and show that males are the cheaper sex. We therefore expect bumblebee colonies to produce more males under resource scarcity. However, the prediction that bumblebee colonies will produce a male-biased investment ratio when resources are scarce cannot explain the extent to which sex allocation is male biased at the population level in bumblebees. In a recent paper, Bourke (1997) showed that the occurrence of protandry (the emergence of males before females) results in male-biased sex investment ratios at the population level. Here, we will extend Bourke's protandry explanation of male-biased population investment ratios in bumblebees, and show that within a protandrous population, both protandrous and protogynous (females emerge before males) colonies exist. The existence of protandrous and protogynous colonies results in split sex ratios in time, because protogynous colonies rely on males produced by protandrous colonies (partial protandry).

In the following, we will elaborate on both arguments that predict split sex ratios in bumblebees in an attempt to explain male-biased investment ratios.

(a) Costs of female and male production and resource-dependent split sex ratios

Male-biased numerical sex ratios are expected when males are the cheaper sex. As bumblebee queens are much larger than males, overproduction of males is expected according to simple Fisherian principles. If queen production requires relatively more resources, one would expect split sex ratios depending on resource availability: male-biased sex ratios when resources are scarce and female-biased sex ratios when resources are abundant (Grafen 1986; Boomsma 1993). This assumes that queens benefit more from increased investment than males. This is valid for bumblebees as queens must be able to hibernate for which they need large fat reserves (Beekman *et al.* 1998a). The reproductive success of a bumblebee queen will therefore increase with the amount of fat reserves, whereas increased reserves will not change the reproductive success of a male.

Crozier & Pamilo (1993, 1996) extended Grafen's (1986) and Boomsma's (1993) argument, and argued that there are different pay-offs for male and female production in colonies of different sizes. This leads to different sex allocations depending on the size (number of workers) of the colony. Crozier & Pamilo (1993, 1996) assume that (i) there is a cost difference between male and queen production, (ii) colonies cannot accurately assess the amount of resources they can invest in sexual production, and (iii) only a proportion of investment made in an individual can be reused. Crozier & Pamilo (1996, from p. 232) calculated the effective ratio of costs for queen to male production. The effective ratio of costs depends on the total amount of resources: for low resource levels, queen production becomes more expensive, whereas at high resource levels, queen production becomes less expensive. Thus, for low resource levels, colonies are forced to specialize on male production as the costs of producing queens is too high, whereas males can be

produced. Moreover, males can be abandoned without losing too much invested capital. This results in small colonies producing mainly males, as a smaller number of workers is available to collect sufficient resources, which has also been observed in bumblebees (Duchateau & Velthuis 1988; Bourke 1997).

If the three assumptions made by Crozier & Pamilo are valid for bumblebees, we would expect differences in sex ratios between field and laboratory populations (assuming that resources are abundant when colonies are reared in the laboratory), as the former will have more difficulties collecting sufficient resources for queen production and will thus produce a more male-biased sex ratio. Thus, the Crozier & Pamilo model predicts male-biased sex ratio as an optimal allocation strategy depending on resource availability.

To test the prediction that colonies produce a more male-biased investment ratio when resources are scarce, we have to calculate investment ratios of bumblebee colonies. Therefore, we need to know the male-to-female cost ratio. Although it is known that queens need more resources, the precise costs for male and queen production have not been determined. Up to now, dry or wet weight of queens and males were used to calculate investment ratios (wet weight ratio for *Bombus melanopygus*, 2.58:1 (Owen & Plowright 1982); wet weight ratio for *B. terrecola*, 2.13:1 (Owen *et al.* 1980); dry weight ratio for *B. terrestris*, 2:1 (Duchateau & Velthuis 1988)). Of course the result depends on the age at which the dry and/or wet weight is determined. Unfortunately, this is not stated in most studies. To overcome this pitfall we have determined the caloric content of both callow and mature queens and males of *B. terrestris*. This will enable a more accurate prediction of investment ratios in bumblebees. The next step will be to determine whether cost differences between queen and male production are reflected in split sex ratios depending on resource abundance, by comparing the sex ratios of field- and laboratory-reared colonies (assuming that resources are not limited in colonies reared in the laboratory). If we do find a difference in sex ratio depending on resource availability, this would indicate that cost differences between male and female production, at least to some extent, determine bumblebee sex ratios at the colony level.

(b) Protandry and split sex ratios in time

Recently, Bourke (1997) explained population male-biased sex investment ratios in bumblebees using models of Bulmer (1981, 1983) on sexual selection for protandry. In his paper, Bourke (1997) emphasized the importance of the relative timing of sexual production in determining sex ratios in social Hymenoptera. As early males have higher mating opportunities than late-emerging males, because they are around for longer, colonies that produce their males early will gain more fitness. As a consequence, sex investment ratios become male biased if early males have a high relative survivorship and early females have a relatively low quality (Bourke 1997). Although Bourke (1997) has shown that protandry at the population level leads to male-biased population investment ratios in bumblebees, we will extend Bourke's argument and show that, within a protandrous population, both protandrous and protogynous (females

emerge before males) colonies exist. This results in split sex ratios between colonies depending on the timing of sexual production: colonies that produce their sexuals relatively early in the season should produce mainly males, whereas late-reproducing colonies should focus on female production.

