REVIEW

David R. Tarpy · David C. Gilley · Thomas D. Seeley

Levels of selection in a social insect: a review of conflict and cooperation during honey bee (*Apis mellifera*) queen replacement

Abstract The extended phenotype of a social insect colony enables selection to act at both the individual level (within-colony selection) and the colony level (between-colony selection). Whether a particular trait persists over time depends on the relative within- and between-colony selection pressures. Queen replacement in honey bee colonies exemplifies how selection may act at these different levels in opposing directions. Normally, a honey bee colony has only one queen, but a colony rears many new queens during the process of colony reproduction. The replacement of the mother queen has two distinct phases: queen rearing, where many queens develop and emerge from their cells, and queen elimination, where most queens die in a series of fatal duels. Which queens are reared to adulthood and which queens ultimately survive the elimination process depends on the strength and direction of selection at both the individual and colony levels. If within-colony selection is predominant, then conflict is expected to occur among nestmates over which queens are produced. If between-colony selection is predominant, then cooperation is expected among nestmates. We review the current evidence for conflict and cooperation during queen replacement in honey bees during both the queen rearing and queen elimination phases. In particular, we examine whether workers of different subfamilies exhibit conflict by acting nepotistically toward queens before and after they have emerged from their cells, and whether workers exhibit cooperation by collectively producing queens of high reproductive quality. We conclude that although workers may weakly compete through nepotism during queen rearing, workers largely cooperate to raise queens of similar reproductive potential so that any queen is suitable to inherit the nest. Thus it appears that potential conflict over queen replacement in honey bees has not translated into actual conflict, suggesting that between-colony selection predominates during these important events in a colony's life cycle.

Keywords Polygyny · Nepotism · Colony reproduction · Reproductive conflict · Levels of selection

D. R. Tarpy (✉)
Department of Entomology,
North Carolina State University,
Campus Box 7613, Raleigh, NC 27695–7613, USA
e-mail: david_tarpy@ncsu.ed

D. C. Gilley
Carl Hayden Bee Research Center,
2000 East Allen Rd., Tucson, AZ 85719–1596, USA

T. D. Seeley
Department of Neurobiology and Behavior,
Cornell University,
Seeley G. Mudd Hall, Ithaca, NY 14853, USA

Introduction

The social insects have long fascinated biologists because of the extreme cooperation among the workers of their colonies (Wilson 1971). The advent of "selfish-gene" thinking, however, has led to the recognition that there are many potential conflicts among colony members (reviewed by Bourke and Franks 1995; Crozier and Pamilo 1996; Keller 1999). To study the interface between cooperation and conflict within social insect colonies, it is helpful to use a multilevel selection approach. Natural selection acts on the phenotype of organisms, and the genes responsible for certain traits are the entities that are ultimately transmitted over generations. However, genes of social insects produce individual-level phenotypes, and individuals produce colony-level phenotypes. The extended phenotype of a social insect colony enables selection to act at the individual level or the colony level, or both (Ratnieks and Reeve 1992; Sundström and Boomsma 2001). For example, genes for a particular selfish behavior may be selected for at the individual level (if they increase the proportional reproductive output of their carriers relative to other colony members) but selected against at the colony level (if they decrease the colony's overall reproductive success). Thus whether or

not such genes will persist, and hence whether conflict or cooperation prevails for a particular trait, depends on the relative magnitudes of within-colony (individual-level) and between-colony (colony-level) selection.

The honey bee (*Apis mellifera*) has been a model organism for studying the interplay between conflict and cooperation. Two aspects of potential intracolony conflict concern the ratio of female and male sexuals and the frequency of male production by workers, both of which have been studied in great detail (reviewed by Bourke and Franks 1995; Crozier and Pamilo 1996). These two potential points of conflict, however, do not translate into actual conflict in most colonies because honey bee queens mate with several males (see Oldroyd et al. 1998; Tarpy and Nielsen 2002). By creating numerous subfamilies within a colony, polyandry alleviates the potential conflict over the sex ratio by aligning the genetic interests of the workers with that of the queen (e.g., Moritz 1985). Similarly, polyandry minimizes worker reproduction within a colony by lowering the genetic value of a worker's nephews compared to her brothers, causing the workers to police each other's egg laying (e.g., Woyciechowski and Lomnicki 1987; Ratnieks 1988). Thus, for these two aspects of reproduction, colony-level selection has minimized intracolony conflict in favor of cooperation.

A third reproductive conflict concerns which queens will represent a colony in the next generation. Workers may potentially benefit by behaving nepotistically, raising their super sisters (G=0.75) rather than their half sisters (G=0.25) as the new queens, thus increasing their inclusive fitness (Hamilton 1964). However, this potential for conflict may not be translated into actual conflict if differential treatment of super and half sisters incurs costs at the colony level that outweigh the benefits to nepotistic individuals (Ratnieks 1989; Carlin et al. 1993; Sundström and Boomsma 2001). For example, if nepotism results in a decrease in colony efficiency (e.g., fewer or poorer queens get produced), then all members within the colony may experience a net decrease in inclusive fitness and

nepotism might be selectively disfavored. It is interesting that while multiple mating decreases potential conflict over those mechanisms of reproduction, it creates potential conflict over queen production by forming multiple subfamilies of workers with different genetic interests. While this aspect of colony reproduction has a direct and obvious influence on colony fitness, the process of queen replacement has been less well studied than other reproductive conflicts.

In this review, we will examine the balance between conflict and cooperation, hence the respective roles of within-colony and between-colony selection, in the context of queen replacement in honey bee colonies. We will do so by considering whether the workers behave conflictually to favor close relatives among the queens produced by their colony, if they behave cooperatively to optimize the reproductive "quality" of the queens, or if they do both.

