Caste in Social Insects: Genetic Influences Over Caste Determination

N. Lo, Australian Museum, Sydney, NSW, Australia; University of Sydney, Sydney, NSW, Australia
M. Beekman and B. P. Oldroyd, University of Sydney, Sydney, NSW, Australia

Introduction

Eusocial insects dominate many ecosystems. Key to their success is the specialization of colony members into royal and worker castes. Royals are specialist reproducers, while workers are typically sterile or subfertile. The question of what determines whether a young social insect will become a royal or a worker has interested biologists for over a century. In the early to mid-twentieth century, there was disagreement on the relative importance of genotype in caste determination. Some proposed that each social insect larva had an equal capacity to develop into either the royal or worker caste, and that environmental factors alone decided their developmental fate. Others believed that an individual's genotype predisposed it to developing into a particular caste. Early experimental evidence supported the first hypothesis. A classic example of 'environmental caste determination' (ECD) was the development of honeybee larvae into queens when fed relatively high amounts of royal jelly, and into workers when fed a diet with low amounts of royal jelly. A variety of studies on caste determination in ants and termites also demonstrated the importance of environmental factors.

These results were in agreement with theoretical predictions that caste determination should be environmentally, rather than genetically, determined. Any allele or gene predisposing an individual to become a worker would be expected to be lost from the population, because of the sterile or subfertile nature of its bearer. Although a few early examples of 'genetically influenced caste determination' (GCD) were reported, by the end of the twentieth century it was widely believed that ECD was the rule in social insects.

Since the turn of the millennium, there has been a sharp increase in the number of demonstrations of GCD. Genotype has been shown to have a strong influence not only on the worker versus royal dichotomy, but also on the development of different forms of workers (e.g., large workers, small workers, and soldiers). This article presents an overview of the various examples of GCD, focusing on the royal versus worker dichotomy, and discusses the factors that may have led to their evolution. The term 'royal' is used only in reference to the winged, dispersing caste that initiates new colonies either alone, with a sexual partner, or with a swarm of workers. In some ant and termite species, a subset of individuals that are morphologically similar to workers (e.g., wingless) may become reproductive; these are termed 'ergatogynes' or 'ergatoids' respectively.

GCD, as defined here, includes a spectrum of caste determination mechanisms, ranging from cases where genotype has a slight, but significant influence on caste, through to cases where an individual's caste is essentially hard-wired on the basis of its genotype. Environmental stimuli thus play an important role in many, if not most, cases of GCD. In ECD, genotype has no significant effect on the probability of becoming one caste or the other.

Empirical evidence for GCD has primarily come from two methods. The first method is comparison of an individual's caste with its genetic profile – usually obtained using microsatellite markers. The advent and widespread use of this relatively straightforward method over the last two decades have enabled most of the examples of GCD to be discovered. A second method for detecting GCD is crossing of royals and ergatogynes/ergatoids and examination of the caste of offspring.

The examples presented here come primarily from the hymenopteran social insects ants and bees. As shown in Figure 1, these insects have a haplodiploid sex determination system. Unfertilized eggs are haploid and become male, while fertilized eggs are diploid and become female. Queens may be monoandrous (mating with only one male) or polyandrous (mating with two or more males).

Stingless Bees of the Genus *Melipona*

The first evidence for a genetically based mechanism of caste determination came from the Meliponini: a tribe of primarily monoandrous, stingless bees from tropical regions. Several species have been domesticated for honey production. In the 1940s, Warwick Kerr noticed that in the genus *Melipona*, queens and workers are reared in identical cells, are fed identical food, weigh the same after emergence, and that queen pupae are scattered randomly through the brood comb. This suggested that caste determination in *Melipona* was not determined by differential feeding or other environmental means.

