THE ROYAL
SOCIETY

doi 10.1098/rspb.2000.1381

Kin-selected conflict in the bumble-bee *Bombus terrestris* (Hymenoptera: Apidae)

A. F. G. Bourke[1]* **and F. L. W. Ratnieks**[2]

[1]*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*
[2]*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK*

Kin selection theory predicts conflict in social Hymenoptera between the queen and workers over male parentage because each party is more closely related to its own male offspring. Some aspects of the reproductive biology of the bumble-bee *Bombus terrestris* support kin selection theory but others arguably do not. We present a novel hypothesis for how conflict over male parentage should unfold in *B. terrestris* colonies. We propose that workers delay laying eggs until they possess information showing that egg laying suits their kin-selected interests. In colonies where queens start to lay haploid eggs early, we hypothesize that this occurs when workers detect the presence of queen-produced male brood in the brood's larval stage. In colonies where queens start to lay haploid eggs late, we hypothesize that it occurs when workers detect a signal from the queen to female larvae to commence development as queens. Our hypothesis accounts for previously unexplained aspects of the timing of reproductive events in *B. terrestris*, provides ultimate explanations for the results of a recent study of mechanisms underlying queen–worker conflict and helps explain this species' characteristic bimodal (split) sex ratios. Therefore, kin selection theory potentially provides a good explanation for reproductive patterns in *B. terrestris*.

Keywords: kin selection; social insect behaviour; sex allocation

1. INTRODUCTION

Kin selection theory shows that potential queen–worker conflict exists over male parentage in social Hymenoptera. In the common case of species with one, singly mated queen per colony, queens are expected to favour their own sons (relatedness, $r = 0.5$) over workers' sons ($r = 0.25$), whereas workers should favour worker-produced males (sons, $r = 0.5$ or nephews, $r = 0.375$) over males produced by the queen (brothers, $r = 0.25$) (Hamilton 1964; Trivers & Hare 1976). Investigating queen–worker conflict over male parentage therefore provides a powerful means of testing kin selection theory (e.g. Trivers & Hare 1976; Ratnieks 1988; Ratnieks & Reeve 1992; Peters *et al.* 1999).

The bumble-bee *Bombus terrestris* provides an excellent system for investigating queen–worker conflict. There is one, singly mated queen per colony and workers can lay haploid, male eggs (Alford 1975; Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000). Colonies are annual, thereby increasing the likelihood of actual conflict because neither queens nor workers have an interest in maintaining the colony for reproduction in later years (Bulmer 1981). Evidence for actual conflict over male parentage in *B. terrestris* in line with kin selection theory is as follows. First, ovary-developed workers in mature colonies attempt to destroy queen-laid eggs and replace them with their own eggs. This leads to physical conflict between workers and the queen and may even result in workers killing the queen (Van Honk *et al.* 1981; Van der Blom 1986; Van Doorn & Heringa 1986; Bourke 1994). Second, the start of worker egg laying and overt queen–worker conflict, i.e. the 'competition point', usually follows the 'switch point', which occurs when the queen stops laying exclusively diploid eggs and starts laying haploid eggs, although some diploid eggs may also be laid after the switch point (Van der Blom 1986; Van Doorn & Heringa 1986; Duchateau & Velthuis 1988). Third, in a recent experimental study of the proximate mechanisms regulating queen–worker conflict in *B. terrestris*, Bloch (1999) showed that adding haploid eggs to colonies accelerated the onset of worker egg laying.

However, several features of the reproductive biology of *B. terrestris* have been suggested not to match the predictions of kin selection theory. The fraction of adult males produced by workers, which is unknown (Beekman & Van Stratum 1998), may be very small because the queen eats many worker-laid eggs (Van Doorn & Heringa 1986; Duchateau & Velthuis 1988; Duchateau 1996). Although the competition point usually follows the switch point, its timing is independent of whether the switch point occurs early or late in colony life and, in some colonies, it occurs three weeks after the switch point, suggesting that the two events are not causally linked (Duchateau & Velthuis 1988). Lastly, in queen-producing colonies the competition point closely follows the laying of the first eggs that will develop into queens (Duchateau & Velthuis 1988; Bloch 1999; Cnaani *et al.* 2000). Experiments have suggested that, before this time, the mother queen produces a pheromone in the presence of which female larvae develop exclusively as workers (Röseler 1970, 1991). Therefore, Duchateau & Velthuis (1988) and Duchateau (1996) proposed that *B. terrestris* workers do not express their kin-selected interests successfully. Instead, these authors argued that, in her own interests, the queen prevents both the development of female larvae as queens and worker egg laying by pheromonal means, but that, towards the end of the colony cycle, the workers' egg laying and aggressive behaviour arise as a side-effect of a general, unexplained loss of dominance by the queen.

In this paper, we propose a novel hypothesis for how queen–worker conflict over male parentage should unfold

---

*Author for correspondence (andrew.bourke@ioz.ac.uk).