Queen replacement in honey bees

A honey bee colony replaces its mother queen when she is old and failing and so needs to be superceded, during "emergencies" when she is the victim of predation or human intervention, or when she leaves in a swarm during colony fission (reproduction). All instances of queen replacement are accomplished in two distinct phases. The first phase is "queen rearing", which is triggered by numerous colony and environmental factors (reviewed by Winston 1987). A colony constructs one to two dozen queen cells, which are larger than worker or drone cells and hang down vertically on the wax combs. Queen cells are initiated when workers either construct special queen "cups" into which the queen lays eggs or when they construct queen cells from worker cells that contain eggs or young larvae (Fig. 1A). The cells are elongated by the workers as the queen larvae grow and develop, and are sealed when their occupants are 5-day-old larvae (Fig. 1B). The total development time of a queen is

Fig. 1A–E The two phases of queen production: queen rearing and queen elimination. **A** A queen cell (*centered*) modified from a worker cell on the brood comb. **B** Queen cells constructed experimentally from "grafted" worker larvae. **C–E** Queen elimination by dueling rivals
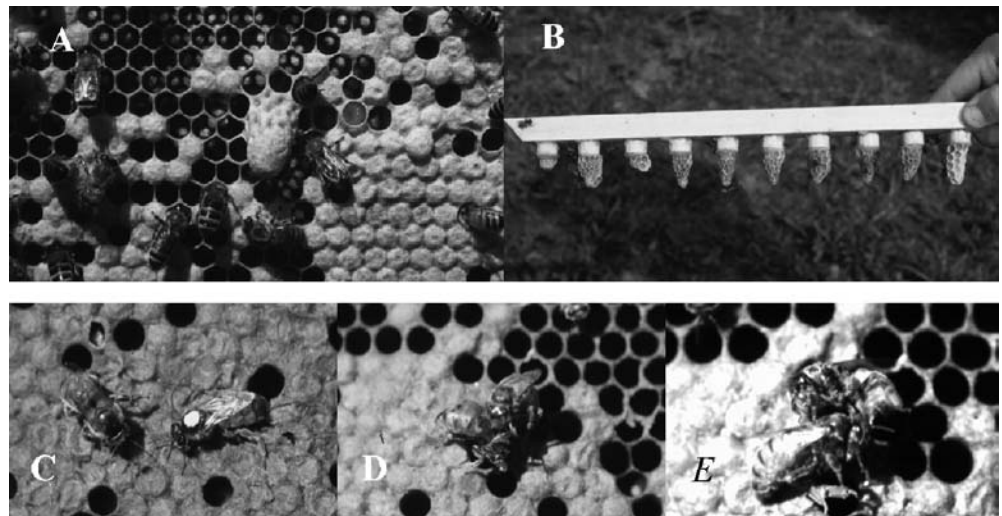
approximately 15 days (e.g., DeGrandi-Hoffman et al. 1998).

The second phase of queen replacement is "queen elimination", where the total number of virgin queens is reduced from many to one (what Tarpy and Fletcher 1998 term "polygyny reduction"). Upon emerging from her cell, a mature queen may either depart the natal nest in a secondary swarm (also known as an afterswarm) with several thousand workers to found a new nest, or she may remain in the natal nest and attempt to kill any other virgin queens in the colony. A queen may kill her rivals either by "assassinating" them before they emerge from their cells (Huber [1926/1792]; Boch 1979; Gilley 2001) or by stinging them during combat (Gilley 2001; Schneider et al. 2001; Fig. 1C–E). In most species of social insects, the workers kill supernumerary queens (Wilson 1971; Michener 1974; Fletcher and Ross 1985; Heinze 1993), but in honey bees, the workers leave it to the rival queens to kill each other (Gilley 2001; Schneider et al. 2001; Gilley and Tarpy, submitted for publication). While this curious fact has been known for centuries (Huber [1926/1792]; Darwin 1859), the interactions among queens and workers—during what Visscher (1993) calls the "afterswarming syndrome"—have been investigated in detail only recently. Anecdotal accounts of queen fights were provided in studies that described communication signals during swarming, such as queen piping and worker shaking (Grooters 1987; Kirchner 1993). Visscher (1993), in an important theoretical paper, outlined the reproductive interests of a queen as she decides to depart in a swarm or engage in fights. He made numerous predictions about the mechanisms regulating queen emergence, queen fighting ability, and swarm departure, and supporting evidence has since been obtained for some of them. Gilley (2001) provides a detailed account of five queen "duels" in observation hives. He describes the pertinent queen behaviors and worker–queen interactions, including queen cell "patrolling", queen piping, worker grooming, worker shaking, and numerous forms of worker aggression toward queens. These acts of aggression can vary greatly among queens. He and others also report that a queen can eject a liquid from her abdomen that the workers react to strongly (Post et al. 1987; Page et al. 1988; Bernasconi et al. 2000) and that this "spraying" behavior may be a fighting tactic that immobilizes rivals in worker aggregations (Gilley 2001; Tarpy and Fletcher 2003). The ultimate outcome of queen replacement is one queen that claims the parental nest and perhaps additional queens in swarms that establish new nests.

It is clear that both the pre- and post-emergence phases of queen replacement involve complex interactions among queens and workers. It is plausible, therefore, that these interactions enable workers to bias the outcomes of these events in favor of certain queens. If the outcomes of queen rearing and queen elimination are biased by workers in favor of more-closely related queens (nepotism), then it would demonstrate actual conflict during queen replacement, indicating that selection has been acting at the individual level (within colonies). If, however, the outcomes favor queens of higher reproductive quality, then it would demonstrate significant cooperation during queen replacement, indicating that selection has been acting at the colony level (between colonies).

The queen rearing phase of queen replacement

Conflict (nepotism) during queen rearing

Nepotism in honey bees has been investigated mostly in the context of queen rearing. Visscher (1998) makes the distinction between studies that have investigated processes (i.e., the social interactions of nurse bees with queen larvae) and those that have investigated products (i.e., the number of queens accepted). Here, we categorize the literature according to the three general methods that have been used in attempts to detect the preferential rearing of queens in favor of more-closely related kin. The first approach has been to perform reciprocal transfers of young worker brood (eggs and larvae) between colonies of varying relatedness to determine if the proportion of queens raised from more-related individuals is higher than the proportion of those raised from less-related individuals (reviewed by Breed et al. 1994; Visscher 1998). A summary of these studies is given in Table 1. The methods of the different experiments vary widely. Some studies simply exchanged combs of brood between colonies and determined how many "emergency" queen cells were constructed on each (Breed et al. 1984; see also Page and Erickson 1986a). Others studies transferred worker eggs (Woyciechowski 1990) or larvae (Breed et al. 1984; Page and Erickson 1984; Tarpy and Fletcher 1998; Mohammedi and Le Conte 2000), or both (Visscher 1986), by employing a commercial practice known as "grafting" (Laidlaw and Page 1997; Fig. 1B). The grafting procedure is accomplished by transferring brood into queen cups that may or may not contain a drop of royal jelly ("wet" vs "dry" grafting, respectively). This procedural difference is worth noting because several authors (e.g., Visscher 1998) have suggested that royal jelly may mask acceptance cues, and may therefore explain why some studies that dry-grafted brood have shown significant results while those that wet-grafted brood have not. Another difference among the studies is the genetic relationships of the developing queens to the workers in the queen-rearing colonies. Some studies used colonies headed by queens that were instrumentally inseminated, hence containing just one or a few subfamilies or "patrilines", while other studies used colonies headed by queens that mated naturally with a (presumably) large, unknown number of males, hence containing many subfamilies. There are two other studies worth noting that are not included in Table 1. Page and Erickson (1986a) reared queens from different subfamilies in two-subfamily colonies that varied in their ratio of workers from each subfamily. While their experimental design could not detect whether more-closely related individuals

