Insect societies and the molecular biology of social behavior

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Summary

This article outlines the rationale for a molecular genetic study of social behavior, and explains why social insects are good models. Summaries of research on brain and behavior in two species, honey bees and fire ants, are presented to illustrate the richness of the behavioral phenomena that can be addressed with social insects and to show how they are beginning to be used to study genes that influence social behavior. We conclude by considering the problems and potential of this emerging field.

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Introduction

The study of behavior involves research on behavioral evolution and underlying neural (and neuroendocrine) mechanisms. Although some topics continue to be studied from an integrative perspective, at the present time the field is largely split into two separate disciplines, neuroscience and behavioral ecology. Neuroscience and behavioral ecology have each enjoyed spectacular success, but our understanding of behavior will be fragmentary as long as they continue solely as divergent disciplines. Fortunately, there are signs of a new rapprochement(1).

Molecular genetic analyses of behavior that occurs in a natural context can catalyze a new synthesis, because both neuroscientists and behavioral ecologists are interested in genes. After all, genes not only encode proteins that build the neuroanatomical, neurochemical, neuroendocrine and neurophysiological mechanisms governing behavior, but also are the units of biological organization upon which natural selection ultimately acts in behavioral evolution. Focusing on genes provides a common language and convergent research themes. An exciting precedent for this idea exists; in developmental biology, the discovery of genes that influence development has sparked just such a fusion of mechanistic and evolutionary approaches(2).

Social behavior is one of the most important forms of behavior upon which to build a gene-based synthesis. Much research already is devoted to social behavior in behavioral ecology, including female choice, kin recognition, reciprocal altruism and the organization of insect societies(3,4). Social behavior has not yet risen to prominence on the neuroscience agenda, but the beginnings of a 'socioneurobiology' can be discerned from studies of bird song learning(5), aggressive behavior in birds(6) and marine arthropods(7), reproductive behavior in fish(8) and socially mediated changes in brain structure in bees(9). These studies highlight the fact that many animal species are especially attuned to their social environment and possess mechanisms that enable them to change their behavior in response to changing conditions.

Molecular genetic studies of social behavior begin with one or both of the following questions. First, is allelic variation correlated with variation in the expression of a social behavior and/or its neural substrates? Results of quantitative genetic studies suggest that such correlations exist for a range of social behaviors(10), but specific genes have yet to be identified. Second, to what extent do changes in gene expression control socially mediated neural and behavioral plasticity? The expression of an early-immediate gene in the brain of a canary(11) or zebra finch(12) is more intense when a novel song is presented but is severely attenuated in response to a familiar song; these findings and those from recent neuroethological studies(7,8) suggest that gene expression in the brain is sensitive to social context. We propose that two-way communication between the nervous system and the genome contributes fundamentally to the control of social behavior. Information acquired by the nervous system on social conditions is likely to induce changes in genomic function that in turn adaptively modify the structure and functioning of the nervous system.

In this paper we first explain why social insects are good models for molecular genetic studies of social behavior. Using two of the more well-studied species, the honey bee, *Apis mellifera*, and the fire ant, *Solenopsis invicta,* we then introduce five areas of research that both illustrate the richness of the behavioral phenomena that can be addressed with social insects and provide a strong foundation for analyses of genes and social behavior. We conclude by considering briefly the problems and potential of this emerging field.

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Social insects: extremists or exemplars?

Among the species of animals most attuned to their social environment are the social insects. They live in societies that rival our own in complexity and internal cohesion. Social insects are characterized by 'eusociality,' which means they live obligately in colonies with overlapping generations, cooperative brood care and a reproductive division of labor(4). The queen reproduces directly, while the workers perform tasks related to colony growth and development and engage in little, if any, reproduction themselves. Advanced eusocial species, including honey bees and fire ants, have the largest colonies, numbering tens or even hundreds of thousands of workers. They also live in the most complex societies, highlighted by an intricate division of labor among workers. Social insects are 'extremists' in their constant expression of social behavior; they coordinate virtually all of their activities with other individuals to ensure colony survival.