Received 31 July 2000 *Accepted* 6 November 2000

© 2001 The Royal Society

Table 1. *Social structure and life-history parameters in* B. terrestris

parameter or stage	value or meaning (reference)
number of queens per colony	one (Alford 1975)
queen mating frequency	single (Estoup *et al.* 1995; Schmid-Hempel & Schmid-Hempel 2000)
colony cycle	annual (only newly produced, mated queens overwinter) (Alford 1975)
caste differences	queens larger than workers and the body size distributions of the castes do not overlap; workers possess ovaries but cannot mate, so can lay only haploid, male eggs (Alford 1975; Röseler 1991)
caste determination	in first- and fourth-instar larvae (Röseler 1991; Cnaani *et al.* 2000)
protandry	present (i.e. males emerge as adults before females): male-specialist colonies (early switchers) produce sexuals before female-specialist ones (late switchers) (Bulmer 1983; Duchateau & Velthuis 1988; Bourke 1997; Beekman & Van Stratum 1998)
split sex ratios	present: half the colonies are early switchers and so specialize in male production (proportion of investment in females is 11–20%) and half are late switchers and so specialize on female production (proportion of investment in females is 59–63%); the relatively few queens produced by early-switching colonies emerge after the males and the relatively few males produced by late-switching colonies emerge after the queens (Duchateau & Velthuis 1988; Beekman & Van Stratum 1998)
switch point	queen starts to lay mainly haploid, male eggs, although some diploid eggs may also be laid after this point; occurs on average ten days after first workers emerge in early-switching colonies and 24 days after in late-switching colonies (Duchateau & Velthuis 1988)
competition point	workers start egg laying, egg eating and aggression; occurs *ca.* 31 days after first workers emerge in both colony types (Duchateau & Velthuis 1988)
immature period	males spend five days as eggs, 13 days as larvae and eight days as pupae, and queens spend five days as eggs, 15 days as larvae and ten days as pupae (Alford 1975; Duchateau & Velthuis 1988; Cnaani *et al.* 1997; Bloch 1999; Ribeiro *et al.* 1999)

during the colony cycle of *B. terrestris*. The hypothesis is that workers begin egg laying when they obtain information that to do so would suit their kin-selected interests. Workers gain this information by either detecting the presence of queen-laid male brood or eavesdropping on signals given by the queen that mark the onset of queen production. This hypothesis provides ultimate reasons for several important yet previously unexplained features of the social biology of *B. terrestris*. These include a number of findings in Bloch's (1999) recent experimental study, as well as those features previously held to challenge kin selection theory (Duchateau & Velthuis 1988; Duchateau 1996). The hypothesis also helps explain bimodal (split) sex ratios in *B. terrestris* (Duchateau & Velthuis 1988; Bourke 1997; Beekman & Van Stratum 1998). Our conclusion is that *B. terrestris* could conform to kin selection theory more strongly than previously realized.

2. THE SOCIAL CONTEXT OF CONFLICT

Conflict in any species takes place in a social context that is defined by a variety of factors. Two important aspects in *B. terrestris* concern the schedule of sexual (male and new queen) production and the system of caste determination (table 1 and figure 1). To begin with, colonies within populations are bimodal with respect to both the timing of sexual production and the sex ratio. In approximately half the colonies (early switchers) queens start to lay haploid eggs (at the switch point) relatively early in the colony cycle and colonies produce mainly males. In the other half (late switchers) queens start to lay haploid eggs relatively late and colonies produce mainly queens that emerge later than the males produced by early-switching colonies (Duchateau & Velthuis 1988; Müller *et al.* 1992; Beekman *et al.* 1998). The competition point occurs at similar times in the colony cycle in both colony types (Duchateau & Velthuis 1988) (table 1). The total sexual biomass produced is equal in the two colony types and whether a colony is an early- or late-switcher appears to be under the control of the queen (Duchateau & Velthuis 1988). As Bourke (1997) described, the likely explanation for protandry (emergence of males before females) in this system comes from a model by Bulmer (1983). Protandry evolves because early-emerging males achieve greater mating success than late-emerging males since they are present in the mating pool for a longer period of female availability. However, although accounting for protandry, Bulmer's (1983) model leaves the reason why colonies have split sex ratios unexplained, since it is conceivable that protandry could coexist with unimodal sex allocation (every colony could produce both sexes, with males emerging before females). We suggest a reason for split sex ratios below (see § 5).

Caste in *B. terrestris* is determined during two critical periods in the development of female larvae (table 1). First, when larvae are two to four days old following hatching from the egg, an irreversible determination towards worker development occurs provided the colony queen is present and the colony has not reached the competition point (Röseler 1970, 1991; Cnaani *et al.* 1997, 2000). Second, in late-switching colonies, larvae aged two to four days around the competition point develop into queens provided they also receive above-average amounts of food in their final (fourth) instar (Röseler 1991; Cnaani & Hefetz 1996; Pereboom 1997, 2000; Ribeiro *et al.* 1999). Such larvae receive extra feeding because of the high worker numbers (and, hence, high worker-to-larva ratio) in mature colonies (Duchateau & Velthuis 1988). The combined result of the sexual production schedule and the caste determination system is that, within populations, most new queens are produced by late-switching colonies in a discrete