Table 1 Summary of the different studies that tested nepotism during queen rearing in honey bees using the reciprocal brood transfer design. Relatedness values are given as the coefficient of relatedness (G). If queens were naturally mated, most workers are half sisters (G=0.25) but an unknown proportion are full sisters (G=0.75). Probabilities are from simple two-tailed binomial tests with p=0.50, the values of which are sometimes different from those in the published reports because different analyses were used

Reference	Exp.	No. colonies	Genetic structure[a]	Graft type[b]	Graft age[c]	Relatedness		No. queens accepted				No. queens reared			
						More related	Less related	More related	Less related	% Related	P	More related	Less related	% Related	P
Breed et al. 1984	1	10	NM	CE	All	0.25[d]	0.00	22	20	52.4	0.64	–	–	–	–
	2	8	NM	Wet	1-day L	0.25[d]	0.00	112	111	50.2	0.89	53	55	49.1	0.92
	3	6	NM	Wet	1-day L	0.25[d]	0.00	94	91	50.8	0.77	34	33	50.8	0.81
Page and Erickson 1984	1	10	SDI	Dry	0-day L	0.75	0.3125 or 0.00	29[e]	18[e]	61.7	0.08	–	–	–	–
Visscher 1986	1	8	NM	Wet	1-day L	0.25[d]	0.00	208	209	49.9	1.00	–	–	–	–
	2	20	NM	Dry	1-day E	0.25[d]	0.00	121	91	57.1	0.03	–	–	–	–
	3	6	SDI	Dry	1-day E	0.50	0.00	26	19	57.8	0.23	–	–	–	–
	4	8	NM	Dry	1-day E	0.22	0.00	28	15	65.1	0.03	–	–	–	–
	5	8	NM[f]	Dry	1-day E	0.25[d]	0.00	75	45	62.5	0.00	–	–	–	–
Woyciechowski 1990	1	5	SDI	Dry	3-day E	0.75	0.00	22	24	47.8	0.88	–	–	–	–
	2	3	MDI	Dry	3-day E	?[g]	0.00	40	51	44.0	0.29	–	–	–	–
Tarpy and Fletcher 1998	1	12	NM	Wet	0-day L	0.25[d]	0.00	107	106	50.2	0.89	31	32	49.2	1.00
Mohammedi and LeConte 2000	1[h]	6	NM	Dry	1-day L	0.25[d]	0.00	583	526	52.6	0.00	–	–	–	–
	1[i]	4	NM	Dry	1-day L	0.25[d]	0.00	232	197	54.1	0.08	–	–	–	–
	2[h]	6	NM	Dry	1-day L[j]	0.25[d]	0.00	–	–	–	–	307	300	50.6	0.74
	2[i]	4	NM	Dry	1-day L[j]	0.25[d]	0.00	–	–	–	–	82	73	52.9	0.42

[a] *NM* Naturally mated with an unknown number of males, *SDI* single-drone inseminated, *MDI* multi-drone inseminated.

[b] *CE* Comb exchange, *Wet* grafting with royal jelly, *Dry* grafting without royal jelly

[c] *All* Worker brood of all ages, *L* worker larvae, *E* worker eggs

[d] Since the queens had mated with a large, unknown number of males, some of the more-related queens may have been super sisters (G=0.75) rather than half sisters (G=0.25)

[e] Only includes number of sealed cells after 6 days, not earlier measures in Table 1

[f] Assumed, but not explicitly stated

[g] Queens were "inseminated with semen from several drones from the same colony." Without knowing the exact number, however, it is impossible to calculate the coefficient of relatedness of the more related individuals

[h] Experiment performed during the summer months

[i] Experiment performed during the winter months

[j] Two larvae were grafted into each queen cup, one more related and one less related

were preferentially raised as queens, they did find that certain subfamilies were preferred over others during queen rearing (see following discussion). More recently, experiment 3 by Mohammedi and Le Conte (2000) involved reciprocal transfers of worker larvae, but they did not measure queen acceptance (as they did in experiments 1 and 2; Table 1). Rather, they compared the combined weights of queen larvae and brood food between sister and unrelated queens, and they did not observe significant differences between the two treatments during either the summer or winter trials.

The second approach to testing for nepotism in queen rearing has been to look for significant associations between queen larvae and workers (particularly the nurse bees that raise them) of the same subfamily, with the expectation that workers will preferentially tend queen larvae to which they are more related (see also Hannonen and Sundström 2003). Noonan (1986) investigated the queen-rearing behaviors of workers in five colonies housed in observation hives. The workers of each of these colonies belonged to one of two phenotypically distinct subfamilies ("cordovan" vs "dark") produced by a recessive genetic marker for cuticular color. While she found no significant differences between the two subfamilies of each colony in the number of queens reared, she observed that workers of the two subfamilies preferentially visited, fed, and maintained queen cells containing larvae of their own subfamily. The results of this study have been questioned, however, because the workers lacked the normal amount of genotypic variability (i.e., they consisted of only two subfamilies) and because there was no control for the recessive cuticular marker (see Breed et al. 1994 for discussion). Page et al. (1989) performed a study similar to Noonan (1986), but the nestmates belonged to one of three subfamilies that were distinguishable only by the malate dehydrogenase (MDH) allozyme. They, too, observed that workers were significantly more associated with developing queens that were more closely related to themselves. However, Oldroyd et al. (1990) reanalyzed their statistical methods and found a systematic flaw that biased their results in favor of rejecting the null hypothesis. An independent analysis by Visscher (1998), on the other hand, showed that Page et al.'s (1989) results are consistent with the predictions of nepotism in three ways. First, he found significant heterogeneity between the counts of nurses and worker larvae in each subfamily, suggesting that nurse bees are not a random sample of the colony's workers (see also Robinson et al. 1994; Pankiw 1997). Second, he found significant heterogeneity between the counts of queen larvae and worker larvae in each subfamily, suggesting that queens are not raised randomly from a colony's brood (see below). Third, he showed that the goodness of fit between the queen and nurse bee distributions to be closer than that of the nurse bee and larvae distributions, which would be expected if nurse bees more closely associate themselves with more-related queens. Thus it seems that the Page et al. (1989) study provides evidence of nepotism in queen rearing after all.