We will calculate fitness for protandrous and protogynous colonies of a laboratory population of *B. terrestris* using a model in which protandrous colonies gain fitness through higher mating chances of early males, and protogynous colonies gain fitness through the production of female-biased sex investment ratios in a population in which the overall numerical sex ratio is male biased. Using this model, we show that male-biased sex investment ratios do not necessarily lead to lower fitness compared to a female-biased investment ratio. Fitness of both protandrous and protogynous colonies depends on the frequency of the two colony types and we will make predictions on equilibrium frequencies (where both protandrous and protogynous colonies have equal fitness). Predicted and observed equilibrium frequencies can then be compared.

2. MATERIAL AND METHODS

(a) *Fitness of protandrous and protogynous colonies*

A total of 41 colonies of *B. terrestris* were reared in a climate room (29 °C; 62% relative humidity (RH); continuous darkness). Of these colonies the following parameters were assessed: rate of worker production; timing of first male and young queen emergence; and total number of workers, males and young queens. Rate of worker production was measured after emergence of the second brood (first brood is raised solely by the queen, second brood by the queen and the first-brood workers), when the queen lays eggs at a constant rate (Duchateau & Velthuis 1988). The rate of worker production was determined until no new workers emerged. This most often coincided with the emergence of the first sexuals. During the total period in which sexuals emerge, queens and males frequently (but not necessarily) emerge simultaneously.

Hereafter, we will refer to protandrous colonies as 'MF' (males first: first adult sexual was a male) and protogynous as 'QF' (queens first: first adult sexual was a female) colonies. If queens and males emerged at the same time, or if males emerged within 4 d after the emergence of the first queen, the colony was classified as QF. This was done because the developmental time (egg to adult) of a queen is 4 d longer than the developmental time of a male (30 versus 26 d (Duchateau & Velthuis 1988)) and thus queen-destined eggs were laid before haploid eggs. In our laboratory population, 23 colonies were MF colonies and 18 were QF colonies (fraction of QF colonies = 0.44).

To determine the dynamics of the emergence of sexuals, time was divided into 12 classes and the number of sexuals appearing in each class assessed. Time classes ranged from 0 d (emergence second brood) until 120 d, with intervals of 10 d. All queens emerging during one time class are assumed to mate and enter diapause within the same time class. So queens are only available for mating in the time class in which they emerged. Males are able to obtain multiple matings and will remain present in subsequent time classes. Thus, no mortality is included.

For the two colony types (QF and MF colonies) fitness (W) was calculated for both queen and workers using:

$$W = r_q(1 + p) \sum_{t=1}^{t=12} q_t + r_m \sum_{t=1}^{t=12} \frac{m_t}{M_t} Q_t, \quad (1)$$

in which r_q and r_m are regression relatedness (see Grafen 1986) to young queens and males, respectively, for the individual of which fitness is determined; p is population-wide fraction of queen-produced males (hence $(1-p)$ is the population-wide fraction of worker-produced males); $q_{QF,t}$ and $q_{MF,t}$ are average number of queens produced in time class t by a QF and an MF colony, respectively; $m_{QF,t}$ and $m_{MF,t}$ are average number of males produced in time class t by a QF and an MF colony, respectively; and Q_t and M_t are total number of queens and males present in time class t .

We incorporate $(1-p)$ into equation (1) because the sex-specific reproductive values of males and females change if workers produce (part of the) males (Crozier & Pamilo 1996). If the queen is the sole producer of haploid eggs ($p=1$), the reproductive value of a female is twice the reproductive value of a male, as males can only pass on their genes by producing daughters whereas females pass on their genes through both daughters and sons. However, as soon as workers produce all the males ($p=0$), the reproductive values of males and females are equal.

Because we assume that the amount of energy that a bumblebee colony can spend on the production of sexuals is limited (Beekman *et al.* 1998b), we have to incorporate the following energy constraint into equation (1):

$$E = c_m \sum_{t=1}^{t=12} m_t + c_q \sum_{t=1}^{t=12} q_t, \quad (2)$$

in which c_m and c_q are the cost of producing a male and a queen, respectively. This yields:

$$W = r_q(1 + p) \left\{ \frac{E - c_m \sum_{t=1}^{t=12} m_t}{c_q} \right\} + r_m \sum_{t=1}^{t=12} \frac{m_t}{M_t} Q_t, \quad (3)$$

which is again calculated for both QF and MF colonies.

(b) *Caloric content of males and females*

Male and queen pupae of *B. terrestris* were collected from colonies and transferred to an incubator (32 °C). 'Callow' individuals were collected immediately after emergence, anaesthetized with CO₂ and killed by freezing. To obtain 'mature' individuals, emerged queens and males were returned to the climate room in which the colonies were reared (29 °C; 62% RH; continuous darkness) and provided with ample pollen and sugar water (Bee-Fit[®]) for 7 d, after which they were also anaesthetized and killed by freezing.

Caloric content was determined by drying the individuals at a temperature of 90 °C for 1 week after which dry weight was assessed. Fat was extracted by placing the individuals in a 1:1 mixture of methanol and chloroform for 6 h, after which they were dried again (1 week) and the lean weight could be determined. Caloric content in kJ was calculated using the method described by Peakin (1972).