Kerr examined the brood of several *Melipona marginata* and *M. quadrifasciata* colonies and noticed that the ratio of

queens to workers in the brood was ~1:3 and 1:7 respectively. He realized that these ratios could be explained by the segregation of two or three caste-determining genes, respectively. Kerr proposed that queens are heterozygous at all caste-determining loci, whereas workers are homozygous at one or more caste-determining loci as shown in **Figure 2**. Notice how all *M. marginata* queens are AaBb and will therefore produce four kinds of gametes at equal frequency from a simple Mendelian segregation: 1 AB: 1 Ab: 1 aB: 1 ab. As with all other Hymenoptera, the Meliponini are haplo-diploid: the females are diploid, but the males are haploid and arise from unfertilized eggs. Therefore, males are produced by the doubly heterozygous *M. marginata* queens in this exact same genetic ratio. This means that there are four kinds of males in the population. For each of the four kinds of males, 1/4 of their diploid female offspring will be queens because they can only mate with doubly heterozygous queens (**Figure 2**). Similarly, for *M. quadrifasciata*, and six other species, 1/8 of female progeny will be queens. Thus, the proportion of potential queens in the population is kept constant because of genic balance at the caste-determining loci. The proportions of each allele are kept equal in the population: any rare allele is at a strong selective advantage because it is likely to be found in heterozygotes and thus passed on by queens, while any common allele is at a disadvantage as it is more likely to be present in homozygous workers and thus not passed on.

What happens to all the extra queens? *Melipona* colonies are founded by swarms that contain large numbers of workers (as opposed to independent colony founding by queens) and there is only one single-mated queen per colony. Thus, even with the three-locus system, where 'only' 1/8 of female brood becomes a queen, an excess of queens is produced. Queens emerge continuously, and whenever there is a laying queen in the colony, they are killed by the workers. Nonetheless, *Melipona* colonies have at least some capacity to reduce the number of queens produced when they are not required, for the number of queens produced declines in unfavorable seasons when food resources are scarce. Thus, there is a strong environmental component to the caste determination mechanism.

Melipona workers have five ventral nerve ganglia, whereas most queens have four, presumably because the two distal ganglia become fused at the pupal stage. Intriguingly, a small proportion of workers show the four ganglia typical of queens, and these were interpreted by Kerr as being genetic queens that had failed to develop phenotypically because of inadequate nutrition. The quantity of food provided to the brood cells by nurse workers seems to be the determinant of whether a genetic queen can develop the full queen phenotype.

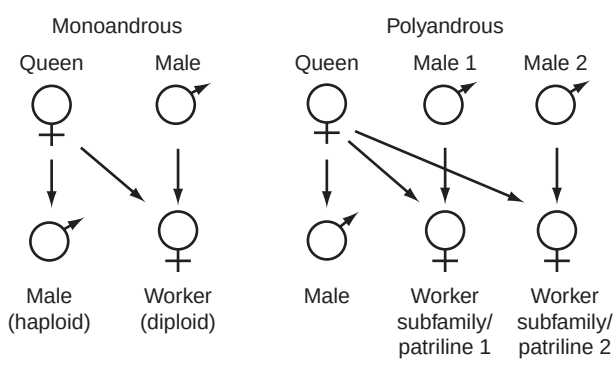

Figure 1 Sex determination and mating systems in ants and bees.

Four kinds of male gametes	Queen gametes: AB	Ab	aB	ab
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	Aabb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aABb
ab	aAbB	aAbb	aaBb	aabb

Figure 2 Model for caste determination in *Melipona marginata*. Individuals that have at least one of two genes homozygous develop into workers, while those with both genes heterozygous (shaded) develop into queens.

Melipona is the only genus of social bees that has equal-sized cells for workers and queens, and the only bees for which GCD has been proposed. Perhaps the reduced ability of workers to influence the caste of larvae developing in sealed cells is the reason why the GCD mechanism has evolved in these bees. It should be noted that Kerr's model has been questioned by some researchers, who suggest that a more complex model may be required.

The Slave-Making Ant *Harpagoxenus sublaevis*

In the slave-making ant *Harpagoxenus sublaevis*, colony initiation involves a queen finding a host colony, killing off all adults, and capturing the brood. Newly hatched workers then tend the eggs that she has laid. The queens' workers are able to raid other colonies for new slave brood.

