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Dedicated to the memory of William Donald Hamilton

Inclusive fitness theory: novel predictions and tests in eusocial Hymenoptera

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Hamilton's first papers concerned social evolution and revolutionized our understanding of reproductive conflict in insect societies. The revolution continues. Recent research at the University of Sheffield has investigated two topics previously not considered from an inclusive fitness perspective: dominance hierarchies and queen–worker caste conflict. The theory makes predictions close to that occurring in hierarchies of *Dinoponera* queenless ants and queen overproduction in *Melipona* bees. An earlier prediction, that multiple paternity favours worker policing, is supported by research on *Vespula* and *Dolichovespula* wasps. However, worker policing also occurs in the hornet, *Vespa crabro*, which has low paternity (effective queen mating 1.1). Worker policing in Vespinae wasps has two novel facultative features. In *Dolichovespula saxonica*, worker policing occurs in colonies headed by a multiple-mated queen but not by a single-mated queen. In *Vespa crabro*, queenless colonies accept worker-laid eggs but reject queen-laid eggs, probably because queen supersedure does not occur but queen parasitism does.

Introduction

In 1964, William Hamilton published his revolutionary two-part paper 'The genetical evolution of social behaviour' in the *Journal of Theoretical Biology* (Hamilton 1964). Part one was a model that showed how natural selection could favour the evolution of traits that caused a reduction in direct reproduction. In his first paragraph, Hamilton stated why this addition to evolutionary theory was needed: "If natural selection followed the classical models exclusively, species would not show any behaviour more positively social than the coming together of the sexes and parental care". According to Hamilton's new theory, the maximizing property of Darwinian fitness is retained in such a way that "each organism appears to be attempting to maximize its inclusive fitness" rather than its classical fitness, the number of offspring it produces.

Part two focused on the implications of the new theory for social evolution and social discrimination and provided "some tentative evidence". Much of this evidence concerned social insects, as insect workers provide an obvious case of reduced direct reproduction. However, Hamilton also discussed other important cases of potentially altruistic behaviour, such as alarm calls and warning coloration. His theory was never restricted to social insects, and neither was his discussion of the evidence.

Insect sociality is only one of many topics revolutionized by inclusive fitness theory's new insights, but it can probably claim to have both received the greatest benefit from, and provided the best evidence for, the new theory. A working knowledge of social insects was one of three factors underlying Hamilton's ability to develop the theory (Wilson 1994: pp. 317–318); the other two were "being bothered" by the problem of altruism and a fascination with the mathematics of kinship.

Hamilton's comment that "…family relationships in Hymenoptera are potentially very favourable to the evolution of reproductive altruism" because of the higher degree of relatedness among haplodiploid full sisters (0.75) than among diploid full siblings (0.5) was initially the theory's most compelling empirical support. J. Maynard Smith (personal communication to FLWR) was sent the 1964 paper for review with a cover letter from the editor to the effect that "two previous referees could not make sense of it, can you?". Maynard Smith found the math hard to follow, but the explanation of how haplodiploidy could predispose Hymenoptera to eusociality made him take notice. Maynard Smith also suggested that the paper be divided into its two parts. In 1965, E. O. Wilson became convinced by the theory's explanation of haplodiploidy and eusociality, and other aspects of hymenopteran social life, such as the fact that males do not work (Wilson 1994: p. 318). These predictions of the theory are favourably presented in *The Insect Societies* (Wilson 1971), although Wilson himself was also circumspect (pp. 333–334), writing "In balance, the Hamilton theory of insect sociality seems to me to be consistent with enough evidence, and to account uniquely for enough phenomena, to justify its provisional acceptance. What this means more precisely is that the factor of haplodiploid bias should be taken into account in future evolutionary interpretations and as a guideline in planning some further empirical research."

Interestingly, the 3/4 relatedness hypothesis for the evolution of eusociality is not generally accepted now (Trivers & Hare 1976, Andersson 1984). However, more compelling social-insect evidence for Hamilton's theory has been forthcoming. In the social Hymenoptera, sex allocation and male production provide excellent evidence for inclusive fitness theory, in that both facultative sex allocation (Boomsma & Grafen 1990, 1991, Ratnieks 1991) and worker policing (Ratnieks 1988) were predicted on inclusive fitness grounds before they were detected (Ratnieks & Visscher 1989, Mueller 1991, Sundström 1994). The prediction of entirely novel phenomena is an important test of any theory. Such predictions illustrate how the theory is able to facilitate discovery, rather than merely provide additional or better explanations for phenomena that are already known.

This paper reviews some of inclusive fitness theory's further insights into the reproductive biology of social insects, particularly reproductive conflict and the processes that may reduce this conflict. The examples given come from recent research carried out at the Laboratory of Apiculture and Social Insects at the University of Sheffield. Such nepotistic choice of material is normally best avoided. However, in this review nepotism still permits us to do justice to the paper's title by providing two examples in which Hamiltonian thinking has been extended into novel areas: the dominance hierarchies of *Dinoponera* queenless ants; and conflict over caste determination in *Melipona* bees. We also give one example of a long-standing prediction — that multiple mating by queen Hymenoptera favours worker policing — that has finally been tested and supported.

Dominance hierarchy length and stability in queenless ponerine ants, *Dinoponera*

In queenless ponerine ants the queen caste has been lost over evolutionary time, and in each colony one or more mated workers play the queen role and carry out most of the reproduction (Peeters 1993, 1997). These mated workers are often referred to as gamergates, rather than queens, to emphasize that they belong to the worker morphological caste (Peeters & Crewe 1984). All queenless ant females have the potential to become gamergates, so a queenless ant colony is an excellent system for studying mechanisms by which reproductive versus working roles are determined in a society of totipotent individuals. In this aspect, queenless ants are similar to eusocial vertebrates.

Dinoponera quadriceps colonies have 35–238 workers (mean 85) and a single gamergate mated to a single unrelated male (Monnin & Peeters 1998). Reproduction is regulated by a dominance hierarchy, and the gamergate has the alpha rank (Monnin & Peeters 1999). Below the gamergate in rank are four to six unmated workers, typically her daughters but sometimes her sisters, who do little work but engage in agonistic interactions. Rank is a strong predictor of the new gamergate. When the mother gamergate dies, she is replaced by one of the three highest-ranking workers (Monnin & Peeters 1999). The new gamergate leaves the nest briefly at night, releases pheromone and mates with one of the attracted males (Monnin & Peeters 1998).