Finally, a recent study by Schneider and DeGrandi-Hoffman (2002) showed that workers within hybrid European and Africanized colonies demonstrated positive kin preferences for queen cell visitations, but not for other measures of queen rearing behavior.

The third approach to testing for nepotism in queen rearing has been to look for significant differences between the distributions of worker subfamilies and queen subfamilies using PCR-based molecular paternity analyses. If nepotism exists, then some subfamilies will be observed more frequently in queen brood than in the worker brood, particularly those subfamilies that have a numerical majority of workers. The first to use this approach was Estoup et al. (1994) in a ground-breaking study utilizing microsatellite DNA markers. They found that the distribution of subfamilies within a single colony's worker brood was the same as the distribution of subfamilies within the colony's queen brood, suggesting an absence of nepotistic queen rearing. The relatively small sample size of genotyped queens, however, greatly limited the possibility that they would be able to detect nepotism. Frank et al. (2002) similarly did not detect any queen rearing biases in six colonies headed by naturally inseminated, mate-limited queens. Tilley and Oldroyd (1997), on the other hand, were able to detect that queens were not reared randomly from worker brood because certain subfamilies were consistently over-represented in queen brood (see also Page and Erickson 1986a; Visscher 1998). They interpret their results as supporting an alternative hypothesis; queens of certain subfamilies are more likely to be reared as queens for reasons other than nepotism (e.g., increased attractiveness to nurse bees). Osborne and Oldroyd (1999) rejected this "royalty alleles" hypothesis in favor of nepotism because they found higher levels of subfamily heterogeneity in colonies related to the queens than in unrelated "discriminator" colonies. Since the subfamily proportions of queen larvae are more skewed towards certain subfamilies when the workers are related to the brood, their findings are consistent with nepotism during queen rearing. Most recently, Châline et al. (2003) found that one subfamily represented a higher proportion of queen brood than expected in a colony undergoing emergency queen replacement. This effect was not detected in a second colony, however, suggesting that nepotism is either weak or not always present.

Despite the accumulated evidence for and against nepotism that has been obtained by these many studies, it is difficult to draw robust conclusions about nepotism during queen rearing in honey bees. The diversity of approaches lends strength to the body of data, but unfortunately it is inappropriate for a meta-analysis (e.g., Arnqvist and Wooster 1995) because the methodologies are so different from one another. In their previous reviews, Breed et al. (1994) conclude that there is little if any nepotism during queen rearing in honey bees, and Visscher (1998) concludes that nepotism does occur, although the data are equivocal and the effect is most likely weak. Thus while the issue of nepotistic queen

rearing remains unresolved, it seems clear that the evidence indicates that strong nepotism is not manifested during queen rearing. Even if weak nepotism does exist, the biological relevance of the behavior during this stage of queen replacement in honey bees is questionable.

Cooperation during queen rearing

Queen rearing by honey bees involves numerous individuals because no single worker can rear a queen on her own; it requires copious amounts of royal jelly to raise a honey bee queen, much more than one worker can secrete over the 5-day period of a queen larva. Even though workers may have divergent genetic interests (see above), they have a common interest in producing queens of high reproductive quality. Determining if workers affect queen quality during queen rearing will reveal the extent of cooperation between different worker subfamilies.

A queen's reproductive "quality" is a function of her mating success, fecundity, and offspring viability (Futuyma 1998; Gilley et al. 2003). While workers influence a queen's mating success or brood viability only indirectly, there are two means by which they can influence a queen's fecundity directly. The first is by feeding a queen larva a diet rich in protein and sugars (reviewed by Herbert 1992; Page and Peng 2001). Royal jelly contains more glucose and fructose than worker brood food, and includes additional substances such as vitamins and 10-HDA (a precursor to the major queen mandibular pheromone). Every queen larva literally floats on a pool of royal jelly; they are always provided with more food than they can consume (Herbert 1992). Thus unless a colony suffers from a serious food shortage (Schmickl and Crailsheim 2002), workers cooperate to ensure that queen larvae are fully fed, thereby ensuring the production of high-quality queens.

The second means by which workers can influence queen fecundity is by initiating queen rearing with brood of an appropriate age. A queen's quality can be quantified by measuring her external morphological characters (e.g., weight, thorax width, and wing lengths) and her internal reproductive organs (e.g., ovary weight, ovariole number, and spermatheca volume), all of which are highly correlated to each other (Eckert 1934; Woyke 1971; Clarke 1989; Hatch et al. 1999; Gilley et al. 2003) and many of which are strongly correlated with brood production (Avetisyan 1961; Makarov 1969). A queen's quality is negatively correlated with the initial age at which an egg or larva is fed royal jelly (Woyke 1971; Hatch et al. 1999; Gilley et al. 2003). For example, a queen that is raised from a 2-day-old worker larva will be of lesser quality compared to a queen raised from a larva that has recently hatched from its egg casing.

Queen rearing is regulated by workers performing two behaviors non-randomly. First, workers build queen cells preferentially from worker cells that contain older worker eggs or from younger larvae (Fletcher 1978; Fell and Morse 1984; Hatch et al. 1999; Fig. 2). Second, workers
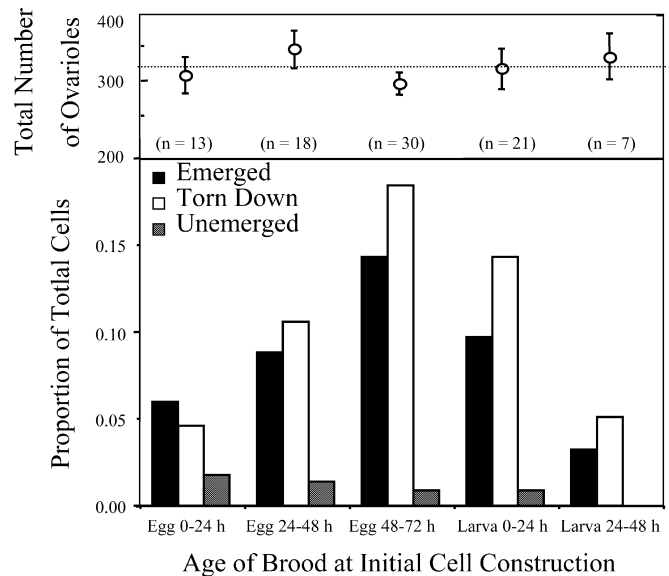