Two forms of reproductives are known in *H. sublaevis*: winged queens, which are relatively rare within populations and wingless 'ergatogynes,' the most common form. Workers are morphologically very similar to ergatogynes, though slightly smaller and anatomically less complex. Following a series of crosses, Alfred Buschinger and coworkers proposed that the wing polymorphism is under the control of a single locus with two alleles: e and E. Queens are always e/e, while ergatogynes are either E/e or E/E. Workers can be of any genotype; however, e/e females have a significantly higher chance of becoming queens than E/e individuals have of becoming ergatogynes. E/E individuals have the lowest chances of becoming reproductives (i.e., ergatogynes). Thus, E is a dominant allele for winglessness and appears to slow development. Genotype thus determines whether a larva will become winged or wingless and whether it can become a queen. As for the case of *Melipona* stingless bees, environmental factors still play an important role in mediating phenotype (i.e., worker or reproductive). However, unlike *Melipona*, the alleles in the population are not balanced, and thus different caste ratios occur depending on the different types of crosses.

It has been speculated that the primitive condition of caste determination in the ancestors of *H. sublaevis* was environmental (i.e., fixation of an 'e'-like allele in populations). Mutation of e to E then occurred at some stage during the transition from the free-living, claustral colony-founding form to the parasitic, slave-making form. *H. sublaevis* initiates colonies via parasitism, whereby the founding queen invades an established colony of a related species. Selection may, therefore, have favored mating by individuals without wings, because of energetic benefits.

The GCD mechanism found in *H. sublaevis* has also been found in the ants *Leptothorax* sp. and *Myrmecina graminicola*, both of which are nonparasitic. The evolution of the equivalent version of the E allele in these cases may have been favored by the presence of patchy habitats, which favor colony foundation in close, rather than distant, sites and therefore do not require founding females to have wings. It remains to be seen whether a GCD mechanism underlies other cases of the queen/ergatogyne dichotomy, which is fairly common in ants.

Harvester Ants of the Genus *Pogonomyrmex*

Two *Pogonomyrmex* species, *P. barbatus* and *P. rugosus*, exist sympatrically in southwestern North America. Previous hybridization events between these two 'parental' species have resulted in three 'lineages' (H1, H2, and J1) with chimaeric genomes. There is little or no gene flow between lineages (or between lineages and the parental species). The three lineages, along with a fourth lineage (J2, which is either a divergent lineage of *P. barbatus*, or another hybrid lineage), have developed a GCD mechanism that differs greatly from the mechanism present in the parental species *P. barbatus* and *P. rugosus*. GCD in these lineages was discovered via microsatellite, allozyme, and mitochondrial genotyping studies.

Like the parental *P. barbatus* and *P. rugosus*, members of all four lineages are polyandrous. When a female and male of the H1 lineage mate, their offspring are able to develop only into queens (**Figure 3**). However, when an H1 female mates with an H2 male, their offspring develop into workers. Thus, an H1 female must mate with males of both H1 and H2 in order to produce both workers and queens. Similarly, matings

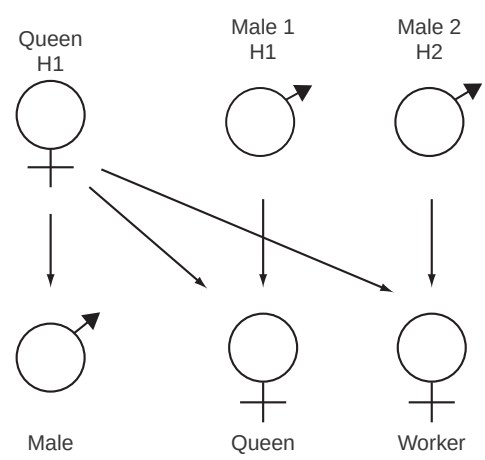

Figure 3 Colony reproduction in *Pogonomyrmex* lineages. The only way that a harvester ant queen (H1) can produce a fully viable colony with both workers and queens is by mating with males of both H1 and H2 lineages. Because she can only produce H1 males, she is reliant on male production by H2 queens for her worker production.

between H2 males and females result in queens, while female H2/male H1 matings result in workers. The same system is found in J1/J2 lineages. Each lineage effectively parasitizes the males of the other lineage to make workers and are therefore known as 'dependent lineages.' Matings between the H and J lineages and between the H, J, and parental lineages lead to inviable offspring.