Monnin and Ratnieks (1999) investigated the inclusive-fitness benefit and cost of high rank in a dominance hierarchy. The benefit is an increased probability of becoming a gamergate following colony fission or death of the current gamergate (Monnin & Peeters 1999). The cost is decreased colony productivity, because high-rankers perform little work. The model determines at which rank it is better to work than to be a hopeful reproductive. That is, the highest rank at which the indirect benefit from the increased colony productivity given by one extra worker exceeds the direct benefit of becoming the next gamergate, discounted by the probability of being the new gamergate. The critical rank depends primarily upon colony size but also upon the linearity of the hierarchy. The more linear the hierarchy (i.e., the greater the probability that beta will be the new gamergate) the shorter the hierarchy of hopeful reproductives.

The importance of this study is twofold. First, most previous studies of dominance hierarchies are descriptive or study proximate mechanisms (e.g., Cole 1981, Appleby 1983, Heinze 1990, Bonabeau *et al.* 1996, De Vries 1998), rather than factors that influence the nature of the hierarchy itself. Second, the prediction is testable. Preliminary data show a close match between predictions and observed hierarchy lengths in *Dinoponera quadriceps* and in *D. australis*, a species with much smaller colonies than *D. quadriceps* (Fig. 1). In particular, the model predicts an increase in hierarchy length of two as colony size rises from 13 to 80, the average sizes of the colonies of *D. australis* and *quadriceps* studied. Future research will compare hierarchy lengths within *D. quadriceps* colonies across the natural range of colony sizes (ca. 35–238), in additional colonies of *D. australis*, and in *D. gigantea* colonies, which are also much smaller than those of *D. quadriceps* (Paiva & Brandão 1995).

This hierarchy-length study (Monnin & Ratnieks 1999) investigated the *patient*, or hopeful reproductive, strategy of a high-ranking worker. The strategy is patient because it involves waiting until a new gamergate is needed, either due to colony fission or death of the mother gamergate. But could an *impatient* strategy be selected for? That is, a strategy in which a high-ranker overthrows the existing gamergate, even while the gamergate is still capable of carrying out her reproductive role. A hopeful reproductive is no more related to her own offspring (0.5) than to

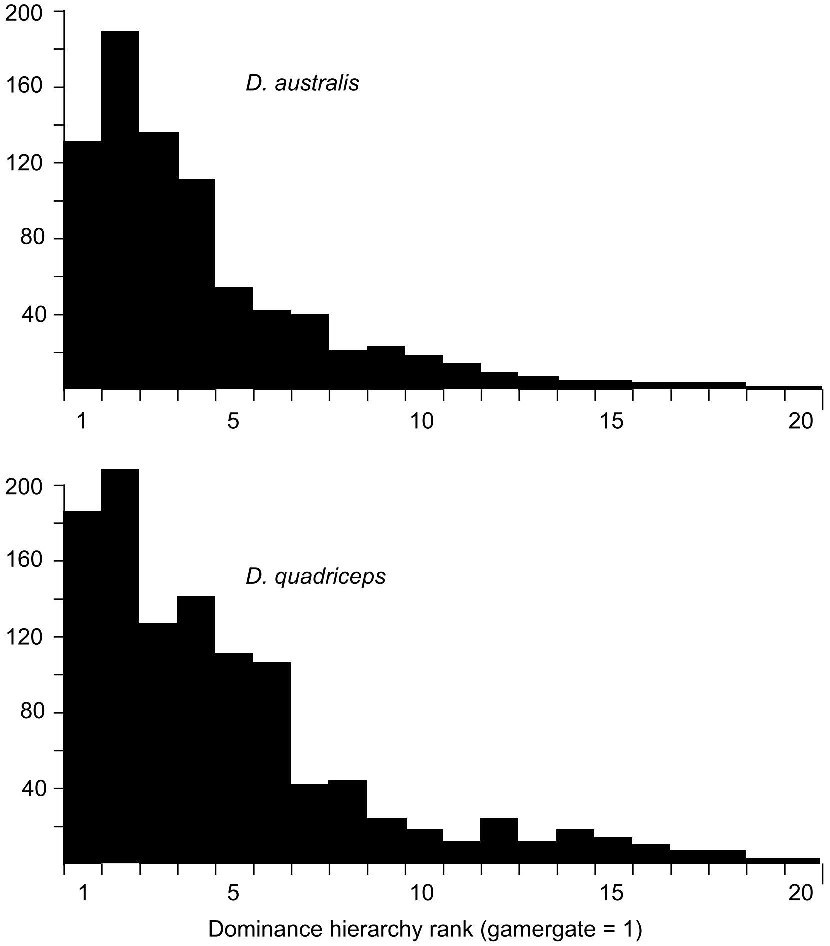


Fig. 1. Number of aggressive behaviours (blocking, gaster rubbing, antennal boxing) performed by workers of ranks one to 20 in four colonies each of *D. quadriceps* (colonies of 80 workers Monnin & Peeters 1999; T. M. Monnin, C. R. F. Brandão, F. L. W. Ratnieks unpubl.) and *D. australis* (average colony size 14 workers T. M. Monnin, C. R. F. Brandão, F. L. W. Ratnieks unpubl.). The sharp decrease in aggressiveness between workers ranking four and five in *D. australis* and workers ranking six and seven in *D. quadriceps* suggests a hierarchy two longer in *D. quadriceps* as predicted by inclusive fitness theory.

her mother gamergate's offspring (0.5, mean of relatednesses of 0.75 and 0.25 to full sisters and brothers). However, there is a relatedness benefit to the impatient pretender from overthrowing her mother, because she is more related to her own offspring (0.5) than to a sister's offspring (0.375). High-ranking workers are young workers and hold high rank only for a short period in relation to the tenure of a gamergate. Thus, it may be better for beta to overthrow alpha early, before she loses her high rank and all chance of reproducing directly (Monnin & Ratnieks 2001).

T. Monnin and F. L. W. Ratnieks (unpubl.) use a single-locus two-allele genetic model to investigate conditions under which gamergate overthrow is favoured. Overthrow is selectively favoured provided that only older gamergates are replaced and that the overthrowing individual (or others of the same genotype) has an above-average chance of benefiting from the vacancy created (Fig. 2). Replacement of an old gamergate is favoured even if the old gamergate is still capable of fulfilling her reproductive role. But other workers and the gamergate should oppose early overthrow (Monnin & Ratnieks 2001). This is consistent with the observation that workers punish (Clutton-Brock & Parker 1995) high-rankers that challenge the gamergate by immobilization (Fig. 3) (Monnin & Peeters 1999). Immobilized workers are spread-eagled for up to several days by relays of low-ranking workers. Following immobilization, the victim loses her high rank and any chance of replacing the gamergate. In preventing gamergate overthrow, immobilization is both a form of worker policing and of punishment (Monnin & Ratnieks 2001).