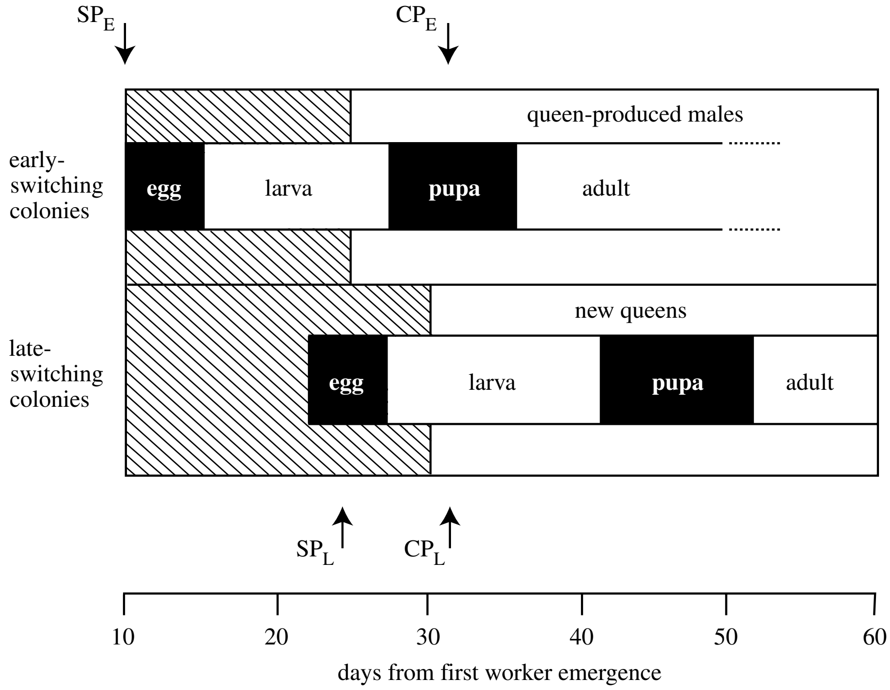

Figure 1. Schematic diagram of the development of the queen's first-laid, sexual-yielding eggs in relation to colony events in early-switching (subscript E) and late-switching (subscript L) colonies of *B. terrestris*. The durations of the successive stages of male and queen brood (egg, larva and pupa) are taken from the references in table 1. The timings (vertical arrows) of the switch points (SP) (first haploid eggs laid by queen) and competition points (CP) (start of worker egg laying and aggression) are taken from Duchateau & Velthuis (1988) (see also table 1). The termination of the colony cycle (beyond day 60) is not shown. We hypothesize that the queen should lose her monopoly of colony reproduction (whose duration is indicated by the hatched areas) following workers' detection of queen-produced, male larvae in early-switching colonies (when the first male larvae are up to ten days old) and their detection of the queen's signal (cessation of pheromone production) to female larvae to commence development as queens in late-switching colonies (when the first queen-destined, female larvae are two to four days old). These hypothesized events are closely followed by the observed competition point in each colony type. *Bombus terrestris* populations may vary in the timing of the switch point in early-switching colonies, the timing of the laying of queen-yielding eggs or the developmental periods of brood (Duchateau & Velthuis 1988; Cnaani *et al.* 1997; Beekman & Van Stratum 1998; Bloch 1999). If so, according to our hypothesis, the predicted timing of the competition points will require modifying accordingly.

reproductive phase starting shortly before the competition point (Duchateau & Velthuis 1988). Most of these queens appear to arise from a single cohort of diploid brood, because the peak of adult queen emergence is sharp (Beekman & Van Stratum 1998).

There is considerable experimental evidence that caste determination in young female larvae of *B. terrestris* is mediated by a non-volatile pheromone produced by the queen and distributed by the workers (earlier evidence reviewed by Michener 1974; Wheeler 1986; Röseler 1991), although such a pheromone has not been chemically characterized. For example, Röseler (1970) and Cnaani *et al.* (1997) found that female larvae younger than the critical age (approximately five days) developed into workers in the presence of a queen during the colony's ergonomic (worker-producing) phase but developed as queens if the colony was made queenless. Larvae separated from the queen by a fine screen developed into queens unless workers were regularly transferred from the queen's side of the screen to the queenless side (Röseler 1970). Duchateau & Velthuis (1988) and Röseler (1991) therefore inferred that queen development occurs in unmanipulated colonies because the colony queen ceases her pheromone production at approximately the time of the competition point due to a 'loss of dominance'. Recent work has confirmed that young female larvae experimentally reared with a queen taken from a colony before its competition point developed into workers, whereas those reared with a queen taken from a colony after its competition point developed into queens, and this result was attributed to the existence of a queen-produced pheromone influencing caste (Cnaani *et al.* 2000).

We follow Duchateau & Velthuis (1988) and Röseler (1991) in inferring that the determination of young female larvae as queens occurs as a result of cessation of pheromone production by the colony queen but, unlike them, do not interpret this as the failure of her direct pheromonal inhibition of queen development. This is because (i) in ultimate terms, the colony queen would not benefit from preventing the production of daughter queens in queen-producing colonies, and (ii) queen-produced pheromones are unlikely to be direct inhibitors of the reproductive interests of nest-mates, their effects being unenforceable, and are more likely to be signals that benefit both senders and receivers (Seeley 1985; Keller & Nonacs 1993). We have therefore previously suggested that the queen's cessation of pheromone production is a signal to female larvae to develop into queens in their own and the queen's interests (Bourke & Ratnieks 1999). Duchateau & Velthuis (1988) argued that the queen's cessation of pheromone production also released workers from direct pheromonal inhibition of egg laying, thereby explaining why the competition point closely follows the laying of the first eggs to develop as queens. Again, for the reasons advanced by Seeley (1985) and Keller & Nonacs (1993), we doubt that the queen's pheromone directly inhibits worker egg laying. Bloch *et al.* (1996), Bloch & Hefetz (1999) and Bloch (1999) also disputed the existence of direct pheromonal inhibition by the queen of worker egg laying on experimental grounds. We propose an alternative reason for the link between the competition point and queen production in § 3.

3. A REINTERPRETATION OF QUEEN–WORKER CONFLICT IN *B. TERRESTRIS*

(a) *Worker interests in early-switching colonies*

Consider the interests of workers in early-switching, male-specialist colonies. Based solely on relatedness, workers should favour worker-produced ($r = 0.375–0.5$) over queen-produced males ($r = 0.25$). Why then does the competition point not occur immediately after the switch point, instead being delayed by *ca.* 21 days (Duchateau & Velthuis 1988) (table 1)?

