Sex ratios in bumble bees

Andrew f. G. Bourke

Phil. Trans. R. Soc. Lond. B 1997 **352**, 1921-1933
doi: 10.1098/rstb.1997.0179

Sex ratios in bumble bees

ANDREW F. G. BOURKE

Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
(`suaaafb@ucl.ac.uk`)

CONTENTS

SUMMARY

The median proportion of investment in females among 11 populations of seven bumble bee (*Bombus*) species was 0.32 (range 0.07–0.64). By contrast, two species of workerless social parasites in the related genus *Psithyrus* had female-biased sex allocation, the reasons for which remain unclear. Male-biased sex allocation in *Bombus* contradicts the predictions of Trivers & Hare's sex ratio model for the social Hymenoptera, which are that the population sex investment ratio should be 0.5 (1:1) under queen control and 0.75 (3:1 females:males) under worker control (assuming single, once-mated, outbred queens and non-reproductive workers). Male bias in *Bombus* does not appear to be either an artefact, or purely the result of symbiotic sex ratio distorters. According to modifications of the Trivers–Hare model, the level of worker male production in *Bombus* is insufficient to account for observed levels of male bias. There is also no evidence that male bias arises from either local resource competition (related females compete for resources) or local mate enhancement (related males cooperate in securing mates). Bulmer presented models predicting sexual selection for protandry (males are produced before females) in annual social Hymenoptera and, as a consequence (given some parameter values), male-biased sex allocation. Bumble bees fit the assumptions of Bulmer's models and are protandrous. These models therefore represent the best current explanation for the bees' male-biased sex investment ratios. This conclusion suggests that the relative timing of the production of the sexes strongly influences sex allocation in the social Hymenoptera.

1. INTRODUCTION

The study of sex ratio evolution in the social Hymenoptera is important for understanding both sex allocation (e.g. Charnov 1982) and the effect of kin selection on social behaviour (Trivers 1985). By combining sex ratio theory (Fisher 1930) with Hamilton's (1964) theory of kin selection, Trivers & Hare (1976) deduced the stable population sex investment ratio for social Hymenoptera with a simple kin structure. They showed that the stable ratio from the queen's viewpoint is 1:1, because queens are equally related to daughters and sons. However, the stable ratio from the workers' viewpoint is 3:1 females:males. This is because haplodiploidy (the production of males from unfertilized, haploid eggs) causes workers to be three times more closely related to sisters than to brothers. Trivers & Hare (1976) argued that workers should usually control sex allocation because they typically outnumber queens and rear the brood. In agreement with the resulting prediction of the Trivers–Hare model, sex investment ratios in many species of social Hymenoptera are female biased (Bourke & Franks 1995; Crozier & Pamilo 1996).

Bumble bees (Hymenoptera: Apidae: *Bombus* species) appear to represent a striking exception to

Phil. Trans. R. Soc. Lond. B (1997) **352**, 1921–1933
Printed in Great Britain

TEX Paper

the Trivers–Hare predictions. This is because they have highly male-biased population sex investment ratios. Bumble bees are annual social Hymenoptera with one queen per colony (monogyny). After overwintering, queens that mated the previous season found colonies by producing workers. Following a growth phase, the colony then produces new queens and males. These mate, the males, old queen and workers die and the new generation of mated queens enters hibernation (Sladen 1912; Plath 1934; Free & Butler 1959; Alford 1975; Morse 1982). Male-biased sex allocation in bumble bees appears to challenge both current sex ratio theory and kin selectionist interpretations of worker behaviour in the social Hymenoptera (Owen *et al.* 1980; Fisher 1987, 1992; Duchateau & Velthuis 1988; Röseler & Van Honk 1990). However, although bumble bee sex ratios have periodically surfaced as a worrying niggle for evolutionary biologists, a comprehensive explanation of them has not been attempted.

This paper aims to investigate the factors affecting sex ratio evolution in the bumble bees. I review the existing data, then examine it in the light of various hypotheses that could account for male bias in population sex investment ratios. Note that the problem posed by male bias in bumble bees is not primarily that of identifying which party controls sex allocation. Extreme male bias is a difficulty for both the queen control and the worker control versions of the standard Trivers–Hare model. In fact, there are cogent reasons for thinking that queen bumble bees should exercise a large degree of sex ratio control. For instance, Müller *et al.* (1992) suggested that queen control is relatively easy in species with short (annual) life cycles, because the level of male production may be largely determined before workers are present. As examples, in all or some colonies of *B. polaris* (Richards 1973), *B. terricola* (Plowright & Plowright 1990) and *B. lucorum* (Müller *et al.* 1992), the queen's male eggs were laid before the first workers emerged. Queens of annual species might also manipulate sex allocation more easily because, not needing to produce workers for later years, they are freer to vary the ratio of haploid to diploid eggs laid (Bulmer 1981). Moreover, the comparatively small size of an annual workforce could make queen control easier (Nonacs 1986*a*). However, since assuming queen control predicts unbiased sex allocation in the standard Trivers–Hare model, it does not immediately solve the problem of why bumble bees have strongly male-biased sex investment ratios.

2. REVIEW OF THE DATA ON SEX INVESTMENT RATIOS IN BUMBLE BEES

I compiled sex ratio data from the literature on 11 populations of seven *Bombus* species and four populations of two parasitic *Psithyrus* species. I calculated sex investment ratios for each population and, where possible, their 95% confidence limits (tables 1, 2). Previous compilations of bumble bee sex ratio data include those of Michener (1974), Trivers & Hare (1976), Strassmann (1984), Helms (1994) and Crozier & Pamilo (1996). The present dataset differs from these by including several studies that they did not cover (Shykoff & Schmid-Hempel 1991; Fisher 1992; Müller & Schmid-Hempel 1992*a*,*b*).

The present dataset also omits Webb's (1961) unpublished sex ratio data on which some previous compilations were based. Webb's (1961) *Bombus* data involved five species (*B. americanorum*, *B. auricomus*, *B. fraternus*, *B. griseocollis* and *B. impatiens*) and showed overall female-biased sex allocation. I omitted these data because this female bias is likely to have been a measurement artefact. There is a trend in *Bombus* for larger, more productive colonies to produce relatively more female-biased sex ratios (Owen *et al.* 1980, § 4). Webb's data came from field collections, where larger, more productive colonies are almost certainly easier to find (Owen *et al.* 1980). If so, this would have led to an overestimate of relative female bias in the population sex ratio. In addition, Webb (1961) probably missed sampling sexual production early in the season when males are more likely to be produced (Trivers & Hare 1976; Owen *et al.* 1980, § 4). The median fraction of investment in females in Webb's (1961) data (as reported by Trivers & Hare (1976)) was 0.68 (range 0.54–0.76). This was significantly different from the median for the 11 *Bombus* populations in table 1 (0.32) (two-tailed Mann–Whitney U-test, $U_{11,5} = 51$, $p < 0.01$). This supports the conclusion that Webb's data are unrepresentative.

Analysis of the current dataset confirmed that population sex investment ratios in *Bombus* tend to be male biased (table 1). Specifically, the median fraction of investment in females across the 11 populations was 0.32 (range 0.07–0.64). The confidence limits of individual population sex investment ratios were wide, either because of small colony sample sizes (e.g. *B. ruderatus*, *B. terrestris sassaricus*) or because of large between-colony variation in sex ratio. Nonetheless, of six *Bombus* datasets in which more than ten colonies were collected, and in which confidence limits could be calculated, population sex investment ratios were significantly male biased in three cases (*B. melanopygus* and the two *B. terricola* populations). The other three cases (*B. affinis*, *B. lucorum*, *B. t. terrestris*) had male-biased mean population sex investment ratios, but these could not be shown to be significantly less than 0.5. In all six cases the population sex investment ratio was significantly less than 0.75 (3:1 females:males), the value expected under worker control in the standard Trivers–Hare model (table 1).