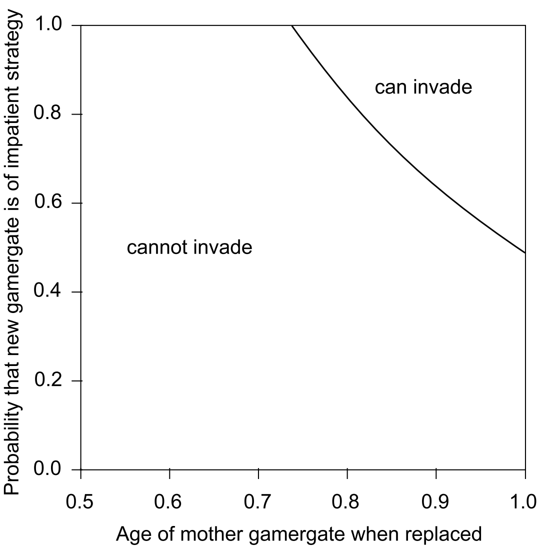


Fig. 2. Results of a genetic model showing conditions under which overthrow of the mother gamergate by a high ranker is favoured in a population in which all colonies are headed by a single once-mated mother gamergate who produces all the colony's offspring. The vertical axis represents the probability that the new gamergate is of the 'impatient' strategy when 50% of the workers are of this genotype (genotype Aa who may attempt to overthrow the gamergate) and 50% are wildtype (AA, do not attempt to overthrow the gamergate). Colonies of this genetic composition occur when a rare allele causing workers to attempt to overthrow the gamergate is maternally transmitted. The horizontal axis is the age of the gamergate, with gamergates having a maximum age of one. Based on T. M. Monnin and F. L. W. Ratnieks (unpubl.).

The fact that a high-ranker benefits only by overthrowing an old gamergate raises mechanistic questions about how gamergate age can be assessed by high-rankers (T. Monnin & F. W. L. Ratnieks unpubl.). It seems likely that dominance behaviours such as blocking and abdomen rubbing (Fig. 3) — performed by the gamergate to high-rankers — are honest signals of vitality, which are then interpreted by a high-ranker as signifying that the gamergate is below the age at which replacing her is beneficial. In blocking there is no direct aggression at all. The gamergate merely faces a high-ranker and moves in step with her, to keep the high-ranker between her outstretched antennae.

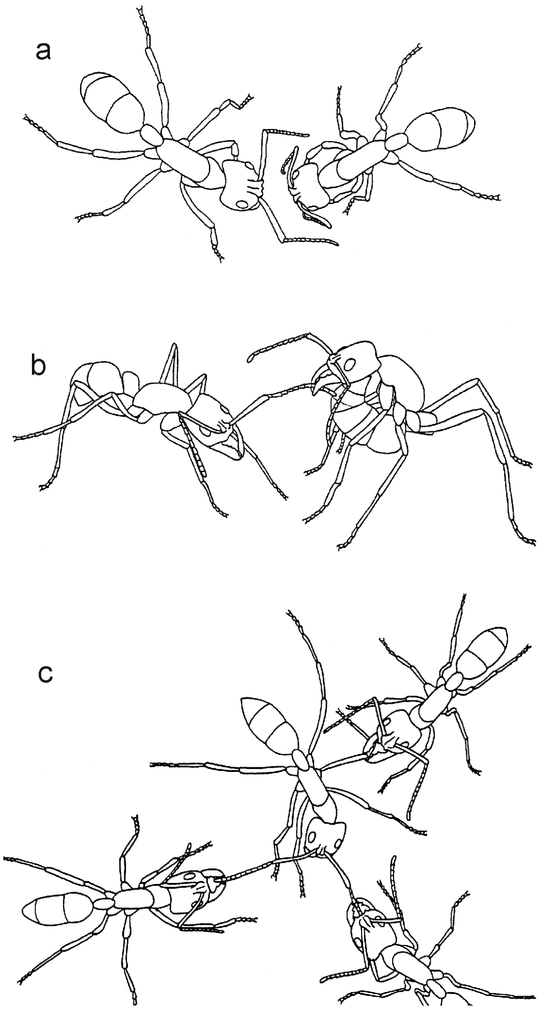


Fig. 3. Agonistic behaviours in *Dinoponera quadriceps*: (**a**) blocking and (**b**) abdomen (gaster) rubbing are normally performed by the gamergate and directed at high-ranking workers; (**c**) immobilization is normally performed by low-ranking workers and directed at high rankers.

Caste-determination conflict: excess queen production in *Melipona* bees

Insect societies with morphologically distinct worker and queen castes normally rear workers and queens in proportions that are adaptive at the colony level. For example, queens are not reared at the wrong time of year, and a small colony rears no queens at all (Bourke & Rat-

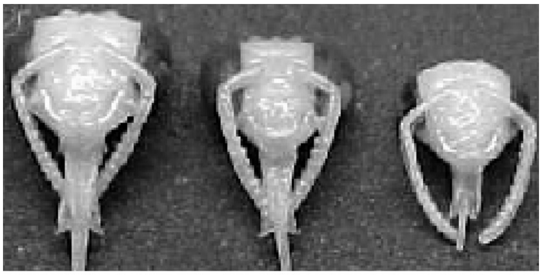


Fig. 4. Heads of *Melipona beecheii* pupae showing the small size of the queen relative to the worker. Original photo by H. Moo-Valle.

nieks 1999, Ratnieks 2001). During the reproductive season, most species rear large numbers of queens, each of which disperses and attempts to found a nest independently. However, in species such as honey bees (*Apis*) (Seeley 1985, Winston 1987) and army ants (*Eciton*) (Hölldobler & Wilson 1990, Gotwald 1995), which have colonies founded by a queen plus a group of workers, relatively few queens are reared. Unlike in species in which queens found nests independently, the rearing of many queens is not beneficial to the adult workers because a queen without workers cannot found a colony. *Melipona* bees are an exception to this pattern, however. In these bees, a queen and a swarm of workers found a new colony, but many excess queens (approximately 22% of all females) (Kerr 1950, Kerr & Nielsen 1966, reviews in Wilson 1971, Michener 1974, Velthuis & Sommeijer 1991) are reared. These excess queens are killed by workers within a few days of emerging from their cells (Engels & Imperatriz-Fonseca 1990, Koedam *et al.* 1995). Why do *Melipona* bees rear so many queens? Far more are reared than would be needed to have a spare queen available in the event of the mother queen's sudden death.

