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# Ant, Bee and Wasp Social Evolution<sup>☆</sup>

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## Introduction and Definitions

Ants, bees, and wasps belong to the Hymenoptera, a 220-My-old monophyletic order, which is among the largest and most diverse in the class Insecta (Fig. 1). Among the characters that are common to all Hymenoptera, perhaps the one that is of greatest interest to the topic of this article (even if it eventually turns out to be only of historical interest) is haplodiploidy, a term that indicates that males are haploid on account of developing parthenogenetically from unfertilized eggs and females are diploid on account of developing from fertilized eggs. In spite of the size of this order (more than 250,000 described species), only a small fraction (<10%) of the species is social, and thus of interest to us here. Even among those that are social, there is a very large variation in the degree of sociality. To focus attention on the phenomena that give rise to the most sophisticated forms of sociality, it has become customary to demarcate the eusocial species from all "lesser" forms of sociality. To qualify as eusocial, a species must exhibit cooperative brood care, differentiation of colony members into fertile reproductive castes (queens) and sterile non-reproductive castes (workers), and an overlap of generations such that offspring assist their parents in brood care and other tasks required for colony maintenance. The discussion in this article thus refers to the evolution of eusociality in ants, bees, and wasps.

It is also customary to distinguish two subgroups within the eusocial species. Some species have relatively small colonies (<100 individuals) and their queens and workers are not morphologically differentiated. These species are often labeled primitively eusocial. In other species by contrast, colony sizes are large (>100 individuals, often in the thousands or hundreds of thousands, sometimes over a million) and queens and workers are morphologically differentiated. These species are often labeled advanced or highly eusocial on the argument that they have acquired more traits that are unlikely to have been present in their solitary ancestors compared to the primitively eusocial species. There is one more remarkable fact about hymenopteran sociality that needs to be mentioned. Feminine monarchy, a phrase used by the cleric Charles Butler in 1634 to describe the honeybee society, applies to all hymenopteran societies, as they are all headed by one or a small number of fertile queens. Indeed, males eclose, usually leave the nest, mate, and die, playing no role in colonial life. As queens do little more than lay eggs, all tasks connected with nest building, brood care, and colony maintenance, are performed by the workers, and this leads to a society of female subjects headed by a female monarch.

## The Paradox of Altruism

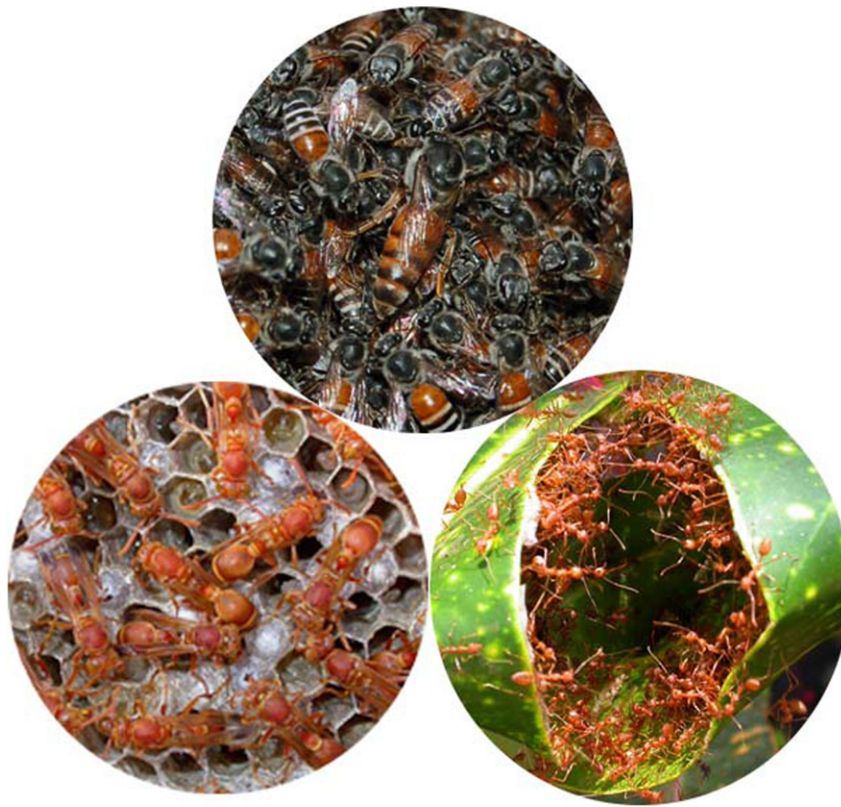
Queens mate and lay both haploid, unfertilized eggs and diploid, fertilized eggs. Workers are, by and large, sterile. In some ant genera, such as *Pheidole*, *Pheidologeton*, and *Solenopsis*, workers have altogether lost their ovaries. In others, they have much smaller ovaries compared to their queens and in some species, workers have lost the ability to mate. In any case, workers never or very rarely lay eggs when their queen is alive so that they usually spend their whole lives working to assist their queen to reproduce, while they themselves die without leaving behind any offspring. Sometimes, the sacrifice of the workers on behalf of their colonies is more dramatic. Honeybee workers defend their colonies by stinging their marauders but such stinging results in the death of the bee, as her sting is armed with barbs pointing outwards, and once inserted, cannot be withdrawn. She flies away leaving behind her sting, the poison gland, and a part of her digestive system only to die within a few minutes. The poison gland continues to pump venom into the body of the victim for up to 60 s, making this a very efficient venom delivery mechanism, but for the owner of the sting, it is an act of suicide. In the Brazilian ant *Forelius busillus*, a small number of workers perform preemptive self-sacrifice every day as they lay down their lives, because the last workers involved in closing the nest entrance in the evening remain outside and die.