Psithyrus is a sister genus of *Bombus* consisting of socially parasitic bees whose workerless queens invade *Bombus* nests and then produce their own sexuals (Sladen 1912; Plath 1934; Free & Butler 1959; Alford 1975; Morse 1982). By contrast with *Bombus*, the four *Psithyrus* populations investigated all had female-biased sex allocation and this female bias was significant in the case of the two *Psithyrus* samples with confidence limits (table 2). Overall, the median fraction of investment in females in *Psithyrus*

Table 1. *Data on population sex investment ratios in* Bombus *bumble bees*

(Notes: (1) Colony number is given as: number of sexual-producing colonies/total number of colonies for which data are reported. (2) The cost ratio used for converting numerical sex ratios to investment sex ratios was a wet weight cost ratio (wet weight of average female divided by wet weight of average male) except in the case of *B. terrestris*, for which none of the studies reports wet weights. Therefore, in this species, the dry weight cost ratio (2.113) given by Duchateau & Velthuis (1988) for *B. t. terrestris* was used. (3) Lumped population mean sex investment ratios (total biomass of females produced divided by the total biomass of all sexuals produced), and (if individual colony data were available) their 95% confidence limits (95% C.L.), were recalculated from the original authors' data using J. J. Boomsma's method described in Bourke & Franks (1995). Blanks (—) indicate lack of data. (4) The degree of protandry could not be quantified consistently from available data. Unless otherwise stated, it is given as the average number of days between the emergence of the first adult males and the emergence of the first adult females. However, for *B. lucorum* (Müller & Schmid-Hempel 1992*b*) and *B. t. terrestris* (Duchateau & Velthuis 1988), the degree of protandry is the separation in days between the dates when the first male egg was laid (the 'switch point') in colonies with male-biased and female-biased sex allocation, respectively, and for *B. terricola* (Owen *et al.* 1980), it is the separation in days between peak adult male and peak adult female production. *B. affinis* (Fisher 1987, 1992) was protogynous (females were produced before males), with a separation of 21 days between the start of adult sexual emergence and the date the weekly sex ratio fell below 0.5. (5) In all but four studies, colonies were kept and fed in the laboratory. The exceptions were *B. lucorum* (Müller & Schmid-Hempel 1992*b*) and *B. terricola* (Owen *et al.* 1980), whose colonies were placed in the field after initiation in the laboratory, and *B. ruderatus* (Pomeroy 1979) and (in 9 of the 21 colonies only) *B. t. terrestris* (Duchateau & Velthuis 1988), where workers had outside access via tubes exiting their laboratory nests. In these cases workers therefore foraged under field conditions. When data were gathered as part of an experimental study (e.g. Shykoff & Schmid-Hempel 1991; Müller & Schmid-Hempel 1992*a*), only sex ratio data for control, unmanipulated colonies are presented in the table. (6) Queen mating frequency is known from genetic evidence for three species in the table: *B. melanopygus* (Owen & Plowright 1980), *B. lucorum* and *B. terrestris* (Estoup *et al.* 1995) all have single mating. Worker male production is known to occur in all 7 *Bombus* species in the table, but its extent was only quantified in one study. In *B. melanopygus*, workers produced 19% of males in queenright colonies and 39% of the males in total (Owen & Plowright 1982).)

species	*N* colonies	*N* females	*N* males	cost ratio (F:M)	population sex investment ratio as the fraction of investment in females (95% C.L.)	protandry (*N* days)	references
B. affinis	22/22	869	3291	3.609	0.488 (0.352–0.592)	absent (−21)	I
B. impatiens	?/10	342	955	3.900	0.583 (—)	—	II
B. lucorum	12/12	51	1455	2.306	0.075 (—)	present (8)	III
B. lucorum	20/36	250	410	1.470	0.473 (0.307–0.620)	present (28)	IV
B. melanopygus	17/17	215	1491	2.580	0.271 (0.119–0.395)	—	V
B. ruderatus	5/5	275	1294	2.000	0.298 (−0.218–0.619)	present (21)	VI
B. terrestris	?/10	345	4316	2.113	0.144 (—)	present (4)	VII
B. t. terrestris	21/21	662	2514	2.113	0.357 (0.122–0.538)	present (14)	VIII
B. t. sassaricus	5/5	142	170	2.113	0.638 (−0.149–0.988)	—	IX
B. terricola	32/32	351	2143	2.127	0.258 (0.145–0.358)	present (28)	X
B. terricola	20/20	148	644	2.056	0.321 (0.213–0.416)	—	XI

References: I, Fisher (1987, 1992); II, Fisher (1992); III, Müller & Schmid-Hempel (1992*a*); IV, Müller & Schmid-Hempel (1992*b*); V, Owen & Plowright (1982); VI, Pomery (1979); VII, Shykoff & Schmid-Hempel (1991); VIII, Duchateau & Velthuis (1988); IX, Duchateau & Velthuis (1988); X, Owen *et al.* (1980); XI, Fisher (1992);

was 0.67 (range 0.60–0.72), a value significantly different from the *Bombus* median of 0.32 (two-tailed Mann–Whitney *U*-test, $U_{11,4} = 43$, $p < 0.01$). A difference in the sex investment ratios of *Bombus* and *Psithyrus* was noted by Trivers & Hare (1976) and Fisher (1992).

It has recently been shown that the Trivers–Hare theory can predict sex ratio splitting (different classes of colony within a population produce systematically different sex ratios) (Grafen 1986; Boomsma & Grafen 1990, 1991). This is expected when workers control sex allocation and their comparative relatedness with the sexes varies among colonies. However, frequency distributions from eight *Bombus* populations suitable for the analysis showed that their colony sex investment ratios were not split bimodally. Instead, within populations, a substantial fraction of colonies (51% of 135 colonies in the pooled dataset) tended to produce all or mainly males, whereas few colonies produced all or mainly females (6% of 135 colonies in the pooled dataset) (figure 1). Both early and recent researchers have noted the occurrence of colonies that produce mainly males within bumble bee populations (e.g. Sladen 1912; Shykoff & Müller 1995). This phenomenon is connected with the fact that unproductive colonies tend to concentrate on male production (§ 4).

I propose that male-biased population sex investment ratios in bumble bees have four conceivable types of explanation. These are that (i) male bias is

Table 2. *Data on population sex investment ratios in* Psithyrus *bumble bees and their* Bombus *hosts*

(Notes: (1) All data came from Fisher (1987, 1992). *P. ashtoni* bees were reared either with *B. affinis* or with *B. terricola* as hosts; in these colonies the hosts produced some of their own sexuals. *P. citrinus* bees were reared either with *B. impatiens* or with *B. vagans* as hosts; in these colonies no host sexuals were reared. All colonies were kept and fed in the laboratory. (2) Colony number is given as: number of sexual-producing colonies/total number of colonies for which data are reported. The mean and confidence limits of the population sex investment ratios were recalculated from Fisher's (1992) original data as described in the notes to table 1. (3) For *P. ashtoni* with *B. affinis* as host, the degree of protandry was quantified as the separation in days between the start of adult sexual emergence and the date the weekly sex ratio (fraction of adult females) exceeded 0.5. The *B. affinis* host bees showed neither protandry nor protogyny (table 1) and instead produced a constant weekly sex ratio over time (Fisher 1987).)

species	*N* colonies	*N* females	*N* males	wet weight cost ratio (F:M)	population sex investment ratio as the fraction of investment in females (95% C.L.)	protandry (*N* days)
P. ashtoni	23/33	749	1037	2.561	0.649 (0.566–0.721)	present (28)
B. affinis host	24/33	147	1871	3.609	0.221 (0.092–0.326)	absent (0)
parasite plus host	33/33	896	2908	—	0.459	
P. ashtoni	25/33	232	328	2.086	0.596 (0.501–0.679)	—
B. terricola host	32/33	119	716	2.056	0.255 (0.152–0.346)	—
parasite plus host	33/33	351	1044	—	0.395	
P. citrinus	6/6	164	229	3.275	0.701 (—)	—
B. impatiens host	0/6	0	0	—	—	—
P. citrinus	5/5	125	172	3.521	0.719 (—)	—
B. vagans host	0/5	0	0	—	—	—

an artefact; (ii) male bias is non-adaptive; (iii) male bias is adaptive for symbionts of bumble bees; and (iv) male bias is adaptive for bumble bees but the assumptions of the standard sex ratio models do not apply; application of the correct model would predict male bias. In this paper I do not discuss explanation (ii) further. This is because, although a non-adaptive explanation possibly exists, it is unlikely to yield general insight into why sex ratios evolve to be male biased. Explanation (iii) relies on the possible occurrence of symbionts in social insects that bias the sex ratio towards male production in their own interests (Crozier & Pamilo 1993). An example of such a sex ratio distorter is the paternal sex ratio factor (*psr*) described from the solitary, parasitoid wasp *Nasonia vitripennis* (Hymenoptera: Pteromalidae) (Werren *et al.* 1988). However, as Crozier & Pamilo (1993) pointed out, an extreme *psr*-analogue would disappear in a monogynous social insect like a bumble bee, because a queen producing all males would be unable to found a colony successfully. Such an agent could therefore persist in bumble bees only by having partial effects, or by somehow affecting just the sexual-yielding brood. In addition, male bias is unlikely to be due exclusively to distorters, which typically create complex polymorphisms in sex ratio across and within populations (Werren *et al.* 1988). Lastly, attributing male bias in bumble bees to sex ratio distorters like *psr* leaves unexplained why *Psithyrus* has female-biased sex allocation despite its being a more likely candidate for infection through not founding its own colonies. In sum, there is as yet no evidence for an influence of sex ratio distorters and it seems an unlikely general explanation for male-biased sex allocation in bumble bees. In the following sections, I discuss the remaining two explanations for male bias in turn.