The probable reason for excess queen production in *Melipona* bees is that it is a selfish strategy for developing female larvae to enhance their inclusive fitness (Bourke & Ratnieks 1999). In a species such as *Melipona* that rears both workers and queens at the same time, there is conflict between a totipotent female larva and the adult workers. A female larva would benefit if she were one of those reared to be a queen. As in the gamergate-overthrow example discussed above, this is because a female is more related to her own offspring than to a sister's offspring. But in a society with a single once-mated queen, the adult workers are indifferent to which females are reared as queens or workers; their inclusive fitness is maximized by rearing the correct proportions of each caste.

Conflict between adult workers and female larvae will usually be won by the adults, because they can control the rearing conditions and larval feeding (Bourke & Ratnieks 1999). In other words, workers can police the caste-fate of totipotent larvae (Wenseleers 2001; T. Wenseleers, F. L. W. Ratnieks & J. Billen unpubl.). However, *Melipona* bees have two aspects of their biology that, in concert, should give larvae the power to determine their own fate. The first concerns the method of feeding. As in other meliponine bees, cells are mass-provisioned. That is, larval food is placed in a cell, an egg is laid on top and the cell is sealed (reviews in Wilson 1971, Michener 1974, Engels & Imperatriz-Fonseca 1990, Velthuis & Sommeijer 1991). Second, unlike other meliponine bees or the queens of most other eusocial Hymenoptera, *Melipona* queens are not larger than workers (Fig. 4). As a result, a provision mass is sufficient to rear either a queen or a worker (reviews in Michener 1974, Velthuis & Sommeijer 1991). Thus, *Melipona* larvae develop in isolation from the workers, in a cell with sufficient food to produce a full-sized queen. In some other meliponine bees and in some ants, dwarf queens occur. Dwarf-queen production may also be a selfish strategy in which a larva develops into a queen on food normally sufficient only to rear a worker (Bourke & Ratnieks 1999).

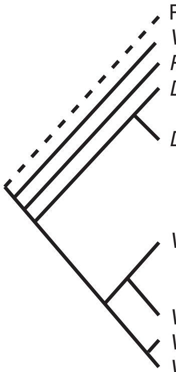
Ratnieks (2001) used a single-locus two-allele model to determine the optimum probability by which female larvae should develop into queens. Clearly, not all females can turn into queens, because some workers are needed for the colony to survive. Under the assumptions that the reproduction of a colony is directly proportional to the number of workers, that the colony is headed by a single, once-mated queen (Peters *et al.* 1999), and that all males are queens' sons, the optimum proportion of females that should develop into queens is 20%. This is remarkably close to the 21%–22% observed, on average (Kerr *et al*. 1966, Velthuis & Sommeijer 1991). When workers produce some of the males, the optimum decreases. When within-colony relatedness declines due to double-mating by queens, the optimum increases. These differences in optima provide a possible means of testing the model's predictions. A key species for further study is *M. bicolor*, which has colonies headed by several related queens (Bego 1989), leading to a mean relatedness of 0.62 among female offspring (Peters *et al.* 1999). This leads to a considerably higher optimal probability (approximately 30%) of developing into a queen.

Worker policing and paternity frequency in Vespinae wasps

In most species of social Hymenoptera with morphologically distinct queens and workers, the workers retain ovaries but cannot mate and so can lay only unfertilized eggs (Ratnieks 1988). Hymenoptera are haplodiploid, so the workers' unfertilized eggs are male. Male production is the subject of much potential conflict, because each worker is more related to her own sons (0.5) than to the sons of her mother (0.25) or sister workers (full-nephews 0.375, half-nephews 0.125) (Ratnieks 1988, Ratnieks & Reeve 1992). However, in many species, worker production by males in queenright colonies is rare. One possible reason for this is worker policing. That is, workers prevent each other from reproducing. In societies with a single queen, worker policing is favoured on relatedness grounds when the effective paternity frequency (Boomsma & Ratnieks 1996) is greater than two, because in these cases workers are more related to the queen's sons (0.25) than to other workers' sons (< 0.25). If the colony is headed by multiple related queens, the paternity frequency at which worker policing is selectively favoured falls below two (Crozier & Pamilo 1996). In addition, worker policing can be selected for at paternity frequencies lower than that dictated by relatedness alone if worker reproduction lowers colony productivity (Ratnieks 1988) or reduces the cost of sex-ratio manipulation by workers (Foster & Ratnieks, accepted).

Comparative data are consistent with the relatedness prediction. Worker policing occurs, and worker production of adult males is rare, in *Apis* (Barron *et al*. accepted), which have multiple-mated queens (Palmer & Oldroyd 2000). However, in bumble bees (*Bombus*) and meliponine bees, which typically have single-mated queens (Owen & Plowright 1982, Peters *et al.* 1999) workers' sons are probably frequent in queenright colonies (Owen & Plowright 1982, Ratnieks 1988). However, although this comparison involves multiple species, the phylogeny of the Apidae shows that it represents only one independent contrast (Schultz *et al*. 1999, Engel 2001), with both multiple paternity and worker policing arising early in the *Apis* clade (Oldroyd *et al*. 2001). However, recent research on Vespinae wasps has added to this data set, and the new data strongly support the hypothesis that worker policing is favoured by higher paternity. Vespinae wasps are a useful group for investigating the effect of paternity frequency on colony reproductive characteristics because paternity frequency is highly variable, ranging from 1 to 7, within a group of four genera with similar basic biology: annual nests headed by a single queen and workers that cannot mate but retain ovaries (Foster & Ratnieks 2001b). In addition, their phylogeny is known (Fig. 5; Carpenter 1987).