## Kin Selection

How can such sterility and sacrifice, seen in tens of thousands of species, arise and persist in nature? Why does natural selection not eliminate these altruistic traits as they do not appear to contribute to the Darwinian fitness of their bearers? These questions remained unanswered and often remained unrecognized as unanswered questions for over 100 years after Darwin had proposed the theory of natural selection. But today, we have a powerful theoretical framework on account of which the evolution of altruism by natural selection is no longer a paradox, at least to the theoreticians, although empiricists may have to wait a bit longer before they can claim to have demystified the evolution of altruism.

The necessary theoretical framework was developed by W.D. Hamilton in 1964 and has come to be known popularly as kin selection. Hamilton argued that self-sacrificing altruism is not necessarily paradoxical if it is directed toward close genetic relatives

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**Fig. 1** A portion of the nest of the Asian dwarf bee *Apis florea* showing the morphological differentiation between the single, large queen and the many small workers surrounding her (top), a portion of the primitively eusocial wasp *Ropalidia marginata*, showing a portion of the paper carton nest and the absence of morphological caste differentiation (bottom left), and a portion of leaf nest and workers of the Asian weaver ant *Oecophylla smaragdina*. Photos: Dr. Thresiamma Varghese.

who share genes with the altruist. This concept is known as inclusive fitness, implying that fitness can be gained not only by producing offspring (direct fitness) but also by aiding genetic relatives (indirect fitness). Hamilton showed that an altruistic trait can be favored by natural selection if the inequality

$$b \cdot r - c > 0$$

is satisfied, where  $b$  is the benefit to the recipient,  $c$  cost to the altruist, and  $r$  coefficient of genetic relatedness between the altruist and recipient. This has come to be known as Hamilton's rule. There is now good evidence that this is a theoretically robust idea, but the hard part has been to show that ants, bees, and wasps, behave as if they obey Hamilton's rule. There was initially a fascinating red herring. On account of haplodiploidy, a hymenopteran female can be more closely related to her full sister ( $r=0.75$ ) than she would be to her own offspring ( $r=0.5$ ). This means that Hamilton's rule can be rather easily satisfied and thus altruistic sterility can evolve rather more easily in the Hymenoptera than in diploid organisms. This haplodiploidy hypothesis had a great appeal for some time because it seemed to have strong empirical support – eusociality was known to have originated at least eleven times independently in the Hymenoptera compared to a single origin (termites) in the rest of the animal kingdom. The euphoria was unfortunately short-lived.

### Testing the Theory

A major problem for the haplodiploidy hypothesis was that although workers can be related to their full sisters by 0.75, they are related to their brothers by 0.25, bringing the average relatedness to siblings back to the diploid value of 0.5. This problem can, in principle, be surmounted if workers invest more in sisters than in brothers. But this would lead to a conflict between workers that would prefer a female-biased investment and their mothers that would prefer equal investment in sons and daughters. How this conflict is resolved and whether its resolution affirms or negates the role of haplodiploidy in social evolution are still being vigorously researched and debated. A more serious problem for the haplodiploidy hypothesis is the increasing evidence for reduction in relatedness among the workers themselves because of both multiple mating (polyandry) by the queens and parallel or serial polygyny (multiple queens). It is now widely accepted that the asymmetries created by haplodiploidy are, by themselves, inadequate to explain the evolution of eusociality in the Hymenoptera.

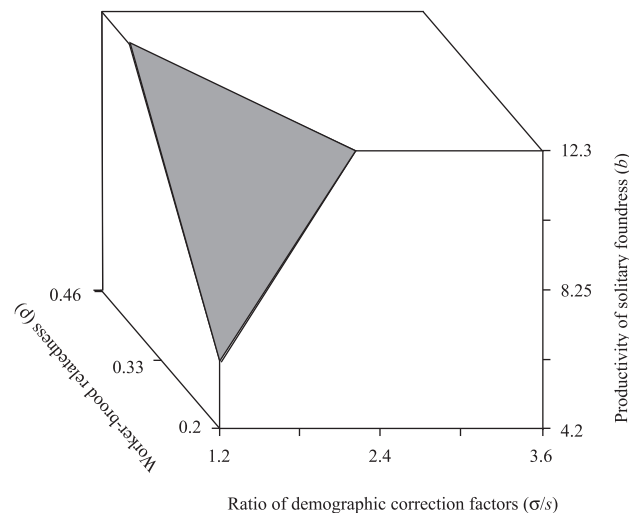
The demise of the haplodiploidy hypothesis by no means weakens Hamilton's rule, which has been often tested. Unfortunately, most tests ignore the benefit and cost terms, and test the limited prediction that altruism should be directed at close rather than distant relatives. This effort, combined with increasingly powerful DNA-based molecular techniques to measure genetic relatedness, has spawned a number of efforts to measure intracolony genetic relatedness. These values tend to be quite variable, often below 0.75 and even below 0.5. Social insect colonies are thus sometimes composed of rather distantly related or even unrelated individuals. Some specific phenomena are elegantly explained by the observed variability in relatedness values. For example, a comparison of intracolony genetic relatedness in honeybees and stingless bees explains why daughter queens inherit the nest and mother queens leave to found their own nests in multiply mated honeybees, while the mother stays and the daughter leaves in singly mated stingless bees. Another well-known example is found in worker policing. In colonies with singly mated queens, workers should prefer to rear nephews ( $r=0.375$ ) rather than brothers ( $r=0.25$ ), while in species with multiply mated queens, workers should prefer to rear brothers ( $r=0.25$ ) rather than nephews ( $r=0.125$ ). There is now good empirical evidence that in stingless bees which mate singly, worker oviposition is common and oophagy by other workers is not common. Conversely, oviposition is rare and worker policing is common in honeybees whose queens mate multiply. A more recent theoretical study reveals that the original prediction that policing should evolve if queens mate multiply and not if queens mate singly, is not robust to small effects of policing on colony efficiency.