Yet despite their special attributes, the problems social insects face are not exceptional. All animals must, to some degree, obtain and process information about their changing ecological and social mileu and act accordingly. Neural and behavioral plasticity is even more contingent upon social context for species with active social lives. In social evolution, the sophistication of behavioral mechanisms for the essentials of life – food, shelter and reproduction – stems from increased abilities to communicate and synchronize behavior with conspecifics. Social insects are thus exemplars of social behavior, and indeed already serve in this capacity for both mechanistic and evolutionary studies(13).

Social insects also provide logistical advantages for genetic studies of social behavior. First, a variety of intricate patterns of behavior can be studied rigorously under natural conditions. Social insect behavior tends to be more stereotyped, and thus more easily assayed, than that of other highly social animals. These are key prerequisites for reliability because the expression of social behavior is notoriously sensitive to the environment, or as E. O. Wilson has noted, *'social behavior comprises the set of phenotypes farthest removed from DNA'*(13). Second, some species, notably the honey bee and several species of ants, can be manipulated extensively in both the field and the laboratory. Third, three of the more well-studied groups of social insects, the ants, bees and wasps, are haplodiploid. A (diploid) queen that mates with just one (haploid) male produces worker offspring that share 3/4, rather than 1/2, of their genes by common descent, which facilitates behavioral genetic analysis. With honey bees, haplodiploidy is exploited further by researchers *via* instrumental insemination technology(14), as discussed in (3) below.

Individual research programs on social insects have already established an integrative approach to social behavior that extends from 'society to gene'. It is our hope that by uniting several of these diverse approaches in a single review, the origins of a new field can be glimpsed.

(1) Social, pheromonal, and genetic control of endocrine-mediated behavioral development in the honey bee

There is an age-related division of labor among adult worker honey bees(15). Young 'nurse' bees primarily feed and care for larvae and the queen; middle-age bees maintain the hive and store food; and the oldest bees forage for nectar and pollen and defend the hive. A bee typically begins to forage at about 21 days of age and then acts in this capacity for the remaining 1-3 weeks of her life.

But behavioral development is not rigid; bees are sensitive to changes in their social environment. Individuals can accelerate, delay, and even reverse their behavioral development in response to changes in colony age demography(16). For example, in a colony deficient in foragers, some individuals develop precociously into foragers about 2 weeks early, when they are as young as 7 days of age.

One molecule involved in the control of behavioral development rates is juvenile hormone, a sesquiterpenoid produced by the corpora allata glands(17). The titer of this hormone is low in bees that work in the hive and high in foragers, and treatment with juvenile hormone or analogs induces precocious foraging. This key insect developmental hormone is thought to influence bee behavior by modulating central nervous system response thresholds to task-related stimuli(16). Plasticity in behavioral development is mediated by the effects of the social environment on juvenile hormone. Precocious foragers have a precociously high titer of juvenile hormone, overage nurses have a low titer, and bees that revert from foraging to nursing show a drop(16-18). These results are consistent with the well-supported belief that the corpora allata occupy a key position in orchestrating an insect's response to environmental factors, similar to neuroendocrine control of the pituitary gland in vertebrates(19). Endocrine-mediated behavioral maturation that is sensitive to social context in bees has striking parallels to phenomena in fish(8) and mammals(20).

Plasticity in honey bee behavioral development is based, at least in part, on social interactions between colony members that involve chemical communication. One pheromone involved is produced by the mandibular glands of the queen. Queen mandibular pheromone is composed of the fatty acids 9-keto-(*E*)2-decenoic acid, *R*-9-hydroxy-(*E*)2-decenoic acid (9-HDA) and *S*-9-HDA, and two aromatics, methyl p-hydroxybenzoate and 4-hydroxy-3-methoxyphenylethanol(21). This pheromone has been known for some time to exert long-lasting primer effects on physiology and behavior by inhibiting the rearing of new queens by workers. More recently, queen mandibular pheromone has been shown to inhibit rates of juvenile hormone biosynthesis(22) and behavioral development(84).