3. MALE BIAS AS A POSSIBLE ARTEFACT

(*a*) *Overestimation of male bias due to measuring sex allocation in laboratory colonies*

The principal reason why male bias could be artefactual is that sex ratios in bumble bees are hard to measure in the field. This is because it is difficult to find all the colonies within a given area and, as was argued in the case of Webb's (1961) data by Owen *et al.* (1980), selective sampling runs the risk of overestimating relative female bias. Investigators have attempted to circumvent this problem in two ways. The first is by measuring sex ratios from colonies started in the laboratory but placed in artificial nests in the field (e.g. Owen *et al.* 1980; Müller & Schmid-Hempel 1992*b*). The second is by raising colonies entirely in the laboratory. However, it is not obvious that, under these conditions, bumble bees rear sexuals as they would in entirely natural circumstances. Conceivably, then, these methods contribute to male-biased sex investment ratios.

I suggest, however, that rearing method is not the main explanation for male bias in bumble bee sex allocation. The studies reported in table 1 involve both cases in which colonies were partially reared in the field and those in which colonies were always kept in the laboratory. Both types of study include examples where population sex investment ratios were signifi-

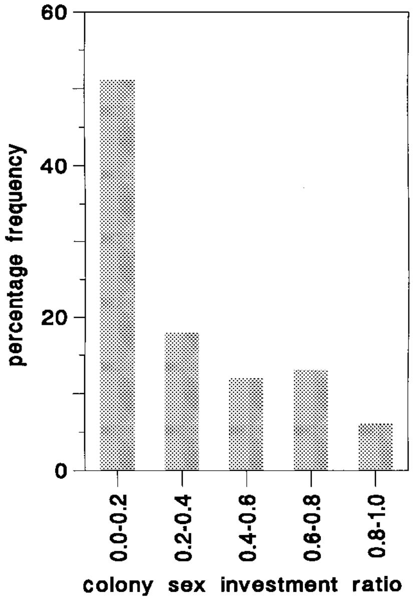

Figure 1. Frequency distribution of the sex investment ratios (fraction of investment in females) of colonies within six *Bombus* species from eight populations ($N = 135$ colonies). The eight datasets used are listed in table 4. Colonies producing five or fewer sexuals were omitted from the analysis (table 4). The frequency distribution of the pooled colony data was strongly shown by four of the six individual populations in the dataset in which more than five colonies were sampled (*B. lucorum*: Müller & Schmid-Hempel 1992*b*; *B. melanopygus*: Owen & Plowright 1982; *B. t. terrestris*: Duchateau & Velthuis 1988; *B. terricola*: Owen *et al.* 1980). The other two did not obviously show this pattern (*B. affinis* and *B. terricola*: Fisher 1992).

cantly male biased and cases where they were not significantly different from 0.5 (table 1). In *B. lucorum*, a laboratory-based study (Müller & Schmid-Hempel 1992*a*) yielded a more male-biased population sex investment ratio than one involving colonies placed in the field (Müller & Schmid-Hempel 1992*b*). However, Shykoff & Müller (1995) found that captive *B. terrestris* colonies allowed to forage in the field were less successful than laboratory colonies at female production and, in *B. terricola*, a similar level of male bias was found among colonies reared in both the laboratory (Fisher 1992) and the field (Owen *et al.* 1980). These findings suggest that rearing method does not bias the sex ratio in one direction consistently.

Müller *et al.* (1992) suggested that the relationship between colony size and the colony sex ratio could be different in laboratory-reared and mainly field-reared colonies, with larger colonies tending to produce more female-biased sex ratios only in the field. However, the laboratory *B. lucorum* data presented by Müller & Schmid-Hempel (1992*a*) appear exceptional in showing a tendency for the colony sex investment ratio to be more male biased as colony productivity increased. In the present data (table 1), the colony sex investment ratio tended to become more female biased with increasing colony productivity, regardless of whether the study was conducted in the laboratory or field (table 4).

(*b*) *Overestimation of male bias from using cost ratios based on weights*

A general problem in studying sex allocation is how to calculate sex investment ratios from data on numerical sex ratios. In the social Hymenoptera, the standard approach has been to use body weights to calculate the cost ratio (the ratio, investment in an average female:investment in an average male) used to convert numerical data to investment data (Trivers & Hare 1976). In ants, this approach underestimates relative investment in males, because males consume more energy per unit weight than females (Boomsma 1989; Boomsma *et al.* 1995). Although this specific problem is therefore unlikely to lead to an overestimation of male investment among bumble bees, it could nonetheless be true that using untransformed wet and dry weights to calculate bumble bee cost ratios (tables 1, 2) somehow biases the measurement of their sex allocation ratios.

Helms (1994) explored the possible occurrence of the bias described by Boomsma (1989) within taxa of solitary and social bees and wasps. He found that, across species and populations, there tended to be a significantly positive regression of the female:male sex investment ratio on the female:male weight-based cost ratio. There also tended to be a regression significantly greater than -1 of the female:male numerical sex ratio on the female:male weight-based cost ratio. These results indicated either an increase in the relative investment in females, or an increase in the error in estimating investment in the sexes, as the level of female:male sexual dimorphism increased (Helms 1994). However, Helms (1994) found neither of these regressions to be significant in bumble bees. I repeated Helms's analysis using the present data on *Bombus* sex ratios and cost ratios (table 1) and again found no significant effects. Therefore, the effects that Helms (1994) identified in other taxa of bees and wasps do not appear to be present in bumble bees.

Another possible complication associated with using weights of bees to estimate relative investment is that the sexes may remain in the nest for different lengths of time before departing to mate and may return to the nest after mating. For example, the interval between emerging as adults and leaving the nest appears slightly longer for young queens than for young males (table 5). In addition, young females sometimes return to their natal nest after mating, whereas males either do not or do so only very briefly (Free & Butler 1959; Alford 1975). If females used their extra time in the nest to gain weight (for example, to prepare for hibernation), then a biomass cost ratio may underestimate relative investment in females, depending on when body weight is measured. Current data do not appear adequate to determine whether such an effect occurs. On the other hand, young queens are also reported to help incubate the brood and occasionally to forage before finally departing (Alford 1975), so it is not clear whether any extra investment they receive is a net gain to them or the colony.