We hypothesize that workers require, but do not have, exact information regarding the timing of their colony's switch point. Workers require this information because to start laying male eggs too early in the colony cycle would be costly to them because it would reduce the extent of colony growth (in both colony types) and divert resources from the production of valuable full sisters (in colonies headed by late-switching queens). We hypothesize that workers lack the required information for several reasons. First, we suggest that workers cannot discriminate whether their colony queen will be an early switcher or a late switcher. This is plausible because the two colony types initially grow at similar rates (Duchateau & Velthuis 1988). Second, the exact time at which the switch point occurs varies within each colony type (Duchateau & Velthuis 1988), making any attempt by workers simply to guess the timing of the switch point liable to error. Third, the queen herself is not expected to signal the occurrence of the switch point to the workers because concealing it makes workers more likely to rear her sons (see below). Fourth, although there is weak evidence that *B. terrestris* queens can discriminate between older larvae by sex (Fisher & Pomeroy 1990), it seems improbable that workers can recognize the sex of eggs or very young larvae when neither queens nor the eggs and larvae have an interest in signalling their sex (Nonacs & Carlin 1990; Beekman 1998; Beekman & Van Stratum 1998). Ayasse *et al.* (1999) found that queen- and worker-laid eggs of *B. terrestris* differed in the composition of their surface chemicals, but they did not examine whether queen-laid, male eggs differed from queen-laid, worker-destined eggs in this way. In *Formica exsecta* ants, workers only eliminated queen-produced males (to bias sex allocation towards females) when the males were late-stage larvae (Chapuisat *et al.* 1997), suggesting that late-occurring discrimination of brood by sex in the context of kin-selected conflicts may be general in social Hymenoptera.

For these reasons, we propose that the earliest stage at which *B. terrestris* workers can detect that the switch point has occurred is when the first queen-laid, male eggs have become older larvae, which would be at least nine to ten days after the switch point (five days before eggs hatch plus four to five days as larvae) (table 1). Workers' detection of the switch point may occur even later than this, since human observers can only readily distinguish larvae by sex in their final instar (Duchateau & Van Leeuwen 1990) and the rates at which workers feed worker and male larvae only diverge appreciably after the tenth day of larval life (Ribeiro *et al.* 1999). This implies that workers detect the presence of male brood *ca.* 15 days after the queen lays the first male eggs.

When they detect the switch point, the decision facing workers is therefore whether to replace the queen's male larvae with their own eggs. This decision will be affected by the consideration that the timing of a male's emergence, as well as relatedness, determines his value to workers (cf. Beekman & Van Stratum 1998). As the occurrence of protandry shows, the mating success of males almost certainly declines over time (Bulmer 1983). If the rate at which male mating success declines is high over the period that workers take to detect and respond to the switch point, workers will not be selected to replace the queen-produced, male larvae when they first detect their presence. The replacement, worker-produced males would emerge later than the replaced, queen-produced males and, thus, be less valuable. Although the rate at which male mating success declines with time is unknown, the fact that the peak of early male emergence is sharp (Beekman & Van Stratum 1998) suggests it is high. After workers have retrospectively detected the switch point, the decision facing them becomes whether to replace any new, queen-laid, male eggs with their own eggs. Since the replacement males would not now be later emerging than those they replace, the decline in male mating success over time no longer affects the workers' decision. Therefore, as they are more closely related to their own sons and nephews, workers should start to lay eggs and this is what is observed.

In short, we propose that the competition point follows the switch point after a delay because workers require time to detect the presence of haploid brood (probably at least nine to ten days and perhaps as much as 15 days after the switch point). Queen-produced, male larvae that are present when detection occurs are not replaced because they enjoy a future mating advantage. We cannot at present quantitatively explain the full magnitude of the delay between the switch and competition points (*ca.* 21 days) because both the rate of decline in male mating success and the exact age of brood at which workers can detect its sex are unknown. However, if it is correct that workers first detect male brood 15 days after the switch point, over 70% of the gap between the switch and competition points would be accounted for (15 out of 21 days). The extra delay in the competition point is not due to workers having to activate their ovaries, because dissections have shown that activation occurs before the switch point (Duchateau & Velthuis 1989).

After they detect the switch point, another factor affecting workers' reproductive decisions is the cost of worker reproduction. First, workers must avoid damaging the developing cohort of queen-produced males (cf. Beekman & Van Stratum 1998). Since the competition point occurs when most of these males have become pupae (table 1), it is likely such damage is largely avoided. Second, workers are only selected to replace queen-laid, male eggs provided the costs to overall productivity are not too high (Cole 1986). Such costs arise in *B. terrestris* because of conflict between workers over the parentage of male eggs, which leads to worker–worker aggression and egg eating in colonies after the competition point (Röseler & Röseler 1977; Van Honk *et al.* 1981; Bloch & Hefetz 1999). However, as Cole (1986) showed, some level of cost to overall productivity (in his models up to 20%) need not impede the evolution of worker male production. Given that such costs would fall with a later start of worker egg laying, since reproductive workers would then have a progressively smaller effect on overall colony output, the presence of these costs may also contribute to the delay in the onset of the competition point.

(b) *Worker interests in late-switching colonies*

Consider next the interests of workers in late-switching, female-specialist colonies. Why does the competition point predictably follow the initiation of queen production (laying of first eggs destined to become queens) by seven to ten days (Duchateau & Velthuis 1988; Bloch 1999)?

