

Caste Determination in Arthropods

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Introduction

Caste is one of the defining features in eusocial insects. At the most basic level, this system is characterized by morphology associated with the reproductive division of labor between sexually reproductive individuals and non-reproductive individuals who care for the reproductive's offspring. In the more derived groups of social insects, developmentally discrete castes fulfill these different behavioral roles. Reproductive division of labor is one of the defining characteristics of eusociality; eusocial insects include termites (Isoptera), ants (Hymenoptera), some bees (Hymenoptera), and some wasps (Hymenoptera).

In Hymenoptera caste divides individuals into one of the three categories: reproductive females (queens), reproductive males, and functionally sterile females (workers). Queens typically disperse, mate, find new colonies, and subsequently provide the reproductive function of the colony. Males disperse and mate with virgin queens; although there are some exceptions, males seldom perform any other activities and die shortly after mating. Workers perform all other tasks, including caring for young, nest building/maintenance, guarding, and foraging. All females are diploid and derived from fertilized eggs, while males are haploid and derived from unfertilized eggs. Female eggs are usually totipotent, that is, they have the potential to develop into either queens or workers. Developmentally, females are highly plastic and their physiology and morphology are influenced by various mechanisms, which result in caste differentiation. Developmental differentiation is primarily governed by nutritional stimuli, but other environmental factors such as climate and social interactions, as well as genetic effects (in some taxonomic groups), are important as well.

Termites (Isoptera) live in eusocial colonies with a similar system of reproductive division of labor to that of Hymenoptera. As with ants, bees, and wasps, a specialized sexual caste performs all reproductive functions for the colony, and a worker caste performs all other nest and foraging tasks. However, both termite sexes are derived from diploid, fertilized eggs, and thus both males and females are capable of developing into either workers or reproductives. Termite colonies are established by a winged queen and a winged king, which are known as primary reproductives. After colony founding, they shed their wings and begin producing workers. In many termite species, colonies produce secondary reproductives; these sexually capable individuals do not have

wings and are capable of superceding sick, injured, or absent parental primary reproductives. The worker caste is wingless and sterile. In some termite species in the families Kalotermitidae and Hodotermitidae, a true worker caste has been replaced by preadult instars (referred to as nymphs or sometimes larvae). These preadult workers are not constrained from developing into reproductives later. Termites are hemimetabolous insects; unlike the holometabolous Hymenoptera, they do not undergo a pupal stage. Nymphs, or larvae, are physically similar to adults, and their metamorphosis is gradual. Thus, termite larvae function as efficient members of the colony workforce even before maturity. While less well-studied than Hymenoptera, termite caste determination appears to be affected by similar mechanisms, such as social interactions and endocrine cues.

In addition to the dichotomy between reproductive and nonreproductive individuals, many ant and termite species produce a polymorphic worker caste (e.g., workers vs. soldiers). In some ant species, extreme morphological differences exist among functionally sterile workers. In one pattern, exemplified by the red imported fire ant, *Solenopsis invicta*, variation is continuous, with workers ranging evenly throughout the entire gamut of body size. In others, like many *Pheidole* species, worker polymorphism is bimodal (or, in some cases, trimodal), and workers develop into small minor workers or large major workers. In termites, many species have distinct worker and soldier castes. Soldiers are generally larger and have more robust heads, usually with structures evolved specifically for defending the colony.

In both ants and termites, strong allometric scaling, in which the head or other structures grow disproportionately large relative to the rest of the body, is also observed. In these cases of allometry, major workers (or soldiers) increase not only in total size, but some characters (e.g., the head) grow nonlinearly, relative to the rest of the body. Most of the developmental determinants of reproductive caste are also key factors controlling worker caste polymorphisms.

Haplodiploid Sex Determination in Hymenoptera

In addition to differentiation between reproductive and nonreproductive castes, Hymenoptera have a unique system of sex determination. All Hymenopteran species are

haplodiploid; males derive from unfertilized eggs, and are thus haploid. Females are derived from fertilized, diploid, eggs. When a reproductive female lays a male-destined egg, no sperm is released for fertilization; however, when a female-destined egg is laid, sperm is released and the egg is fertilized prior to oviposition (Figure 1). Eusocial Hymenoptera colonies are female-dominated, and male tasks are usually limited only to dispersal and mating. Thus, it is important that the queens regulate the sex of their eggs, or the colony will be burdened with useless males at inopportune times of the colony life cycle.