Table 3. *Predicted sex investment ratios with worker reproduction*

(Notes: (1) Queenright colonies are assumed to be headed by single, once-mated, outbred queens. m is the fraction of males produced by queenright colonies that is produced by workers in those colonies. β is the fraction of all males in the population that is produced by workers in queenless colonies. Worker control refers to control by non-laying workers (or by multiple laying workers). If there is a single laying worker and worker control, the case (i) formula becomes $(6-3m)/(8-m)$. (2) In cases (ii) and (iii), it is assumed that the frequency of queenless colonies is less than 67% (queen control case) or 40% (worker control case). Above these critical frequencies, the population sex ratio (fraction of females) simply equals $(1-w)$, where w is the frequency of queenless colonies (Pamilo 1991; Crozier & Pamilo 1996). (3). The original models from which these formulae derive are in Benford (1978) (case (i)), Taylor (1981) (case (ii), queen control), Nonacs (1986*a*) (case (ii), worker control) and Pamilo (1991) (case (iii)). See also Boomsma & Grafen (1991), Bourke & Franks (1995) and Crozier & Pamilo (1996).)

colony type with worker reproduction	population sex investment ratio (fraction of investment in females)	
	queen control	worker control
(i) queenright	$\frac{1}{2}$	$(6-3m)/(8-2m)$
(ii) queenless	$(2-\beta)/(4-\beta)$	$(6-3\beta)/(8-3\beta)$
(iii) both	$\dfrac{1+(1-m)(1-\beta)}{2+(1-m)(2-\beta)}$	$\dfrac{6-3\beta(1-m)-3m}{8-3\beta(1-m)-2m}$

Table 4. *Correlations and partial correlations of colony sex investment ratio, worker number and sexual productivity in* Bombus *bumble bees*

(Notes: (1) Abbreviations as follows: S, colony sex investment ratio, measured as the fraction of T invested in females (angular transformed); T, total sexual production of colony, i.e. biomass of females produced plus biomass of males produced ($\log_{10}$ transformed); W, number of workers in colony ($\log_{10}$ transformed); X, Y, correlation of X and Y. $X, Y.Z$, partial correlation of X and Y (Z held constant). (2) Significance of correlation coefficients: no asterisk, $p > 0.05$, NS; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; three asterisks, $p < 0.001$. (3) All coefficients were calculated from the original authors' data. Only datasets with data on individual colony sexual production could be included in the analysis. Within datasets, colonies producing five sexuals or fewer were excluded, because extreme sex ratios are more likely to occur by chance in these colonies. T and W were logarithmically transformed to ensure normality of the data. S and T were calculated using wet weights, except in the case of *B. t. terrestris* and *B. t. sassaricus*, where only dry weight data were available. Blanks (—) indicate lack of data due to the absence of published information on colony size (W).)

species	N colonies	correlation coefficients			partial correlation coefficients			reference
		S,T	S,W	T,W	$S,T.W$	$S,W.T$	$T,W.S$	
B. affinis	21	0.455^{*}	0.246	0.163	0.434	0.196	0.059	I
B. lucorum	14	0.708^{**}	0.749^{**}	0.813^{***}	0.257	0.422	0.604^{*}	II
B. melanopygus	17	0.461	0.260	0.190	0.434	0.198	0.082	III
B. ruderatus	5	0.848	0.771	0.940^{*}	0.567	−0.144	0.848	IV
B. t. terrestris	21	0.061	—	—	—	—	—	V
B. t. sassaricus	5	0.899^{*}	—	—	—	—	—	VI
B. terricola	32	0.441^{*}	0.539^{**}	0.762^{***}	0.055	0.349	0.693^{***}	VII
B. terricola	20	0.096	−0.156	0.685^{***}	0.282	−0.306	0.712^{***}	VIII

References: I, Fisher (1992); II, Müller & Schmid-Hempel (1992*b*); III, Owen & Plowright (1982); IV, Pomeroy (1979); V, Duchateau & Velthuis (1988); VI, Duchateau & Velthuis (1988); VII, Owen *et al.* (1980); VIII, Fisher (1992).

(*c*) *Overestimation of male bias due to diploid male production*

A final factor that possibly contributes to the appearance of male-biased sex allocation in bumble bees is diploid male production. In the Hymenoptera, complementary sex determination can lead to the production of sterile, diploid males if there is inbreeding (e.g. Cook & Crozier 1995). Conceivably, many males in bumble bees are diploid males that workers 'perceive' as females and to which workers therefore allocate investment that was destined for genuine females (Crozier & Pamilo 1996). Male bias in sex allocation would then be partly illusory. However, although diploid males can be produced in bumble bees by forcing matings between nestmates (e.g. Plowright & Pallett 1979; Duchateau *et al.* 1994;

Table 5. *Do Bombus bumble bees match the assumptions of models for the evolution of protandry?*

Assumption 1: adult sexuals are released continuously over a long period of time.
Male flight times are typically June–July to September–October in *B. hortorum*, *B. pascuorum*, *B. pratorum* and *B. terrestris* in England (Prŷs-Jones & Corbet 1987). In Switzerland, Schmid-Hempel & Durrer (1991) found *B. pascuorum* males emerged over the period mid-July to mid-October.

Assumption 2: females leave the nest and mate once shortly after emergence as adults.
Young queens are reported to leave the nest for mating when 2–8 days old (Free & Butler 1959), five days old (Alford 1975) or seven days old (Richards 1973).

Queens do tend to mate once in bumble bees (Crozier & Pamilo 1994, § 6).

Assumption 3: males are ready to mate shortly after emergence as adults, can mate multiply, and can live following emergence for an appreciable period.
All authors agree that young male bumble bees leave the nest at an early adult age, typically 2–4 days (e.g. Sladen 1912; Free & Butler 1959; Michener 1974; Alford 1975). Specific examples are *B. polaris* (Richards 1973) and *B. atratus* (Garofalo *et al.* 1986).

Foster (1992) describes mating experiments with four Canadian *Bombus* species suggesting that males can mate when 3–5 days old. This implies that males are ready to mate upon leaving the nest. On the other hand, *B. terrestris* males started mating aged 10 days (Duchateau & Mariën 1995), indicating a slight maturation period in this species.

Multiple mating by males was reported by Garofalo *et al.* (1986) in *B. atratus*, Foster (1992) in four Canadian *Bombus* species, and Duchateau & Mariën (1995) in *B. terrestris*. However, sperm depletion among multiply mating males occurred in *B. atratus* and *B. terrestris*. In addition, as a cautionary note, multiple mating may have been induced artefactually in these studies, because all pairings occurred with the bees confined to cages.

After their departure from the nest, males live in the field for up to several weeks, maintaining themselves by feeding at flowers (e.g. Sladen 1912; Alford 1975). Male adult longevity was measured in the field by Alcock & Alcock (1983) and O'Neill *et al.* (1991), who marked individual territory-holders of North American *Bombus* species. They found that some individual males lived up to 26 days. In the laboratory, some *B. terrestris* males lived for 55 days (Duchateau & Mariën 1995).

Duchateau & Mariën 1995), there is no reason to think that their frequency is high in natural populations. For example, Duchateau *et al.* (1994) found no diploid males among the laboratory-reared progeny of 86 wild-caught *B. terrestris* queens. In addition, population genetic studies suggest that bumble bees lack inbreeding (*B. melanopygus*: Owen & Plowright (1980); *B. terrestris*: Estoup *et al.* (1996)).

Summing up, none of the factors discussed in this section seems likely to explain male bias in bumble bee sex investment ratios. I conclude that male bias is not an artefact but a genuine biological phenomenon.

4. MALE BIAS IS ADAPTIVE FOR BUMBLE BEES

The remaining general explanation for male-biased population sex investment ratios in bumble bees is that selection on the bees themselves results in male bias, but that previously the standard Trivers–Hare model has been inappropriately applied. This section therefore discusses factors that, when used to modify the standard model, predict male bias.

An apparent candidate for this type of factor is multiple mating by queens, which lowers the expected level of female bias in the Trivers–Hare model assuming worker control (Bourke & Franks 1995; Crozier & Pamilo 1996). However, even with high mating frequencies, the workers' stable sex investment ratio (as the fraction of investment in females) never falls below 0.5 (e.g. Benford 1978). In addition, current data suggest that queens in most *Bombus* species are singly mated and that, if not, their average mating frequencies are low (below two) (Crozier & Pamilo 1996). Furthermore, a population of *B. melanopygus* exhibited male-biased sex allocation, yet from genetic evidence this species has single mating (Owen & Plowright 1980, 1982, table 1). The rest of this section discusses four more promising hypotheses for why there might be selection on bumble bees for male-biased sex investment.

(*a*) *Worker reproduction*

The standard Trivers–Hare predictions assume, along with monogyny, single queen mating and outbreeding, that workers are sterile. But worker reproduction involving the production of males from unfertilized haploid eggs is well known in bumble bees (e.g. Morse 1982; Plowright & Laverty 1984; Bourke 1988, 1994) and can in theory lead to male bias in population sex investment ratios (e.g. Benford 1978; Boomsma & Grafen 1991). The scale of this effect depends on the fraction of males contributed by workers and on whether workers reproduce (i) in queenright colonies (colonies containing a queen) alone; (ii) in queenless colonies alone; or (iii) in both (table 3).