We hypothesize that workers detect or eavesdrop on the colony queen's signal (cessation of pheromone production) to female larvae that determination towards the queen developmental pathway can commence. Workers are then selected to start egg laying as, with respect to relatedness, workers in late-switching colonies value the colony queen only as a producer of new queens (full sisters, $r = 0.75$), since they are more closely related to worker-produced males than to the queen's sons. In addition, given that half the colonies are early-switching male specialists (table 1), both queens and workers agree that late-switching colonies should concentrate on female production (see § 5). Therefore, once workers have learned from the queen's signal that most of the diploid larvae that will yield queens have embarked on development as queens, workers' interests lie in producing their own male offspring. Workers should be able to detect the queen's signal with relatively little delay since the queen benefits from signalling to the larvae unambiguously. Female larvae receiving the signal react by changing to the queen developmental pathway when aged two to four days after hatching (see § 2) and their egg stage lasts five days (table 1). The sum of these periods is seven to nine days. Therefore, our hypothesis explains both why the competition point invariably follows the laying of the first eggs destined to be queens and almost all of the duration of the gap (seven to ten days) between these events.

Our hypothesis assumes that worker egg laying and aggression after the competition point do not greatly harm the developing queen-destined larvae (cf. Cole 1986). This assumption appears justified (Duchateau & Velthuis 1988). It also predicts that workers should react differently to queens before and after the competition point. This prediction appears contradicted by experimental findings that queens taken from colonies after the competition point inhibited worker ovarian development as effectively as queens taken before it (Bloch *et al.* 1996). However, the experimental groups of workers in this study were small (three to ten workers), consisted of callow (young) workers and lacked developing female brood. This meant that important aspects of the social context necessary for the workers' response (large colony size, experienced workers and female larvae) may have been lacking.

(c) *Summary, assumptions and forerunners of the hypothesis*

In summary, our overall hypothesis is that *B. terrestris* workers only value the colony queen as an early-male producer, conditionally on the queen being able to exploit the workers' delay in detecting the switch point, and unconditionally as a producer of new queens. Once workers have information that the queen has lost her value to them with respect to either of these attributes (after workers detect the switch point in early-switching colonies and after they detect the point of caste determination in late-switching colonies), workers are selected to lay their own male eggs (figure 1). This hypothesis assumes that workers have a non-zero, if low, chance of producing adult males that obtain successful matings despite these males' late emergence. However, the number of such males need not be high. It is only necessary that worker-produced males are sufficiently numerous and successful for the workers' fitness pay-off to exceed that from the workers' alternative courses of action. These alternatives are to rear less closely related, queen-produced males in early-switching colonies and, effectively, to do nothing in late-switching colonies, since most queen-destined brood is produced in a single batch and colonies are annual.

Several previous authors have proposed hypotheses resembling ours. First, Beekman (1998) also argued that workers' behaviour should depend on the colony type. She proposed that workers in male-specialist colonies should be more inclined to lay male eggs than in female-specialist ones, in which workers should prevent one another's reproduction by worker policing (Ratnieks 1988). However, this suggestion does not explain the delay in egg laying by workers in male-specialist colonies or the high frequency of worker egg laying in female-specialist ones. Second, Bloch (1999) supported the idea that common social mechanisms affect both the timing of the competition point and of queen production, although he did not provide an ultimate explanation for this link. Third, Cnaani *et al.* (2000) have recently independently proposed that workers perceive the onset of queen development (possibly via changes in the queen's pheromone) and react by starting to lay eggs. However, these authors suggested a different ultimate explanation for the workers' behaviour to ours, namely that workers use the onset of queen development to infer the impending end of the colony and, hence, their last opportunity to reproduce. We favour our explanation because that of Cnaani *et al.* (2000) does not explain why workers do not reproduce earlier. We attribute this to the argument that reproduction before the time when queen-destined brood have started their development is not in workers' kin-selected interests, thus explaining the tight, observed link between the onset of queen production and the competition point.

Table 2. *Results of Bloch's (1999) study of* B. terrestris *and present authors' ultimate interpretation*

finding number (Bloch's (1999) observation or manipulation)	Bloch's (1999) result	our interpretation of result with respect to kin conflict hypothesis
finding 1 (observation of unmanipulated colonies)	the competition point preceded the switch point in 4–9% of colonies; when the switch point occurred first, its timing was significantly but weakly correlated with the timing of the competition point, accounting for only 22% of the variation in the timing of the competition point	supports hypothesis: the correlation in the timing of the switch and competition points was not stronger because, across early-switching colonies, workers vary in their ability to detect the switch point and, in late-switching colonies, the onset of queen production is the prime determinant of the timing of the competition point; the competition point preceding the switch point is due to worker error
finding 2 (observation of unmanipulated colonies)	the timing of the competition point was most strongly correlated with the onset of queen production	supports hypothesis: workers eavesdrop on the queen's signal to female larvae to commence development as queens and use it as a cue to start egg laying
finding 3 (experimental doubling of worker number prior to the switch point; worker number kept constant in controls)	the fecundity of the colony queen and the timing of the switch point were unaffected; the onset of queen production and the competition point occurred significantly earlier relative to controls	supports hypothesis: the queen responds to the increase in colony size by signalling to female larvae to develop as queens earlier than in control colonies, so bringing the competition point forward
finding 4 (experimental replacement of colony queen's eggs prior to the switch point with haploid eggs laid by a donor queen; replacement eggs were diploid in controls)	the colony queen's fecundity and the timing of her switch point were again unaffected; the onset of queen production and the competition point again occurred significantly earlier relative to controls	supports hypothesis, as Bloch (1999) suggested: workers in colonies receiving male eggs perceive an earlier switch point, bringing forward the competition point as the hypothesis predicts; why queen production started earlier needs explaining too—conceivably, early-switching queens wait a set time between the switch point and laying their first (few) queen-yielding eggs, so if queens perceive an earlier switch point, queen production is brought forward
finding 5 (experimental manipulation as in finding 4)	in control colonies, the worker-to-larva ratio accounted for a significant fraction (50%) of the variation in the timing of the competition point (high worker-to-larva ratio was associated with an earlier competition point), whereas in colonies receiving male eggs this ratio had no significant effect on the timing of the competition point	supports hypothesis: the worker-to-larva ratio can only strongly influence the timing of the onset of queen production and, hence, have a large effect on the timing of the competition point, in colonies receiving female eggs