3. RESULTS

(a) *Caloric content of males and females*

Measurement of caloric content of callow and mature queens and males (table 1) show that the male-to-queen

Table 1. *Caloric content (kJ) of callow and mature queens and males*

(Callows did not feed after emergence, whereas mature individuals were fed ample pollen and sugarwater for one week.)

	queens		males	
	callow	mature	callow	mature
average (kJ)	3.01	7.83	1.44	2.35
<i>n</i>	46	222	101	201
s.d.	0.56	0.61	0.39	0.65
ratio callow:mature	1:2.60		1:1.63	
ratio callow female:male			2.09:1	
ratio mature female:male			3.33:1	

cost ratio depends on when the caloric content is determined: 1:2.09 (0.48) for callow and 1:3.33 (0.30) for mature individuals. This means that when numerical sex ratios are translated into investment ratios, the result depends on the moment at which the cost ratio was assessed.

It seems more accurate to use the cost ratio of mature individuals, as queens remain inside the colony until they are 5–7 days old (e.g. Bourke 1997). However, even if we use the 1:3.33 cost ratio (measured 7 days after emergence; table 1) to calculate investment ratios, we still find a male-biased investment ratio. Müller & Schmid-Hempel (1999) found a 1:13 (female:male; fraction of males=0.93) numerical sex ratio in a field population of *B. terrestris*, and we found a 1:5 (fraction of males=0.83) numerical sex ratio in the laboratory (table 2: 41 colonies produced a total number of 12588 males and 2488 queens). Using the 1:3.33 cost ratio, this yields a 1:3.9 (field) and 1:1.5 (lab) investment ratio, which is still male biased. One explanation may be that males require even less resources because they stay for a shorter time in the nest than assumed: Bertsch (1984) showed that males leave the nest immediately after emergence. Others mention a period of 2–4 days (e.g. Bourke 1997). If males leave the colony much sooner than assumed, investment in males becomes less, which results in a less male-biased investment ratio. It must be noted however, that Boomsma (1989) and Boomsma *et al.* (1995) showed that, in ants, male-production costs are underestimated because males consume more energy per unit weight than females. However, this was not the case in bumblebees (Helms 1994; Bourke 1997).

Our data do show that a bumblebee colony has to invest more energy into queens after emergence than in males: table 1 shows that queens gain more in caloric content within one week than males (ratio callow:mature queen, 1:2.60; ratio callow:mature male, 1:1.63). The colony has to collect enough resources to make this increase possible in a relatively short period. This could make the production of (a large number of) queens even more difficult.

If we assume that the male-to-female mature cost ratio of 0.30 is a reasonable estimate, the observed numerical sex ratio of 0.83 found in our laboratory population is

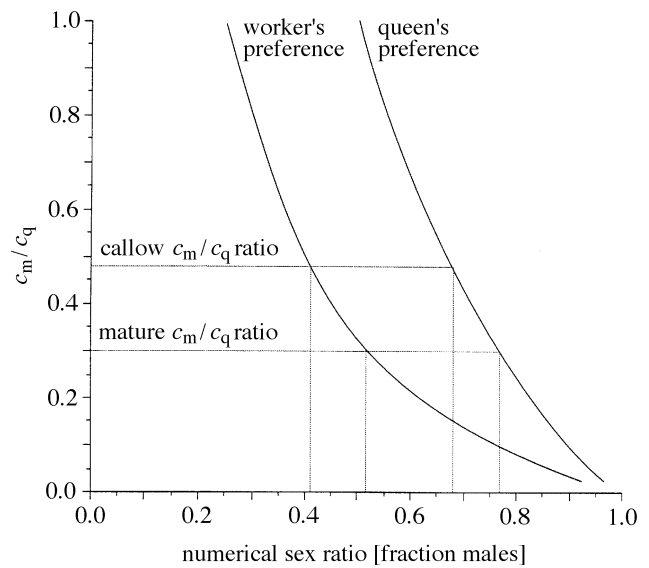


Figure 1. Numerical sex ratio (fraction of males) under queen control (equal investment in both sexes) and worker control (3:1 female-biased investment ratio) for different male-to-female cost ratios. Expected numerical sex ratios using callow and mature cost ratios are shown by the vertical lines.

close to the predicted numerical sex ratio (0.78) under queen control (figure 1). The male-to-female cost ratio that corresponds with the observed numerical sex ratio of the field population (0.93) is 0.03 and 0.05 under worker and queen control, respectively.

(b) *Fitness of protandrous and protogynous colonies*

In figure 2, the timing of the emergence of the first male and first queen is plotted for protogynous (QF) and protandrous (MF) colonies, and table 2 shows the characteristics of both colony types. Both colony types do not differ in growth rate (rate of worker production) and colony size (total number of workers produced). They do differ in sex ratio, mainly owing to the high number of queens produced in the QF colonies (on average 104 versus 27). The relative number of males produced by both colony types differs less (on average 238 versus 361). However, the total amount of energy spent on sexuals (in kJ) is not significantly different for QF and MF colonies (*t*-test for independent samples: *t*=1.3179, *p*=0.1952).

Besides differences in sex ratio, there is a large difference in timing of male and queen production (after emergence of the second brood), as expected. The MF colonies produce males early, whereas QF colonies produce their males much later (table 2). This results in an early peak of MF males, followed by a smaller peak of QF males (figure 3). The reverse is true for queen production (figure 3). Because mortality is not included, early males will have more mating opportunities than males that emerge later. Although zero mortality is unrealistic, it is generally assumed that male bumblebees can live up to several weeks in the field (see references in Bourke (1997)). Moreover, males have been shown to attain multiple matings (see references in Bourke (1997)). However, at present it is unknown if subsequent matings are fecund. In both Bourke's and this paper it is therefore assumed that males do transfer sperm during later matings.