The primary modulator of behavioral development, however, appears to be a signal that originates from the workers

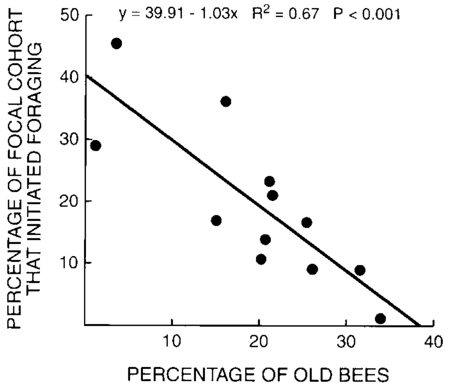

Fig. 1. Relationship between colony age demography and individual behavioral development in honey bees. The graph is a compilation of results from several experiments; each data point refers to a cohort of bees of known age (N = 400-500) that lived in a colony with a known age structure. Rate of behavioral development was measured by determining the percentage of bees in the focal cohort in each colony that initated foraging when they were 14-32 days old(23).

themselves. Older bees inhibit the behavioral development of younger bees. This has been shown by 'social engineering' experiments in which colony age demography is manipulated in precise ways while maintaining constant other potentially important characteristics such as the size of the colony, the amount of food and brood it contains, and the age and genotype of the queen. Rate of behavioral development is negatively correlated with the proportion of older bees in a colony (Fig. 1). Precocious foraging can be induced by depleting colonies of foragers, but depleting control colonies of an equal number of bees of all ages has no such effect(23). Transplants of foragers from a typical colony to experimental single-cohort colonies, initially composed of all 1-day-old bees, inhibited precocious foraging in the resident bees(24). Inhibition occurred even if transplanted foragers were not allowed to forage; this means that the resident bees probably sensed the foragers directly, rather than sensing some change in the hive environment such as the odor or actual amount of freshly collected food.

Pheromonal mediation of foraging age is also suspected in worker-worker interactions. The mandibular glands of workers contain compounds similar to those found in queen mandibular glands, including the predominant worker mandibular acid, 10-HDA. Perhaps tactile aspects of food exchange or antennal contact are important or a pheromone is exchanged during these interactions, as with queen mandibular pheromone (Fig. 2).

Identification of compounds that regulate socially mediated changes in behavioral development in honey bees can be used as a starting point for molecular genetic studies of social behavior. One potentially fertile line of investigation is to identify enzymes and other regulatory proteins involved in the production of juvenile hormone and mandibular gland pheromone(s)(25), and their genes, and study their regulation as a function of colony age demography. Another gene that may be involved is *period* (*per*). There is an intriguing correlation between genetic variation for rate of behavioral development(26) and the ontogeny and periodicity of behavioral circadian rhythms in honey bees (D. Moore, T. Giray, S.E. Fahrbach and G.E. Robinson, unpublished observations), results that are reminiscent of the pleiotropic effects of *per* in the fruit fly, *Drosophila melanogaster*(27). The role of *per* in honey bee behavioral development is now being examined (D.P. Toma and G.E. Robinson, unpublished).

(2) Brain plasticity and behavioral development in honey bees

As the worker honey bee matures, she also exhibits structural changes in the brain that are similar to those reported in vertebrates reared in specific environments or exposed to seasonal fluctuations in reproductive hormones. These changes in regional brain volume occur only in the antennal lobes and the mushroom bodies; the latter have been studied more extensively and are considered here exclusively (Fig. 3).

The mushroom bodies comprise a set of extremely densely packed neurons (the Kenyon cells) and a set of associated neuropils, the calyces and the alpha and beta lobes(28). Inputs to the mushroom bodies arise primarily in the visual and olfactory centers of the brain and form synapses onto processes of the Kenyon cells in the calyces. Protocerebral 'extrinsic neurons' of the mushroom bodies provide a pathway by which information processed through the mushroom bodies can modulate behavior *via* descending projections to motor centers.

Fig. 2. Communication in the beehive: two honey bees antennating and exchanging food with one another. These behaviors have been implicated in the distribution of pheromones that help coordinate rate of individual behavioral maturation with the needs of the colony. Photograph by K. Lorenzen.

