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# Social Interactions in "Simple" Model Systems

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**Deciphering the genetic and neurobiological underpinnings of social behavior is a difficult task. Simple model organisms such as *C. elegans*, *Drosophila*, and social insects display a wealth of social behaviors similar to those in more complex animals, including social dominance, group decision making, learning from experienced individuals, and foraging in groups. Although the study of social interactions is still in its infancy, the ability to assess the contributions of gene expression, neural circuitry, and the environment in response to social context in these simple model organisms is unsurpassed. Here, I take a comparative approach, discussing selected examples of social behavior across species and highlighting the common themes that emerge.**

## Social Behavior

The social environment affects behavior across species, from microbes to humans (Benabentos et al., 2009). Social behavior is broadly defined here as an interaction between members of the same species that changes their subsequent behavior. Investigating the mechanisms underlying social behavior becomes increasingly more challenging as you move up the phylogenetic tree. Simple animals such as nematodes, flies, and bees have simpler behaviors, smaller genomes, and simpler nervous systems than more complex animals such as mammals. And yet, simple animal models have much to tell us about social behavior.

Simple and more complex animals share many common behaviors, including courtship and mating, aggression, parenting, foraging, learning, and memory. Many of the social behaviors exhibited by simple animals are reminiscent of social behaviors in more complex animals. For example, mate copying is observed in both complex animals (for example see Godin et al., 2005; White and Galef, 2000) and the fruit fly (Mery et al., 2009). Additionally, aggressive interactions in the fruit fly could lead to the formation of dominance hierarchies. After an inexperienced male watches two males fight, he alters his subsequent behavior accordingly, depending on whether he encounters the loser or winner (Yurkovic et al., 2006). Behavioral changes can depend on group size and composition. Sleep need varies with group size (Ganguly-Fitzgerald et al., 2006), and individuals are affected by the food choices made by other animals (Tinette et al., 2004). The circadian clock is also affected by social signals that vary with group membership (Levine et al., 2002; Fujii et al., 2007). And finally, the means by which thousands of honey bees select a nest site shares common themes with group decision making in humans. Clearly, simple animals show interesting and relevant social behaviors.

Biological factors that influence social behaviors are similar to those that influence individual behaviors. Genetic contributions to social behavior involve the encoding of molecules with important structural and functional roles in the tissues (e.g., the brain) that influence behavior. Behavior is also strongly influenced by the environment, which has profound effects on development and physiological function. The environment can also act directly on the genome to change both the abundance and spatiotemporal expression pattern of molecules that influence behavior (Robinson et al., 2008). Thus, variation in social behavior within and between individuals arises from interdependencies between genes and the environment. Additionally, epigenetics may be particularly relevant for social behavior, as it provides a mechanism through which the consequences of experience are passed along to shape patterns of gene transcription without affecting genotype (reviewed in Bird, 2007). The recent discovery of epigenetic processes in simple animals makes it possible to study the extent of epigenetic patterning and its "inheritance" in organisms where the genome and epigenome can be easily manipulated (Lyko et al., 2000; Kronforst et al., 2008). While the role of epigenetics in social behavior across species is yet to be determined, simple model organisms allow us to address this question under a variety of social contexts.

While simple animals have smaller brains and behavioral repertoires, they are still able to exhibit plastic responses to the environment. Their behaviors are not hard wired! *Drosophila* and honey bees show learning and memory and attention-like processes (van Swinderen and Greenspan, 2003) and use social learning in their every day lives (Chittka and Niven, 2009). Like mammals (Cacioppo and Hawkley, 2009), simple animals are affected by social isolation. Isolating *C. elegans* during development reduces the behavioral response to touch, slows development, and alters neuronal connectivity (Rose et al., 2005). Social isolation in *Drosophila* reduces lifespan (Ruan and Wu, 2008), increases aggression (Hoffmann, 1990; Zhou et al., 2008), reduces the need for sleep (Ganguly-Fitzgerald et al., 2006; Donlea and Shaw, 2009), and decreases fiber number in the mushroom bodies, the functional equivalent to the mammalian hippocampus (Technau, 2007). Social isolation also reduces mushroom body volume in honey bees (Maleszka et al., 2009). Although rarely studied in simple animals, critical periods during development may also be important for the development of normal social behavior (but see Rai and Rankin, 2007; Svetec and Ferveur, 2005).