Table 2. Characteristics of queens-first (QF) and males-first (MF) colonies

(The 41 colonies used were reared in the laboratory under standardized conditions (29 °C; 62% RH; continuous darkness). Average values and standard deviation (in parentheses) are given. Time starts at emergence of the second brood. Significance values are based on Mann–Whitney *U*-tests.)

	no. of workers	no. of queens	no. of males	numerical sex ratio ($M/(M+Q)$)	rate of worker production (workers d ⁻¹)	emergence first queen (d)	emergence first male (d)
queens first ($n=18$)	120 (26.5)	104 (64.2)	238 (213)	0.61 (0.184)	5.26 (1.67)	18.9 (4.48)	27.2 (7.28)
males first ($n=23$)	141 (56.1)	27 (34.8)	361 (203)	0.94 (0.075)	5.62 (2.16)	31.1 (15.2)	12.7 (5.77)
	n.s.	$p < 0.01$	$p < 0.05$	not tested	n.s.	$p < 0.01$	$p < 0.01$

Figure 4 shows the fitness of QF and MF colonies for the queen and workers, respectively. Fitness is calculated using equation (3) for $p=1$ (all males in the population are produced by the queen) and $p=0$ (all males in the population are produced by one worker) for different fractions of QF colonies. Varying the fraction of QF colonies results in different values for $q_{QF,t}$, $q_{MF,t}$, $m_{QF,t}$, $m_{MF,t}$, Q_t and M_t . Using these different values, fitness for MF and QF colonies is calculated for each fraction of QF colonies. A mature male-to-female cost ratio (0.30) was used. Because the amount of energy spent on the production of sexuals is the same for QF and MF colonies, E enters equation (3) as a constant. As p is the population-wide fraction of worker-produced males, one can only compare fitness curves of QF and MF colonies for the same value of p .

If $p=1$, the fitness pay-off through queens is larger because of the sex-specific reproductive values of females and males (see equation (3)). Because QF colonies produce a female-biased investment ratio, workers in QF colonies gain much fitness through queen production because the sex-specific value for queens is twice the sex-specific value for males if $p=1$. Thus, theoretically, workers of QF colonies should refrain from producing haploid eggs, as lowering p will devalue queens. This only holds if workers of all QF colonies refrain from laying haploid eggs, which is unlikely because the effect on p will be insignificant if workers of some QF colonies do produce male eggs (unless the effective population size is small). Therefore, we do not expect workers to refrain from laying haploid eggs because of its possible effect on p . However, since QF males emerge when most of the queens are already mated (figure 3), the reproductive success of these late males will be low. Hence, even if workers of QF colonies produce sons and raise nephews instead of brothers, their fitness gain is only small. In addition, if worker reproduction results in a lower number of queens raised by a colony (for instance owing to aggressive acts or lower foraging activities) relative to a colony in which workers refrain from producing haploid eggs, worker policing (workers preventing each other from reproducing (Ratnieks 1988)) could evolve in QF colonies. Workers of MF colonies should try to take over male production, and worker policing is not likely to evolve in these colonies. MF colonies gain fitness through the production of early males and, because these early

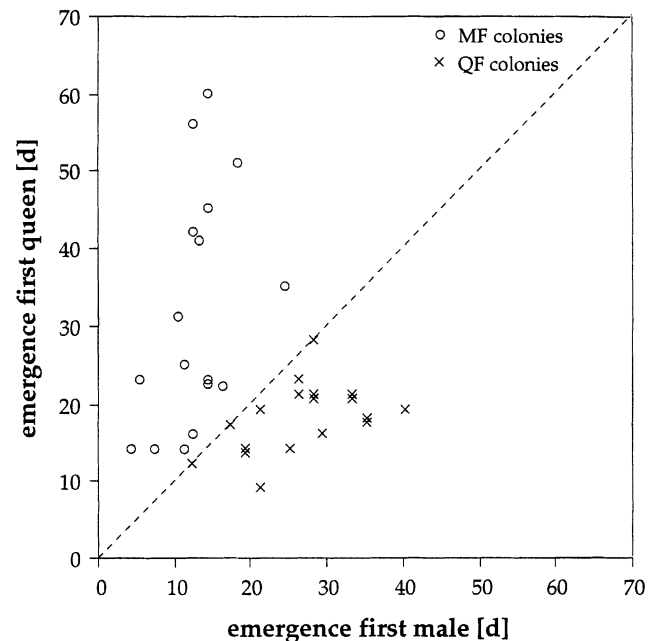


Figure 2. Timing of emergence of the first male and first queen in QF and MF colonies. Time starts after emergence of the second brood.

males have more mating opportunities than males that emerge later, early males are valuable. Thus, workers of MF colonies gain much fitness by producing these early males themselves. Therefore, workers of MF colonies have highest fitness if $p=0$ and should try to take over male production. Queens, both in MF and QF colonies, always have highest fitness if they are the sole producer of haploid eggs ($p=1$; figure 4).