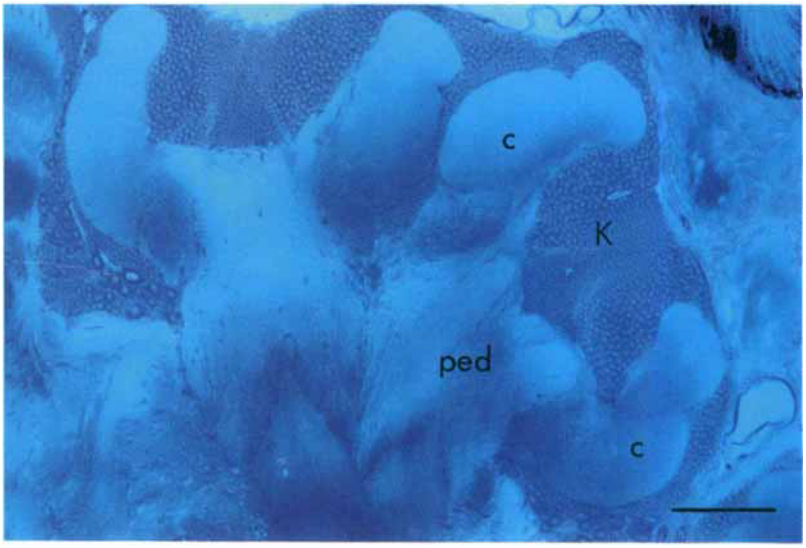

Fig. 3. The paired calyces of the mushroom bodies of the worker honey bee brain, shown in transverse section. The mushroom bodies of the bee lie dorsal and medial within the protocerebrum. The dendrites of the Kenyon cells (K, neuronal somata are stained purple) form the cup-like calyces (C, neuropil is stained blue); their axons form the 'stalk' or peduncle (ped). Scale bar, 100 μm.

The mushroom bodies are larger in the brains of social insects than nonsocial insects, suggesting that they play a special role in regulating social life(29). They also are essential for olfactory learning in several species(30), including the honey bee(31), and in the cockroach, for success in a spatial learning task analogous to the Morris water maze(32).

The volume of the neuropil of the mushroom bodies is significantly larger in foragers than in 1-day-old bees or nurse bees(33). This increase is associated with a decrease in the volume occupied by the somata of the Kenyon cells, so these changes produce a reorganization of space within the brain of the maturing bee. In bees undergoing normal behavioral development, the changes are not evident until the end of the third week of life (normal age at onset of foraging). They can be easily detected in the brains of precocious foragers(33-35), however, which means that the reorganization of the mushroom bodies, too, is sensitive to changes in social context. This does not necessarily mean, however, that changes in the mushroom bodies are directly related to the act of foraging.

The mushroom bodies of queen bees exhibit a pattern of structural change comparable to that seen in workers(36), but unlike workers, queens never forage. Queens do, however, fly from the nest to mate. These results suggested that plasticity of the mushroom bodies is associated with learning the location of the hive in relationship to other landmarks. Workers return to their hive with each load of nectar or pollen while queens return after a mating flight; both castes take a series of orientation flights prior to the onset of foraging and mating, respectively. Ongoing studies of male honey bees (drones) will provide important insight into the hypothesized relationship between the mushroom bodies and spatial learning. Drones are genetically and developmentally less similar to workers than are queens, but also must learn their hive's location to return to it after a mating flight. For worker bees, the implication of this hypothesis is that plasticity of the mushroom bodies helps support the shift from working in the hive to foraging, whenever it occurs. These considerations do not eliminate the possibility that this plasticity also contributes in some way to the actual performance of the cognitively demanding task of foraging.

What cellular and molecular changes occur during the reorganization of the mushroom bodies, and how can they be linked to social context? It appears that the population of Kenyon cells is highly stable in adult life. The production of new neurons or glial cells is not detectable using bromodeoxyuridine labeling(37) and there is no evidence for cell death using the TUNEL method, which histologically assays DNA fragmentation (J.E. Mehren, G.E. Robinson and S.E. Fahrbach, unpublished observations). Neuropil expansion therefore probably represents increased arborization of the Kenyon cells within the calyces, outgrowth of new processes from pre-existing Kenyon cells, ingrowth of new afferents, or some combination of these events. Based on results from both vertebrates and invertebrates(38), presumably any one of these changes could alter the number of synapses per Kenyon cell, a change that would in turn impact the processing of information through the mushroom bodies. One goal of current research on the honey bee brain is an ultrastructural examination of synaptic organization within the calyces at different stages of behavioral development. Another goal is to delineate the molecular mechanisms contributing to neuropil expansion.

At the present time, neither exposure to juvenile hormone alone nor flight experience in and of itself appear to be essential for volume changes within the mushroom bodies(34). This should not be surprising given the apparent complexity of the social context that regulates behavioral development in colonies of honey bees [see (1) above]. Precocious foraging, however, can be viewed as a particularly efficient tool for studying structural plasticity, and the brains of precocious foragers can be assayed for altered patterns of gene expression that differentiate them from their hive-bound counterparts.