Another final factor to consider is the evolutionary consequences of social behavior. The theory of indirect genes states that variation in phenotype is shaped by social experience and that this has consequences for population allelic frequencies (Moore et al., 1997; Wolf et al., 1998). In doing so, it partitions

the variation in phenotype by genotype, physical environment, social environment, and their interactions. The division of the environmental variable into physical and social components is the important part of the theory, and it provides a quantitative measure of the social component of phenotypic variation. Demonstration of indirect genetic effects have been shown in the fire ant (Ross and Keller, 1998, 2002), the fruit fly (Petfield et al., 2005; Kent et al., 2008), and the honey bee (Linksvayer et al., 2009); however, a link to changes in allele frequency has not been established.

Species comparisons provide a panoramic view of social behavior, enabling us to uncover common themes. Here, I discuss how studies of simple model organisms, with their easy-to-manipulate genes, genomes, and nervous systems, can provide insight into mechanisms involved in social behavior both within and between species. This paper is not meant to provide an exhaustive review of research on social behavior in simple animals and its history. Rather, I have selected illustrative examples of behaviors that have parallels in more complex animals to give the readers a flavor of some of the recent research in the field, and unfortunately, many excellent papers are not discussed here.

There are many unanswered questions about social behavior. Does social behavior differ from individual behavior at the mechanistic level? Is there a ''social brain'' that is common to all social species? Do social behaviors have distinct signatures in the brain or the genome? Are social cues sensed, integrated, and processed differently than abiotic cues? Why are elements of social behavior conserved across species? Many of these questions await further development of the field of social behavior.

## The Nematode Worm Caenorhabditis elegans

The nematode *C. elegans* is highly amenable to behavior and neurogenetic analyses. Many behaviors have been studied in *C. elegans*, including response to touch and odors, heat sensitivity, feeding, locomotion, mating, learning, aggregation, and stress responses (reviewed in de Bono and Maricq, 2005). Its genome has been sequenced, and a great number of mutants and transgenic lines have been generated. The molecular components of each neuron can be manipulated using targeted expression, individual neurons can be ablated or activated, and molecular expression levels can be manipulated with RNAi to address the importance of the molecule in the behavioral phenotype of interest. With genetic and molecular approaches, it is straightforward to determine the neurons involved in a particular behavior and their patterns of interaction, making *C. elegans* a superb model for neurogenetic analyses of behavior.

*Social Isolation.* The presence of conspecific animals provides important sensory input for *C. elegans* (Rose et al., 2005). The responses to social isolation are influenced by *glr-1*, a glutamate receptor subunit gene that affects the development of normal behavior and the neurocircuitry used for transduction of mechanosensory stimulation, and *egl-4*, a cGMP-dependent protein kinase gene that affects body size. Mechanical stimulation of isolated worms restores normal mechanosensory behavior and circuitry but not body size, suggesting that development of normal body size requires the presence of other worms. Interestingly, there is a critical period in development whereby interactions between worms affect adult body size (Rai and Rankin, 2007). Thus, social isolation during development can have multiple phenotypic effects on adult functions, some of which require sensory input during critical periods of development.

*Social Aggregation.* Two *C. elegans* behaviors known to have a social component are male-female hermaphrodite mating (Srinivasan et al., 2008; Liu and Sternberg, 1995) and aggregation behavior (de Bono and Bargmann, 1998), both of which have naturally varying polymorphisms (Ardiel and Rankin, 2009). Here, I discuss *C. elegans* aggregation behavior in more detail. Variation in the *npr-1* gene accounts for what has been called social foraging behavior (de Bono and Bargmann, 1998). Interestingly, recent work has suggested that variation in *npr-1* arose in the same strain background, suggesting that it may have arisen as a laboratory mutation (McGrath et al., 2009). While feeding on a bacterial lawn, social worms aggregate, forming clumps at the border of the food and exhibit rapid locomotion, whereas solitary worms feed alone (Figure 1A). The difference between social and solitary strains arises from a single amino acid change in the *npr-1* gene, which encodes a receptor with similarity to the members of the mammalian neuropeptide Y receptor family (reviewed in de Bono and Sokolowski, 2007). Social strains have a lower activity form of NPR-1 than solitary ones, and null mutants are hypersocial. NPR-1 is expressed mainly in neurons, where it localizes to cell bodies, axons, and dendrites (Coates and de Bono, 2002). Although NPR-1 is found in most developmental stages, manipulation of its temporal expression pattern indicates that it exerts an acute rather than developmental affect on aggregation behavior.