In *Dolichovespula saxonica*, queens show considerable variation in paternity frequency, ranging from one to more than two (mean 1.35) (Foster & Ratnieks 2000, Foster *et al*. 2001). In nine field colonies collected during the reproductive stage of their life cycle, there was a significant negative correlation between the proportion of adult and pupal males that were workers' sons and queen paternity frequency (Fig. 6). This correlation is consistent with the hypothesis that worker policing is facultative and occurs only in colonies headed by a multiple-mated queen. The hypothesis is further supported by observations of oviposition in the four of these nine colonies that were kept in glass-sided nest boxes, which allowed observation of the combs but still permitted the wasps to forage freely (Fig. 6) (Foster 2000). The initial proportions of workers' sons (egg stage) were high in all four colonies. In the one observation colony with a single-paternity queen, the final (adult)



Clade	Species	Paternity			% males sons of workers	% workers with active ovaries	% reproductive nests		Refs.
		Effective	Range	(*n*)			Queenless	(*n*)	
Polistinae	*Polistes*	< 1.05	1–2	–	–	–	–		1,2
Vespa	*crabro*	1.11	1–3	(14)	0	1	14	(14)	3,4
Provespa	*anomola*	1.00	1	(1)	–	–	–		5
D. maculata grp	*maculata*	1.00	1	(10)	21	–	74	(19)	6
	media	1.08	1–2	(10)	7	5	32	(19)	6
D. norwegica grp	*arenaria*	1.09	1–4	(20)	17	4	54	(13)	7
	sylvestris	1.15	1–2	(10)	10	7	36	(14)	6
	norwegica	1.08	1–3	(10)	3	10	86	(14)	6
	saxonica	1.35	1–3	(10)	35	11	20	(10)	6
V. vulgaris grp	*germanica*	2.35	1–7	(55)	–	0.6	0	(25)	8,9,13
	maculifrons	7.14	–	(30)	0	2	–		9,10
	vulgaris	1.90	1–4	(17)	0	0.4	6	(50)	11,13
V. koreensis grp	no data	–	–	–	–	–	–		–
V. rufa grp	*rufa*	–	> 1	(1)	–	–	–		12
V. squamosa grp	*squamosa*	3.33	–	(17)	0	–	–		10

Fig. 5. Paternity frequency, worker reproduction and ovary development, and frequency of queenless reproductive-stage colonies in Vespinae wasps (*Vespa, Provespa, Dolichovespula, Vespula*) with outgroup comparison (*Polistes*). The phylogeny is from Carpenter 1987. Active ovaries are those containing one or more full-sized eggs. Paternitry frequencies and origin of males determined using allozymes or DNA microsatellites. References: (1) Field *et al.* 1998; (2) Peters *et al.* 1995; (3) Foster *et al.* 1999; (4) Foster *et al.* 2000; (5) K. R. Foster (unpubl.); (6) Foster *et al.* 2001; (7) F. L. W. Ratnieks & J. J. Boomsma (unpubl.); (8) M. A. D. Goodisman, R. W. Matthews & R. H. Crozier (unpubl.); (9) Ross (1985); (10) Ross (1986); (11) Foster and Ratnieks (2001a); (12) Thorén *et al.* (1995); (13) Spradberry (1971). Figure modified from Foster and Ratnieks (2001b).

and initial (egg) proportions of workers' sons were equal. However, in the three colonies with a multiple-paternity queen, the final proportions of workers' sons were significantly lower than the initial proportions. As in studies of facultative sex allocation (Boomsma & Grafen 1991, Mueller 1991, Sundström 1994), the use of colony rather than species (Trivers & Hare 1976) or clade as the unit of comparison results in much greater statistical power.

In *Vespula vulgaris* a different research protocol was used to detect worker policing (Foster & Ratnieks 2001a) Following honey-bee methods (Ratnieks & Visscher 1989), worker-laid eggs were obtained from queenless colonies with laying workers. The eggs were then transferred into a test comb taken from a queenright discriminator colony. Queen-laid eggs were placed in adjacent cells, and the test combs were returned to the discriminator colonies. The results were similar to those from honey-bee research. Worker-laid eggs were rapidly removed, but most queen-laid eggs were spared (4/120 versus 106/120 remaining after one hour, and 0/120 versus 80/120 remaining after 16 hours; $n = 3$ trials with $n = 2$ discriminator colonies). This removal of worker-laid eggs was even more rapid in *Vespula vulgaris* than in the honey bee (Ratnieks 1995).

The above methods were also used to determine whether worker policing occurs in the hornet *Vespa crabro* (Foster 2000; K. R. Foster, J. Gulliver & F. L. W. Ratnieks unpubl.). This species has an effective paternity frequency of only 1.1, and all colonies have effective paternity below two (Foster *et al.* 1999, Foster *et al.* 2000). Worker policing by egg removal also occurred in the hornet. Queenright hornet colonies removed worker-laid eggs. After 16 hours, 1/79 worker-laid eggs versus 46/72 queen-laid eggs remained ($n = 3$ trials with $n = 2$ discriminator colonies; $P < 0.001$ per trial). However, in queenless colonies, policing was in the opposite direction. After 16 hours, 36/44 worker-laid eggs versus 20/41 queen-laid eggs remained ($n = 2$ trials with 2 discriminator colonies; $P < 0.01$

per trial). Why does this ‘reverse’ policing occur? A plausible adaptive reason is that queen supersedure with a related queen does not occur as it does in the honey bee (Seeley 1985, Foster 2000, K. R. Foster, J. Gulliver & F. L. W. Ratnieks unpubl.). Conversely, queen parasitism probably does occur in the hornet, given that colonies with a single queen but two matrilines occur (Foster *et al.* 2000). Thus, in the hornet, queen-laid eggs in a queenless colony can come only from an unrelated queen, and removing them increases the inclusive fitness of the workers, who are the offspring of the now-dead queen and highly related to each others’ male offspring (ca. 0.35).

Worker policing in other Vespinae species has not yet been investigated. However, it seems likely that worker policing does not occur or is ineffective in most *Dolichovespula*, given that workers’ sons are readily detected in many queenright colonies (Foster *et al.* 2001) (Fig. 5). Conversely, workers’ sons have not been detected in three species of *Vespula* that have been examined (Fig. 5) (Foster & Ratnieks 2001b). All *Vespula* species studied so far have paternity frequencies close to or greater than two, and all *Dolichovespula* species have paternity frequencies close to one (Fig. 5) (Foster & Ratnieks 2001b, Foster *et al.* 2001). The comparison between *Vespula* and *Dolichovespula* is as predicted by inclusive fitness theory based on relatedness. Workers’ sons were not detected in *Vespula* but were detected in *Dolichovespula*. However, the situation in *Vespa crabro* is not as predicted based on relatedness alone, because worker policing occurs in a species with low paternity (Foster & Ratnieks 2001b). *Dolichovespula* and *Vespa crabro* have very similar paternity frequencies, yet the reproductive biology of *Vespa crabro* is more similar to that of *Vespula* species, which have multiple paternity. Why is *Vespa* the exception? Currently, we do not know (Foster & Ratnieks 2001b).