Nevertheless, the low values of genetic relatedness are somewhat embarrassing for the general idea of kin selection, but some investigators now argue that lifetime monogamy is a fundamental condition for the evolution of eusociality, and processes such as polyandry and polygyny that lead to lower relatedness are later elaborations. Others have given a near burial to kin selection itself by arguing that kin selection is only a weak force and that high genetic relatedness is more likely to be a consequence of eusociality rather than a factor in its origin. A potential problem is that Hamilton's rule is seldom tested in its entirety, by simultaneously measuring  $b$ ,  $r$ , and  $c$  and when that is done, it does appear to have impressive explanatory power. It is another matter though that such tests point to the greater importance of  $b$  and  $c$ , over  $r$ , which is tantamount to greater importance for ecology and demography over genetic relatedness (Fig. 2).

Somewhat surprisingly, a major controversy regarding the role and validity of kin selection and a call for a return to standard population genetic modeling in lieu of inclusive fitness models, has now taken the field by storm and radically divided the scientific community into kin selectionists and non-kin selectionists. There is no sign of any middle ground yet and we seem to be in for a period of speaking past each other and defensive posturing, rather than much real progress in solving the paradox of altruism.

## The Proximate Causation of Social Behavior

Debates about the relative importance of understanding the so-called ultimate (evolutionary) factors and proximate (causation) factors in the evolution of eusociality have waxed and waned much as they have in many other fields of animal behavior. By and



**Fig. 2** A graphical illustration of a unified model showing the parameter space where worker behavior is selected (unshaded) and the missing chip of the block where solitary nesting behavior is favored in the primitively eusocial wasp *Ropalidia marginata*. The model simultaneously considers the benefit, cost, and relatedness parameters in Hamilton's rule and therefore incorporates genetic, ecological as well as demographic factors in the evolution of eusociality. The unified model predicts that about 95% of the wasps in the population studied should opt for the altruistic, sterile worker role and only about 5% of the wasps in the population should opt for the selfish solitary nest founding role. In striking confirmation of the predictions of the model, empirical field data indicate that about 92–96% of the wasps choose to nest in groups (where most of them will end up as altruistic sterile workers) and only about 4–8% of the wasps choose to nest solitarily and reproduce. Reprinted with permission from Gadagkar, R., 2001. *The Social Biology of Ropalidia marginata – Toward Understanding the Evolution of Eusociality*. Cambridge, MA: Harvard University Press. Copyright © 2001 by the President and Fellows of Harvard College.

large, investigations relating to the proximate causation of social behavior have lagged behind evolutionary studies, especially since the advent of kin selection. There is, however, a strong attempt to redress the imbalance in the past decade or so.

### **Division of Labor**

The remarkable ecological success of social insects is attributed primarily to their sociality and in turn largely to the division of labor that social insects achieve in organizing their work. Division of labor is therefore a topic that has successfully rivaled kin selection in attracting the attention of social insect researchers. A major early interest concerned how the morphologically differentiated subcastes among some species of ant workers increase the ergonomic efficiency of the colony by pursuing parallel rather than serial processing of tasks. Later, attention shifted to how a worker decides what it needs to do at any given time. There is considerable evidence that division of labor, especially in species that lack morphologically differentiated worker subcastes such as honeybees, is based on age polyethism. Thus, there is a strong correlation between what a bee does and its age – young bees begin their adult careers as cleaner bees and gradually move through the tasks of building the nest, nursing the larvae, unloading and processing the food, guarding the nest, and finally go out of the nest in search of food. In the 1990s, there developed an interesting debate about whether internal physiological factors such as age and hormone levels drive task allocation of the workers or whether it is governed by external factors such as prior experience and work availability at any given time. Supporters of the latter idea came up with the interesting phrase “tasks allocate workers” to bring out the contrast with the idea that workers allocate themselves to different tasks based on their physiology. Like so many debates, this one too appears to have died down with time, without settling many of the interesting issues raised during the heat of the debate. Instead, discussion has moved on to other, perhaps more productive, topics.