But is aggregation behavior due to interactions between individuals, or do social worms prefer to aggregate around particular abiotic factors (e.g., reduced oxygen levels) more so than the solitary worms? The answer is yes to both questions. Atypical soluble guanyl cyclases (sGCs), which are thought to act as oxygen sensors, interact with *npr-1* (Gray et al., 2004; Cheung et al., 2005; Persson et al., 2009; Zimmer et al., 2009). Mutations in these sGCs and reductions in ambient oxygen suppress aggregation and bordering behavior in social worms (Cheung et al., 2004; Gray et al., 2004). Rogers et al. (2006) suggest that anterior and posterior oxygen sensors mediate aggregation behavior by responding to the rise in oxygen levels as the worm moves away from the aggregate. These results suggest that the aggregation behavior is a response to oxygen levels and, therefore, is not a social behavior according to our definition.

However, the discovery of *C. elegans* mating pheromones suggests that chemical communication can influence behavior (Srinivasan et al., 2008). In a study assessing the role of pheromonal communication in aggregation behavior, Macosko et al. (2009) showed that aggregation involves direct responses to other animals and not just a shared preference for environments with low oxygen levels. Solitary animals are repelled by ascaroside pheromones produced by other animals, whereas social animals are attracted to them. They also discovered that the RMG inter/motor neuron acts as a hub of integration for the diverse cues known to affect social behavior. Low *npr-1* activity in RMG correlates with social behavior, while high *npr-1* activity is associated with solitary behavior. High RMG activity is necessary for all components of social behavior. Their model

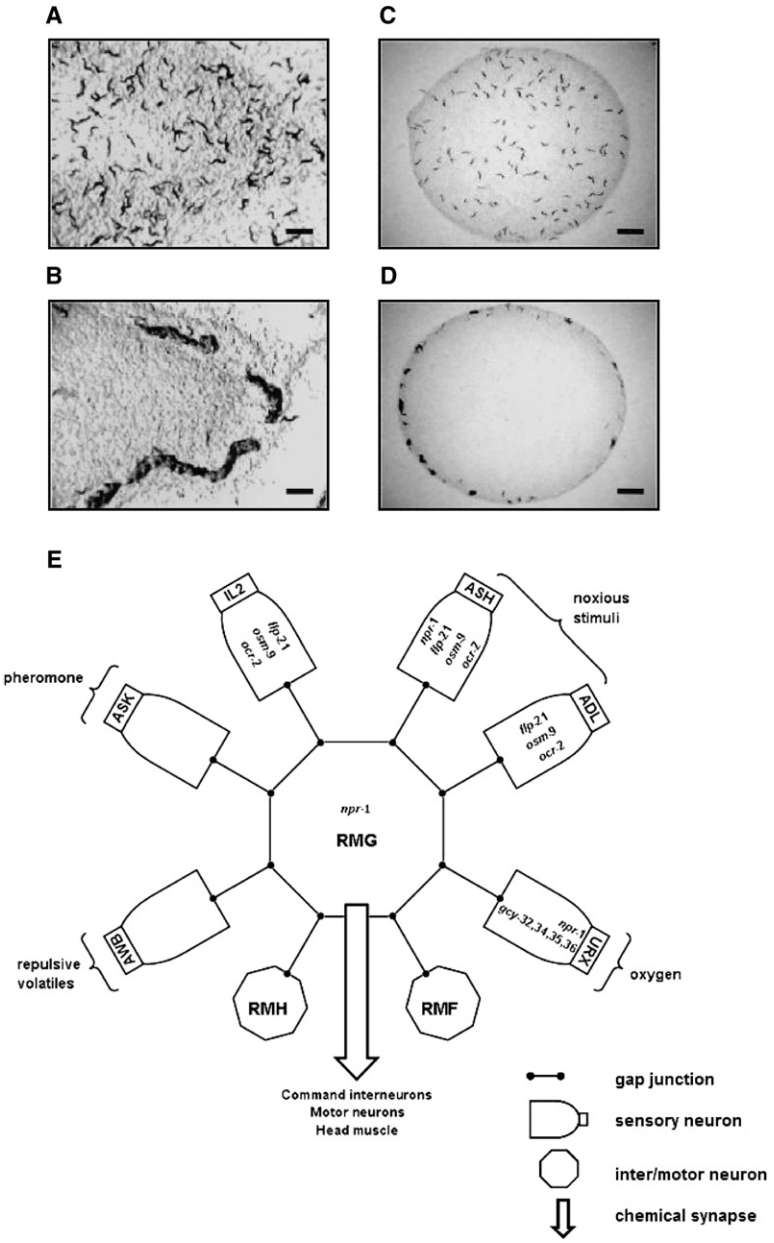


**Figure 1. Solitary and Social Worms**

Solitary worms disperse over a lawn of *E. coli* bacteria (A and C), whereas social worms aggregate and form clumps (B and D). Scale bars represent 2 mm in (A) and (B) and 2.5 mm in (C) and (D). Image is reprinted from de Bono and Bargmann (1998). (E) A hub and spoke circuit diagram of neurons with gap junctions to RMG; stimuli detected by sensory neurons are shown when known as well as the names of genes that are expressed in these neurons. For further information, see text. Modified from Ardiel and Rankin (2009), who adapted it from Macosko et al. (2009).