Molecular studies of the mushroom bodies in insects other than *Drosophila* have been few. We believe that there can be a useful symbiosis between molecular studies of the mushroom bodies in *Drosophila* and the honey bee, especially with respect to the following four classes of molecules: cell adhesion molecules, neuronal transcription factors, molecules involved in synaptic plasticity and neurotransmitter-associated enzymes. The first three categories include molecules likely to be involved directly in the observed structural changes, and which may be common to synaptic modulation in all animals. The fourth category links specific neural circuits in the honey bee brain responsive to environmental cues, including social cues, to the activation of mechanisms of synaptic plasticity. Ongoing studies on the transcription factor *mef2* (S.M. Farris, R.L. Davis, G.E. Robinson and S.E. Fahrbach, unpublished) and *acetylcholinesterase* gene expression (M. Shapira, M. Korner, H. Soreq and G.E. Robinson, unpublished) suggest that such molecular analyses will be profitable. The opportunities are many; the identification of a brain region responsive to social cues and the exploration of the synaptic changes that occur in this region are the first steps toward integrating socially mediated behavioral development into emerging molecular models of learning and memory.

(3) Quantitative trait loci for food collection preferences in honey bees

Bees can perform several different jobs during a particular phase of behavioral development(16), and genetic variation affects which of them they specialize in(39). There is a great deal of genetic diversity in a honey bee colony. It arises not only from recombination within the queen but from polyandrous mating. Queens mate with 10-17 drones and use the sperm from at least several of them simultaneously. Honey bee colonies are thus ensembles of 'subfamilies', each subfamily being a group of workers descended from the queen and one of her mates. By instrumentally inseminating queens with sperm from three drones bearing different allozyme markers at the *malate dehydrogenase* (*Mdh*) locus, it was found that workers belonging to different subfamilies often differ in their likelihood of performing certain jobs(39,40). For example, some foragers specialize in collecting nectar while others prefer pollen, and subfamily membership is an important determinant of this behavioral difference(41).

Preference for collecting nectar or pollen does not reflect a personal dietary choice; bees collect nectar and pollen for their colony, not for themselves. Nectar is used to make honey, which is consumed by all adult bees in a colony. Pollen is consumed by nurse bees, who then synthesize a proteinaceous 'jelly' that is fed to both the brood and older adults(42). A shortage of honey or pollen in the hive has profound effects on the behavior of foragers(43). For example, foragers in a colony that lacks honey are more likely to collect nectar of lower sugar concentration and then to recruit their nestmates to it (with the famous dance language(44)) than if their colony is well stocked with honey. These responses are mediated by social interactions and not by direct assessments of the honey stores. Foragers sense how long it takes for them to be relieved of their load of freshly collected nectar by a younger bee; quick handling signifies a food shortage while a longer wait reflects ample food stores.

Artificial selection, which has been used extensively in behavioral genetic studies of honey bees beginning with the pioneering studies of Rothenbuhler(45), was employed to study the genetics of food collection preferences. This research is facilitated by the ability to inseminate a queen bee with the sperm of a single selected drone, which, as discussed above, results in the production of thousands of very closely related worker progeny. Selecting for differences in the amount of stored pollen in the hive, two strains of bees were established. Bees in the high strain are more likely to collect pollen and bees in the low strain are more likely to collect nectar, even when co-fostered in a wild-type colony(46). Interestingly, the behavior of bees from these strains is not only influenced by their own genotype, but by the genotypes of other colony members. For example, when individuals from the low strain lived in a low-strain colony, they returned with pollen on about 35% of their foraging trips, but in high-strain colonies the percentage of pollen foraging trips for low-strain bees dropped to less than 3%(47). The reason for this drastic change is thought to be that relatively large stores of pollen in high-strain colonies somehow dampen the stimuli for pollen collecting for most low-strain foragers.