In honeybees (*Apis mellifera*), the decision to fertilize an egg is based on the cell type in which the egg is to be laid; in the comb, workers create different cell types for males, workers, and queens. Therefore, the workers produce appropriate numbers of each cell type and the queen must be able to differentiate among them and then lay a fertilized or nonfertilized egg as necessary. In other Hymenoptera, the mechanism underlying the queen's decision is not completely clear.

Physical Features of Caste

In most eusocial Hymenoptera, caste differentiation is based primarily on environmental factors experienced during egg and larval development. Morphological differences

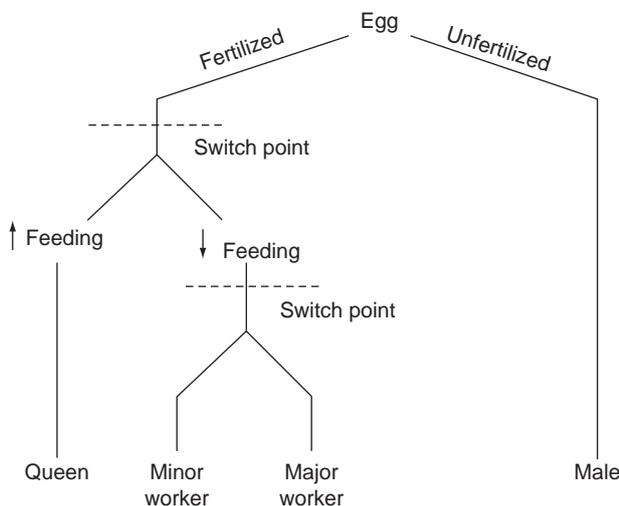


Figure 1 Model of differentiation possibilities of a totipotent Hymenoptera egg. If the egg is left unfertilized, it will develop into a haploid male. However, if fertilized, the egg can become a queen or a worker. The switch point between these caste fates is predominantly due to differential feeding regimens, with queen-destined larvae receiving more, and better quality, food. In some species, another switch point exists in which differentiation between worker morphological castes occurs (e.g., minor (small) vs. major (large, a.k.a. soldier)). In addition to a relationship with feeding regimen, hormonal changes also affect these switch points (e.g., Figure 2).

related to caste-specific tasks arise due to developmental differences triggered during growth and development. In general, reproductive females are larger, have larger ovaries with more ovarioles, and possess a spermatheca (an organ used for long-term storage of sperm). Workers are smaller, have smaller ovaries with fewer ovarioles, lack spermathecae (in more highly derived species), and have features more useful in nonreproductive tasks (e.g., the corbicula, or 'pollen basket' leg structure of honeybee workers).

Termite primary reproductives are generally larger, possess autotomous wings, and have larger and more well-developed eyes and a more heavily sclerotized body than nonreproductives. After shedding their wings, primary reproductive queens enlarge their ovaries, causing abdominal swelling. Workers usually lack compound eyes, are more lightly sclerotized, and are smaller. These developmental differences can result from a combination of several environmental factors, such as nutrition, the social environment of the colony, and climatological effects, as well as genetic factors.

Nutritional and Endocrine Controls of Caste Determination

With a few important exceptions, female eggs of eusocial Hymenoptera are not predestined for a specific caste fate and are genetically and morphologically indistinguishable from each other. After oviposition, however, environmental stimuli come into play to determine caste-specific developmental trajectory. In species across the spectrum of eusocial evolution, the predominant environmental factor in caste determination is nutrition. Specifically, a nutritional switch during development is the point of divergence between the reproductive and nonreproductive castes. The developmental timing of this switch is highly variable between species; generally, species with an earlier switch exhibit larger differences between the castes, since more developmental time is allotted towards caste-specific growth.

Nutritional determination of caste is best known from studies of the honeybee. As pointed out above, castes are reared in specific cell types in the comb. The cell type is important for determining the treatment of the larvae by workers; the nutrition delivered to the larvae determines the caste fate of any given individual. Honeybee larvae are fed with a mixture of mandibular gland secretions, hypopharyngeal gland secretions, pollen (a protein source), and sugar (from honey, a carbohydrate source); both queen- and worker-destined larvae are fed with all of these substances, but quantity and ratio differ. Queen-destined larvae are fed in high quantity with what is commonly referred to as royal jelly, a mixture of these four components that contains a higher concentration of mandibular gland secretion and sugar than what is provided

to worker-destined larvae. Thus, queen-destined larvae are provided with a larger amount and higher quality food source, and the nutritional switch is flipped to push their development towards the reproductive phenotype (Figure 1). While ant, wasp, and other eusocial bee larvae vary in diet, this same basic pattern of nutritional caste determination applies in these groups.