A situation with more than one reproductive strategy can only persist if, at some point, the different strategies have equal fitness. We use the expression 'equilibrium frequencies' to describe the fractions of QF colonies where QF and MF colonies have equal fitness. Because fitness is different for the distinct parties and depends on who lays the haploid eggs, five equilibrium frequencies are present: 0.44 and 0.76 for the queen (for $p=0$ and $p=1$, respectively; figure 4a) and 0.33, 0.44 and 0.89 for the workers (for $p=0$ for egg-laying workers and non-laying workers, respectively, and for $p=1$; figure 4b). Because it is not realistic to assume that all the haploid eggs are produced either by the workers ($p=0$) or by the queen ($p=1$), we

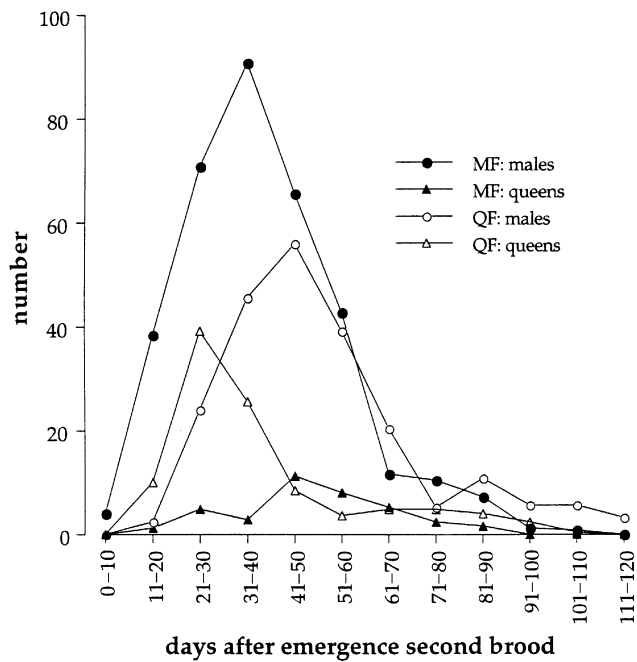


Figure 3. Average number of males and queens produced by males-first (MF) and queens-first (QF) colonies at different times after emergence of the second brood.

have plotted the expected equilibrium frequencies (where QF and MF colonies have equal fitness) for different values of p in figure 5. Note that only two lines remain as the equilibrium for the workers (W_w) cannot be calculated for $p < 1$ (if $p < 1$ we have to make a distinction between laying and non-laying workers), and the equilibrium frequencies for the queen and non-laying workers are the same for all values of p except for $p = 1$ (in this case all haploid eggs are produced by the queen and thus no distinction is made between non-laying and laying workers).

Equilibrium frequencies can also be calculated for different male-to-female cost ratios, the result of which is shown in figure 6. Again, the equilibria for the queen and non-laying workers are equal. The observed fraction QF colonies of 0.44 in our bumblebee population predicts a male-to-female cost ratio of 0.30 seen from the point of view of the queen and the non-laying workers ($p = 0$), and 0.56 for the egg laying workers ($p = 0$). If the male-to-female cost ratio exceeds 0.79, no equilibrium frequency is present for W_w .

4. DISCUSSION

Our data presented in table 2 show that the protandrous and protogynous colonies have different sex investment ratios: protandrous colonies (MF) produce a male-biased sex investment ratio (fraction of males = 0.94, which corresponds to a ratio of 1:13) and protogynous colonies (QF) produce a female-biased sex investment ratio (fraction males = 0.61 or 1:2). MF colonies produce their males early and these males have a larger chance to mate (table 2 and figure 3). QF colonies rely on the males produced by MF colonies but gain fitness by producing a female-biased investment ratio in a population in which the overall numerical sex ratio is male biased.

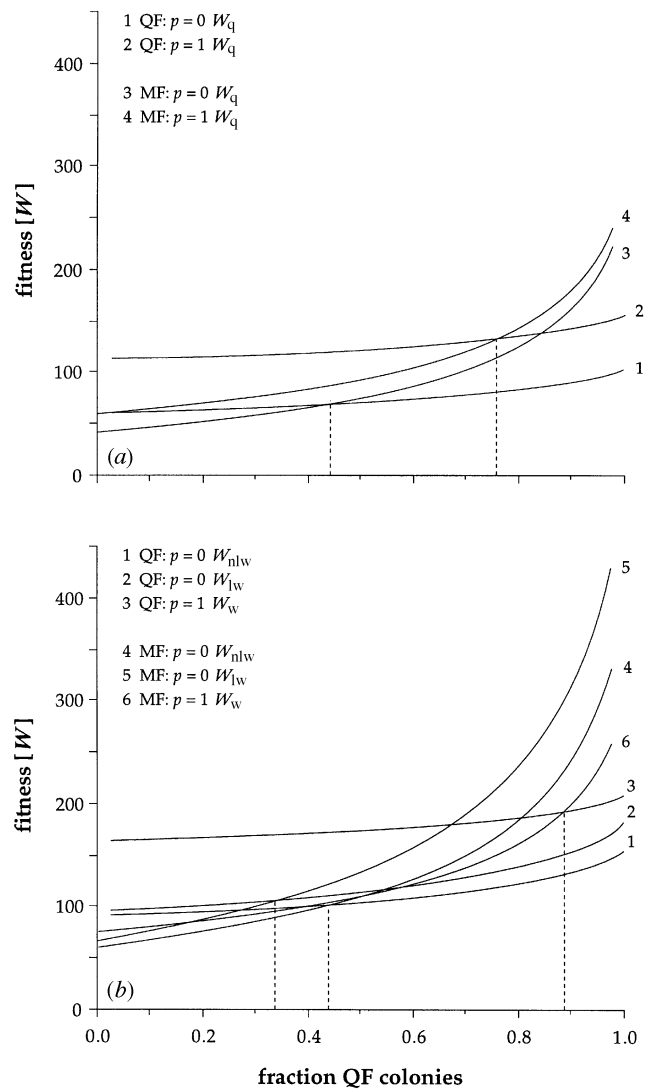


Figure 4. Fitness curves for mother queens (a), and workers (b), of queens-first (QF) and males-first (MF) colonies with all males produced by the queen ($p = 1$) and all males produced by one worker ($p = 0$). In the latter case, fitness is calculated for the egg-laying worker (W_{lw}) as well as for the non-laying workers (W_{nlw}). The vertical lines represent the fraction of QF colonies where fitness of QF and MF colonies is equal. See text for further explanation.