Fig. 2 The number of queen cells constructed from modified worker cells depends on the age of the egg or larva from which it is constructed. Furthermore, the proportion of initiated cells which are torn down increases as a function of age. This may suggest that workers are selectively destroying queen cells with poorer quality queens. The remaining queens have similar reproductive potentials, as measured by their similar ovariole numbers (means±SE; adopted from Hatch et al. 1999)

tear down queens cells after they have been sealed (Allen 1956; Gary and Morse 1962; Fletcher 1978; Winston and Taylor 1980; Melathopoulos et al. 1996; Schneider et al. 2001), and cells that are started with older brood are destroyed significantly more often than cells initiated with eggs (Hatch et al. 1999; Schneider and DeGrandi-Hoffman 2002). The bias in cell destruction suggests that workers are affecting the outcome of queen rearing by decreasing the variation in queen reproductive potential (Winston 1987). Indeed, the resultant queens in the Hatch et al. (1999) study did not differ significantly with respect to several measures of fecundity. Most notably, there were no significant differences in ovariole number between the queens produced from different age cohorts (Fig. 2), which is arguably the most direct measure of a queen's reproductive value. Thus workers may be "weeding out" low-quality queens during queen rearing by preferentially destroying their cells. However, the conclusive experiment of this hypothesis has yet to be performed (e.g., comparing queens from cells destined to be destroyed to those that are not destroyed). Thus the current evidence, perhaps intuitively, suggests that colonies have evolved elaborate mechanisms to maximize the reproductive quality of the queens that they rear.

The queen elimination phase of queen replacement

Nepotism (conflict) during queen elimination

Honey bee colonies raise many more queens than will survive to head the colonies they produce. Studies of queen rearing, therefore, have investigated only the first phase of queen replacement. The queen elimination phase involves complex interactions among queens, as well as between workers and queens (see above). The intense interactions between workers and virgin queens creates a high potential for selection to act on colony members to bias the outcomes in favor of certain queens. Given that queen survival is the ultimate end of queen replacement, it is surprising that so few studies have examined the mechanisms by which the final replacement queen is determined.

As in studies of queen rearing, supporting evidence for nepotism during queen elimination would be observed if queens that are more related to the workers of a colony have a higher probability of surviving to head a colony than queens that are less related to the workers. The first study to test this hypothesis (albeit indirectly) was Page and Erickson (1986b), who established numerous observation arenas in 135 ml cardboard cups, each of which contained ten super sister workers and one queen that was either a half sister or a super sister to the workers. A second queen, which was either a half sister or a super sister to the workers and resident queen, was added to the arenas after 0.5 to 6.0 h. They found that queens had a survival advantage after 8 h if they were more related to the workers and less related to their rival queen. However, they did not demonstrate a significant survival advantage of queens that were super sisters to the workers (42 alive vs 36 dead) compared to those that were half sisters to the workers (58 alive vs 26 dead). As the authors point out, their experimental design may not reflect the full suite of behaviors during polygyny reduction because it involved an artificial environment, not a normal colony.

Another experiment, by Tarpy and Fletcher (1998), simultaneously placed pairs of virgin queens that were reared in a common colony into four-frame "nucleus" colonies. Each pair of queens consisted of one that was a half sister or a super sister of the colony's workers and one queen that was unrelated to the colony's workers. They found that queens that were related to the workers had a significantly higher probability of surviving after 48 h compared to queens that were unrelated to the workers. Their study, however, tested queens that were genetically unrelated to the workers in colonies that lacked control over the number of subfamilies within them. Since nestmate recognition has a (small) genetic component (reviewed by Breed 1998), it is unclear whether their observed advantage of related queens is due to nepotism or nestmate recognition.

A third study, by Schneider and DeGrandi-Hoffman (2003), demonstrated that virgin queens of African paternity (when reared in European colonies) exhibited greater piping activity, eliminated more of their rival queens, and were more likely to become the replacement queen than their half sisters of European paternity. They also showed that African-patriline queens received more shaking signals from workers, which is associated with longer survival and greater fighting success (Schneider et al. 2001; Schneider and DeGrandi-Hoffman 2003). Workers of African paternity were more likely to perform these signals, and they exhibited a greater tendency to perform shaking signals on super sister queens. They conclude, however, that a queen's paternity has a stronger influence on shaking signaling than relatedness because African-patriline queens were preferentially shaken by workers independent of whether the workers were super or half sisters. Thus while these results indicate that workers may bias the queen elimination phase in favor of particular queens, they do not necessarily demonstrate nepotism during queen elimination.

Most recently, Gilley (2003) used a powerful experimental design to test nepotism in unmanipulated colonies undergoing natural queen replacement. Using microsatellite DNA markers, he genotyped hundreds of workers that were involved in aggressive worker–queen interactions with 27 dueling queens in swarming colonies in observation hives. He found that workers that harassed queens were neither more-closely nor more-distantly related to the queens than were workers selected at random. A subsequent power analysis showed that these results are unlikely to be due to a type II error (false negative). While it is possible that nepotism may occur via other worker–queen interactions (such as infrequent trophallactic interactions), this experiment is strong evidence that nepotism is not a factor during the queen elimination phase.

In fire ants (*Solenopsis invicta*), workers do not favor their mother during fights between co-foundress queens (Balas and Adams 1996; Bernasconi and Keller 1996; Bernasconi et al. 1997). Despite some conflicting evidence, it is most likely that honey bee workers also do not favor more-closely related sister queens. Clearly more studies are required in the context of queen elimination before any robust conclusions can be drawn. The complete lack of kin-biased interactions between workers and queens during queen duels strongly suggests that workers do not bias the queen elimination process in favor of super sisters. Thus it is unlikely that nepotism occurs during the queen elimination process, again suggesting that there is minimal conflict during queen replacement.