In case (i), the population sex investment ratio is unaffected by worker reproduction under queen control (table 3). Under worker control, it becomes relatively more male biased, but absolute male bias (a fraction of investment in females less than 0.5) cannot arise (table 3). This is assuming that no one worker can monopolize worker male production within a colony. However, even relaxing this assumption, more than 80% of males must come from a single laying worker for there to be any absolute male bias (table 3). This seems much too high. For example, Owen & Plowright (1982) found that only 39% of all males came from workers in *B. melanopygus* (and they came from both queenright and queenless colonies). In other species, it appears that the fraction of worker-produced males successfully developing and entering the mating pool is far lower (e.g. Richards 1977; Duchateau & Velthuis 1988).

In cases (ii) and (iii), the population sex investment ratio is always male biased under queen control (table 3). However, to generate male bias of the order of 0.3–0.4 (as the fraction of investment in females) requires high levels of worker male production. For example, in case (ii), the population sex investment ratio falls to 0.4 only when the fraction of worker-produced males reaches 67% (table 3). Under worker control in cases (ii) and (iii), the population sex investment ratio is never male biased, unless the fraction of queenless colonies is 50% or more (in which case workers in queenless colonies are producing all the males) (table 3). These values again seem too high. No studies showing male bias of 0.3–0.4 or below reported such high levels of worker reproduction or of orphaning (table 1), and at least one explicitly stated that few worker-produced males were successfully raised (Duchateau & Velthuis 1988).

Owen & Plowright (1982) found that 17 *B. melanopygus* colonies produced 1491 males in total. Of these, 1125 came from queenright colonies and 366 from queenless ones. Of the queenright males, 19% were from workers. These results allow one to test the case (iii) model quantitatively. Applying the formulae in table 3 with the fraction of worker-produced males in queenright colonies (m) = 0.19 and the fraction of males from queenless colonies (β) = 366/1491 = 0.25, the expected population sex investment ratios are 0.47 under queen control and 0.69 (i.e. remaining female biased) under worker control. The actual value found was 0.27 (table 1). In sum, the scale of worker reproduction in bumble bees does not seem great enough to account for the degree of male bias observed in population sex investment ratios under any of the above models.

(*b*) *Local resource competition*

Local resource competition occurs when relatives compete for resources. This represents a violation of the assumption in Fisher's (1930) sex ratio theory that relatives compete at random. Modifying the theory predicts that, when competing relatives are females, there should be male bias in the population sex investment ratio (Clark 1978). The basic reason is that the genetic return on producing females falls with rising investment, in contrast to the case for males (providing these engage in population-wide competition for mates). The exact degree of bias depends on the number of females that a given patch of habitat can support. Theoretically, if a colony queen could only hope to replace herself within her patch, the optimal sex ratio would involve producing a single non-dispersing daughter and as many dispersing males as resources allow (Charnov 1982).

It is therefore conceivable that local resource competition among each colony's female progeny accounts for male-biased sex investment in bumble bees. This has not previously been proposed for these insects, although Bulmer (1983*a*) speculated that local resource competition (for nest sites) accounted for male bias in *Lasioglossum* bees (Hymenoptera: Halictidae). In bumble bees, I suggest that related queens could compete in two ways. The first involves competition over hibernation sites following departure from the natal nest and mating. The second involves competition for nest sites in spring. However, there is little evidence for preferential competition among relatives in either of these contexts. In *B. impatiens*, queens overwinter in short burrows near the natal nest (Plath 1934; Szabo & Pengelly 1973), so competition between sisters for the same overwintering sites is a possibility. However, in most species, queens apparently disperse widely from the natal nest before hibernating (Plath 1934; Free & Butler 1959; Szabo & Pengelly 1973; Morse 1982). Similarly, evidence from marking experiments (Bowers 1985) and observations of migrations by queens (Mikkola 1984) suggest that dispersal in spring is also widespread. This implies that the fierce intraspecific competition for nest sites that is frequently observed among bumble bee queens (e.g. Sladen 1912; Plath 1934; Hobbs 1965, 1967; Richards 1973, 1978) is usually between non-relatives. This idea deserves testing directly by performing genetic analyses (e.g. using microsatellite DNA markers) on the corpses of queens found dead together within early nests.

Another way to test the local resource competition hypothesis is to examine variation in colony sex investment ratios as a function of colony productivity. The hypothesis predicts that colony sex investment ratios should grow more male biased as sexual productivity increases. This is because local resource competition affects an individual colony's sex ratio more strongly as the colony's proportionate contribution to the population's overall female output rises (Frank 1987). A complication is that worker number is likely to covary with colony productivity and may independently affect sex allocation because of queen–worker kin conflict over the sex ratio (Nonacs 1986*a*,*b*). The modified prediction is therefore that the partial correlation of sex investment ratio (proportion of investment in females) and total sexual production, with workforce size held statistically constant, should be negative across colonies (cf. Nonacs

1986*b*). This was tested with the *Bombus* sex ratio data from the studies in table 1.

The results did not support the local resource competition hypothesis (table 4). In eight suitable datasets, the correlations of colony sex investment ratio and colony sexual productivity were positive (significantly so in four cases). This was also true of the partial correlations in all six datasets permitting their calculation (table 4), although in no case was the partial correlation significant. The reason why more productive colonies tended to produce more female-biased sex investment ratios is unclear, although it is suggested below that this could stem from selection for protandry. Note also that the frequency distribution of colony sex investment ratios (figure 1) would be explained at the proximate level if, given the association of female bias and productivity, few colonies achieve a large reproductive output within populations.

The analysis additionally showed that worker number was always positively associated with colony sexual productivity (significantly in four cases) (table 4). In other words, as one would expect, colonies with bigger workforces produced more sexuals. However, there was no consistent association between worker number and colony sex investment ratio when effects of colony productivity were statistically controlled (there were four positive partial correlations of sex ratio and worker number and two negative ones, and none of the correlations was significant) (table 4). Therefore, workers did not appear to gain more control of sex allocation in proportion to their numbers (Nonacs 1986*b*). This finding is consistent with the suggestion that queen bumble bees exert a large degree of sex ratio control (Müller *et al.* 1992; § 1).

(*c*) *Local mate enhancement*

Local mate enhancement involves cooperation for mate acquisition among related males (Packer & Pusey 1987). If this occurs, there are increasing returns on investment in sons, leading again to an expected male bias in the population sex investment ratio. Local mate enhancement could explain male bias in the sex ratios of lions, in which related males form alliances for taking over prides (Packer & Pusey 1987). There is no reason in theory why similar phenomena cannot occur in social insects (Crozier & Pamilo 1993), although this suggestion has not previously been made for bumble bees.

Male bumble bees find mates either by waiting at one spot for a passing queen, or by waiting for emerging females outside foreign nests, or by patrolling flight paths with scent marks placed at intervals to which females are attracted (Sladen 1912; Free & Butler 1959; Michener 1974; Alford 1975; Morse 1982; Free 1987). At least in the second and third of these situations, more than one male may occur together (e.g. Alford 1975; Lloyd 1981). If related males cooperated to obtain matings in either case (for example, by defending from unrelated males a foreign nest with emerging queens or a network of flight circuits), there could be selection for male-biased sex investment ratios through local mate enhancement. However, existing reports of bumble bee mating behaviour describe only male–male competition (e.g. *B. subterraneus*: Alford (1975); *B. fervidus*: Lloyd (1981)). No authors describe cooperation among male subgroups. On the other hand, such behaviour may have been overlooked because it was not expected. The issue could be addressed by detailed behavioural observations of mating sites in the field and genetic analyses of any potentially cooperating males to check if they are related. But the available evidence is against any suggestion that local mate enhancement occurs.

(*d*) *Protandry*

A final explanation for male bias in bumble bee sex investment ratios is that it stems from selection for protandry (the production of adult males before the production of adult females). Wiklund & Fagerström (1977) and Bulmer (1983*b*) showed that, given the right conditions, protandry may evolve through sexual selection. Bulmer (1983*a*) extended these models to annual social Hymenoptera. In Bulmer's (1983*a*) models, one result was male bias in the overall population sex investment ratio (i.e. as measured over the whole season of sexual production). This occurred under queen control for some parameter values (see below); however, other values led to unbiased sex ratios or female bias. Bulmer (1983*a*) explicitly suggested his models as a reason for protandry in bumble bees. Bulmer (1981) had earlier modelled queen–worker conflict over sex allocation and male parentage in annual social Hymenoptera. One conclusion was that protandry could evolve because queens were selected to produce males in the penultimate brood to force the workers to rear the queen's sons rather than their own. However, Bulmer (1983*a*) argued that, instead, protandry first evolved through sexual selection and that it acted only as a 'preadaptation' for the queen to gain greater power over sex allocation and male parentage. Bulmer (1983*a*) did not suggest sexually selected protandry as an explanation for male-biased sex allocation in bumble bees, of which he was apparently unaware. Duchateau & Velthuis (1988) attributed their finding of a class of *B. terrestris* colonies that produce sexuals early and have male-biased sex ratios to selection for protandry, but did not cite Bulmer's (1983*a*) model.