A theme that has gained currency and has produced a large body of extremely interesting, and indeed, practically useful knowledge, relates to the self-organization of work in large colonies. It is now recognized that workers in social insect colonies accomplish rather complex tasks not by any top-down control by a leader or foreman but by bottom-up control. In this scenario, individual workers follow simple local rules but their collective labor leads to the emergence of complex patterns such as an architecturally sophisticated nest or the choice of the shortest foraging path. Perhaps the most elegant studies of self-organization concern nest construction behavior in ants, bees and wasps (and termites). This has come to be known as swarm intelligence, also known as distributed or collective intelligence. Swarm intelligence is flexible, robust, and self-organized. Flexible because the locally available workers can quickly respond to a change in their local environment without waiting for the central authority to perceive that change or without a potential conflict with some physiologically programmed universal algorithm. Robust because even if some individuals fail to perform, there are others who can substitute for them. And self-organized because there is no need for supervision. The concept of swarm intelligence has led not only to a better understanding of how social insects achieve the remarkable feats they do but also to a surprising degree of practical application in the context of the performance of human tasks. Based on our understanding of how ants, bees, and wasps utilize swarm intelligence, so-called ant colony optimization algorithms (AOL) are in regular use in the telecommunication industry, on the Internet, and in the cargo industry, and the number of such applications is growing.

### **Regulation of Reproduction**

From a physiological point of view and especially for someone interested in controlling insect reproduction, the fact that a single queen maintains reproductive monopoly in colonies consisting of hundreds or thousands of potentially reproductive workers, is even more remarkable than anything we have discussed so far. Unfortunately, we do not understand the mechanism behind this feat in any degree of detail. Traditionally, it has been thought that queens in small colonies of primitively eusocial species achieve reproductive monopoly by suppressing worker reproduction through physical aggression and intimidation. Workers are thought to succumb to such suppression even if they might get more fitness by laying a few of their own eggs because they have no choice – they are physically too weak to fight back and leaving the nest is worse than staying on and attempting to get indirect fitness. Queens in large colonies of highly eusocial species cannot obviously physically aggress against every worker and hence behavioral dominance is not an option for them. They are known in many cases to produce pheromones that might serve the same purpose. In imitative language, queens of highly eusocial species have long been said to suppress worker reproduction by means of pheromones. In a thoughtful essay, Laurent Keller and Peter Nonacs (see Further Reading) pointed out that this idea is untenable. It is hard to imagine how queens can suppress worker reproduction against their interests by means of pheromones because workers can fight back by evolving enzymes or other chemical weapons that would neutralize the queen pheromone. Hence, it must be assumed that it is in the evolutionary interest of the workers themselves to refrain from reproduction and strive to increase the productivity of their colonies. The direct fitness they thus lose would be small, as they are no match to their large physogastric queens in terms of egg laying.

This has led people to be cautious of the language they use, but even more importantly, it has led to the idea that the queen pheromone must be an honest signal not only of their superior fertility but also of their health and vigor at any given time. This has in turn spawned a plethora of studies attempting to detect and understand these signals. While honeybee queen pheromones were long thought to be volatile compounds produced by the queen's mandibular glands, ant and wasp researchers have now drawn attention to cuticular hydrocarbons (CHC), mostly linear or branched long-chain hydrocarbons, present adsorbed to the

wax coating on the cuticles of the insects. The primary function of the CHCs appears to be to protect them from dehydration and since they are highly variable, they are thought to have been coopted to serve the function of signals. Each individual may have a unique CHC profile that has led to the phrase “cuticular hydrocarbon signature.”

Ironically, it is not the honeybees or the ant species with large-sized queens and large numbers of workers that have been at the forefront of the CHC research. Instead, queenless ponerine ants in which mated workers (gamergates) serve as the sole egg layers of their colonies, bumble bees in which the queens only modestly outsize their workers, and even primitively eusocial wasps without any morphological caste differentiation, have led this research from the front. This has had two consequences. First, CHCs have also been implicated in nestmate recognition, a function of crucial importance to all social insects (see Section “Kin and Nestmate Discrimination”). Second, honest signaling of fertility is also being attributed to the queens of primitively eusocial species without morphologically differentiated queens. The whole field of CHC research is in its infancy; and there is rather scanty evidence yet that the insects themselves perceive the diversity in the CHC cocktail to a degree of precision and sophistication that can begin to match the increasingly sophisticated gas chromatograms and multivariate statistical analysis tools that researchers now use to detect the compounds and discriminate different individuals. On the other hand, it might well be that the true suppression of worker reproduction by physical aggression and intimidation, even in the small colonies of primitively eusocial species, may be a myth, and the regulation of reproduction in all species of social insects may depend on CHCs and other similar honest chemical signals. It must be admitted that there is really no direct experimental evidence that physical aggression and intimidation are necessary and sufficient to suppress worker reproduction. Future research in this area is eagerly awaited. On the other hand, the idea that of pheromonal regulation of reproduction has gained in strength following a study of 64 species of social insects that reveals a conserved class of queen pheromones evolved from similarly conserved signals in solitary ancestors.