(Figure 1E) proposes that distributed sensory inputs coordinated through gap junctions via connections with RMG produce particular synaptic outputs affecting *C. elegans* movement patterns. High RMG activity increases social aggregation and the response of the ASK sensory neuron to ascarosides. ASK activity is reduced by high levels of NPR-1 activity. This work identifies an anatomical circuit underlying social behavior and suggests that *npr-1* changes the properties of the circuit by modulating RMG neuron activity. The involvement of a single class of pheromones in multiple complex behaviors raises a number of questions. How do these pheromones differentially affect mating, social aggregation, and dauer formation? How is this regulated at the molecular level and through what circuitry?

### *The Fruit Fly Drosophila melanogaster*

*Drosophila* has recently emerged as model for studies of social behavior, and although there is not yet any clear conceptual framework that integrates the results of this work, it is clear that this research will provide genetic and molecular insights that translate to investigations of social behavior in other species. And for many of us, there is much pleasure in understanding the workings of the fly itself.

*Drosophila* has a broader set of social behaviors than *C. elegans*, presumably because of its more complex nervous system and its more complex physical and social environments (Reaume and Sokolowski, 2006). *Drosophila*'s long history as a genetic model, along with its cadre of genome resources, makes it an ideal organism to identify genes and molecules involved in normal social behavior. Transgenic manipulation of nervous system function further facilitates the identification of brain structures and neural circuits important for social behavior. Given the feasibility of generating genetic mutants in any phenotype of interest, I expect that researchers will soon undertake genetic screens for mutants that disrupt or enhance social behavior.

*Drosophila* has a tremendous tool kit for neurogenetic analysis of social behavior, and much of its genome is covered by deletion and insertional mutants and a library of RNAi lines (Dietzl et al., 2007). Additionally, targeted expression of a gene to a cell, tissue, or group of tissues is possible, allowing for manipulation of cells and circuitry (Brand and Perrimon, 1993; Venken and Bellen, 2005). Optogenetic approaches have been utilized in flies, allowing precise spatial and temporal manipulation of neural activity in behaving flies (Miesenböck, 2009).

Natural genetic variation for behavior can also be an excellent resource for identifying genes (de Belle and Sokolowski, 1987; de Belle et al., 1989; Dierick and Greenspan, 2006; Edwards et al., 2006). Genome sequences of numerous inbred *D. melanogaster* lines are now available, which will enable identification of naturally varying genes and nucleotides for any number of phenotypes as well as investigations of pleiotropy and epistatic interaction networks. Together, the many intra- and interpopulation and species genomes will enable us to understand

the evolution of social interactions in *Drosophila* at the molecular level.

*Courtship and Mating.* Courtship involves social interactions; the male performs a sequence of behaviors (orient, tap, sing using wing vibration, attempted copulation, copulation) and the female responds by rejection (a kick in the head or extrusion of her ovipositor) or acceptance (she allows him to mount her and copulate). *Drosophila* courtship and mating have been reviewed extensively from the mechanistic (Villella and Hall, 2008) and evolutionary perspective (Markow and O'Grady, 2005).

Social interactions during courtship and mating are mediated in part through pheromonal communication. The characterization of *Drosophila* pheromones is a deep and rich field initiated by the early work of Jean-Marc Jallon (Antony and Jallon, 1982) along with his students and colleagues (Ferveur et al., 1996, 1997; Ferveur and Sureau, 1996; Wicker-Thomas et al., 2009; Chertemps et al., 2007; Ueyama et al., 2005). Many of these chemical cues are hydrocarbons found on the waxy cuticle of the fly. The cuticular hydrocarbon profile mediates the effects of courtship conditioning, a classic behavioral paradigm where male courtship behavior is altered by exposure to an unreceptive female (Siwicki et al., 2005; Ejima et al., 2007). While some of these pheromones have been characterized, other unidentified compounds are thought to contribute to pheromonal signaling. Toward the goal of monitoring simultaneous changes in chemical signaling and behavior, Yew et al. (2008) developed the direct analysis in real-time (DART) mass spectrometry technique to analyze cuticular hydrocarbons in living animals. They were able to detect known pheromones and also discovered six additional cuticular hydrocarbons. More recently, the authors identified a novel class of oxygen-containing compounds on the cuticular surface and demonstrated a role for the previously uncharacterized hydrocarbon CH503 in courtship-related pheromonal communication (Yew et al., 2009).