Of course, the fitness pay-offs through males and queens are frequency dependent, i.e. they depend on the number of QF and MF colonies in the population. In figure 4 we show the fitness curves for the two reproductive strategies for both the queen (figure 4a) and workers (figure 4b) depending on who produces the male eggs ($p = 1$, all males produced by the queen; $p = 0$, all males produced by one worker). The QF and MF colonies occurred in more or less equal numbers in our laboratory population: 18 QF colonies and 23 MF colonies (fraction of QF = 0.44; table 2). If we look at the worker fitness curves in figure 4b, we see that below a fraction of QF colonies of about 0.68, workers always have a higher fitness when they are in a QF colony if the queen produces all the male eggs. The optimal reproductive strategy for workers is therefore reached when they are in a QF colony. However, the queen determines if she lays diploid or haploid eggs and therefore whether a colony will be a

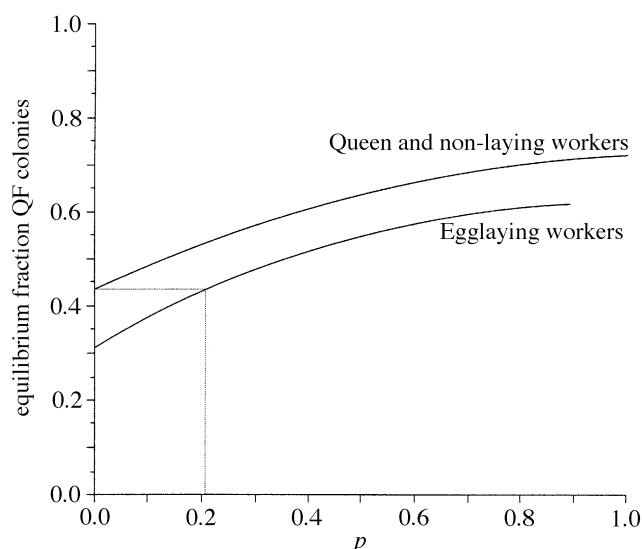


Figure 5. Equilibrium fraction of queens-first colonies as a function of p (fraction of queen-produced haploid eggs in the population). Only two lines are present as the equilibrium fractions cannot be calculated for the workers (W_w) (because $p=1$ in that case), and the equilibria of the queen (W_q) and the non-laying workers (W_{nlw}) are the same except for when $p=1$ (non-laying workers present in that case). Using the value of 0.44 for the fraction of QF colonies observed, the expected values for p are 0 and 0.22 (vertical line).

QF or MF colony. By the time workers can assess that they are in an MF colony they can only increase their fitness by producing haploid eggs themselves and by raising nephews instead of brothers.

If more males in the population are produced by the queen (p increases), the equilibrium frequency shifts towards more QF colonies (figure 5). The frequency observed in our population was 0.44 and this corresponds to $p=0$ for the queen and the non-laying workers, and $p=0.22$ for the laying workers (figure 5). This assumes that a large fraction of the males in our population are worker produced, if our population is in equilibrium and thus MF and QF colonies have equal fitness. A situation in which most of the haploid eggs are worker produced is not optimal from the queen's point of view, as her fitness is highest if she produces all the males ($p=1$; figure 4a).

Earlier we concluded that the observed numerical sex ratio of our laboratory population was very close to the stable sex ratio under queen control using the 0.30 male-to-female cost ratio (figure 1). This seems to be in conflict with the above-mentioned inference that most of the males in our population are worker produced. However, if workers control male production ($p=0$), the stable population sex investment ratio is 1:1 for both the queen and her workers (Crozier & Pamilo 1996). This suggests that the real queen-worker conflict in bumblebee colonies is not over the sex investment ratio but over male parentage. For reasons already mentioned, workers of QF colonies should not try to take over male production, whereas workers of MF colonies should. Consequently, workers must be able to assess whether they are in a MF or QF colony and thus be able to discriminate between male and female brood. Because sex discrimination by the workers is not in the interest of the queen, queens will be

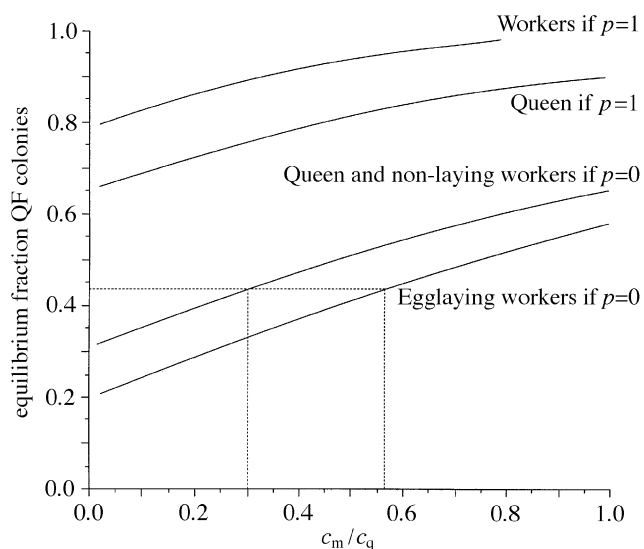


Figure 6. Equilibrium fraction of queens-first colonies as a function of male-to-female cost ratio. Again the equilibria for the queen (W_q) and non-laying workers (W_{nlw}) are equal. Vertical lines represent expected male-to-female cost ratios for the observed fraction of QF colonies of 0.44.