It has been speculated that this GCD mechanism evolved as a result of queen–daughter conflict. To maximize their inclusive fitness, daughters are selected to avoid becoming workers and to enhance their chance of becoming queens. On the other hand, queens are selected to coerce their daughters into becoming workers rather than queens, thus enabling their young colonies to develop more rapidly. Indeed, in parental *P. rugosus* as well as dependent lineages, a maternal effect in incipient colony queens results only in workers being produced; overwintering is necessary before the queen caste can develop. The H and J lineages may represent cases in which the queen is no longer able to stop members of her own lineage from developing into queens. However, she is still able to influence hybrid offspring of the other lineage into becoming workers. An outcome of this is that intralineage offspring, which are able to become only queens, either die or do not develop properly during colony foundation.

The caste determination system of one of the parent species, *P. rugosus*, has recently been shown to be under genetic influence; however, it is very different from that present in the dependent lineages. In *P. rugosus*, the probability of becoming one caste or the other depends on the compatibility between the parental genomes. Thus, the offspring of a female who has mated with several different males have significantly different probabilities of becoming a queen. However, if the same males mate with a different female, these probabilities change. It is the combination of the two genomes that influences caste. Environmental factors play a much larger role in this system than in the dependent lineages: caste totipotency is retained in the former, but has been lost in the latter (at least in the case of intralineage matings).

A somewhat similar system to that found in the *Pogonomyrmex* dependent lineages has been discovered in a hybrid system involving the fire ants *Solenopsis geminata* and *S. xyloni*. Workers were hybrids of these two species, while almost all the queens were of pure *S. xyloni* ancestry. *S. xyloni* females have become developmentally constrained to become queens and are unable to become workers, even if there are no hybrid workers in the colony. On the other hand, hybrids can still become queens, but this happens only rarely, in the absence of pure *S. xyloni* offspring. In contrast to the *Pogonomyrmex* lineages, reciprocal hybridization is rare or does not occur: offspring of *S. geminata* queens are invariably fathered by other *S. geminata* males, rather than *S. xyloni* males. Another difference is that *Solenopsis* colonies contain multiple, singly mated queens, rather than single, multiply mated queens. To produce both workers and queens, a colony must therefore contain both heterospecifically and conspecifically mated queens. This sets the stage for conflict between queens, since heterospecifically mated queens can gain direct fitness only via males.

The Japanese Termite *Reticulitermes speratus*

The majority (~80%) of termite species have an irreversibly wingless worker caste; larvae can follow one of two main developmental lines: the 'nymph' line (leading to alates) or the worker line (**Figure 4**). Since termites are hemimetabolous, two forms of immature 'neotenic' reproductives (one from each developmental line) can arise under certain conditions (e.g., in the absence of the queen or king). Laboratory crossing experiments of neotenic reproductives from the Japanese wood-feeding species *Reticulitermes speratus*, collected in Eastern Japan (Ibaraki), have shown an influence of genotype on caste determination in this species. Following four different crosses of nymphoids and ergatoids, eggs from each cross were reared under identical conditions (in the absence of the king and queen), and the caste and sex of offspring were examined. The same experiments were performed on offspring from parthenogenetic nymphoids and ergatoids. Parthenogenesis occurs via thelytokous automixis, resulting in highly homozygous offspring.

The four neotenic crosses and two parthenogenetic treatments resulted in very different offspring types; this was neatly explained by the presence of an X-linked locus named *worker* (*wk*) with two alleles A and B (**Figure 5**; unlike ants and bees, termites are diplo-diploid, and usually have an XY sex-determination system).

In the model, females inheriting two copies of A (i.e., wk^{AA}) become nymphs (i.e., future queens), while those inheriting one copy of A and B (wk^{AB}) become workers. Males inheriting a single copy of A (wk^{A}Y) become workers, while those inheriting a single copy of B (wk^{B}Y) become nymphs (i.e., future kings). The genotype wk^{BB} is lethal. Environmental factors still play an important role in the GCD mechanism. When reproductives are present during development, individuals with a worker genotype remain workers; however, 25% of females with a nymph genotype are modified into workers (male nymph genotypes have not been tested). Environmental stimuli, most likely pheromones, also control the development of nymphoids, ergatoids, and soldiers from their precursor castes.

The selective advantage of the GCD system may be enhanced inhibition of selfish reproduction by offspring.