The assumptions of the protandry models are as follows: (i) adult sexuals are released continuously over a long period of time; (ii) females leave the nest and mate once shortly after emergence as adults; and (iii) males are ready to mate shortly after emergence as adults, can mate multiply and can live following emergence for an appreciable period. Under these conditions, protandry evolves because early-emerging males receive more mating opportunities than late-emerging ones, since they are alive over longer periods of female availability (Wiklund & Fagerström 1977; Bulmer 1983*a*,*b*). Evidence exists that non-social insects meeting these conditions, for example some butterflies and solitary Hymenoptera, are protandrous as predicted (Wiklund & Fagerström 1977; Thornhill & Alcock 1983).

In Bulmer's (1983*a*) models (which assumed queen control), protandry also led to male bias in the population sex investment ratio in some circumstances. For example, in one model, protandry and male bias were promoted by a high relative survivorship of early males and by a low relative quality of early females. The ultimate cause of the male bias appeared to be a higher overall relative reproductive value of males stemming from the way their fitness depended on the timing of their production (Bulmer 1983*a*; cf. Werren & Charnov 1978; Seger 1983). In quantitative terms, the absolute lower limit of the expected sex ratio (fraction of females) in the models was 0.25. Bulmer (1983*a*) did not publish results for a worker control version of his models, but one can assume that workers always favour greater relative female bias than the queen because of their higher comparative relatedness with females. Bulmer's (1983*a*) queen control models can clearly generate male bias in the sex investment ratio of the order of the observed levels (table 1). In addition, Bulmer's (1983*a*) assumption of queen control in bumble bees seems largely justified (§ 1).

If the protandry hypothesis applies to bumble bees, then of course their features should fit the models' assumptions. This turns out to be the case (table 5). Specifically, adult sexuals are released over a period of several months; young queens leave the nest within a few days and usually mate once; and males also leave the nest as young adults and thereafter have sufficiently high survivorship to permit them multiple mating opportunities, of which laboratory studies suggest they are capable of taking advantage (table 5).

The next test of the protandry hypothesis is whether in fact bumble bees are generally protandrous. Again, the data suggest that they are. Protandry appears usual in bumble bees, although its extent varies and there is one exception (*B. affinis*, which also lacked a strongly male-biased sex investment ratio) (table 1). Studies reporting protandry aside from those in table 1 include Hobbs (1964) (*B. balteatus*), Richards (1973) (*B. polaris*), Pomeroy & Plowright (1982) (*B. perplexus*), Shelly *et al.* (1991) (*B. pennsylvanicus sonorus*), Foster (1992) (various Canadian *Bombus* species), and Müller *et al.* (1992) (*B. lucorum* and *B. terrestris*, but not *B. terricola*).

Note, however, that protandry could occur at two levels in bumble bees. At the within-colony level, individual mixed-sex producing colonies could produce first males as adults and then females as adults. Alternatively, at the within-population level, colonies producing sexuals early could produce mainly males and colonies producing sexuals late could produce mainly females. An example of within-colony protandry is *B. ruderatus*, in which male adult emergence started before that of females within each of four mixed-sex producing colonies (Pomeroy 1979). An example of protandry at the population level is *B. t. terrestris*. In this case, colonies consisted of 'early-switching' colonies that produced sexuals early and had male-biased sex allocation and 'late-switching' ones that produced sexuals late and had female-biased sex allocation (Duchateau & Velthuis 1988). A similar pattern was found in *B. lucorum* (Müller & Schmid-Hempel 1992*b*). The occurrence of this pattern suggests that, if small and unproductive colonies generally concentrate on male production (table 4, see above), this is not simply because they are short of resources. Rather, it might be a deliberate strategy to grow for a shorter time and produce males protandrously with respect to the rest of the population. Colonies may also opt for protandrous sexual production in the absence of productivity differences, as Duchateau & Velthuis (1988) found that early- and late-switching colonies of *B. t. terrestris* had equal overall sexual productivities.

Summing up, bumble bees meet the assumptions of Bulmer's (1983*a*) sexually selected protandry models and are typically protandrous. These models are also capable of explaining male-biased sex allocation. Therefore, unlike the previous selectionist hypotheses, Bulmer's (1983*a*) models seem a likely possible explanation for male bias in bumble bees.

5. SEX ALLOCATION IN *PSITHYRUS*

Sex ratio evolution in the socially parasitic genus *Psithyrus* provides an important contrast with the situation in *Bombus*. To start with, *Psithyrus* has no worker caste, so worker control cannot occur (Trivers & Hare 1976; Fisher 1992). In addition, population sex investment ratios in *Psithyrus* are consistently female biased (Fisher 1992, table 2), although the present data come from only two species. Therefore, a comprehensive account of bumble bee sex allocation has to explain why *Bombus* and *Psithyrus* differ. For example, *P. ashtoni* (when parasitizing *B. affinis*) had significantly female-biased sex allocation, yet was protandrous (table 2). It therefore represents an apparent counter-example to Bulmer's (1983*a*) protandry model. On the other hand, protandry in *P. ashtoni* may not be sexually selected, but a specific adaptation of the parasite to avoid late-season aggression of the host workers towards male larvae (Fisher 1987).

Fisher (1992) proposed local mate competition as a possible explanation for female-biased sex allocation in *Psithyrus*. Local mate competition represents another violation of assumptions in the standard sex ratio models and typically occurs when related males compete for mates (Hamilton 1967). It predicts female bias because it causes diminishing returns on investment in males. Socially parasitic species would seem particularly prone to local mate competition, because they are necessarily more sparsely distributed than their hosts, and this might encourage within-nest mating (cf. Nonacs 1986*a*). However, at least some *Psithyrus* species have males that mate in the open or along flight paths, or both, just as in *Bombus* males (e.g. Sladen 1912; Free & Butler 1959;

Alford 1975; Morse 1982). So it is not obvious that *Psithyrus* has a mating system conducive to local mate competition, whereas *Bombus* does not.

The occurrence of local mate competition in *Psithyrus* would predict that, as colony sexual productivity among the parasites increases, the parasites' colony sex investment ratio should grow more female biased (Frank 1987). This follows from the same argument, but with reversed effect, as was used in the previous section to predict increasing male bias with greater sexual productivity under local resource competition. However, in *P. ashtoni* (with *B. affinis* as host), there was no significant positive correlation of colony sex investment ratio (fraction of investment in females) with total sexual productivity ($r = 0.051$, d.f. $= 21$, $p > 0.1$). This was also the case for *P. ashtoni* with *B. terricola* as host ($r = -0.140$, d.f. $= 19$, $p > 0.1$). (In this analysis, all data came from Fisher (1992), colony sex investment ratio was angular transformed, colony productivity was $\log_{10}$-transformed and colonies producing five or fewer sexuals were omitted.) Therefore, the data do not support the idea that female bias stems from local mate competition in *Psithyrus*.

A totally different explanation is that the sex investment ratio in *Psithyrus* is largely determined by the *Bombus* hosts. In some cases, for example *P. ashtoni*, the host queen in a colony parasitized by *Psithyrus* may survive and reproduce, and the host workers may also attempt to reproduce (Fisher 1987, 1992). In *P. ashtoni*, Fisher (1992) pointed out that the 'combined' sex investment ratios (i.e. those calculated by including both the parasite and the host sexuals) were similar to the sex investment ratios of the unparasitized hosts. Specifically, in *B. affinis* parasitized by *P. ashtoni*, the host bees contributed 44% of all investment in sexuals; the 'combined' fraction of investment in females was 0.46 and the sex investment ratio of the unparasitized *B. affinis* bees was 0.49. In *B. terricola* parasitized by *P. ashtoni*, the host bees contributed 59% of all investment in sexuals; the 'combined' sex investment ratio was 0.39 and the sex investment ratio of the unparasitized *B. terricola* was 0.32 (tables 1, 2). This suggests that *P. ashtoni* sex ratios represent the 'balance' left when workers rear an overall sex ratio (of hosts and parasites) appropriate to the unparasitized colony (Fisher 1992).