Two-way selection with a different base population of colonies was repeated(48) to confirm these results and to search for quantitative trait loci (QTLs) involved in food collection preferences. A genetic linkage map was made using random amplified polymorphic DNA (RAPD) markers(49,50). The map was constructed with the (haploid) male offspring of a hybrid queen, which increased mapping efficiency because it made it easier to identify fragment-length polymorphisms and dominant/recessive markers. Backcrosses were performed by instrumentally inseminating inbred, high-strain, virgin queens each with the sperm of a son of a high/low hybrid queen. The resultant colonies of backcross workers were then assayed for amount of pollen stored in the hive. These colony-level analyses identified two QTLs, *pln1* and *pln2*; variation in both of these chromosomal regions was significantly correlated with variation in the amount of stored pollen(51). To extend these analyses to the individual bee level, a hybrid queen was backcrossed to a high-strain drone(51,52). The resulting worker progeny differed only in the QTL marker alleles inherited from the queen (due to recombination). Behavioral observations and *pln1* and *pln2* determinations revealed a significant effect of both QTLs on the food collection preferences of individual bees (Fig. 4). Reciprocal results were obtained for pollen and nectar foragers, again suggesting that genes within *pln1* and *pln2* influence the foraging decision itself.

Because *Apis mellifera* has an extremely broad distribution throughout the world, determining the frequency of high and low marker alleles at *pln1* and *pln2* in different environments may give insight into how ecological forces shape the genetics of social organization. In temperate climates, large quantities of honey are required for colonies to survive the long winters, suggesting that temperate-evolved honey bees should have relatively high frequencies of low-pollen (high-nectar) QTL alleles. In contrast, bees of the tropics emphasize brood rearing rather than honey storage(15), which suggests that they should have higher frequencies of high-pollen alleles.

These results also may lead to the identification of specific genes for food-collection preferences by positional cloning, especially as the honey bee linkage map becomes more saturated. The estimate of average map distance to physical distance is 52 kb/cM; honey bees have a very high rate of recombination(50) and a low level of repetitive DNA(53), which are helpful characteristics for map-based cloning.

(4) Molecular population genetics and Africanized ('killer') bees

The introduction of African honey bees into South America had many repercussions for the bee industry and public perception about bees(54), but overlooked in the media-driven panic attack has been the fascinating biological story unfolding in the New World. A subspecies of the European honey bee from south-central Africa, *Apis mellifera scutellata*, was brought to Brazil in 1956 to cross with European subspecies previously introduced to both North and South America. The goal was to create a bee for the tropics with the gentleness of European subspecies and the reputed good honey-producing characteristics of the African subspecies. Unfortunately, the introduced African bees escaped into the wild prematurely, creating a new feral bee population that has caused considerable damage to beekeeping and public health. 'Africanized' bees have since spread throughout the neotropics, from northern Argentina to the southern United States. Remarkably, molecular population genetic analyses reveal that they have remained largely African in genotype despite numerous opportunities to hybridize with European bees.

There are clear, repeatable, differences in numerous social behaviors and colony-level attributes between European and Africanized bees. Also, there are easily recognizable molecular differences in mitochondrial and nuclear genomes. This provides the variation needed to pursue studies of molecular genetics and behavior and makes it simple to identify both types of bees and their hybrids.

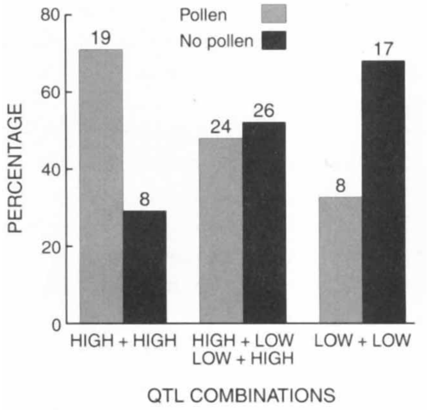

Fig. 4. Percentage of pollen and nectar foragers found to have the following pairs of pollen foraging behavior QTL marker alleles: both high, one high and one low, or both low. The number of individual bees for each category is shown above each bar(52).

The differences between European and Africanized bees cover the full range of within-species variation. For example, Africanized bees are smaller, have shorter brood development times and adult life spans(55), exhibit unusually high metabolic rates for their body size(56), have faster rates of behavioral development(57), are more aggressive in colony defense(58), and may do more pollen foraging(59). Colonies of Africanized bees reproduce more often(60), maintain smaller populations(61), and more readily abandon their nest when environmental conditions are poor(62).