As shown in **Figure 5**, offspring produced in developing king–queen *R. speratus* colonies have a genetic bias toward the worker, rather than the nymph, pathway. Workers have a relatively low propensity to develop into 'selfish' neotenics, requiring two molts and ~30 days, compared with one molt and ~12 days for nymphs. The evolution of genetically biased workers with a reduced capacity to become reproductive may have facilitated increased foraging behavior, permitting increased colony size and ecological dominance.

The predictions of the model in **Figure 5** have yet to be examined in field colonies from Ibaraki; a subsequent study of colonies in Central Japan (Kyoto), which employed microsatellite genotyping of colony members, showed that the model does not hold for colonies of this population. These colonies were almost always headed by a single king and numerous nymphoid neotenics, the latter of which parthenogenetically produced by the founding queen. No ergatoids were found, and both workers and nymphs in these colonies were shown to have

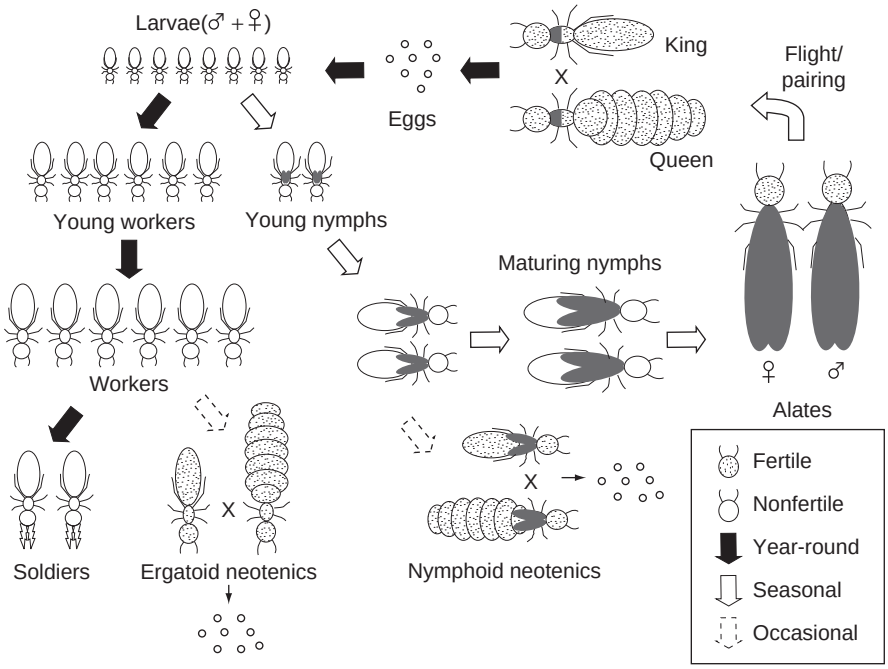

Figure 4 Generally accepted life cycle of termites with a worker caste. Reprinted Lo N, Hayashi Y, and Kitade O (2009) Should environmental caste determination be assumed for termites? *American Naturalist* 173: 848–853, with permission from The University of Chicago Press.

	wk^{B} Y ♂ Nymphoid	*wk*^{A} Y ♂ Ergatoid	Parthenogenesis
wk^{AA} ♀ Nymphoid	*wk*^{AB} ♀ Worker	*wk*^{A} Y ♂ Worker	*wk*^{AA} ♀ Nymph	*wk*^{A} Y ♂ Worker	*wk*^{AA} ♀ Nymph
wk^{AB} ♀ Ergatoid	*wk*^{AB} ♀ Worker	*wk*^{A} Y ♂ Worker	*wk*^{AA} ♀ Nymph	*wk*^{A} Y ♂ Worker	*wk*^{AA} ♀ Nymph
	wk^{BB} ♀ Lethal	*wk*^{B} Y ♂ Nymph	*wk*^{AB} ♀ Worker	*wk*^{B} Y ♂ Nymph	*wk*^{BB} ♀ Lethal

Figure 5 One-locus-two-allele model of caste determination in *Reticulitermes speratus*. Genotype matches phenotype under the experimental rearing conditions used (i.e., an absence of reproductive individuals). See text for details. Reprinted Hayashi Y, Lo N, Miyata H, and Kitade O (2007) Sex-linked genetic influence on caste determination in a termite. *Science* 318: 985–987, with permission from the American Association for the Advancement of Science.

alleles from both the king and the nymphoid queens. These results are not congruent with the predictions of the model shown in Figure 5, since inbreeding is not required to produce nymphs. The breeding biology of *R. speratus* in different parts of Japan is known to be variable. Colonies from some areas have been shown to contain ergatoid reproductives, which differs from the situation in populations from Kyoto. The caste determination systems Kyoto and Ibaraki may, therefore, have somewhat different caste determination mechanisms. Alternatively, the system proposed in Figure 5 may be strongly overridden under field conditions.