In a recent study, Billeter et al. (2009) developed flies lacking oenocytes, the cells responsible for hydrocarbon production, as a pheromonal blank slate on which to examine the effect of single and multiple pheromones. They discovered that hydrocarbons are involved in both the recognition of an individual's sex and its species (Billeter et al., 2009). Perfuming virgin females lacking oenocytes with the aversive male pheromone cVA causes a delay in mating, whereas adding a single female aphrodisiac (7,11-HD) to the mix restores the time to mate, indicating that the effect of male aversive pheromones can be overcome with a single female aphrodisiac pheromone. Amazingly, the same pheromone is sufficient to provide species identification. Without pheromones, *D. simulans* males court and mate *D. melanogaster*; however, application of 7,11-HD prevents this, suggesting that reproductive isolation can be accomplished through differences in a single pheromone. Moreover, this study demonstrated that cuticular hydrocarbon pheromones do not only act to signal attractiveness but also to inhibit certain ''nonadaptive'' interactions, such as male-male and heterospecific courtship. One question that arises from these studies is whether flies are able to perform individual recognition. New behavioral paradigms and imaging technology that can quantify the behavior of individual flies interacting in social groups will soon make it possible to answer these challenging questions (Branson et al., 2009; Dankert et al., 2009). These flies lacking oenoytes are a valuable tool to investigate many other social phenomena that involve chemical communication.

Pheromones are detected by the olfactory system, and activation of a single class of olfactory receptor neurons is thought to be sufficient to mediate behavioral responses to pheromones. This hypothesis was confirmed in a study by Kurtovic et al. (2007) where the authors found that the *Drosophila* male-specific pheromone cVA acts through the olfactory receptor Or67d to regulate mating behavior in both males and females. Interestingly, cVA appears to have opposite effects in the two sexes: inhibiting male mating behavior but promoting female mating behavior. How does a single pheromone acting through the same class of neurons trigger a different behavioral response in females and males? The recent identification of sexually dimorphic projections between the DA1 glomerulus and the protocerebrum indicates that cVA mediates these sex-specific effects via activation of a sexually dimorphic circuit (Datta et al., 2008). Classically, detection of pheromones was thought to involve direct activation of olfactory receptors; however, detection of cVA has been shown to be mediated by the extracellular pheromone-binding protein LUSH, which undergoes a conformational change upon pheromone binding that stimulates neuronal firing (Laughlin et al., 2008). The gustatory system also appears to play a role in *Drosophila* courtship. Male files with mutations in the gustatory receptor gene *Gr32a* have altered courtship behavior, specifically high courtship toward males and mated females, suggesting that GR32a acts as a receptor for an inhibitory pheromone (Miyamoto and Amrein, 2008).

During and after mating, a number of molecular, physiological, and behavioral changes occur in female flies (reviewed in Sirot et al., 2009). Seminal fluid proteins (Sfps) are made in the male reproductive tract and transferred along with sperm to females during mating. Males that transfer larger quantities of specific Sfps have been shown to have a significant competitive advantage, suggesting that Sfp production is crucial for male fitness in competitive environments (Wigby et al., 2009). Once inside the female, interactions between Sfps and female-specific proteins trigger multiple short- and long-acting phenotypes at the physiological (increased rates of oogenesis, ovulation after mating, reduction in female lifespan), behavioral (reduced mating, increased egg-laying and food intake), and gene expression (increase in expression of antimicrobial peptide genes, reduced immune response) levels. How these changes occur, which molecules are involved, and how they exert their effects outside the reproductive tract are exciting questions for future investigation. Clearly, these molecular interactions are important from an evolutionary perspective, as they affect reproductive success and, thus, an animal's lifetime fitness.

Over 100 Sfps have been shown to transfer to the female during mating. The best known of these is the Sex Peptide (SP), which interacts with the SP receptor (SPR) expressed in the female reproductive tract and the nervous system (Yapici et al., 2008). Sex peptide has a critical role in regulating female behavior and infers a mating cost to the female by decreasing female fitness (Wigby and Chapman, 2005). The control of post-mating behaviors by SPR activation is mediated by a small subset of internal sensory neurons innervating the uterus and

oviduct (Häsemeyer et al., 2009; Yang et al., 2009). Social context also influences the transfer of Sfps; males will transfer more SP during mating when they are in the presence of other males than when they are alone (Wigby et al., 2009).