Another male production prediction concerns matricide (Ratnieks 1988, Bourke 1994). In annual species such as the Vespinae, it is not necessary to keep the queen alive to ensure the survival of the colony, because the colony will die at the end of the season anyway. Thus, the workers in societies with paternity frequency below 2 may benefit from killing the queen to remove her competition over male production. Data from the Vespinae strongly support the hypothesis that matricide is affected by paternity frequency in the predicted way (Fig. 5), because the proportion of queenless reproductive-stage colonies is low in *Vespula* (3%, mean of two species) and high in *Dolichovespula* (50%, mean of six species) (Foster & Ratnieks 2001b). Because *Vespula* colonies have a longer annual cycle than *Dolichovespula*, it is very unlikely

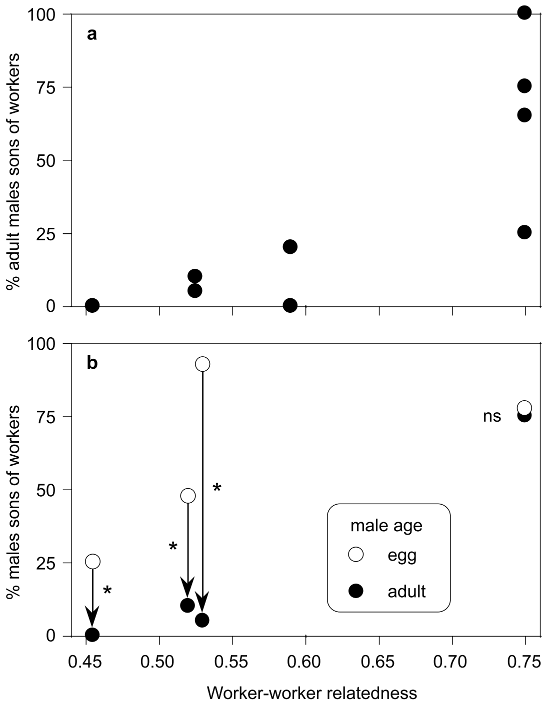


Fig. 6. Male production in *Dolichovespula saxonica* as revealed by DNA microsatellite analysis and observations of egg laying by queen and workers in four observation colonies. — **a**: Proportions of males that are workers’ sons; the trend to lower workers’ sons with lower worker–worker relatedness is significant ($P < 0.004$, Spearman’s rank correlation). — **b**: Change in workers’ sons from the egg to adult stage; changes in proportion are significant for the three colonies with lowest relatedness ($P < 0.001$, Chi-square) but non-significant for the colony with 0.75 relatedness ($P > 0.39$). Data for egg maternity assume that queens lay eggs at an even sex ratio. Initial ratios of worker-laid versus queen-laid eggs were not significantly different among these four colonies ($P > 0.86$, Chi-square). Based on data in Foster and Ratnieks (2000).

that this difference is caused by queens dying of old age. If death by old age were the reason, a higher proportion of queenless reproductive-stage *Vespula* versus *Dolichovespula* colonies would be expected. Once again, *Vespa crabro*, with only 14% queenless reproductive-phase colonies, is more similar to *Vespula* than to *Dolichovespula*. *Vespa crabro* has the paternity of *Dolichovespula* but the male production, matricide and worker policing of *Vespula*.

Discussion

The above models and results show that the Hamiltonian approach to studying reproductive conflict in insect societies is still in a very productive phase. New models have been developed to investigate important yet novel areas of colony reproduction, such as dominance hierarchies and caste-determination conflict, and the predictions from these models are in close agreement with empirical data. Earlier models relating to worker production of males, worker policing and matricide have been tested in Vespinae wasps, and these results are also in close agreement with predictions. Some results are unexpected, such as ‘reverse’ policing in the hornet. With hindsight, they are compatible with inclusive fitness theory. However, it would have been preferable for them to be predicted *a priori*, as have most of the results discussed above, to avoid the criticism of *post hoc* rationalization (Gould & Lewontin 1981).

How do the new models described here relate to Hamilton’s original model? The big difference is that the new models are more narrowly focused. Hamilton (1964) provided a general model that showed that traits that reduce the reproduction of an individual can be selected for, provided that the traits also increase the reproduction of relatives. Hamilton also provided a rule, now known as Hamilton’s rule, which provided the general condition under which a social action would be selected for in terms of the costs and benefits to actor and recipient and their genetic relatedness. The new models described here, and in fact most models of social behaviour, are more narrowly focused than this. Each considers a specific question in a specific range of organisms or conditions. The models are often constructed in conjunction with ongoing empirical studies, and together they go a long way to doing what could only be dreamed of 30 years ago (Hamilton 1972): “Although the argument [from concepts of relatedness and inclusive fitness] is potentially quantitative, social biology is still very far from providing the multiple measurements of fitness and the coefficients of relatedness that would permit exact tests of the theory.”

Not all the results that we have obtained are as expected — the existence of worker policing in the hornet, despite a paternity frequency of only 1.1, for example. What does this say about the validity of inclusive fitness as a predictor of reproductive behaviour? The existence of worker policing in the hornet is in keeping with inclusive fitness predictions for a situation in which worker reproduction has a cost. The classical worker-policing model, the result of which is that worker policing is favoured at paternity frequencies above two (Ratnieks 1988), considers relatedness but ignores costs of worker reproduction and worker policing. Because worker reproduction is likely to be more costly to the colony in terms of reduced productivity than is worker policing, the effect of these costs will typically be to reduce the paternity threshold at which worker policing is selectively favoured (Ratnieks 1988). So finding worker policing at paternities below two is expected, although we have little idea of why *Vespa crabro* should be so different from *Dolichovespula* in this respect, given their great similarities in colony kin structure, size and life cycle. Conversely, finding the non-existence of worker policing at paternities above two is not as likely, although it is possible for various mechanistic reasons. For example, it could be that there is no mechanism for policing, or there is a successful mechanism for evading worker policing (Oldroyd & Ratnieks 2000, Barron *et al*. accepted), or worker policing could be more costly than worker reproduction. In biological systems it is almost impossible to know all the factors of importance that may cause some trait to occur or not. As a result, testing of theories needs to be done broadly using the accumulated evidence of many studies. The testing of inclusive fitness theory predictions has in recent years been greatly facilitated by the technological breakthrough provided by DNA microsatellites, which can be used to determine relatedness and kinship to almost any desired degree of accuracy. In 1964, Hamilton was sure of the paternity frequency of only one species, the honey bee *Apis mellifera*, and also moderately sure of *Atta*. Ironically, the data showed that both had multiple-mated queens. There is unlikely to be any technological breakthrough in measuring costs. The likely solution is more prosaic: well-chosen and tractable model systems in careful field studies. In this respect we have recently been studying worker policing in *Apis mellifera capensis* (P. Neumann, C. Pirk & F. L. W. Ratnieks unpubl.). Because the workers of this subspecies reproduce by thelytoky there is no relatedness benefit from worker policing. But worker policing still occurs, suggesting that it enhances colony productivity.