The Fungus-Growing Ant *Acromyrmex echinatior*

The leaf-cutting and fungus-growing species *Acromyrmex echinatior* is found between Mexico and Panama. Queens of *A. echinatior* mate with multiple males, and, as a result, their offspring (queens or workers) consist of a number of full-sister lineages (patrilines; Figure 1). Ants of different patrilines are half-sisters. Two different-sized workers exist: small (SW), which specialize in tending the fungus garden, and large (LW), which forage outside the nest for plant material to feed the fungus with. It has been shown that one fifth of patrilines cheat on their nestmates by having a much higher chance of becoming a queen than a worker. These royal patrilines are shown to be rare both within individual colonies and in the population as a whole.

'Normal' patrilines are represented roughly equally among small and large workers and queens, whereas 'royal' patrilines show a bias toward queens and a bias toward either large workers (royal-LW patrilines) or small workers (royal-SW patrilines). As larvae destined to become LW also need to be fed more, the slightly higher than expected contribution of royal-LW patrilines toward queens is most likely a result of some larvae of these patrilines being fed extra to allow them to become queens. These patrilines are equally abundant as 'normal' patrilines within colonies. The real cheats are the royal-SW patrilines, which hardly contribute to the production of workers, and, if they do, produce mostly SW. Hence, feeding does not explain the bias toward queens in royal-SW patrilines. Cues that lead a larva to develop into one caste or another may be more complex than feeding alone. Royal-SW patrilines were shown to have an increased fitness of almost 500% relative to normal patrilines, whereas the fitness of royal-LW was not much higher than that of normal patrilines.

Despite the enormous increase in fitness, royal-SW patrilines are rare, as evolutionary theory would predict for real cheats. Most likely, the frequency of cheating genotypes is constrained by colony-level selection: colonies that contain too many royal cheats suffer a reduction in reproductive output. In addition, the direct feeding of ant larvae by workers gives nurse workers some control over the caste fate of larvae by evolving the capacity to recognize potential cheats (although such recognition has not yet been demonstrated; the rarer the cheats are, the more error-prone such recognition will be).

The Parthenogenetic Ants *Cataglyphis cursor*, *Wasmannia auropunctata*, and *Vollenhovia emeryi*

The ants *Cataglyphis cursor*, *Wasmannia auropunctata*, and *Vollenhovia emeryi* each have an unusual breeding system, whereby queens are produced parthenogenetically and workers are produced by sexual reproduction (queens are multiply mated in each case). In the case of *C. cursor*, and probably *V. emeryi*, automictic parthenogenesis occurs, leading to increased homozygosity of offspring, but not clonally produced offspring. In *W. auropunctata*, no meiosis occurs, resulting in clonally produced queens. Additionally, in *W. auropunctata* and *V. emeryi*, males (haploid) are clonally produced, apparently via elimination of the maternal genome in eggs. In all three cases, parthenogenesis allows queens to take advantage of the twofold increase of asexual production when producing further queens, while sexual production of workers provides the benefits of a genetically diverse worker population.

Although the genotypes of workers and queens in these species are clearly differentiated by high versus low heterozygosity, they do not necessarily have GCD, as defined here. In *C. cursor*, the caste determination system appears to be environmental. Studies of larval development in laboratory colonies indicate that both parthenogenetically and sexually produced eggs are not biased toward one caste or the other. Rather, it is the physiological state of nurse workers that determines the caste of larvae. In the field, queens lay parthenogenetic eggs in the spring, which then become queens. At other times, eggs are fertilized and develop into workers. Thus, cold temperatures are important in the switch of development of larvae from workers into queens.