Cooperation during queen elimination

If selection has acted on colonies to maximize the reproductive value of replacement queens, it is plausible that workers would bias polygyny reduction in favor of queens with higher reproductive potential. Unfortunately, only a few studies have investigated this possibility.

Using the same experimental assay as Tarpy and Fletcher (1998), Tarpy et al. (2000) placed into nucleus colonies pairs of queens that differed in either their actual or initial-rearing ages. In their first experiment, they

found that 3-day-old ("old") queens survived significantly more often than 1-day-old ("young") queens, which suggests that either older queens have an inherent survival advantage or that workers bias fights in favor of older queens. In their second experiment, they found that queens reared from <12-h-old larvae ("high quality" queens) did not survive significantly more often than queens reared from 2-day-old larvae ("low quality" queens), which suggests that higher quality queens do not have an inherent survival advantage and are not favored by workers over lower quality, same-aged sister rivals. The low-quality queens in their study, however, were not significantly different from their high-quality counterparts with respect to many morphological measures (and none if a Bonferroni correction is implemented). It is possible that a greater difference in queen quality between the two treatments would have yielded different results.

Schneider and DeGrandi-Hoffman (2003) investigated the relationship between queen size and survival, although it was not the primary focus of their study. They reported no difference in the dry weights between winning and losing queens. Likewise, queens that eventually survived or were killed did not differ in thorax length or width (S. Schneider, personal communication). However, they observed significant, positive correlations between queen size, the number of rivals eliminated, piping activity, and the rate at which workers performed shaking signals on queens (S. Schneider, personal communication). Smaller queens, however, survived significantly longer, which most likely accounted for the overall lack of a significant relationship between queen size and success. Thus, while queen quality (as measured by dry weight) does not determine the ultimate outcome of queen elimination, it may influence the dynamics of the process.

Most recently, Gilley et al. (2003) examined cooperation during queen elimination by using methods similar to those of Tarpy et al. (2000). They created queens of high quality, low quality, and very low quality by rearing queens from newly hatched eggs, 2-day-old larvae, and 3-day-old larvae, respectively. They placed the developing queens in swarming colonies in observation hives and, upon the queens' emergence, recorded the rates of six worker–queen interactions. They predicted that workers would have more frequent antagonistic interactions (e.g., "chasing" and "grabbing") and fewer beneficent interactions (e.g., trophallaxis and grooming) with lower-quality queens than with higher-quality queens. However, they found no consistent effect of queen quality on the rates of worker–queen interactions, suggesting that workers do not actively cooperate to choose high-quality queens during queen elimination. This study has several strengths that make this finding unlikely to be a false negative: (1) genetic variation among the experimental queens was reduced by rearing them from the brood of one singly inseminated queen, (2) there was a significant effect of the queen treatment on both external morphology and internal organs of the experimental queens, and (3) a power analysis showed that even small differences in

interaction rates would have been detected given the experiment's sample size and the variance in the data.

It appears, from the few studies available, that a queen's quality has little bearing on the outcomes of the queen elimination phase of queen replacement, but more data are needed before definitive conclusions can be drawn.

Conclusions

Much remains to be learned about conflict and cooperation during queen replacement in honey bees, since much of the evidence presented above is negative or equivocal. Nonetheless, it is possible to draw tentative conclusions about the relative roles of individual- versus colony-level selection during this important reproductive process in honey bees (Table 2). When colonies rear queens, there may exist a small amount of conflict over which individuals to raise as queens based on genetic relatedness, but the workers mostly cooperate in both constructing and destroying queen cells so that the queens which emerge are of high reproductive quality. When the queens mature and emerge from their cells, they interact significantly with the workers and engage in mortal combat with each other, but it is unclear whether these interactions favor queens of certain subfamilies or of higher reproductive quality. Thus, it appears that queen replacement in honey bees is largely a cooperative venture, where workers cooperate to raise high-quality queens so that whichever queen eventually survives the elimination phase is a suitable replacement for the departed mother queen. Just as potential conflict over male production and the sex ratio does not translate into actual conflict, the potential conflict over queen production appears to be minimized in favor of cooperation.

Theoretically, colonies should always maximize the reproductive quality of queens because they are under constant selective pressure—both at the individual and colony levels—to ensure that replacement queens are of the highest reproductive value. It is less clear, however, if selection should always favor individuals that behave nepotistically (e.g., Griffin and West 2002; Queller and Strassmann 2002). For example, Breed et al. (1994) point out that directional selection for nepotistic queen rearing would cause the trait to become fixed in the population. Thus, in the absence of other costs, selection would maintain the trait even though the net effect of the preference is neutral because all subfamilies would express it. So far, the evidence seems to be consistent with these arguments.

The largest gaps in our knowledge of the queen replacement process relate to the following questions. Do workers preferentially tear down queens cells of lower quality queens? and Why do workers interact so intensely with queens during queen duels? One possibility is that these interactions enable the workers to limit the selfish sororicide of the queens, thus ensuring sufficient queens for the colony, including secondary swarms it might

Table 2 Summary of evidence for conflict and cooperation during the queen rearing and queen elimination phases of queen replacement in honey bee colonies

	Queen rearing		Queen elimination	
	Conflict	Cooperation	Conflict	Cooperation
Relative number of studies devoted to the issue	Many	Many	Few	Very few
General conclusion from the current body of evidence	Workers exhibit little, if any, nepotism toward developing queens	Workers strongly cooperate to raise queens of high reproductive quality	Workers may bias the outcomes of queen duels, but probably not in favor of more-related queens	Workers do not differentially interact with queens of different reproductive quality
Adaptive significance	Colony-level selection is predominant over individual-level selection, so queens of high reproductive potential are reared		Colony-level selection is predominant over individual-level selection, so neither too many nor too few queens are eliminated	

produce. In future investigations into these questions, it will be important to study the queen replacement process in its entirety (i.e., both the queen rearing and queen elimination phases), and to bear in mind how different levels of selection can shape the respective behaviors of queens and workers.

> "...We ought to admire the savage instinctive hatred of the queen-bee, which urges her to destroy the young queens, her daughters [sic], as soon as they are born, or to perish herself in the combat; for undoubtedly this is for the good of the community; and maternal love or maternal hatred, though the latter fortunately is most rare, is all the same to the inexorable principle of natural selection" (Darwin 1859).