*Social Influences on Circadian Function*. Social interactions during courtship and mating can change gene expression and behavior. Recent studies have shown that the social context of the fly can significantly change the temporal pattern of pheromone expression and mating in male flies (Krupp et al., 2008; Kent et al., 2008). Additionally, the authors discovered that social interactions affected the circadian clock by altering the temporal pattern of gene expression of the clock genes *period*, *timeless*, and *clock.* The amplitude and accumulation of clock gene transcripts in the head and abdominal oenocytes, the site of hydrocarbon production, are altered by social context. Similar changes are seen for RNA expression of the *dsat1* gene, which encodes an enzyme involved in hydrocarbon pheromone production in the oenocytes. These data indicate that chemical signaling and clock function are plastic and dependent on an individual's social environment and that flies are able to acquire information about their group membership. This work provides an entry point for studies of the cellular basis of how clock function is affected by the social context. Additionally, there is great interest in understanding the mechanisms and circuitry underlying social group phenomena, including the sensory systems involved in determining group membership and the brain regions involved in integrating the sensory input and the ''decision'' making involved in determining group membership.

*Aggression.* Aggression is widespread in the animal world. Although aggression in flies was originally observed by Sturtevant in 1915 (Sturtevant, 1915) and then by Jacobs in 1960 (Jacobs, 1960), studies of the mechanisms involved in fly aggression have only emerged in the past few years (Lee and Hall, 2000; Certel et al., 2007; Chan and Kravitz, 2007; Mundiyanapurath et al., 2009). Analysis of male-male interactions in the presence of food revealed a spectrum of behaviors, including offensive behaviors such as chasing, lunging, and boxing, and defensive behaviors, such as walking, running, or flying away (Chen et al., 2002). Lunging behavior is performed more often by the more aggressive fly and predicts who is the winner or loser of an aggressive interaction. Quantification of lunging with automated video analysis demonstrated that an 8% difference in the size of competitors could predict dominance for the larger fly (Hoyer et al., 2008). The biogenic amine octopamine is required for appropriate behavioral responses in males, and lack of octopamine reduces the transition from courtship to aggression (Certel et al., 2007; Hoyer et al., 2008). Octopamine is thought to be the insect equivalent of norepinephrine, a molecule that increases aggression in vertebrates, and octopamine receptors in insects are related to mammalian adrenoceptors that are also involved in aggression (Roeder, 2005). Interestingly, aggression was only partially restored in octopamine mutant flies when octopamine levels were increased by feeding or transgenic expression, suggesting that octopamine levels in the brain may be sensitive to the dose and timing of expression. Further research is needed to better understand octopamine's role in the neurocircuitry of aggression in the male brain.

Along with octopamine, serotonin increases aggression in flies, whereas the *Drosophila* neuropeptide Y acts to decrease aggression (Dierick and Greenspan, 2007). These modulatory systems also affect aggression in the mouse, suggesting conservation of the biochemistry underlying aggression in these distantly related species (Dierick and Greenspan, 2007).

Other studies have also shown an interaction between genes and the social environment in aggression (Zhou et al., 2008; Wang et al., 2008). In both mammals and *Drosophila*, social grouping reduces aggression whereas social isolation increases it (Hoffmann, 1987). Flies reared in isolation and subsequently exposed to social experience show a fighting frequency resembling that of socially reared flies, indicating a resiliency of the fly to early social isolation (Wang et al., 2008). Increasing octopamine increases aggression only in socially grouped flies, not socially isolated flies (Zhou et al., 2008). Transgenic manipulations of octopaminergic signaling identified just five octopaminergic neurons in the subesophageal ganglion of the *Drosophila* brain as critical for increased aggression.

Natural allelic variation in the *Cyp6a20* gene, a cytochrome P450, also plays a role in male aggression in *Drosophila* (Dierick and Greenspan, 2006). This finding was replicated in a study by Wang et al. (2008), who identified the *Cyp6a20* gene in a screen for differentially expressed genes in flies reared alone or in groups. The authors found that social experience increases *Cyp6a20* expression and decreases aggression. Additionally, aggression is only increased in socially reared *Cyp6a20* mutants, not socially isolated flies, suggesting that *Cyp6a20* mediates the suppressive effects of social rearing on aggression. Together, these studies demonstrate that both genetic variation and the social environment influence aggression and that social context is important for the regulation of aggression by *Cyp6a20*. Additionally, in vertebrates, one member of the P450 family of enzymes has been linked to male-male aggression and is affected by social experience, suggesting that a role for some of these P450 genes in aggression might be conserved even if their signaling pathways are distinct (Matsumoto et al., 2003).