The underlying causes of these trajectory shifts are likely due to changes in the larval endocrine system in response to nutritional stimulation during key periods. As larvae pass a critical size due to increased food intake, physiological changes that influence development are triggered, and the larvae proceed down the queen-specific developmental pathway. For example, juvenile hormone (JH), a sesquiterpenoid hormone known to govern developmental functions across insect taxa, is upregulated in response to increased nutrition. As food intake increases, so does body size and JH level; thus, larvae reach a critical threshold and the developmental pathway is changed to the larger form (e.g., from worker to queen, or from minor to major worker; see Figure 2).

In addition to JH, the IGF-1 IIS pathways (insulin/insulin-like growth factor-1-like signaling) have been implicated in controlling queen-worker differentiation. In the honeybee, several IIS genes are upregulated in queen-destined larvae compared to worker larvae. Also in honeybees, knockdown of the expression of *target of rapamycin (tor)*, a central component in growth regulation response to nutritional stimuli, results in prevention of queen development, even in the presence of queen-specific nutritional cues. This emerging research focus

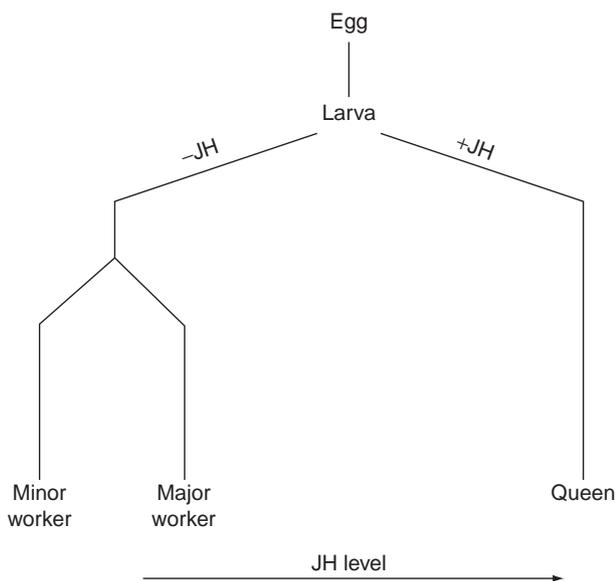


Figure 2 General diagram of developmental control points where juvenile hormone (JH) is known or suspected to play a role in caste determination. Those in larval or early pupal development are probably associated with nutritional switches.

on the molecular level of metabolic pathways should shed light onto how the nutritionally mediated orchestration of caste determination is operating at the endocrine, genetic, and molecular levels.

This focus on the endocrine and physiological relationships in social Hymenoptera has led to new ideas relating to the evolution of eusociality in insects. Work by West-Eberhard, and later Amdam and Page, has led to the presentation of what is known as the reproductive ground plan hypothesis, a framework that explains how the physiological mechanisms regulating reproductive behaviors in solitary ancestors may have been co-opted for the regulation of nonreproductive behavior in workers of extant social insects. In line with this hypothesis is the idea that, for eusocial societies to operate, a finely tuned relationship between both social environment and developmental responsiveness must evolve.

In many primitively eusocial bees and wasps, all females are potentially queens. Despite the variation in their size, all have complete reproductive systems and can lay both fertilized and unfertilized eggs after mating. In these systems, caste is determined less by larval development and more by behavioral dominance among adults. Some individuals may be larger, and can thus more capably dominate nestmates, but this is the point at which any developmental caste-differentiation ends. Since increased size usually denotes queen behavior/ placement in the dominance hierarchy, larval nutrition and growth are also keys to the development of different caste roles. Recent research by Hunt has shown that, at least in the paper wasp *Polistes metricus*, some developing larvae facultatively go through diapause, resulting in increased storage protein synthesis and sequestration, and are thus more likely to express the queen phenotype as adults. In addition, the mechanisms of *Polistes* diapause have been proposed as an antecedent to sociality; similar to the predictions of the reproductive ground plan hypothesis, this work explains how the dichotomy between *Polistes* workers and queens may be based on the co-option of the bivoltine wasp life history seen in solitary species, which is split between early season nondiapausing individuals and late season diapausing individuals, for regulation of the social systems seen in *Polistes* wasps.