The sex investment ratio among just the *B. affinis* host sexuals in the *P. ashtoni*-parasitized colonies was highly male biased (0.22) and more so than in unparasitized *B. affinis* colonies (0.49) (tables 1, 2). This was consistent with a high level of male production by the host workers arising from the ineffectiveness of the *P. ashtoni* queen in suppressing them (Fisher 1987, 1992). A similar pattern occurred in *B. terricola* parasitized by *P. ashtoni* (table 2). This would automatically lead to female bias in *P. ashtoni* if its sex investment ratio represented just a 'balance' as Fisher (1992) suggested. On the other hand, the production of *Bombus* reproductives in laboratory colonies parasitized by *P. ashtoni* could be partly artefactual (Fisher 1987, 1992), as Plath (1934) found field colonies producing no *Bombus* sexuals. In addition, both *P. ashtoni* (which had host reproduction) and *P. citrinus* (which did not) exhibited female-biased sex allocation (table 2). Furthermore, in *P. ashtoni* parasitizing *B. affinis*, the parasites' sex ratio did not differ significantly in colonies with and without host reproduction (t-test, $t = 0.21$, d.f. $= 21$, $p > 0.8$) (this analysis was not possible in *P. ashtoni* parasitizing *B. terricola*, where nearly all colonies had host reproduction). Therefore, it remains unclear whether reproduction by the hosts truly affects the parasites' sex investment ratio. More field information on colony reproductive output and the mating system is required before the sex allocation strategy of *Psithyrus* bees can be fully understood.

6. CONCLUSIONS

The data and analysis presented in this paper suggest that male bias in the sex investment ratios of *Bombus* bumble bees is a genuine biological phenomenon. Male bias challenges the expectation from Trivers & Hare's (1976) standard sex ratio model for the social Hymenoptera that sex investment ratios should be either even or female biased. I propose that the selectionist hypothesis most consistent with existing information is Bulmer's (1983*a*) idea that male bias stems from sexual selection for protandry.

If correct, this conclusion demonstrates that the relative timing of sexual production can be a key factor in determining sex investment ratios in the social Hymenoptera. This point has been made (for different reasons) for another group of annual social insects, the polistine wasps (Hymenoptera: Polistinae), by Strassmann (1984), Strassmann & Hughes (1986) and Suzuki (1986) (see also Crozier & Pamilo 1996). In addition, since Bulmer's (1983*a*) models assumed queen control of sex allocation, the present conclusion supports the view that queen control is a standard feature of bumble bees, unlike the case in some other social insects (Bourke & Franks 1995; Crozier & Pamilo 1996). This reinforces Trivers & Hare's (1976) argument that the distribution of practical power is an important determinant of the outcome of kin conflicts. On the other hand, bumble bee workers are apparently still able to exert some control over colony reproduction through laying male eggs (e.g. Plowright & Laverty 1984; Bourke 1994).

The above conclusions require additional testing with field data. It needs confirming that, as current evidence suggests, bumble bees fit the assumptions of the protandry hypothesis and are generally protandrous. Next, given this, it needs establishing that the relative survivorships and qualities of the sexes are such as to lead to male-biased sex investment under the protandry models. Any further sex ratio studies of bumble bees should also preferably involve the collection of genetic data on the queen mating frequency, the level of diploid male production and the amount of worker male production in queenright and queenless colonies. They should include a search for local resource competition among females and local mate enhancement among males, if only to eliminate these factors decisively. Laboratory investigations of the possible occurrence of sex ratio distorters in bumble bees would also prove useful. Lastly, the issue of which caste controls sex allocation could be addressed directly by looking for differences in the primary (egg) sex ratio among sexual-destined broods and the adult sex ratio, as has been done revealingly in ants (e.g. Sundström *et al.* 1996). This would help answer the crucial question of whether queens or workers discriminate against brood by sex. Given the ease with which bumble bees may be observed in the field and kept and manipulated in the laboratory, a definitive answer to the puzzles posed by their male-biased sex investment ratios seems entirely attainable.

I thank the following for comments: S. D. Albon, M. Beekman, M. G. Bulmer, M.-J. Duchateau, C. Müller, P. Schmid-Hempel, R. Schmid-Hempel, H. H. W. Velthuis and the anonymous referees.

REFERENCES

Alcock, J. & Alcock, J. P. 1983 Male behaviour in two bumblebees, *Bombus nevadensis auricomus* and *B. griseicollis* (Hymenoptera: Apidae). *J. Zool. Lond.* **200**, 561–570.

Alford, D. V. 1975 *Bumblebees.* London: Davis-Poynter.

Benford, F. A. 1978 Fisher's theory of the sex ratio applied to the social Hymenoptera. *J. Theor. Biol.* **72**, 701–727.

Boomsma, J. J. 1989 Sex-investment ratios in ants: has female bias been systematically overestimated? *Am. Nat.* **133**, 517–532.

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. 1988 Worker reproduction in the higher eusocial Hymenoptera. *Q. Rev. Biol.* **63**, 291–311.

Bourke, A. F. G. 1994 Worker matricide in social bees and wasps. *J. Theor. Biol.* **167**, 283–292.

Bourke, A. F. G. & Franks, N. R. 1995 *Social evolution in ants.* Princeton University Press.

Bowers, M. A. 1985 Bumble bee colonization, extinction, and reproduction in subalpine meadows in northeastern Utah. *Ecology* **66**, 914–927.

Bulmer, M. G. 1981 Worker-queen conflict in annual social Hymenoptera. *J. Theor. Biol.* **93**, 239–251.

Bulmer, M. G. 1983*a* The significance of protandry in social Hymenoptera. *Am. Nat.* **121**, 540–551.

Bulmer, M. G. 1983*b* Models for the evolution of protandry in insects. *Theor. Pop. Biol.* **23**, 314–322.

Charnov, E. L. 1982 *The theory of sex allocation.* Princeton University Press.

Clark, A. B. 1978 Sex ratio and local resource competition in a prosimian primate. *Science* **201**, 163–165.

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

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 and Hall.

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

Duchateau, M. J. & Mariën, J. 1995 Sexual biology of haploid and diploid males in the bumble bee *Bombus terrestris. Insectes Soc.* **42**, 255–266.

Duchateau, M. J. & Velthuis, H. H. W. 1988 Development and reproductive strategies in *Bombus terrestris* colonies. *Behaviour* **107**, 186–207.

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

Estoup, A., Scholl, A., Pouvreau, A. & Solignac, M. 1995 Monoandry and polyandry in bumble bees (Hymenoptera; Bombinae) as evidenced by highly variable microsatellites. *Mol. Ecol.* **4**, 89–93.

Estoup, A., Solignac, M., Cornuet, J.-M., Goudet, J. & Scholl, A. 1996 Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe. *Mol. Ecol.* **5**, 19–31.

Fisher, R. A. 1930 *The genetical theory of natural selection.* Oxford: Clarendon Press.

Fisher, R. M. 1987 Queen-worker conflict and social parasitism in bumble bees (Hymenoptera: Apidae). *Anim. Behav.* **35**, 1026–1036.

Fisher, R. M. 1992 Sex ratios in bumble bee social parasites: support for queen-worker conflict theory? (Hymenoptera: Apidae). *Sociobiology* **20**, 205–217.

Foster, R. L. 1992 Nestmate recognition as an inbreeding avoidance mechanism in bumble bees (Hymenoptera: Apidae). *J. Kans. Entomol. Soc.* **65**, 238–243.

Frank, S. A. 1987 Variable sex ratio among colonies of ants. *Behav. Ecol. Sociobiol.* **20**, 195–201.

Free, J. B. 1987 *Pheromones of social bees.* London: Chapman and Hall.

Free, J. B. & Butler, C. G. 1959 *Bumblebees.* London: Collins.

Garofalo, C. A., Zucchi, R. & Muccillo, G. 1986 Reproductive studies of a Neotropical bumblebee, *Bombus atratus* (Hymenoptera, Apidae). *Rev. Brasil. Genet.* **9**, 231–243.

Grafen, A. 1986 Split sex ratios and the evolutionary origins of eusociality. *J. Theor. Biol.* **122**, 95–121.