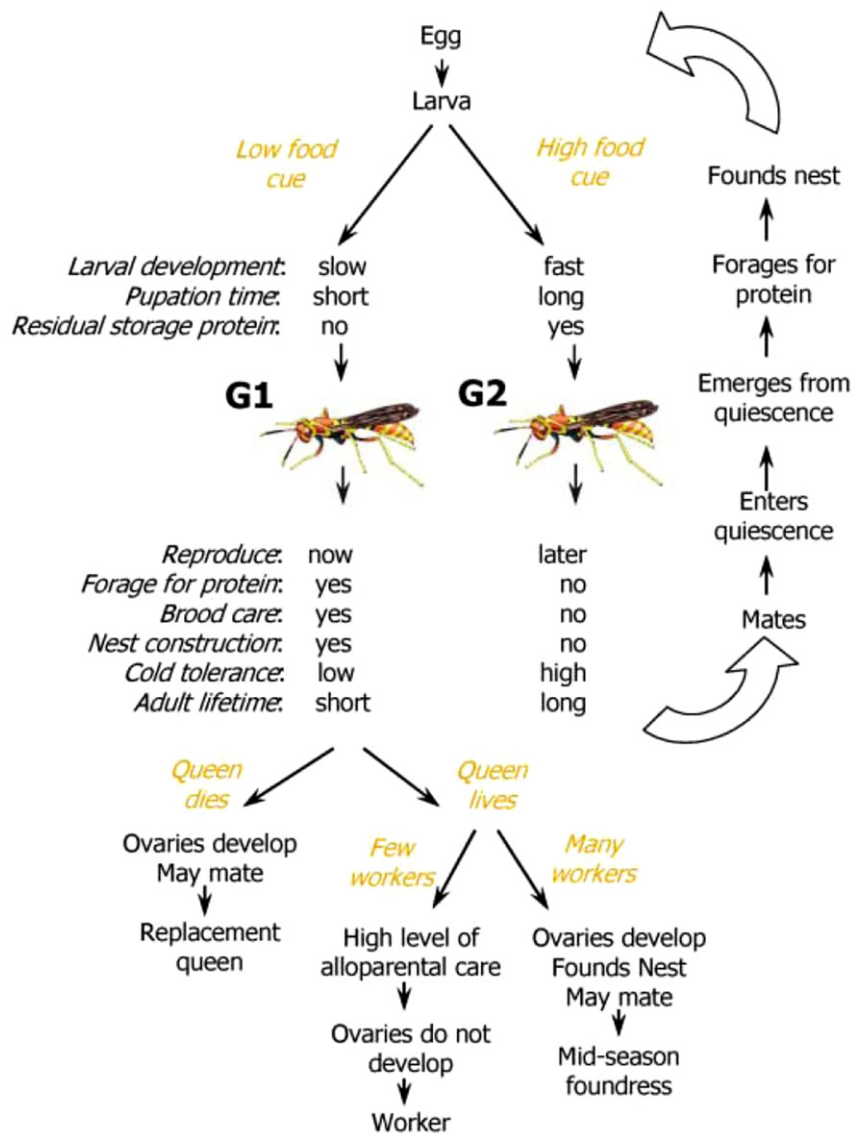
### **Kin and Nestmate Discrimination**

Low average intracolony genetic relatedness, if accompanied by high variance, is not really a difficulty for kin selection if there is good intracolony kin recognition so that altruism can be selectively directed to close kin. Hence, there has been an earnest search for the evidence for kin recognition. But that search has yielded nothing but disappointment. No really incontrovertible evidence for intracolony kin recognition has been uncovered in any ants, bees, or wasps. On the other hand, social insects have very well-developed abilities for nestmate discrimination. This suggests that keeping away nonnestmates and thus maintaining colony integrity are more important in the daily lives of social insects than to pursue intracolony nepotism. For some 15 years after Hamilton proposed the idea of kin selection, there was no attempt to test whether animals had direct kin recognition abilities. Then the deluge began and the first pieces of evidence for nestmate recognition was enthusiastically welcomed as evidence for kin recognition and as further vindication of the kin selection theory. This error in judgment was soon realized and fortunately, it did not dampen the enthusiasm for extending studies of nestmate discrimination to scores of other species of social insects. Today, CHCs, mentioned earlier, in connection with the regulation of reproduction, are also thought to mediate nestmate discrimination. Whether the same set of molecules can simultaneously mediate both the discrimination of nestmates and nonnestmates and queens and workers within a colony, is still a matter of debate. Perhaps, the evidence today for the role of CHCs in nestmate discrimination is stronger than the evidence for their role in reproductive regulation.

### **The Social Behavior Toolkit**

Theoreticians modeling the origin and evolution of social behavior posit one or more genes (alleles) for altruism or other social traits and investigate how they might fare against competition with their selfish or other ancestral counterparts. The implied idea of an allele for altruism should perhaps remain a metaphor. But it is not uncommon for empiricists to take this concept literally and begin to look for genes for altruism, sterility, dominance, etc. To help disengage from this trend, Mary Jane West-Eberhard explicitly suggested what has come to be known as the Ovarian Groundplan hypothesis. The idea she emphasized is that apparently new traits shown by social insects may be a result of coopting phenotypically plastic traits already existing in their solitary ancestors. This has now been developed into the Diapause Groundplan hypothesis, which argues that the worker and gyne castes of a primitively eusocial wasp such as *Polistes* arise from the developmental pathway already present in bivoltine solitary insects. More generally, investigators refer to the Reproductive Groundplan Hypothesis, which can encompass species that do not diapause.