Termite caste differentiation is fundamentally different than that of Hymenoptera because the hemimetabolous developmental cycle of termites allows for larvae to be active members of the colony work force. However, like Hymenoptera, hormones are important regulators of caste differentiation. When larvae molt into their next instar, variation in endocrine activity can alter gene expression and produce caste-specific phenotypes. This system is poorly understood because studies on endocrine influences in termite caste determination are rare. The available evidence suggests that immature individuals that molt with low JH titers are able to express the

reproductive phenotype, but those with higher JH titers have constrained gonad development and retain a worker phenotype. For example, in *Zootermopsis angusticollis*, winged queens require an increase in ecdysteroid levels and a decrease in JH levels to begin actual reproductive activity. While most studies have focused on JH, the underlying controls of caste fate in termites are most likely a complicated network of processes driven by JH, ecdysteroids, and other factors. Future research is needed, however, to elucidate the exact mechanisms behind these developmental changes. While this is true of all insect groups, termites are particularly understudied, especially compared to eusocial Hymenoptera.

Climate Effects on Caste

In many ant species, such as some *Formica* and *Myrmica*, larvae are more likely to develop into queens if they are exposed to winter or winter-like chilling effects. This is most likely an adaptation to prevent production of sexual castes during inopportune times of the year. If larvae that have not overwintered are less likely to develop into queens, the colony will be less likely to produce unnecessary virgin queens during nonmating seasons when the investment would be wasted.

Social and Maternal Influences on Caste

Since caste determination is usually based on nutritional and endocrine factors, the ultimate determinant is the treatment of the developing larvae. As larvae are completely dependent on workers for care, worker behavior is a key factor influencing caste determination. In many species of bees, wasps, and ants, worker behavior is modulated in some way by the mature queen. The queen's influence can change worker treatment of developing larvae and prevent the necessary dietary stimuli during key periods of larval development. In eusocial insects with relatively small colonies, queens can affect worker behavior with direct physical domination or intimidation, and thus influence the rearing/fate of developing larvae. More often, however, colonies are too large for a queen (or queens) to physically interact in such a manner. In these cases, queens instead release pheromonal signals that prevent workers from treating developing larvae appropriately to produce sexuals, or by inhibiting larval development directly.

For example, honeybees are noted for their use of queen pheromone to affect caste determination. When the queen produces this pheromone, workers will not build queen cells; without these larger, queen-specific structures, the expression of queen-rearing behavior cannot or will not occur. Queen pheromones, which are

produced in the queen's mandibular gland, are primarily made up of 9-keto-(*E*)-2-decenoic acid (9ODA) and 9-hydroxy-(*E*)-2-decenoic acid (9HDA), both of which are highly specific in their effects on biological activity. The production of these compounds varies over a queen's lifetime, depending on her age, mating status, as well as the seasonality of the colony. For example, older queens will change their production to allow the workers to raise virgin queens and prepare for colony fission.

Thus, in honeybees, the queen influences caste determination even before egg-laying, let alone larval development by using pheromonal secretions to prevent the worker behaviors necessary for queen-destined larval development.

A similar method of worker control exists in vespine wasps, although the effects are somewhat reversed. Again, queen development is contingent on the production of appropriate cells by workers. Whereas queen pheromones prevent this from occurring in honeybees, Ikan's work showed that these cells are only produced when the wasp queen produces pheromonal secretions.

In some ant species, such as *Plagiolepis pygmaea*, *Tetraponera anthracina*, and *Myrmica rubra*, pheromones have other caste determining effects on larvae. Pheromones released by the queen can inhibit the development of queen-destined larvae via inhibition at a critical developmental window during the larval cycle. In species that require overwintering for queen production, pheromones can prevent larval hibernation from occurring. By preventing this key stage, the queen effectively removes the potential for queen development. Bumble bees, *Bombus terrestris*, exhibit a similar system in which exposure of developing larvae to a queen pheromone also precludes any chance of those larvae developing into queens themselves. Only when this pheromone is no longer produced can queen-destined larvae be reared.