Molecular differences between Africanized and European bees are also pronounced. Both mitochondrial(63) and nuclear(64) genomic variation is sufficient to be used as sub-specific diagnostic tools. Mitochondrial DNA restriction fragment length polymorphisms at cleavage sites using the restriction enzymes *Eco*RI and *Xba*I have been particularly useful, indicating differences in the large ribosomal, CO-I and CO-II subunits(65). This work has been facilitated by the sequencing of the entire honey bee mitochondrial genome(66). Allelic frequencies of *Mdh* and *hexokinase*, as revealed by protein electrophoresis, also distinguish Africanized from European subspecies(67). It is suspected that some of the striking behavioral differences between European and Africanized bees are involved in the predominance of African genes in the neotropics, but conclusive links have not been demonstrated. Comparative studies of Africanized and European bees have great potential to integrate molecular genetics, social behavior and ecology.

(5) Colonial rule in fire ants, Pgm-3 *and* Gp-9

In insect societies, queens (and kings, in termites) reproduce, while workers largely serve their genetic interests by caring for their younger siblings. The 'altruistic' behavior of workers is seemingly at odds with that of most other animals on earth and has captivated many evolutionary biologists, beginning with Darwin himself. There is an extensive literature seeking to explain the evolutionary paradox of the largely sterile social insect worker that nevertheless enjoys spectacular ecological success when measured in terms of species persistence, species number, or even biomass(5). Some of these theories, although developed with social insects in mind, have shed light on many different types of cooperative behavior throughout the animal kingdom.

One interesting feature of reproductive division of labor in insect societies is the variation in queen number. Although in most species (including the honey bee) a colony contains just one egg-laying queen (gyne), in some species polygyny, not monogyny, is the rule(68). The existence of more than one queen in an insect colony, sometimes on a facultative basis, raises a host of both evolutionary and mechanistic questions. What are the ecological and social factors that favor polygyny rather than monogyny? How is queen number regulated, and by whom, queens or workers? Implicit in both types of questions is the realization that queens, like workers, are behaviorally sensitive to social context.

Fire ants display striking intraspecific variation in queen number(69). Originally only monogynous colonies of fire ants were found in the United States. Polygynous colonies were only discovered in the 1970s, but in some areas this form of social organization is now dominant. Monogynous and polygynous fire ant colonies differ in many ways. For example, polygynous colonies are established by colony fission, with several queens and a contingent of workers leaving the natal nest, while monogynous colonies are established by a solitary queen, without workers. Polygynous colonies also are larger than monogynous colonies, but egg production per queen is lower in the former(70).

Colonies of fire ants in northern Georgia, USA, were surveyed for variation in ten polymorphic enzyme loci by protein electrophoresis(71). In all but one locus, allele frequencies were in accord with Hardy-Weinberg frequencies, indicating no effects of natural selection. Moreover, there were no differences in allele frequencies between polygynous and monogynous colonies. Allele frequencies at the *phosphoglucomutase* (*Pgm-3*) locus, however, showed significant deviations from those predicted under Hardy-Weinberg equilibrium. These deviations were explained by comparing monogynous and polygynous colonies; the most common genotype for monogynous queens, accounting for about half of the individuals sampled, was $Pgm\text{-}3^{AA}$ (new nomenclature(72)), but absolutely no queens in polygynous colonies were of this genotype. $Pgm\text{-}3^{AA}$ workers and pre-reproductive queens were found in polygynous colonies, but no mature $Pgm\text{-}3^{AA}$ queens. *Pgm-3* is inherited as the product of a single mendelian locus(71). A high level of polymorphism at the *Pgm-3* locus is maintained in polygynous populations of fire ants in the face of strong directional selection because polygynous queens often mate with males from monogynous populations, in which the frequency of the $Pgm\text{-}3^{A}$ allele is high(73).