Perhaps the greatest effect of Hamilton's theory is neither that it has enabled us to understand social evolution, nor that it has made predictions that have led to the discovery of novel phenomena. Although both these effects are of very great importance, the most important is that we now look at social phenomena in an entirely different way. Indeed, we see social phenomena much more widely now. The theory has changed the way we see nature. Nowhere is this change in perspective more important than in understanding the potential for conflict that is inherent in biological systems, whether among individuals in insect societies or among genetic elements within a genome. (The latter situation was also investigated by Hamilton [1967] in another highly influential paper.)

In the case of the social insects, the transition in perspective has been from an uncritical acceptance of cooperation as a given of their biology to a deeper understanding based upon knowledge of the potential conflicts that exist among individuals and the processes that can reduce these conflicts. Thus, *Dinoponera* ants have societies in which any worker could, potentially, replace the gamergate. In fact, this apparent recipe for anarchy results in stable societies in which the gamergate is rarely replaced. This is because it is not usually in the interests of a high-ranking worker to overthrow the gamergate. And even when gamergate-overthrow is in the interests of a high-ranking worker, it is usually in the interests of other workers and the gamergate to prevent overthrow. Similarly, in species with morphologically distinct queens and workers it is often in the interests of a totipotent female larva to develop into a queen. But when queen-rearing is not also in the interests of the adult workers, they can usually prevent a larva from becoming a queen via control of brood nutrition. So pervasive is this adult power over brood that only in *Melipona*, an exception that proves the rule, do larvae have great power of self-determination. When self determination occurs, the result — the rearing and execution of excess queens — is something that is inexplicable from a perspective that views a colony as a collection of cooperating individuals but is readily explicable from a Hamiltonian perspective in which individuals in colonies have selfish, as well as collective, interests. Oster and Wilson (1979) discussed the relative importance of colony-level and individual-level selection and commented (p. 23) that "The interaction of individual-level and colony-level selection complicates the evolution of caste in ways that are just beginning to be explored". The *Melipona* example suggests that it is only in rare cases that the individual level wins out against the colony level because the adult workers, representing the colony-level interest, collectively hold too much power over the larvae. In Vespinae wasps, the data presented here show that kinship has a great effect on the resolution of reproductive conflicts. There appear to be three endpoint syndromes. In one, found in *Vespa crabro* and *Vespula*, worker policing enforces cooperation by preventing the workers from benefiting from attempted reproduction. These societies are characterized by workers that rarely activate their ovaries. In the other extreme endpoint, found in most *Dolichovespula*, workers probably do not police each other, frequently have active ovaries, produce many of the colony's males and often overthrow the queen. *Dolichovespula saxonica* is intermediate between these two extremes, because it has worker policing in some colonies but also much egg-laying by workers.

In an apparently obvious but typically insightful comment, Hamilton (1964) wrote "The colonies of the social insects are remarkable in having true genetic diversity in the co-operating individuals". Nearly 40 years later, and through the application of his theory, we now better understand how genetic diversity is compatible with — and may even enhance — cooperation.

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References

Andersson, M. 1984: The evolution of eusociality. — *Annu. Rev. Ecol. Syst.* 15: 165–189.

Appleby, M. C. 1983: The probability of linearity in hierarchies. — *Anim. Behav.* 31: 600–608.

Barron, A., Oldroyd, B. P. & Ratnieks, F. L. W. [accepted]: Worker reproduction in honey-bees (*Apis*) and the anarchic syndrome: a review. — *Behav. Ecol. Sociobiol.*

Bego, L. R. 1989: Behavioral interactions among queens of the polygynic stingless *bee Melipona bicolor bicolor* Lepeltier (Hymenoptera, Apidae). — *Bras. J. Med. Biol. Res.* 22: 587–596.

Bonabeau, E., Théraulaz, G., Deneubourg, J.-L., Aron. S. & Camazine, S. 1996: Mathematical model of self-organizing hierarchies in animal societies. — *Bull. Math. Biol.* 58: 661–717.

Boomsma, J. J. & Grafen, A. 1990: Intraspecific variation 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.* 4: 383–407.

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

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

Bourke, A. F. G. & Ratnieks, F. L. W. 1999: Conflict and caste determination in social insects. — *Behav. Ecol. Sociobiol.* 46: 287–297.

Carpenter, J. M. 1987: Phylogenetic relationships and classification of the Vespinae (Hymenoptera: Vespidae). — *Syst. Entomol.* 12: 413–431.

Clutton-Brock,T. & Parker, G. A. 1995: Punishment in animal societies. — *Nature*. 373: 209–216.

Cole, B. J. 1981: Dominance hierarchies in *Leptothorax* ants. — *Science*. 212: 83–84.

Crozier, R. H. & Pamilo, P. 1996: *Evolution of social insect colonies*. — Oxford University Press, Oxford.

De Vries, H. 1998: Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. — *Anim. Behav.* 55: 827–843.

Engel, M. S. 2001: Monophyly and extensive extinction of advanced eusocial bees: insights from an unexpected Eocene diversity. — *Proc. Natl. Acad. Sci.* 98: 1661–1664.

Engels, W. & Imperatriz-Fonseca, V. L. 1990: Caste development, reproductive strategies, and control of fertility in honey bees and stingless bees. — In: Engels, W. (ed), *Social insects: an evolutionary approach to castes and reproduction*: 167–230. Springer Verlag, Berlin.

Field, J., Solís, C. R., Queller, D. C. & Strassmann, J. E. 1998: Social and genetic structure of paper wasp cofoundress associations: tests of reproductive skew. — *Am. Nat.* 151: 545–563.

Foster, K. R. 2000: The conflict over male production in the Vespinae wasps. — Ph.D. thesis, University of Sheffield, U.K.

Foster, K. R. & Ratnieks, F. L. W. 2000: Facultative policing in a social wasp. — *Nature* 407: 692–693

Foster, K. R. & Ratnieks, F. L. W. 2001a: Convergent evolution of worker policing by egg eating in the honey bee and common wasp. — *Proc. R. Soc. Lond.* B 268: 169–174.

Foster, K. R. & Ratnieks, F. L. W. 2001b: Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. — *Behav. Ecol. Sociobiol.* 50: 1–8.

Foster, K. R. & Ratnieks, F. L. W. [accepted]: The effect of sex allocation biasing on the evolution of worker policing. — *Am. Nat.*

Foster, K. R., Ratnieks, F. L. W. & Raybould, A. F. 2000: Do hornets have zombie workers*?* — *Mol. Ecol.* 9: 735–742.