In addition to pheromonally mediated inhibition of sexual development, queens can also influence the caste fate of their offspring during the egg stage. In some ant species, such as *Formica polyctena*, queens can bias otherwise totipotent eggs towards queen development by giving them a nutritional boost before oviposition. These eggs are larger and have better nutritional content than worker-destined eggs, which appear to lower the further nutritional stimulation necessary to switch larvae to queen development. Another method of maternal influence on egg fate is the sequestering of variable hormone quantities in eggs. In *Pogonomyrax* harvester ants, for example, queen-destined eggs have significantly lower levels of ecdysteroid hormones. This is in accordance with previous research, which has shown that JH application to a queen or her eggs results in significantly more queens, because JH depresses ecdysteroid level. In any case, larvae in species in which this occurs are not capable of as much caste plasticity, since their caste fate is determined in the egg stage.

Termites are poorly understood when compared to the eusocial Hymenoptera, but mechanisms of social control of caste determination appear to exist in termite colonies. The social interactions influencing reproductive status are often complex due to the presence or potential presence of both primary and secondary reproductives in termite societies. In some species, production of primary reproductives is thought to be regulated by worker manipulation of the developing larvae. In several species, workers destroy the wing buds of developing primary reproductives, removing their ability to disperse. Evidence in more primitive termite species, however, has shown that, like in many Hymenoptera, reproductive development may be mediated by inhibitory stimuli produced by current adult reproductives, though the mechanism of this inhibition is still unclear.

The production of secondary reproductives in established termite colonies can vary widely between species. In species that lack a true worker caste, in which nonreproductive tasks are performed only by immature individuals (pseudergates), secondary reproductives seem to derive from either older larvae or immature primary reproductives. The switch of an individual to a secondary reproductive role may result from changes in developmental timing, which produces an incomplete primary reproductive (i.e., wingless). In termite species with a true worker caste (that is, a caste of individuals that, even as adults, perform worker tasks), secondary reproductives can be derived from reproductively inactive primary reproductive offspring, or by the subsequent development of larvae or workers into reproductive morphology. The control over the sexual maturation of these individuals appears to belong to the functioning reproductives present in the colony, but, again, the mechanisms are unknown. In some species, primary reproductives physically dominate potential secondary reproductives, changing their caste fate before maturation. In other cases, functioning reproductives may inhibit sexual development via pheromone action on developing larvae, but there is no evidence to support this. Additionally, functional reproductives may also influence worker treatment of larvae, thus indirectly influencing caste determination. For example, workers of some species are known to remove extra secondary reproductives from the nest.

Genetic Effects

While there is extensive evidence for the environmental basis of caste determination across many taxa, findings in some taxonomic groups show that genetic factors can have significant effects on adult caste. Heritable variation in caste is seen in some ant, bee, and termite species.

Perhaps the best known case is the genetic caste determination seen in some *Pogonomyrmex* harvester ants.

Research on *Pogonomyrmex* exhibiting genetic caste determination revealed two distinct genetic lineages within individual colonies; queens are homozygous and belong to one of the two possible lineages, while workers are heterozygotes derived from cross-lineage mating. When the queen produces fertilized eggs, the lineage of the male whose sperm is used determines the caste of the newly-laid egg; one lineage of male results in queen development, while the other results in worker development. While the mechanism is still unclear, incompatibilities between the lineages that prevent queen production from heterozygous matings are proposed to exist. This system is particularly interesting because, for a queen to found a successful colony, she must mate with males from both lineages in order to produce both female castes.

Another example of genetic differences between castes is seen in the little fire ant, *Wasmannia auropunctata*. In this species, workers are produced normally, from fertilized eggs; queens, however, are produced from thelytokous parthenogenesis in which meiotic cells are fused in order to parthenogenetically produce unfertilized diploid eggs. Thus, diploid eggs are produced solely from queen gametes. This life history strategy has caused an almost complete separation of queen and male gene pools.

In some species of ants, such as *Dinoponera australis*, a true queen caste has been completely lost. Similarly, in the Cape bee, *Apis mellifera capensis*, colonies can reproductively operate in the absence of a queen. Without a queen present, some workers must reproduce. In many ant species with this trait, workers retain a spermatheca, and can thus successfully mate and reproduce normally. In the Cape bee, however, a reproductive worker produces males via unfertilized eggs and females via thelytokous parthenogenesis, similarly to *W. auropunctata* queens.