The HoneyBee *Apis mellifera*

In large measure, the honeybee (*Apis mellifera*) was responsible for the development of the paradigm that caste determination is environmentally determined – indeed the queen bee production industry depends on ECD for the conversion of worker larvae into queens for sale. But even in the honeybee, it now appears that not all female larvae are created equal. When a queen honeybee dies, the workers select 5–20 female larvae (out of the

several thousand that are available) and lavishly feed them so that they develop into queens. All the rest develop into workers. This seems strong evidence for ECD, but recent pedigree analyses using genetic markers show that some patrilines are strongly over represented in the queen larvae. Some scientists now believe that there are genetic determinants that make these larvae more attractive for rearing as queens. So even in honeybees, there may be a bit of GCD after all.

Conclusions

Evolutionary theory predicts that all individuals will behave selfishly, provided the cost of expressing such behavior (e.g., colony level costs) does not outweigh the benefits in terms of inclusive fitness. Hence, when female brood are totipotent, every individual 'prefers' to develop into a queen and prefers that her sisters develop into workers. This is so because an individual's relatedness to its own offspring is higher than its relatedness to those of sisters. Because the expression of selfish behavior by a large number of individuals will result in a reduction in inclusive fitness of most individuals, the outcome of selection at multiple levels (individual- and colony-level) will be the suppression of selfish behavior to a sustainable, low level.

How can the expression of selfish behavior be suppressed? In the case of Hymenopteran social insects, it is generally argued that adult workers hold the greatest overall power for several reasons. Because workers are mobile and numerous, they have access to more and better information than larvae. This is especially important when timing of queen production plays a role. Workers are the main brood-rearers and therefore have more opportunities to manipulate developing females by controlling larval nutrition and rearing temperature. For example, such manipulation prevents female larvae of the ant *Hypoponera* from cannibalizing other larvae and developing as queens (this is possible as size differences between queens and workers are small), since workers actively keep brood of different sizes separated to reduce such cannibalism. Being physically stronger gives workers the ability to kill noncompliant female brood. In *Myrmica* ants, workers not only feed larvae a spartan diet but also physically harass the larvae by biting them so that they metamorphose and develop into workers. Manipulation by workers also occurs in termites, although it is not known how frequently. In the termite *Pterotermes occidentis*, immature individuals are prevented from metamorphosing into alate reproductives by other colony members, which bite and destroy their wing buds. Lastly, workers act as a collective, sharing an interest as they are equally related to female brood or, if within-colony relatedness varies, are assumed to be incapable of discriminating among different classes of kin. Being united into a 'community of interest' is likely to tip the balance of power further toward the workers.

GCD may have evolved as a means of curtailing selfish behavior by totipotent female brood, in cases where the collective workers are less able to directly influence the caste fate of female brood. This may be relevant to the cases of *Melipona*, *Pogonomyrmex*, and *Reticulitermes*, but not to *Harpagoxenus laevis* and other ants with a similar caste determination mechanism. The understanding of the molecular genetic basis of the cases of GCD described here may provide additional clues as to the selective forces governing its evolution.

See also: Caste Determination in Arthropods; Cooperation and Sociality; Kin Selection and Relatedness; Social Insects: Behavioral Genetics.

Further Reading

Anderson KE, Linksvayer TA and Smith CR (2008) The causes and consequences of genetic caste determination in ants (Hymenoptera: Formicidae). *Myrmecological News* 11: 119–132.

Crozier RH (1979) Genetics of sociality. In: Hermann HR (ed.) *Social Insects*, pp. 223–286. New York, NY: Academic Press.

Keller L (2007) Uncovering the biodiversity of genetic and reproductive systems: Time for a more open approach. *The American Naturalist* 169: 1–8.

Kerr WE (1950) Evolution of the mechanism of caste determination in the genus *Melipona*. *Evolution* 4: 7–13.

Matsuura K, Vargo EL, Kawatsu K, et al. (2009) Queen succession through asexual reproduction in termites. *Science* 323: 1687.

Queller DC and Strassman JE (1998) Kin selection and social insects. *Bioscience* 48: 165–175.

Wheeler DE (1986) Developmental and physiological determinants of caste in social Hymenoptera – evolutionary implications. *The American Naturalist* 128: 13–34.

Winter U and Buschinger A (1986) Genetically mediated queen polymorphism and caste determination in the slave-making ant, *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). *Entomologia Generalis* 11: 125–137.