Gyne is the term used for wasps eclosing in the fall in the annual colonies of social wasps such as *Polistes*, which overwinter and found new colonies in the following spring. In solitary bivoltine species, there are two generations of females produced per year: a first generation of females (G1) that reproduce soon after eclosion and a second generation of females (G2) that undergo diapause before reproduction. This hypothesis leads to the prediction that *Polistes* workers (who do not reproduce) correspond to G1 females who are programmed to reproduce and the gynes (who are the future reproductives) correspond to G2 females that have their reproduction turned off (Fig. 3). This prediction at first seems counter-intuitive because workers are generally thought of as sterile and gynes as fertile. But these predictions are testable and there is now considerable evidence to support the diapause groundplan hypothesis. The idea that the social behavior toolkit of the ants, bees, and wasps is borrowed with some modification (and that modification is made possible because of phenotypic plasticity) from their solitary ancestors, is not only powerful but also one that suggests many new lines of investigation.



**Fig. 3** The *Polistes* life cycle incorporates the fundamental elements of the bivoltine groundplan, larvae respond during development to a food cue and diverge onto one of two trajectories. Scanty provisioning leads to the G1 pathway, which is signaled by slow larval development (due to low nutrient inflow), short pupation time, and no storage protein residuum in emerging adults. More abundant provisioning leads to more rapid larval development, longer pupation time, and residual storage protein in emerging G2 adults. G1 females have a “reproduce now” phenotype, and they forage for protein, care for the brood, and construct nests. The expression of these behaviors is conditional, as indicated by branching points in the G1 sequence. If the queen is lost, a G1 female can develop her ovaries, mate if males are present, and become a replacement queen. If a queen is present but the number of workers is low, a G1 female will alloparentally express maternal behaviors (i.e., nest construction, nest defense, brood care, and foraging) as a worker at her natal nest. Finally, if a queen is present and the number of workers is high, a G1 female may depart from the natal nest and found a satellite nest in midseason. Because the cold tolerance of G1 females is low, they do not survive quiescence, and lifetimes are short. In contrast, G2 females have a “reproduce later” phenotype. They express no maternal behaviors the first year, but after emerging from quiescence, they break reproductive diapause and shift to the “reproduce now” phenotype. Figure and legend reprinted with permission from Hunt, J.H., Amdam, G.V., 2005. Bivoltinism as an antecedent to eusociality in the paper wasp genus *Polistes*. *Science* 308, 264–266.

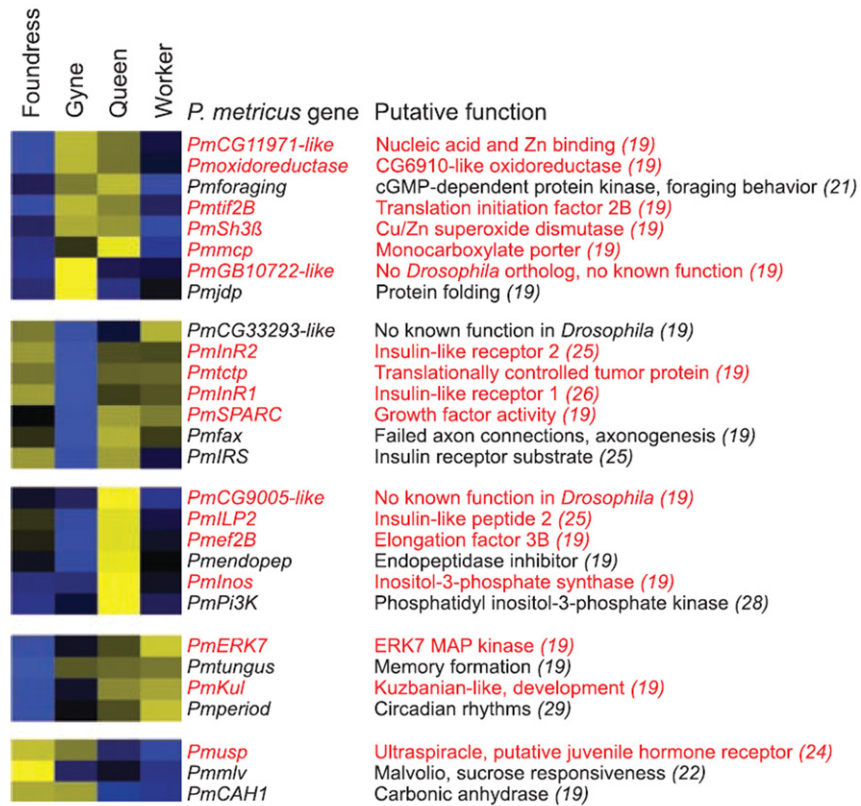
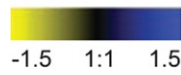
### How Does Social Behavior Develop?