Even though he mistook sister–sister competition for mother–daughter competition, Darwin recognized the importance of colony-level selection. Indeed, it was natural selection acting on the colony phenotype that enabled Darwin to reconcile the evolution of worker sterility in social insects with his fledgling theory of natural selection. This group-selection thinking has been rejected historically almost wholesale by biologists in favor of selection acting solely at the individual level (Wilson 1997). As Dawkins (1982, 1989) argued, however, the distinction between individual- and group-selection is a matter of which level of biological organization serves, in his words, as the "vehicle" of gene propagation. While most higher-level groups of organisms, such as flocks of birds or schools of fish, lack sufficient cohesion to serve as effective vehicles of gene propagation, many authors have argued that colonies of social insects may be well suited to do so because their members have common genetic interests (reviewed by Keller and Reeve 1999). Unlike the cells of a metazoan organism, however, the genetic interests of colony members can differ, leading to a complex mix of conflict and cooperation. Queen replacement in honey bees exemplifies this mix of conflict and cooperation because colony members simultaneously have different genetic interests yet share a common goal. By examining this interface, and hence the relative within- and between-group selection pressures, we can better understand how selection has shaped the behavior of nestmates during a critical point in a colony's life cycle.

References

Allen MD (1956) The behavior of honeybees preparing to swarm. Anim Behav 4:14–22

Arnqvist G, Wooster D (1995) Meta-analysis: synthesizing research findings in ecology and evolution. Trends Ecol Evol 10:236–240

Avetisyan GA (1961) The relation between interior and exterior characteristics of the queen and fertility and productivity of the bee colony. In: XVIII International Beekeeping Congress, Madrid, Spain, pp 44–53

Balas MT, Adams ES (1996) The dissolution of cooperative groups: mechanisms of queen mortality in incipient fire ant colonies. Behav Ecol Sociobiol 38:391–399

Bernasconi G, Keller L (1996) Reproductive conflicts in cooperative associations of fire ant queens (Solenopsis invicta). Proc R Soc Lond B 263:509–513

Bernasconi G, Krieger MJB, Keller L (1997) Unequal partitioning of reproduction and investment between cooperating queens in the fire ant, Solenopsis invicta, as revealed by microsatellites. Proc R Soc Lond B 264:1331–1336

Bernasconi G, Ratnieks FLW, Rand E (2000) Effect of "spraying" by fighting honey bee queens (Apis mellifera L.) on the temporal structure of fights. Insectes Soc 47:21–26

Boch R (1979) Queen substance pheromone produced by immature queen honeybees. J Apic Res 18:12–15

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, N.J.

Breed MD (1998) Recognition pheromones of the honey bee. Bioscience 48:463–470

Breed MD, Velthuis HHW, Robinson GE (1984) Do worker honey bees discriminate among unrelated and related larval phenotypes? Ann Entomol Soc Am 77:737–739

Breed MD, Welch CK, Cruz R (1994) Kin discrimination within honey bee (Apis mellifera) colonies: an analysis of the evidence. Behav Process 33:25–40

Carlin NF, Reeve HK, Cover SP (1993) Kin discrimination and division of labour among matrilines in the polygynous carpenter ant, Camponotus planatus. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 362–401

Châline N, Arnold G, Papin C, Ratnieks FLW (2003) Patriline differences in emergency queens rearing in the honey bee, Apis mellifera. Insectes Soc 50:234–236

Clarke RJ (1989) Queen physiological quality. A discussion paper for New Zealand queen producers. Sunflora Apiaries, Blenheim, New Zealand, pp 1–9

Crozier RH, Pamilo P (1996) Evolution of social insect colonies: sex allocation and kin selection. Oxford University Press, Oxford

Darwin C (1859) On the origin of species. Murray, London

Dawkins R (1982) The extended phenotype. Freeman, Oxford

Dawkins R (1989) The selfish gene, 2nd edn. Oxford University Press, Oxford

DeGrandi-Hoffman G, Watkins JC, Collins AM, Loper GM, Martin JH, Arias MC, Sheppard WS (1998) Queen developmental time as a factor in the Africanization of European honey bee (Hymenoptera: Apidae) populations. Ann Entomol Soc Am 91:52–58

Eckert JE (1934) Studies in the number of ovarioles in queen honeybees in relation to body size. J Econ Entomol 27:629–635

Estoup A, Solignac M, Cornuet J (1994) Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. Proc R Soc Lond B 258:1–7

Fell RD, Morse RA (1984) Emergency queen cell production in the honey bee colony. Insectes Soc 31:221–237

Fletcher DJC (1978) Vibration of queen cells by worker honeybees and its relation to the issue of swarms with virgin queens. J Apic Res 17:14–26

Fletcher DJC, Ross KG (1985) Regulation of reproduction in eusocial Hymenoptera. Annu Rev Entomol 30:319–343

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

Futuyma DJ (1998) Evolutionary biology. Sinauer, Sunderland, Mass.

Gary NE, Morse RA (1962) The events following queen cell construction in honeybee colonies. J Apic Res 1:3–5

Gilley DC (2001) The behavior of honey bees (Apis mellifera ligustica) during queen duels. Ethology 107:601–622

Gilley DC (2003) Absence of nepotism in the aggressive interactions between workers and dueling queen honey bees. Proc R Soc Lond B. 270:2045–2049

Gilley DC, Tarpy DR, Land BB (2003) The effect of queen quality on the interactions of workers and dueling queen honey bees (Apis mellifera L.). Behav Ecol Sociobiol 55:190–196

Griffin AS, West SA (2002) Kin selection: fact and fiction. Trends Ecol Evol 17:15–21

Grooters HJ (1987) Influences of queen piping and worker behaviour on the timing of emergence of honey bee queens. Insectes Soc 34:181–193

Hamilton WD (1964) The genetical evolution of social behaviour. I and II. J Theoret Biol 7:1–52

Hannonen M, Sundström L (2003) Worker nepotism among polygynous ants. Nature 421:910–910

Hatch S, Tarpy DR, Fletcher DJC (1999) Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insectes Soc 46:372–377

Heinze J (1993) Queen–queen interactions in polygynous ants. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, New York, pp 334–361

Herbert EW Jr (1992) Honey bee nutrition. In: The hive and the honey bee. Dadant, Hamilton, Ill., pp 197–233

Huber F (1926) New observations on bees, vol 1. Translated from the French by C.P. Dadant. American Bee Journal, Hamilton IL [First published in 1792]

Keller L (1999) Levels of selection in evolution. Princeton University Press, Princeton, N.J.