Foster, K. R., Seppä, P., Ratnieks, F. L. W. & Thorén, P. A. 1999: Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. — *Behav. Ecol. Sociobiol.* 46: 252–257.

Foster, K. R., Ratnieks, F. L. W., Gyllenstrand, N. & Thorén, P. A. 2001: Colony kin structure and male production in *Dolichovespula* wasp*s*. — *Mol. Ecol.* 10: 1003–1010.

Gotwald, W. H. Jr. 1995: *Army ants: The biology of social predation*. — Cornell University Press, Ithaca, NY.

Gould S. J. & Lewontin, R. C. 1981: The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. — *Proc. R. Soc. Lond.* B 205: 581–598.

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.

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

Heinze, J. 1990: Dominance behavior among ant females. — *Naturwissenschaften* 77: 41–43.

Hölldobler, B. & Wilson, E. O. 1990: *The ants*. — Harvard University Press, Cambridge, MA.

Kerr, W. E. 1950: Genetic determination of castes in the genus *Melipona*. — *Genetics* 35: 143–152.

Kerr, W. E. & Nielsen, R. A. 1966: Evidence that genetically determined *Melipona* queens can become workers. — *Genetics* 54: 859–865.

Kerr, W. E., Stort, A. C. & Montenegro, M. J. 1966: Importância de alguns fatôres ambientais na determinação das castas do gênero *Melipona*. — *An. Acad. Bras. Cien.* 38: 149–168.

Koedam, D., Aguilar Monge, I. & Sommeijer, M. J. 1995: Social interactions of gynes and their longevity in queenright colonies of *Melipona favosa* (Apidae: Meliponinae). — *Neth. J. Zool.* 45: 480–494.

Michener, C. D. 1974: *The social behavior of the bees*. — Harvard University Press, Cambridge, MA.

Monnin, T. & Peeters, C. 1998: Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadriceps*. — *Anim. Behav.* 55: 299–306.

Monnin, T. & Peeters, C. 1999: Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. — *Behav. Ecol.* 10: 323–332.

Monnin, T. & Ratnieks, F. L. W. 1999: Reproduction versus work in queenless ants: when to join a hierarchy of hopeful reproductives? — *Behav. Ecol. Sociobiol.* 46: 413–422.

Monnin, T. & Ratnieks, F. L. W. 2001: Policing in queenless ponerine ants. — *Behav. Ecol. Sociobiol.* 50. [In press].

Mueller, U. G. 1991: Haplodiploidy and the evolution of facultative sex-ratios in a primitively eusocial bee. — *Science* 254: 442–444.

Oldroyd, B. P., Halling, L. A., Good, G., Wattanachaiyingcharoen, W., Barron, A. B., Nanork, P., Wongsiri, S. & Ratnieks, F. L. W. 2001: Worker policing and worker reproduction in *Apis cerana*. — *Behav. Ecol. Sociobiol.* [In press].

Oster, G. F. & Wilson, E. O. 1978: *Caste and ecology in the social insects*. — Princeton University Press, Princeton, NJ.

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

Paiva, R. V. S. & Brandão, C. R. F. 1995: Nests, worker population, and reproductive status of workers, in the true giant queenless ponerine ant *Dinoponera* Roger (Hymenoptera: Formicidae). — *Ethol. Ecol. Evol.* 7: 297–312.

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

Peeters, C. 1993: Monogyny and polygyny in ponerine ants with or without queens. — In: Keller, L. (ed.), *Queen number and sociality in insects*: 234–261. Oxford University Press, Oxford.

Peeters, C. 1997: Morphologically "primitive" ants: comparative review of social characters, and the importance of queen-worker dimorphism. — In: Choe, J. & Crespi, B. (eds), *The evolution of social behavior in insects and arachnids*: 372–391. Cambridge University Press, Cambridge.

Peeters, C. & Crewe, R. 1984: Insemination controls the reproductive division of labour in a ponerine ant. — *Naturwissenschaften* 71: 50–51.

Peters, J. M., Queller, D. C., Strassmann, J. E. & Solís, C. R. 1995: Maternity assignment and queen replacement in a social wasp. — *Proc. R. Soc. Lond.* B 260: 7–12.

Peters, J. M., Queller, D. C., Imperatriz-Fonseca, V. C., 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. 1995: Evidence for a queen-produced egg-marking pheromone and its use in worker policing in the honey bee. — *J. Apicult. Res.* 34: 31–37.

Ratnieks, F. L. W. 2001: Heirs and spares: caste conflict and excess queen production in *Melipona* bees. — *Behav. Ecol. Sociobiol.* [In press].

Ratnieks, F. L. W. & Reeve, H. K. 1992: The structure of conflict in hymenopteran societies: processes that reduce conflict in advanced eusocial species. — *J. Theor. Biol.* 158: 33–65.

Ratnieks, F. L. W. & Visscher, P. K. 1989: Worker policing in the honey bee. — *Nature* 342: 796–797.

Ross, K. G. 1985: Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). — *J. Zool. Lond. A.* 205: 411–424.

Ross, K. G. 1986: Kin selection and the problem of sperm utilization in social insects. — *Nature* 323: 798–800.

Schultz, T. R., Engel, M. S. & Prentice, M. 1999: Resolving conflict between morphological and molecular evidence for the origin of eusociality in the “Corbiculate” bees (Hymenoptera: Apidae): a hypothesis-testing approach. — *Univ. Kansas Nat. Hist. Mus. Spec. Publ.* 24: 125–138.

Seeley, T. D. 1985: *Honeybee ecology*. — Harvard University Press, Cambridge, MA.

Spradberry, J. P. 1971: Seasonal changes in the population structure of wasp colonies (Hymenoptera: Vespidae). — *J. Anim. Ecol.* 40: 501–523.

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

Thorén, P. A., Paxton, R. J. & Estoup, A. 1995: Unusually high frequency of (CT)n and (GT)n microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). — *Ins. Mol. Biol.* 4: 141–148.

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

Velthuis, H. H. W. & Sommeijer M. J. 1991: Roles of morphogenetic hormones in caste polymorphism in stingless bees. — In: Gupta, A. P. (ed), *Morphogenetic hormones of arthropods*: 346–383. Rutgers University Press, Rutgers, NJ.

Wenseleers, T. 2001. Conflict from cell to colony. — Ph.D. thesis. Catholic University of Leuven, Belgium.

Wilson, E. O. 1971. *The insect societies*. — Harvard University Press, Cambridge, MA.

Wilson. E. O. 1994. *Naturalist*. — Penguin Books, London.

Winston, M. R. 1987. *The biology of the honey bee*. — Harvard University Press, Cambridge, MA.