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

Hamilton, W. D. 1967 Extraordinary sex ratios. *Science* **156**, 477–488.

Helms, K. R. 1994 Sexual size dimorphism and sex ratios in bees and wasps. *Am. Nat.* **143**, 418–434.

Hobbs, G. A. 1964 Ecology of species of *Bombus* Latr. (Hymenoptera: Apidae) in southern Alberta. I. Subgenus *Alpinobombus* Skor. *Can. Entomol.* **96**, 1465–1470.

Hobbs, G. A. 1965 Ecology of species of *Bombus* Latr. (Hymenoptera: Apidae) in southern Alberta. III. Subgenus *Cullumanobombus* Vogt. *Can. Entomol.* **97**, 1293–1302.

Hobbs, G. A. 1967 Ecology of species of *Bombus* (Hymenoptera: Apidae) in southern Alberta. VI. Subgenus *Pyrobombus. Can. Entomol.* **99**, 1271–1292.

Lloyd, J. E. 1981 Sexual selection: individuality, identification, and recognition in a bumblebee and other insects. *Fla. Entomol.* **64**, 89–118.

Michener, C. D. 1974 *The social behavior of the bees: a comparative study*. Cambridge, MA: Belknap Press of Harvard University Press.

Mikkola, K. 1984 Migration of wasp and bumble bee queens across the Gulf of Finland (Hymenoptera: Vespidae and Apidae). *Notulae Entomologicae* **64**, 125–128.

Morse, D. H. 1982 Behavior and ecology of bumble bees. In *Social insects*, vol. III (ed. H. R. Hermann), pp. 245–322. New York: Academic.

Müller, C. B. & Schmid-Hempel, P. 1992*a* Variation in life-history pattern in relation to worker mortality in the bumble-bee, *Bombus lucorum*. *Funct. Ecol.* **6**, 48–56.

Müller, C. B. & Schmid-Hempel, P. 1992*b* Correlates of reproductive success among field colonies of *Bombus lucorum*: the importance of growth and parasites. *Ecol. Entomol.* **17**, 343–353.

Müller, C. B., Shykoff, J. A. & Sutcliffe, G. H. 1992 Life history patterns and opportunities for queen-worker conflict in bumblebees (Hymenoptera: Apidae). *Oikos* **65**, 242–248.

Nonacs, P. 1986*a* Ant reproductive strategies and sex allocation theory. *Q. Rev. Biol.* **61**, 1–21.

Nonacs, P. 1986*b* Sex-ratio determination within colonies of ants. *Evolution* **40**, 199–204.

O'Neill, K. M., Evans, H. E. & Bjostad, L. B. 1991 Territorial behaviour in males of three North American species of bumblebees (Hymenoptera: Apidae, *Bombus*). *Can. J. Zool.* **69**, 604–613.

Owen, R. E. & Plowright, R. C. 1980 Abdominal pile color dimorphism in the bumble bee, *Bombus melanopygus*. *J. Hered.* **71**, 241–247.

Owen, R. E. & Plowright, R. C. 1982 Worker-queen conflict and male parentage in bumble bees. *Behav. Ecol. Sociobiol.* **11**, 91–99.

Owen, R. E., Rodd, F. H. & Plowright, R. C. 1980 Sex ratios in bumble bee colonies: complications due to orphaning? *Behav. Ecol. Sociobiol.* **7**, 287–291.

Packer, C. & Pusey, A. E. 1987 Intrasexual cooperation and the sex ratio in African lions. *Am. Nat.* **130**, 636–642.

Pamilo, P. 1991 Evolution of colony characteristics in social insects. I. Sex allocation. *Am. Nat.* **137**, 83–107.

Plath, O. E. 1934 *Bumblebees and their ways*. New York: Macmillan.

Plowright, R. C. & Laverty, T. M. 1984 The ecology and sociobiology of bumble bees. *A. Rev. Entomol.* **29**, 175–199.

Plowright, R. C. & Pallett, M. J. 1979 Worker-male conflict and inbreeding in bumble bees (Hymenoptera: Apidae). *Can. Entomol.* **111**, 289–294.

Plowright, R. C. & Plowright, C. M. S. 1990 The laying of male eggs by bumble bee queens: an experimental reappraisal and a new hypothesis. *Can. J. Zool.* **68**, 493–497.

Pomeroy, N. 1979 Brood bionomics of *Bombus ruderatus* in New Zealand (Hymenoptera: Apidae). *Can. Entomol.* **111**, 865–874.

Pomeroy, N. & Plowright, R. C. 1982 The relation between worker numbers and the production of males and queens in the bumble bee *Bombus perplexus*. *Can. J. Zool.* **60**, 954–957.

Prŷs-Jones, O. E. & Corbet, S. A. 1987 *Bumblebees*. Naturalists' Handbooks 6. Cambridge University Press.

Richards, K. W. 1973 Biology of *Bombus polaris* Curtis and *B. hyperboreus* Schönherr at Lake Hazen, Northwest Territories (Hymenoptera: Bombini). *Quaest. Entomol.* **9**, 115–157.

Richards, K. W. 1977 Ovarian development of queen and worker bumble bees (Hymenoptera: Apidae) in southern Alberta. *Can. Entomol.* **109**, 109–116.

Richards, K. W. 1978 Nest site selection by bumble bees (Hymenoptera: Apidae) in southern Alberta. *Can. Entomol.* **110**, 301–318.

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.

Seger, J. 1983 Partial bivoltinism may cause alternating sex-ratio biases that favour eusociality. *Nature* **301**, 59–62.

Schmid-Hempel, P. & Durrer, S. 1991 Parasites, floral resources and reproduction in natural populations of bumblebees. *Oikos* **62**, 342–350.

Shelly, T. E., Buchmann, S. L., Villalobos, E. M. & O'Rourke, M. K. 1991 Colony ergonomics for a desert-dwelling bumblebee species (Hymenoptera: Apidae). *Ecol. Entomol.* **16**, 361–370.

Shykoff, J. A. & Müller, C. B. 1995 Reproductive decisions in bumble-bee colonies: the influence of worker mortality in *Bombus terrestris* (Hymenoptera, Apidae). *Funct. Ecol.* **9**, 106–112.

Shykoff, J. A. & Schmid-Hempel, P. 1991 Parasites delay worker reproduction in bumblebees: consequences for eusociality. *Behav. Ecol.* **2**, 242–248.

Sladen, F. W. L. 1912 *The humble-bee. Its life history and how to domesticate it*. London: Macmillan.

Strassmann, J. E. 1984 Female-biased sex ratios in social insects lacking morphological castes. *Evolution* **38**, 256–266.

Strassmann, J. E. & Hughes, C. R. 1986 Latitudinal variation in protandry and protogyny in polistine wasps. *Monit. Zool. Ital. (N.S.)* **20**, 87–100.

Sundström, L., Chapuisat, M. & Keller, L. 1996 Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. *Science* **274**, 993–995.

Suzuki, T. 1986 Production schedules of males and reproductive females, investment sex ratios, and worker-queen conflict in paper wasps. *Am. Nat.* **128**, 366–378.

Szabo, T. I. & Pengelly, D. H. 1973 The over-wintering and emergence of *Bombus (Pyrobombus) impatiens* (Cresson) (Hymenoptera: Apidae) in southern Ontario. *Insectes Soc.* **20**, 125–132.

Taylor, P. D. 1981 Sex ratio compensation in ant populations. *Evolution* **35**, 1250–1251.

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

Trivers, R. 1985 *Social evolution*. Menlo Park, CA: Benjamin/Cummings.

Trivers, R. L. & Hare, H. 1976 Haplodiploidy and the evolution of the social insects. *Science* **191**, 249–263.

Webb, M. C. 1961 The biology of the bumblebees of a limited area in eastern Nebraska. Ph.D. thesis, University of Nebraska, Lincoln.

Werren, J. H. & Charnov, E. L. 1978 Facultative sex ratios and population dynamics. *Nature* **272**, 349–350.

Werren, J. H., Nur, U. & Wu, C.-I. 1988 Selfish genetic elements. *Trends Ecol. Evol.* **3**, 297–302.

Wiklund, C. & Fagerström, T. 1977 Why do males emerge before females? A hypothesis to explain the incidence of protandry in butterflies. *Oecologia* **31**, 153–158.

Received 20 September 1996; accepted 27 January 1997