4. APPLICATION TO BLOCH'S (1999) FINDINGS

Bloch (1999) performed both observations and manipulations of *B. terrestris* colonies in order to investigate the proximate mechanisms underlying the timing of the competition point. In this section, we use Bloch's findings as data with which to test our hypothesis. We argue that they support the hypothesis and, hence, the application of kin selection theory to the reproductive behaviour of *B. terrestris* (table 2).

To begin with, Bloch found a significant correlation between the timing of the switch point and the competition point in unmanipulated colonies. This finding was as predicted by kin selection theory (workers react to the queen's all-male production by egg laying), as Bloch himself noted. However, Bloch suggested that the weakness of the observed correlation needed explaining (finding 1 in table 2) and attributed it to an effect of colony size on worker behaviour (see below). Our hypothesis in the previous section suggests another, not mutually exclusive explanation. In early-switching colonies there is unpredictability over when the switch point occurs and variation across colonies in the workers' ability to detect it, and in late-switching colonies the more immediate determinant of the competition point is the time at which female larvae commence development as queens (table 2). Variation across colonies in the workers' ability to detect the switch point would also account for the small minority of cases in which the competition point preceded the switch point (finding 1 in table 2) since it implies that workers should occasionally make errors in their assessment of their colony's state (cf. Ratnieks 1991; Sundström 1994).

Next, as § 3 showed, our hypothesis provides an ultimate explanation for the strong correlation in unmanipulated, late-switching colonies between the timing of the initiation of queen production and the competition point, as well as for the length (seven to ten days) of the delay between these events (finding 2 of Bloch 1999) (see also Duchateau & Velthuis 1988). Regarding Bloch's (1999) finding 3, we propose that the primary effect of experimentally doubling worker number was to cause the colony queen to signal earlier to female larvae that determination towards the queen developmental pathway should occur. This would account for both the observed earlier onset of queen production, which was left unexplained by Bloch (1999) and, as a consequence, according to the prediction of our hypothesis, the earlier competition point. Our explanation makes the plausible assumption that the queen assesses her colony's position in the annual cycle from the number of workers. If so, doubling worker number causes the queen to perceive an earlier attainment of the critical colony size for sexual production and, hence, to give her signal earlier. This effect would be reinforced by the fact that, at the proximate level, a high worker-to-larva ratio is needed for queen production because queen larvae require extra feeding (see § 2). Lastly, this reasoning also explains Bloch's (1999) finding 5, since only in colonies receiving female eggs could the worker-to-larva ratio have strongly influenced the timing of the onset of queen production and, hence, have had a large effect on the timing of the competition point (table 2).

5. SEX RATIO EVOLUTION IN *B. TERRESTRIS*

Our hypothesis for the timing of worker egg laying (see § 3) also helps explain patterns of sex allocation in *B. terrestris*. Helms (1999) recently investigated an ant (*Pheidole desertorum*) in which queens can lay worker-biased diploid eggs. Half of the colonies were male specialists and half were female specialists and the population sex investment ratio was at the queen optimum of 1:1. The colonies did not differ in either productivity or the workers' relatedness asymmetry, so split sex ratios could not be attributed to these factors (Crozier & Pamilo 1996). Helms (1999) proposed that with a probability of 0.5 (that is, in half the colonies) queens achieve control of sex allocation by choosing to lay only worker-determined eggs and haploid eggs, thus forcing the sterile workers to raise males. This means that, in the other half of the colonies, the optimum sex ratio for both workers and the queen is all females (Pamilo 1982; Boomsma & Grafen 1991). In short, queens achieve their optimal population sex investment ratio by having a means of effectively preventing queen production in half the colonies. This provides an explanation for the observed bimodality in *P. desertorum* colony sex ratios.

We suggest that sex ratio evolution in *B. terrestris* resembles that proposed for *P. desertorum* in both its outcome and biological foundations, with the difference being that *B. terrestris* also exhibits protandry and worker egg laying. Regarding the outcome, *B. terrestris* populations also contain approximately equal frequencies of male- and female-specialist colonies that do not differ in sexual productivity or relatedness asymmetry (see § 2). The population sex investment ratio is in some cases male-biased and in others not significantly different from 1:1 (Bourke 1997; Beekman & Van Stratum 1998). Regarding the underlying biological conditions, we propose that, in line with our hypothesis in § 3, *B. terrestris* queens prevent queen production in half the colonies by opting for an early switch point. This presents workers with a brood of early males that, by the time workers detect them, cannot profitably be replaced with workers' sons. Replacing the queen's sons with new queens is also not feasible for workers because the colony queen has power over queen production through switching to laying haploid eggs. In short, *B. terrestris* queens have a means of ensuring all-male broods analogous to that of *P. desertorum*. Therefore, a queen strategy of adopting an early switch point with a probability of 0.5 would cause the queen and workers in the other half of the colonies to maximize fitness by agreeing on all-female production and, thus, could spread in the population because it would lead to the queens' optimal population sex ratio. As in *P. desertorum*, it would also result in split sex allocation. This explanation for split sex ratios resembles a suggestion by Bulmer (1981) that queens in annual colonies may coerce workers into raising queen-laid males by restricting the supply of diploid eggs in the penultimate generation, although Bulmer (1981) did not use this idea to account for split sex ratios explicitly.