Debates between the practitioners of the proximate and ultimate questions have by no means died down, and indeed they sometimes threaten to go out of hand. One way to reduce conflict is to introduce more players into the ring. Help to do precisely that and thus to channel these debates into more productive directions may come from an unexpected quarter – ethology’s Nobel laureate Niko Tinbergen. In an influential paper in the early 1960s, Tinbergen argued that we should simultaneously be asking four different kinds of questions concerning any behavior: What is the current adaptive value of the behavior? What are the proximate

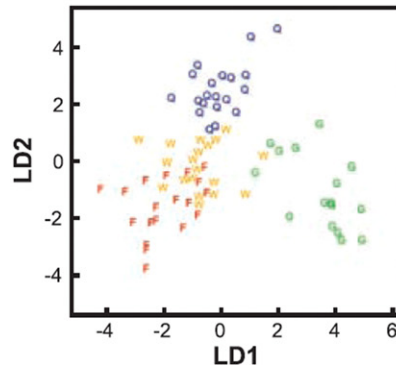
**A**  
 Reproductive and Provisioning Status of  
*P. metricus* Foundresses, Queens, Workers  
 and Gynes

	Provisioning	Non-provisioning
Reproductive	<b>Foundress</b> (maternal care)	<b>Queen</b>
Non-reproductive	<b>Worker</b> (sibling care)	<b>Gyne</b>

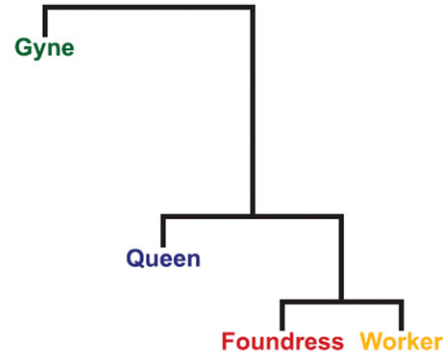
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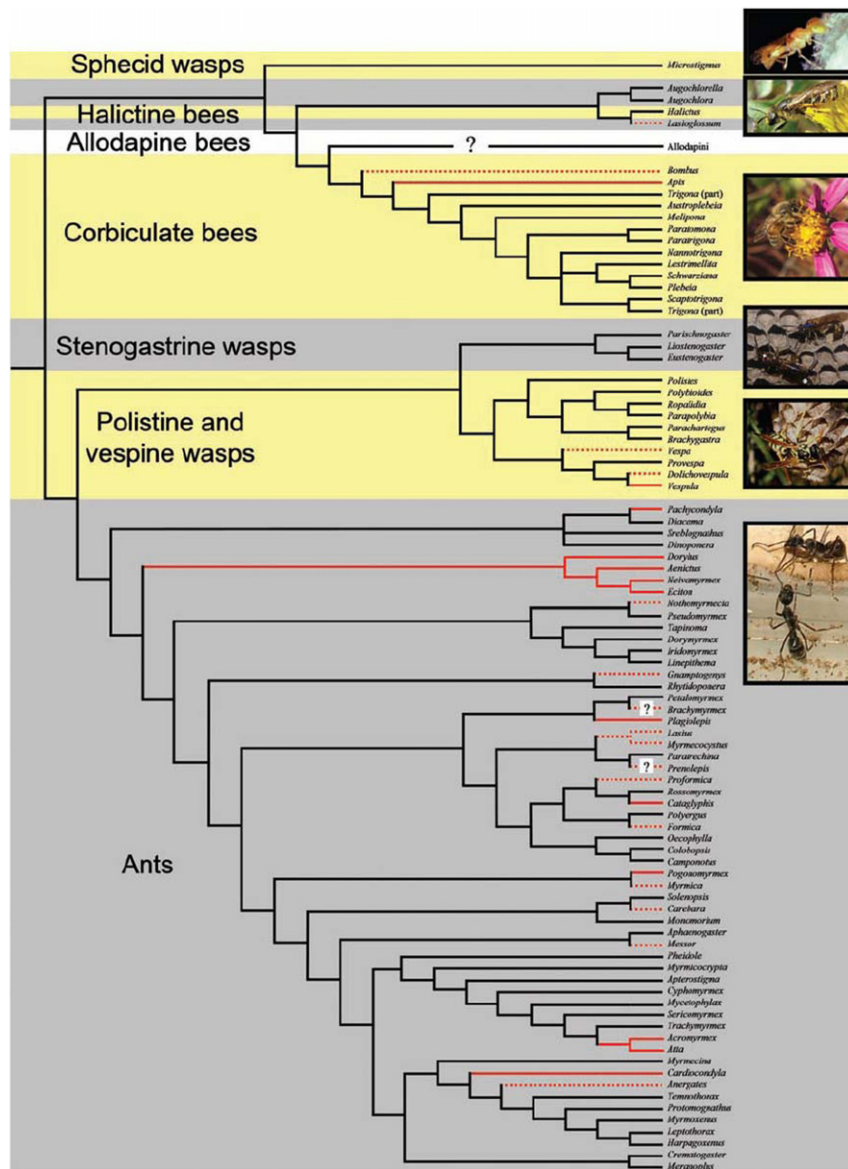
**C**