How might *Cyp6a20* exert its effects on aggression? *Cyp6a20* is expressed in support cells associated with pheromone-sensitive sensilla in the insect olfactory pathway. These non-neuronal cells express an odorant binding protein called LUSH (Xu et al., 2005) that is required for detection of the cVA pheromone (Antony and Jallon, 1982; Laughlin et al., 2008). A recent study has demonstrated that exposure to synthetic cVA, which acts through olfactory sensory neurons expressing the receptor Or67d, increases lunging and other aggressive behaviors in flies (Wang and Anderson, 2010). The authors propose that cVA may play a role in density-dependent processes in *Drosophila*, with higher density of flies leading to higher levels of cVA and increased aggression. Further studies are needed to understand how cVA might differentially affect aggression, mating, and aggregation in *Drosophila* and the mechanisms underlying its seemingly specific effects on these behaviors.

Only male *Drosophila* establish dominance relationships with one another in competitive situations (Nilsen et al., 2004). The intensity of aggressive interactions is higher in male pairs who use lunging and boxing more often than female pairs who use shoving and head-butting behaviors. Sex-specific splicing of

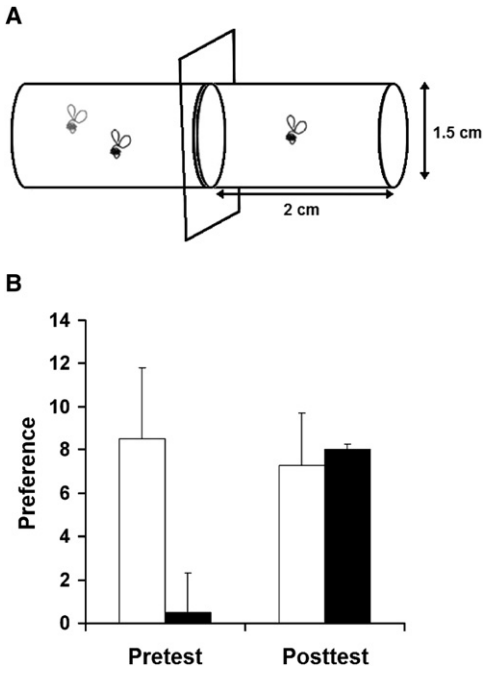


**Figure 2. Mate Copying in *Drosophila***
(A) Apparatus used in the Mery et al. (2009) copying experiment and (B) histogram showing that mate copying can shift preference for a male in good condition to one in poor condition.

the *fruitless* gene, which is known to play an important role in courtship behavior, contributes to gender-specific differences in aggression demonstrating pleiotropy of this gene in courtship and aggression (Vrontou et al., 2006). Sex-specific behavioral characteristics during aggression are mediated by specific subgroups of neurons expressing male forms of *fruitless* (Chan and Kravitz, 2007).

To investigate genes affecting male aggression in natural populations and their interactions in a transcriptional network, Edwards et al. (2009) correlated natural variation in aggression across 40 wild-derived inbred lines with variation in genome-wide transcript abundance. They found 266 novel candidate genes associated with aggressive behavior, many of which have pleiotropic effects on other metabolic, developmental, and behavioral traits. For example, locomotor reactivity was genetically correlated with aggression. The aggression network consists of nine modules of correlated transcripts that differ in their gene ontology designations. Full DNA sequencing of these 40 lines will enable the identification of *cis*- and *trans*-acting polymorphisms and help in determining network correlations and information flow. Combined analysis of natural variation in DNA sequence, the transcriptome, metabolome, and proteome has great potential for dissection of complex traits such as aggression (Edwards et al., 2009; Kent et al., 2009).

*Mate Copying.* Social learning is defined here as the ability of an individual to acquire new information from observing or interacting with other animals, usually conspecifics. Until recently, social learning was mostly studied in vertebrates (reviewed in Heyes and Galef, 1996) and eusocial insects (reviewed in Leadbeater and Chittka, 2007). The first evidence of mate copying, a form of social learning, in invertebrates has recently been described (Mery et al., 2009). The fly provides the opportunity to uncover the molecules and neural substrates involved in mate copying, which is relevant to aspects of social learning in other species, including humans. Mate copying may provide an inexperienced female with evidence of the quality of a particular male. Mery et al. (2009) allowed an inexperienced female to watch an experienced female choose a partner from a selection of males that were reared in poor or good conditions (Figure 2A). Female flies normally prefer males in good condition, which predicts greater and better quality sperm (Fricke et al., 2009). However, when naive flies watch another female mate with a poor condition male, the naive fly will change her innate preference and prefer poor condition males (Figure 2B). Thus, the experience of simply watching other females make mating choices changes the mate preferences of naive females. This behavioral change was not limited to good and bad condition males. When the researchers dusted equally good condition male flies with pink or green fluorescent dust, the naive female once again preferred the male who she had observed with the experienced female. It is not known how long the naive fly remembers this experience or if this type of copying applies to other possible social interactions, including, for example, aggressive interactions, food, and oviposition choices. It is also not known whether the watcher fly can apply the criteria learned from this experience to other social situations or whether the mate copying experience has boosted the social status of the previously undesirable male. From an evolutionary perspective, it would be informative to have measures of the fitness consequences of social learning in flies (Sarin and Dukas, 2009).