Bulmer (1983) modelled sex allocation under queen control and protandry and predicted population male bias for some parameter values. This could be why population sex investment ratios in *B. terrestris* (and other *Bombus* species) sometimes exhibit male bias (Bourke 1997). Worker male production potentially also contributes to male-biased population sex investment ratios in *B. terrestris* (Bourke 1997; Beekman & Van Stratum 1998). However, although we have argued that an above-zero chance of producing adult sons has a strong effect on worker behaviour, the degree of worker male production may still not achieve the relatively high levels that sex ratio models predict are needed to affect sex investment ratios appreciably (Crozier & Pamilo 1996; Bourke 1997).

6. CONCLUSION

We conclude that kin selection theory potentially provides a detailed explanation for a suite of previously puzzling features of the social biology of *B. terrestris*, some of which have been held to challenge the theory. Both the assumptions and predictions of our hypothesis for how conflict unfolds in *B. terrestris* (see § 3) require testing and are testable experimentally in *B. terrestris* and comparatively by reference to other *Bombus* species. For example, based on existing evidence, the hypothesis assumes a queen-produced pheromone influencing caste determination in young female larvae. It should therefore be possible to isolate and identify and then perform bioassays with this pheromone. Our hypothesis predicts that the addition of male larvae to early-switching *B. terrestris* colonies should accelerate the onset of the competition point more than the addition of haploid eggs (because workers should assess the switch point as having occurred earlier). It also predicts that, if worker number is experimentally increased, late-switching queens cease pheromone production (so signalling to female larvae to develop as queens) earlier than in unmanipulated colonies. Some *Bombus* species lack determination to the queen developmental pathway in early-instar female larvae and, therefore, probably lack a queen-produced, caste-signalling pheromone. Instead, caste is likely to be determined solely by the amount of larval food (Röseler 1991). In these species, we predict that there should be no tight correlation between the laying of the first eggs that will become queens and the outbreak of worker aggression, because workers lack the information available to them in *B. terrestris*. Finally, our analysis of worker behaviour in *B. terrestris* underscores an important general point in social evolution, namely that the outcome of kin-selected conflict should depend on asymmetries in the level of power and information available to opposing parties (Trivers & Hare 1976; Ratnieks 1998; Bourke & Ratnieks 1999).

We thank the following for comments: Madeleine Beekman, Marie José Duchateau, Jeremy Field, Laurent Keller and an anonymous referee. We dedicate this paper to the memory of W. D. Hamilton, to whose genius all students of social behaviour and the social insects are indebted.

REFERENCES

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

Ayasse, M., Birnbaum, J., Tengö, J., Van Doorn, A., Taghizadeh, T. & Francke, W. 1999 Caste- and colony-specific chemical signals on eggs of the bumble bee, *Bombus terrestris* L. (Hymenoptera: Apidae). *Chemoecology* **9**, 119–126.

Beekman, M. 1998 Reproductive strategies in bumblebees: within colony conflicts and cooperation. In *Social insects at the turn of the millennium* (ed. M. P. Schwarz & K. Hogendoorn), p. 52. Adelaide, Australia: XIII Congress of IUSSI.

Beekman, M. & Van Stratum, P. 1998 Bumblebee sex ratios: why do bumblebees produce so many males? *Proc. R. Soc. Lond.* B **265**, 1535–1543.

Beekman, M., Lingeman, R., Kleijne, F. M. & Sabelis, M. W. 1998 Optimal timing of the production of sexuals in bumblebee colonies. *Entomol. Exp. Appl.* **88**, 147–154.

Bloch, G. 1999 Regulation of queen–worker conflict in bumble-bee (*Bombus terrestris*) colonies. *Proc. R. Soc. Lond.* B **266**, 2465–2469.

Bloch, G. & Hefetz, A. 1999 Regulation of reproduction by dominant workers in bumblebee (*Bombus terrestris*) queenright colonies. *Behav. Ecol. Sociobiol.* **45**, 125–135.

Bloch, G., Borst, D. W., Huang, Z.-Y., Robinson, G. E. & Hefetz, A. 1996 Effects of social conditions on juvenile hormone mediated reproductive development in *Bombus terrestris* workers. *Physiol. Entomol.* **21**, 257–267.

Boomsma, J. J. & Grafen, A. 1991 Colony-level sex ratio selection in the eusocial Hymenoptera. *J. Evol. Biol.* **4**, 383–407.

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

Bourke, A. F. G. 1997 Sex ratios in bumble bees. *Phil. Trans. R. Soc. Lond.* B **352**, 1921–1933.

Bourke, A. F. G. & Ratnieks, F. L. W. 1999 Kin conflict over caste determination in social Hymenoptera. *Behav. Ecol. Sociobiol.* **46**, 287–297.

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.

Chapuisat, M., Sundström, L. & Keller, L. 1997 Sex-ratio regulation: the economics of fratricide in ants. *Proc. R. Soc. Lond.* B **264**, 1255–1260.