**D**







**Fig. 5** Phylogeny of genera of eusocial Hymenoptera (ants, bees, and wasps) for which female mating frequency data are available. Each independent origin of eusociality is indicated by alternately colored clades. Clades exhibiting high polyandry (<2 effective mates) have solid red branches, those exhibiting facultative low polyandry (<1 but <2 effective mates) have dotted red branches, and entirely monandrous genera have solid black branches. Mating frequency data are not available for the allodapine bees. Reprinted with permission from Hughes, W.O.H., Oldroyd, B. P., Beekman, M., Ratnieks, F.L.W., 2008. Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320, 1213–1216.

factors that cause the behavior? How does the behavior develop in the lifetime of an individual animal? What is the evolutionary history of the behavior? The first two questions correspond to the ultimate and proximate questions we have already discussed. Tinbergen's third question, which concerns the ontogeny of behavior, has only very recently begun to be asked in the context of

**Fig. 4** *Polistes metricus* wasp brain gene expression analysis tests the prediction that maternal and worker (eusocial) behavior share a common molecular basis. (a) Similarities and differences in reproductive and brood provisioning status for the four behavioral groups analyzed in this study: foundresses, gynes, queens, and workers. Each individual wasp (total of 87) was assigned to a behavioral group on the basis of physiological measurements (b)–(d). Results for 28 genes selected for their known involvement in worker (honeybee) behavior. (b) Heatmap of mean expression values by group and a summary of analysis of variance (ANOVA) results for each gene. Genes were clustered by K-means clustering; those in red show significant differences between the behavioral groups. *P. metricus* gene names were assigned on the basis of orthology to honeybee genes; putative functions were assigned on the basis of similarity to *Drosophila melanogaster* genes. (c) Results of linear discriminant analysis show that foundress and worker brain profiles are more similar to each other than to the other groups. (d) Results of hierarchical clustering show the same result (based on group mean expression value for each gene). Figure and legend reprinted with permission from Toth, A.L., Varala, K., Newman, T.C., et al., 2007. Wasp gene expression supports an evolutionary link between maternal behavior and eusociality. *Science* 318, 441–443.

social behavior in insects. Not surprisingly, ontogenetic questions have first and foremost been applied to understand how some individuals in insect societies come to develop and behave as fertile queens, while others come to develop and behave as sterile workers. It has long been assumed that caste determination is not genetic but entirely environmental. And there is plenty of evidence that environmental factors, especially nutrition in the early larval stage, influence the future caste of the individual. What is surprising, however, is that more than a negligible number of cases of genetic determination of caste, or at least genetic influences on caste ratios, are being thrown up when genetic, and more recently, molecular genetic techniques, are applied. These genetic influences remain poorly understood at the molecular or physiological level. But the mechanisms involved in nutritional influence on caste have recently begun to be unraveled in an impressively sophisticated experimental paradigm. It is well known that honeybee larvae fed with royal jelly develop into queens, while those denied royal jelly develop as workers. Gene expression profiles determined using microarray analysis have shown that queen- and worker-destined larvae differ greatly in which genes they upregulate and which they downregulate, paving the way for tracing the ontogenetic development of caste-specific morphology, physiology, and behavior. A similar study but one that used adults of the primitively eusocial wasp *Polistes canadensis* also helps identify genes that are involved in producing caste-specific phenotypes in queens and workers. A more recent microarray study shows that gene expression in the brains of worker-destined wasps is similar to that of nest foundresses, both of which specialize in maternal behavior, compared to queens and gynes, which do not display maternal behavior (see Fig. 4). These kinds of studies accompanied by full genome sequences are expected to usher in a new era in the study of insect sociality. If integrated into the more classical approaches involving ultimate and proximate causation they should revolutionize the study of insect sociality.

## The Phylogeny of Social Behavior

The good news is that we have also begun to put Tinbergen's fourth question to work for us. In the last decade or two, DNA sequencing has become routine, and powerful statistical techniques to construct molecular phylogenetic trees are being developed. This has made it possible to reconstruct the phylogeny of social insect lineages and trace the evolutionary history of social behavior to a level of sophistication that Tinbergen could hardly have imagined. It is gradually becoming fashionable to investigate the salience of different traits to the origin of eusociality in the context of a phylogenetic tree. The first such study made on eusocial wasps identified nesting, oviposition into an empty nest cell, progressive provisioning of larvae, adult nourishment when they feed larvae, and inequitable distribution of food among nestmates, as the most important traits in the origin of vespid eusociality. Interestingly, no role was obvious for the asymmetries in genetic relatedness created by haplodiploidy, although haplodiploidy was found to be important as a mechanism that permitted females to choose the sex of their offspring and produce an all-female brood. In contrast, a similar study that includes many more eusocial lineages in addition to the wasps says nothing explicit about haplodiploidy but shows that high relatedness expected from monogamy is ancestral to all the eusocial lineages considered. It therefore suggested a high salience for genetic relatedness in the origin of eusociality (Fig. 5). That these studies may, in the beginning, yield a contradictory and confusing picture is a minor problem compared to the expected long-term benefits of taking this approach. A more impressive and less controversial study of attine ants has helped trace the origin of five different agricultural systems of ants. There can be little doubt that these studies will multiply rapidly in the near future. What is less likely to happen automatically, which may therefore require some special effort, is the preservation of the climate required for a simultaneous pursuit of all of Tinbergen's four questions and a meaningful integration of the knowledge gained for these diverse approaches.

## Further Reading

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