selected to mask the sex of the brood. This has been called sexual deception (Nonacs & Carlin 1990). At present it is unknown whether bumblebee workers are able to discriminate between young male and female brood. But even if workers could detect the presence of haploid eggs and take over male production, this will not necessarily decrease the queen's fitness for she will certainly be the mother of the first MF males, which have the largest mating success. It is even debatable whether workers are able to increase their inclusive fitness by replacing brothers with sons and/or nephews. If the queen is more efficient in producing haploid eggs than the workers, the latter run a chance of replacing a large number of eggs to which they are less related with just a few eggs to which they are more related. How frequently workers produce males in bumblebees is unknown; estimates of the proportion of males that are worker produced in *B. terrestris* populations range from negligible (M. J. Duchateau, personal communication) to 0.82 (van Honk *et al.* 1981).

From the above it is clear that in a protandrous bumblebee population, both protandrous and protogynous colonies can coexist depending on the frequency of both colony types. The existence of both colony types could even be a strategy to avoid inbreeding as males of MF colonies will most often mate with queens produced by QF colonies. What remains is to explain the difference observed between sex investment ratios of colonies raised in the field and laboratory.

We have shown that bumblebee queens are not only more costly to produce (measured as 'callows') but also need more investment after emergence (measured as 'matures') than males (table 1). Therefore, queen production is likely to be more expensive at low resource levels, as predicted by Crozier & Pamilo (1993, 1996). This would result in small colonies producing mainly males because a smaller number of workers is available to collect sufficient resources. Contrary to other authors

Table 3. Average number and standard deviation of queens produced in field colonies ('field'), colonies reared in the laboratory for several generations ('lab'), and colonies produced by field-caught queens collected in spring ('spring') and reared in the laboratory

	field ^a	lab	spring
average no. of queens	4	61	67
s.d.	14.9	62.7	78.5
total no. of colonies	26	41	18

^aData for queens produced in field colonies from Müller & Schmid-Hempel (1999).

(Duchateau & Velthuis 1988; Bourke 1997), we did not find any relation between colony size and sex ratio in our laboratory population of *B. terrestris* (table 2). However, in the field this relation between colony size and queen production is present: colonies producing queens had on average 209 workers, whereas colonies that produced only males had 104 (Müller & Schmid-Hempel 1999). That the sex ratios also differ between field (1:13) and laboratory (1:5) populations indicates that resource scarcity biases sex ratio towards males in the field, whereas in the laboratory large numbers of queens can be produced independent of colony size. Table 3 shows that the number of queens that emerge from laboratory colonies is not a result of selection for genotypes that produce more queens: colonies produced by queens caught in spring produced even more queens when kept under identical conditions as the laboratory colonies that were reared under artificial conditions for many generations.

Comparing the sex investment ratios of colonies reared in the field with the sex investment ratio of colonies reared in the laboratory is only a rough indication that resource scarcity shifts sex allocation towards a male bias. A more elegant method would be to rear bumblebee colonies in the laboratory and to vary the amount of resources.

An alternative model that predicts different sex ratios depending on resource availability is multifaceted parental care (Rosenheim *et al.* 1996). As with the model of Crozier & Pamilo (1993, 1996), multifaceted parental care also predicts female-biased sex ratios when resources are abundant, although for different reasons. When resources are abundant, no more brood can be produced because egg or brood constraints come into play (e.g. if the supply of mature oocytes or the available space in the nest is limited). As a result, excess resources will be fed to females that will develop into queens. If resources are scarce, colonies produce male-biased sex ratios because females will develop into workers instead of queens. Thus, by making females into workers instead of queens, investment made in females can be partly regained, whereas investment made in males can never be regained. Multifaceted parental care also predicts that queens produced under high resource levels will be larger than queens produced under low resource levels. As the Crozier & Pamilo model does not predict differences in weight between queens reared under high and low resource conditions, comparing the weight of both can discriminate between the two models.

In conclusion, we have shown that the occurrence of male-biased investment ratios in the bumblebee *B. terrestris* does not necessarily lead to a lower fitness for the queen or her workers. A male-biased investment ratio can be the result of an optimal allocation of resources under low resource conditions, as is often the case in field populations. This then leads to split sex ratios depending on the amount of resources available to a bumblebee colony, with colonies under low resource conditions producing mainly males, and colonies under high resource conditions focusing more on female production. However, the extent to which bumblebee populations show a male-biased sex allocation cannot be explained by cost differences between male and female production alone. As Bourke (1997) has already explained, male-biased investment ratios in populations of bumblebees are a by-product of the occurrence of protandry. In our paper, we have extended Bourke's argument and showed that two reproductive strategies, a protandrous and a protogynous strategy, exist within the same male-biased protandrous population, whereby both specialize on producing one sex.