In termites, genetic caste determination has been identified in the species *Reticulitermes speratus*; a single sex-linked locus with two alleles produces sex-specific traits. These alleles play a key role in the caste fate of the offspring.

The existence of the genetically influenced systems represents a puzzling evolutionary question. What is the benefit of reduced flexibility in caste production? The loss of totipotency results in a reduction in the ability of the colony to more fluidly adapt to quickly changing scenarios by changing/controlling caste ratios. For example, in the *Pogonomyrmex* genetic caste determination systems, queens are produced at proportions in line with predicted genotypic ratios independent of the age and colony size. In this scenario, colonies are unable to stop queen egg production, even at a very young colony age, when queen production is unnecessary and unwanted. While it has been shown that nascent colonies of *Pogonomyrmex* do not actually raise queen-lineage eggs to adulthood, the laying of these eggs at all still represents a potential waste of resources during a key time in the colony life cycle, and how this strategy is evolutionarily stable is puzzling.

Polymorphic Worker Castes

Among the eusocial Hymenoptera, only ants, and to a lesser extent, bumble bees, produce workers with morphologically complex worker castes. While relatively few ant genera produce polymorphic workers, some of the most ecologically successful often produce highly polymorphic work forces (e.g., *Pheidole* and *Camponotus*). Species with a broad spectrum of worker morphs tend to have a very early developmental switch for queen–worker differentiation, providing a maximum developmental time frame for intra-worker caste differences to develop (Figure 3).

The extreme variation seen between workers in ant species with a polymorphic worker caste has been explained by the reprogramming of the developmental pathways present in all eusocial Hymenoptera, specifically a revision of critical size and growth rules. While the framework is reprogrammed, development is still influenced by many of the same environmental stimuli that affect queen–worker differentiation, primarily larval nutrition.

The critical size of a larva is simply the size threshold it must reach to begin the developmental pathway into the next largest morph. Variation in the critical size needed for this transition (due to variable reaction norms of larvae) can give rise to polymorphic worker castes with continuous variation. Additionally, more strictly controlled thresholds exist, which can result in the production of more discrete worker size classes. Growth rules correspond to the allocation of energy to the growth and development of different body structures, which results in differences in the growth rate and final size of body characters, producing allometries. Both critical size threshold and the patterning of growth rules can be influenced during sensitive windows during development, and thus produce

wide variations in worker morphology and size, even in colonies of highly related individuals. The location in developmental time where larvae actually make the switch to the larger caste-type is highly variable between species. Generally, the later the switch occurs during larval development, the more flexible the system; in species where the switch point occurs during the final instar, decisions about caste ratio can be made even at the figurative last minute of larval development (Figure 1).

Many species of termites also produce a soldier caste in addition to normal workers. In several species, increased JH levels in circulating hemolymph promote expression of the presoldier or soldier phenotype. This increase seems to coincide with the initiation of molting, caused by peaking levels of ecdysteroid hormones. These changes have been proposed to promote mandibular development and a more robust cuticle; recent evidence has also shown that these hormonal changes during development induce shifts in gene expression patterns to produce the soldier phenotype.

In addition to environmental factors like presence of predators or competitors and food availability, soldier production may also be limited by the same types of social influences that affect reproductive caste development. Soldiers, possibly through secretory products from the head, are able to use pheromones to inhibit the differentiation of developing larvae into new soldiers. In addition, there is some evidence that functioning reproductives are able to stimulate soldier production. The ability of the colony to regulate soldier number in these ways is most likely an adaptation to regulate caste ratio to maximize colony efficiency.

Conclusion

As noted, one of the defining features of social insect biology is the variation in caste between highly related, if not effectively identical, individuals within a colony. Undoubtedly, the determination of caste during development is affected by many factors, both within and between taxonomic groups. However, research into this field has led to many advances in our understanding of the various roles played by nutrition, hormones, climate, social organization, and genetics. While many questions remain unanswered as to the complexities underlying caste determination in insects, new tools in these fields of research continue to emerge and should prove invaluable in providing future information about the underlying factors influencing caste.

See also: *Pheidole*: Sociobiology of a Highly Diverse Genus; Termites: Social Evolution.



Figure 3 Example of different castes developed from totipotent eggs of the harvester ant *Pogonomyrmex badius*. (a) Queen (unmated, with wings still attached); (b) minor worker; (c) major worker; (d) male. Photo courtesy of Adrian A. Smith.

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