Keller L, Reeve HK (1999) Dynamics of conflicts in insect societies. In: Keller L (ed) Levels of selection in evolution. Princeton University Press, Princeton, N.J., pp 153–175

Kirchner WH (1993) Vibrational signals in the tremble dance of the honeybee, Apis mellifera. Behav Ecol Sociobiol 33:169–172

Laidlaw HH Jr, Page RE Jr (1997) Queen rearing and bee breeding. Wicwas, Cheshire, Conn.

Makarov YI (1969) Biologically and economically useful characters of far eastern bees and their selection. PhD thesis, Timiryazev Academy of Agriculture, Moscow

Melathopoulos AP, Winston ML, Pettis JS, Pankiw T (1996) Effect of queen mandibular pheromone on initiation and maintenance of queen cells in the honey bee (Apis mellifera L.). Can Entomol 128:263–272

Michener C, D, (1974) The social behavior of the bees. Harvard University Press, Cambridge, Mass.

Mohammedi A, Le Conte Y (2000) Do environmental conditions exert an effect on nest-mate recognition in queen rearing honey bees? Insectes Soc 47:307–312

Moritz RFA (1985) The effects of multiple mating on the worker-queen conflict in Apis mellifera. Behav Ecol Sociobiol 16:1986

Noonan KC (1986) Recognition of queen larvae by worker honey bees (Apis mellifera). Ethology 73:295–306

Oldroyd BP, Clifton MJ, Parker K, Wongsiri S, Rinderer TE, Crozier RH (1998) Evolution of mating behavior in the genus Apis and an estimate of mating frequency in Apis cerana (Hymenoptera: Apidae). Ann Entomol Soc Am 91:700–709

Oldroyd BP, Rinderer TE, Buco SM (1990) Nepotism in the honey bee. Nature 346:707–708

Osborne KE, Oldroyd BP (1999) Possible causes of reproductive dominance during emergency queen rearing by honeybees. Anim Behav 58:267–272

Page RE Jr, Erickson EH (1984) Selective queen rearing by worker honeybees: kin or nestmate recognition. Ann Entomol Soc Am 77:578–580

Page RE Jr, Erickson EH Jr (1986a) Kin recognition during emergency queen rearing by worker honey bees (Hymenoptera: Apidae). Ann Entomol Soc Am 79:460–467

Page RE Jr, Erickson EH (1986b) Kin recognition of virgin queen acceptance by worker honey bees (*Apis mellifera* L.). Anim Behav 34:1061–1069

Page RE Jr, Peng CYS (2001) Aging and development in social insects with emphasis on the honey bee, *Apis mellifera* L. Exp Gerontol 36:695–711

Page RE Jr, Blum MS, Fales HM (1988) *o*-Aminoacetophenone, a pheromone that repels honeybees (*Apis mellifera* L.). Experientia 44:270–271

Page RE Jr, Robinson GE, Fondrk MK (1989) Genetic specialists, kin recognition and nepotism in honeybee colonies. Nature 339:576–579

Pankiw T (1997) Queen rearing by high and low queen mandibular pheromone responding worker honey bees (*Apis mellifera* L.). Can Entomol 129:679–690

Post DC, Page RE Jr, Erickson EH (1987) Honeybee (*Apis mellifera* L.) queen feces: source of a pheromone that repels worker bees. J Chem Ecol 13:583–591

Queller DC, Strassmann JE (2002) The many selves of social insects. Science 296:311–313

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

Ratnieks FLW (1989) Conflict and cooperation in insect societies. PhD thesis, Cornell University, Ithaca, N.Y.

Ratnieks FLW, Reeve HK (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

Robinson GE, Page RE, Jr., Arensen N (1994) Genotypic differences in brood rearing in honey bee colonies: context-specific? Behav Ecol Sociobiol 34:125–137

Schmickl T, Crailsheim K (2002) How honeybees (*Apis mellifera* L.) change their broodcare behaviour in response to non-foraging conditions and poor pollen conditions. Behav Ecol Sociobiol 51:415–425

Schneider SS, DeGrandi-Hoffman G (2002) The influence of worker behavior and paternity on the development and emergence of honey bee queens. Insectes Soc 49:306–314

Schneider SS, DeGrandi-Hoffman G (2003) The influence of paternity on virgin queen success in hybrid colonies of European and African honeybees. Anim Behav 65:883–892

Schneider SS, Painter Kurt S, Degrandi-Hoffman G (2001) The role of the vibration signal during queen competition in colonies of the honeybee, *Apis mellifera*. Anim Behav 61:1173–1180

Sundström L, Boomsma JJ (2001) Conflicts and alliances in insect families. Heredity 86:515–521

Tarpy DR, Fletcher DJC (1998) Effects of relatedness on queen competition within honey bee colonies. Anim Behav 55:537–543

Tarpy DR, Fletcher DJC (2003) 'Spraying' behavior during queen competition in honey bees. J Insect Behav 16:425–437

Tarpy DR, Nielsen DI (2002) Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera:Apidae). Ann Entomol Soc Am 95:513–528

Tarpy DR, Hatch S, Fletcher DJC (2000) The influence of queen age and quality during queen replacement in honeybee colonies. Anim Behav 59:97–101

Tilley CA, Oldroyd BP (1997) Unequal subfamily proportions among honey bee queen and worker brood. Anim Behav 54:1483–1490

Visscher PK (1986) Kinship discrimination in queen rearing by honey bees (*Apis mellifera*). Behav Ecol Sociobiol 18:453–460

Visscher PK (1993) A theoretical analysis of individual interests and intracolony conflict during swarming of honey bee colonies. J Theor Biol 165:191–212

Visscher PK (1998) Colony integration and reproductive conflict in honey bees. Apidologie 29:23–45

Wilson DS (1997) Multilevel selection theory comes of age—introduction. Am Nat 150:S1–S4

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge, Mass.

Winston ML (1987) The biology of the honey bee. Harvard University Press, Cambridge, Mass.

Winston ML, Taylor OR (1980) Factors preceding queen rearing in the Africanized honeybee (*Apis melifera*) in South America. Insectes Soc 27:289–304

Woyciechowski M (1990) Do honey bee, *Apis mellifera* L., workers favour sibling eggs and larvae in queen rearing? Anim Behav 39:1220–1222

Woyciechowski M, Lomnicki A (1987) Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. J Theor Biol 128:317–328

Woyke J (1971) Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens, and results of insemination. J Apic Res 10:45–55