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REFERENCES

- Beekman, M., van Stratum, P. & Lingeman, R. 1998a Diapause survival and post-diapause performance in bumblebee queens (*Bombus terrestris*). (Submitted.)
- Beekman, M., Lingeman, R., Kleijne, F. M. & Sabelis, M. W. 1998b Optimal timing of the production of sexuals in bumblebee colonies. *Entomol. Exp. Appl.* (In the press.)
- Bertsch, A. 1984 Foraging in male bumblebees (*Bombus lucorum* L.): maximizing energy or minimizing water load? *Oecologia* **62**, 325–336.
- Boomsma, J. J. 1989 Sex-investment in ants: has female bias been systematically overestimated? *Am. Nat.* **133**, 517–532.
- Boomsma, J. J. 1993 Sex ratio variation in polygynous ants. In *Queen number and sociality in insects* (ed. L. Keller), pp. 86–109. Oxford University Press.
- Boomsma, J. J. & Grafen, A. 1990 Intraspecific variation in ant sex ratios and the Trivers–Hare hypothesis. *Evolution* **44**, 1026–1034.
- Boomsma, J. J. & Grafen, A. 1991 Colony-level sex ratio selection in the eusocial Hymenoptera. *J. Evol. Biol.* **3**, 383–407.
- Boomsma, J. J., Keller, L. & Nielsen, M. G. 1995 A comparative analysis of sex ratio investment parameters in ants. *Funct. Ecol.* **9**, 743–753.
- Bourke, A. F. G. 1997 Sex ratios in bumblebees. *Phil. Trans. R. Soc. Lond. B* **352**, 1921–1933.
- Bulmer, M. G. 1981 Worker–queen conflict in annual social Hymenoptera. *J. Theor. Biol.* **93**, 239–251.
- Bulmer, M. G. 1983 The significance of protandry in social Hymenoptera. *Am. Nat.* **121**, 540–551.
- Crozier, R. H. & Pamilo, P. 1993 Sex allocation in social insects: problems in prediction and estimation. In *Evolution and diversity of sex ratio in insects and mites* (ed. D. L. Wrensch & M. A. Ebbert), pp. 369–383. New York: Chapman & Hall.
- Crozier, R. H. & Pamilo, P. 1996 *Evolution of social insect colonies: sex allocation and kin selection*. Oxford University Press.

- Duchateau, M. J. & Velthuis, H. H. W. 1988 Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour* **107**, 186–207.
- Estoup, A., Scholl, A., Pouvreau, A. & Solignac, M. 1995 Monoandry and polyandry in bumblebees (Hymenoptera: Bombinae) as evidenced by highly variable microsatellites. *Molec. Ecol.* **4**, 89–93.
- Fisher, R. A. 1930 *The genetical theory of natural selection*. Oxford: Clarendon Press.
- Fisher, R. M. 1987 Queen–worker conflict and social parasitism in bumblebees (Hymenoptera: Apidae). *Anim. Behav.* **35**, 1026–1036.
- Fisher, R. M. 1992 Sex ratios in bumblebee social parasites: support for queen–worker conflict theory? (Hymenoptera: Apidae). *Sociobiology* **20**, 205–217.
- Grafen, A. 1986 Split sex ratios and the evolutionary origins of eusociality. *J. Theor. Biol.* **122**, 95–121.
- Hamilton, W. D. 1964a The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**, 1–16.
- Hamilton, W. D. 1964b The genetical evolution of social behaviour. II. *J. Theor. Biol.* **7**, 17–52.
- Helms, K. R. 1994 Sexual size dimorphism and sex ratios in bees and wasps. *Am. Nat.* **143**, 418–434.
- Müller, C. B. & Schmid-Hempel, P. 1992 Variation in life-history pattern in relation to worker mortality in the bumblebee *Bombus lucorum*. *Funct. Ecol.* **6**, 48–56.
- Müller, C. B. & Schmid-Hempel, P. 1999 Effects of conopid parasitoids on the performance of bumblebee colonies. (In preparation.)
- Nonacs, P. & Carlin, N. F. 1990 When can ants discriminate the sex of brood? A new aspect of queen–worker conflict. *Proc. Natn. Acad. Sci. USA* **87**, 9670–9673.
- Owen, R. E. & Plowright, R. C. 1982 Worker–queen conflict and male parentage in bumblebees. *Behav. Ecol. Sociobiol.* **11**, 91–99.
- Owen, R. E., Rodd, F. H. & Plowright, R. C. 1980 Sex ratios in bumblebee colonies: complications due to orphaning? *Behav. Ecol. Sociobiol.* **7**, 287–291.
- Peakin, G. J. 1972 Aspects of productivity in *Tetramorium caespitum*. *Ecol. Pol.* **20**, 55–63.
- Ratnieks, F. L. W. 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**, 217–236.
- Röseler, P. F. & van Honk, C. G. J. 1990 Castes and reproduction in bumblebees. In *Social insects: an evolutionary approach to castes and reproduction* (ed. W. Engels), pp. 147–166. Berlin: Springer.
- Rosenheim, J. A., Nonacs, P. & Mangel, M. 1996 Sex ratios and multifaceted parental investment. *Am. Nat.* **148**, 501–535.
- Trivers, R. L. & Hare, H. 1976 Haplodiploidy and the evolution of the social insects. *Science* **191**, 249–263.
- van Honk, C. G. J., Röseler, P. F., Velthuis, H. H. W. & Hoogeveen, J. C. 1981 Factors influencing the egg laying of workers in a captive *Bombus terrestris* colony. *Behav. Ecol. Sociobiol.* **9**, 9–14.

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