$Pgm\text{-}3^{AA}$ queens show advanced states of reproductive development, relative to $Pgm\text{-}3^{Aa}$ and $Pgm\text{-}3^{aa}$ queens(74), but this for some reason places them at risk when confronted by ants from polygynous colonies in laboratory assays. $Pgm\text{-}3^{AA}$ queens are killed by the workers around the time oogenesis is initiated, but $Pgm\text{-}3^{Aa}$ and $Pgm\text{-}3^{aa}$ queens are not. These results suggest a potent interaction between queen genotype and social environment, because $Pgm\text{-}3^{AA}$ queens are plentiful in monogynous colonies in the field. Queen fecundity apparently is correlated with pheromone production(75), suggesting that workers in polygynous colonies use pheromone cues to discriminate against the more fecund $Pgm\text{-}3^{AA}$ genotype. In other animals, including insects, *Pgm-3* is known to play a critical role in glucose metabolism, and variation in *Pgm* genes can affect growth rates, fecundity and survival(76). These results suggest that variation in this gene (or closely linked genes) causes variation in physiological and behavioral aspects of queen reproductive development. Recently, *General protein-9* (*Gp-9*), another co-dominant mendelian locus that may be linked to *Pgm-3*, was implicated in the regulation of queen number in fire ant colonies(72,76); perhaps others await discovery as well. The mystery of why workers in polygynous colonies seemingly favor less fecund queens provides an intriguing socioecological context for studies of genes and proteins that are involved in both queen reproductive development and worker discrimination.

Conclusions

The accounts presented here reveal two strategies for connecting genes to social behavior. In the studies of behavioral and brain development, emphasis has been placed on understanding underlying sociophysiological mechanisms that can then be used to motivate research on genes expressed in association with particular social signals. In the studies of foraging preferences, Africanized bees and queen number, the predominant strategy uses genetic markers to correlate genetic variation with behavioral variation in populations, thereby providing a foundation for the search for specific genes. It is no surprise that genes that influence social behavior from bees and ants that await discovery with either approach are likely to be involved in endocrine and neural processes, perhaps with an emphasis on those associated with chemical signaling among colony members. Communication is a central element in the integration of behavior in all animal societies; pathways of signal transduction both between and within individual society members should provide rich substrates for molecular genetic analyses.

There are two current shortcomings in molecular genetic research on social insect behavior. First, although an emphasis on natural contexts is important when studying social behavior, it is logistically more difficult to link genes to behavior that is studied in the field rather than in the laboratory. This problem is partially addressed by developing reductionistic assays that reflect behavior in nature at least to some degree (sections 1 and 5), or by establishing neuroanatomical or neurochemical correlates of a particular behavioral state (section 2). Second, it is not yet possible to genetically engineer social insects, so even if a specific gene were implicated in a social behavior, the techniques of gene addition, deletion and substitution are not available and one cannot yet go beyond establishing a correlation. It is expected that in the near future it will be possible to make transgenic insects besides *Drosophila*(77), including honey bees(78). But a truly rigorous molecular biology of social behavior requires the ability to turn on or off specific genes in specific brain regions at specific points in an animal's life, as is currently employed in studies of learning and memory(79) and chronobiology(80). Perhaps techniques under development for human genomic manipulation, such as antisense therapy(81), can be used to test hypotheses of gene function in animal species that are favorable for studies of social behavior but lack the genetic resources associated with *Drosophila, Caenorhabditis elegans* and the laboratory mouse. Other emerging technologies inspired by the Human Genome Project, such as chip-based genome screening for expression and sequence variation analysis(82), may also contribute significantly to gene discovery in social insects if they can be applied to species that will not have their genomes sequenced extensively.

Despite these limitations, the time is right to develop a molecular biology of social behavior. Social behavior not only provides rich material for analyses that integrate neuroscience and evolutionary biology, but it is exceptionally well suited to demonstrate, in molecular terms, the fundamental principle that the phenotype is a product of the genotype and the environment. It is especially important to develop and communicate to the lay public such demonstrations, to address the concerns raised by those who invoke the specter of 'biological determinism'. Ironically, although sociobiology was initially attacked as a form of biological determinism, the research in behavioral ecology that it inspired has shown repeatedly that the expression of complex patterns of social behavior is not rigid, but rather depends heavily on the environment.

Social insects can contribute profoundly to the development of a molecular biology of social behavior because their social environments, though complex, can be extensively and precisely manipulated. Given the striking conservation of function for genes involved in development(83), it is likely that some genes identified from social insects will also be important in vertebrate societies. Using social insects for integrated ecological, behavioral, physiological and genetic analyses of social behavior can make for a comprehensive 'molecular sociobiology' that considers both the genotype and the environment, the immediate causes of social behavior and the evolutionary as well, and thus ultimately may make a contribution far beyond the study of molecular genetics.

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