Cnaani, J. & Hefetz, A. 1996 The effect of social environment in the colony on caste determination and JH synthesis in *Bombus terrestris* larvae. In *Proceedings of the XXth International Congress of Entomology*, p. 390. Florence, Italy.

Cnaani, J., Borst, D. W., Huang, Z. Y., Robinson, G. E. & Hefetz, A. 1997 Caste determination in *Bombus terrestris*: differences in development and rates of JH biosynthesis between queen and worker larvae. *J. Insect Physiol.* **43**, 373–381.

Cnaani, J., Robinson, G. E., Bloch, G., Borst, D. & Hefetz, A. 2000 The effect of queen–worker conflict on caste determination in the bumblebee *Bombus terrestris*. *Behav. Ecol. Sociobiol.* **47**, 346–352.

Cole, B. J. 1986 The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behav. Ecol. Sociobiol.* **18**, 165–173.

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

Duchateau, M. J. 1996 Is kin conflict expressed in the colony cycle of the bumble bee *Bombus terrestris*? In *Proceedings of the XXth International Congress of Entomology*, p. 404. Florence, Italy.

Duchateau, M. J. & Van Leeuwen, P. 1990 Early sex determination in larvae of *Bombus terrestris*. *Insectes Soc.* **37**, 232–235.

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

Duchateau, M. J. & Velthuis, H. H. W. 1989 Ovarian development and egg laying in workers of *Bombus terrestris*. *Entomol. Exp. Appl.* **51**, 199–213.

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.

Fisher, R. M. & Pomeroy, N. 1990 Sex discrimination and infanticide by queens of the bumble bee *Bombus terrestris* (Hymenoptera: Apidae). *Anim. Behav.* **39**, 801–802.

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

Helms, K. R. 1999 Colony sex ratios, conflict between queens and workers, and apparent queen control in the ant *Pheidole desertorum*. *Evolution* **53**, 1470–1478.

Keller, L. & Nonacs, P. 1993 The role of queen pheromones in social insects: queen control or queen signal? *Anim. Behav.* **45**, 787–794.

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

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. & Carlin, N. F. 1990 When can ants discriminate the sex of brood? A new aspect of queen–worker conflict. *Proc. Natl Acad. Sci. USA* **87**, 9670–9673.

Pamilo, P. 1982 Genetic evolution of sex ratios in eusocial Hymenoptera: allele frequency simulations. *Am. Nat.* **119**, 638–656.

Pereboom, J. J. M. 1997 'While they banquet splendidly the future mother': the significance of trophogenic and social factors on caste determination and differentiation in the bumblebee *Bombus terrestris*. PhD thesis, University of Utrecht, The Netherlands.

Pereboom, J. J. M. 2000 The composition of larval food and the significance of exocrine secretions in the bumblebee *Bombus terrestris*. *Insectes Soc.* **47**, 11–20.

Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. L., Roubik, D. W. & Strassmann, J. E. 1999 Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proc R. Soc. Lond.* B **266**, 379–384.

Ratnieks, F. L. W. 1988 Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am. Nat.* **132**, 217–236.

Ratnieks, F. L. W. 1991 Facultative sex allocation biasing by workers in social Hymenoptera. *Evolution* **45**, 281–292.

Ratnieks, F. L. W. 1998 Conflict and cooperation in insect societies. In *Social insects at the turn of the millennium* (ed. M. P. Schwarz & K. Hogendoorn), pp. 14–17. Adelaide, Australia: XIII Congress of IUSSI.

Ratnieks, F. L. W. & Reeve, H. K. 1992 Conflict in single-queen Hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J. Theor. Biol.* **158**, 33–65.

Ribeiro, M. F., Velthuis, H. H. W., Duchateau, M. J. & Van der Tweel, I. 1999 Feeding frequency and caste differentiation in *Bombus terrestris* larvae. *Insectes Soc.* **46**, 306–314.

Röseler, P.-F. 1970 Unterschiede in der Kastendetermination zwischen den Hummelarten *Bombus hypnorum* und *Bombus terrestris*. *Z. Naturforsch.* **25b**, 543–548.

Röseler, P.-F. 1991 Roles of morphogenetic hormones in caste polymorphism in bumble bees. In *Morphogenetic hormones in arthropods: roles in histogenesis, organogenesis, and morphogenesis* (ed. A. P. Gupta), pp. 384–399. New Brunswick, NJ: Rutgers University Press.

Röseler, P.-F. & Röseler, I. 1977 Dominance in bumble bees. In *Proceedings of the VIIIth International Congress of the International Union for the Study of Social Insects*, pp. 232–235. Wageningen. The Netherlands.

Schmid-Hempel, R. & Schmid-Hempel, P. 2000 Female mating frequencies in *Bombus* spp. from Central Europe. *Insectes Soc.* **47**, 36–41.

Seeley, T. D. 1985 *Honeybee ecology.* Princeton University Press.

Sundström, L. 1994 Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* **367**, 266–268.

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

Van der Blom, J. 1986 Reproductive dominance within colonies of *Bombus terrestris* (L.). *Behaviour* **97**, 37–49.

Van Doorn, A. & Heringa, J. 1986 The ontogeny of a dominance hierarchy in colonies of the bumble bee *Bombus terrestris* (Hymenoptera, Apidae). *Insectes Soc.* **33**, 3–25.

Van Honk, C. J. G., 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.

Wheeler, D. E. 1986 Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications. *Am. Nat.* **128**, 13–34.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.