*Courtship Conditioning.* Flies learn and remember certain aspects of their courtship experience (reviewed in Villella and Hall, 2008; Griffith and Ejima, 2009). When a naive male is placed with a nonreceptive mature mated female, he initially courts her vigorously. During this interaction, the nonreceptive female extrudes her ovipositor when the naive male tries to copulate with her, and the naive male is exposed to the pheromone profile of the mated female, causing him to subsequently reduce his courtship toward the mated female. When this male is then placed with a receptive virgin female, the male continues to show a suppression of his courtship even though the virgin female is receptive. Courtship conditioning affects both short- and long-term memory, and it can last for hours to days, depending on the amount of training given to the male by the mated female. Interestingly, learning mutants such as *dunce* and *amnesiac* fail to show courtship suppression or show significantly less of it, respectively. This suppression of courtship as a result of a previous "frustrating" experience is called courtship conditioning; it was discovered more than 30 years ago. There are various versions of the courtship conditioning assay (experiencing flies with males, headless females), but the basic idea is the same. What is known about the neural substrates for courtship conditioning? Unlike negative olfactory associative learning and memory in the fly that has been mapped primarily to the mushroom bodies of the fly brain, the spatial distribution pattern for conditioned courtship is distributed in the nervous system (Figure 3; reviewed in Villella and Hall, 2008). The mushroom

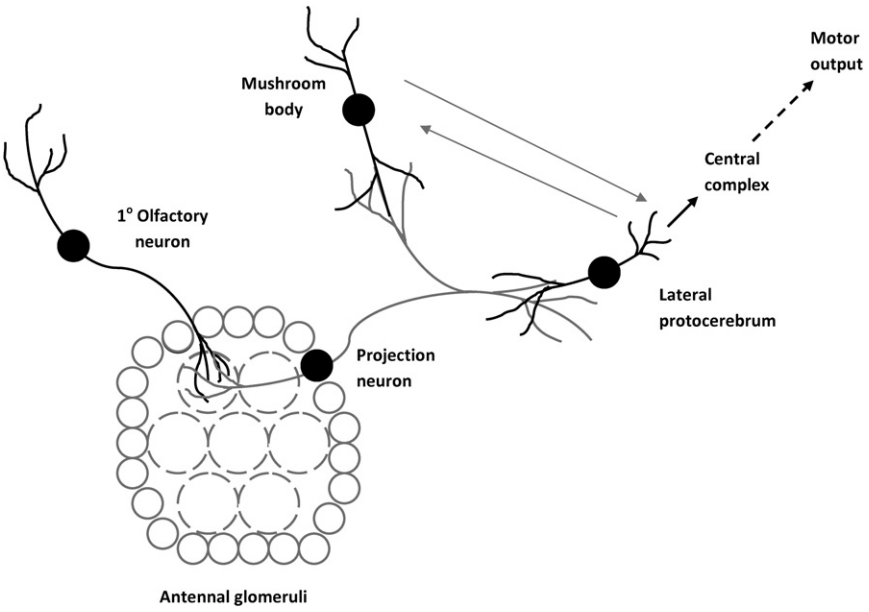


**Figure 3. Neural Circuitry for Courtship Conditioning**
Taken with permission from Mehren et al., 2004.

bodies, central complex, antennal lobes, lateral protocerebrum, and optic lobes are important for courtship conditioning.

*Learning Alone or in Groups.* Many genes common to most animals are known to affect various aspects of learning and memory in flies (reviewed in Berry et al., 2008; Griffith and Ejima, 2009). Flies have short-term (STM), middle-term (MTM), long-term (LTM), and anesthesia-resistant memory (ARM). Social facilitation of learning in flies has not been well studied. However, a study by Chabaud et al. (2009) showed that social interactions between flies enhance their performance in ARM, likely by the facilitation of memory retrieval. Social facilitation of memory was only found when flies had ARM and when flies were tested for their memory in groups; it did not matter whether flies were trained alone or in groups. Flies improve their memory performance when they are tested in groups, even if members of the group were trained to avoid different odors, suggesting that flies do not increase their test score by simply following one another. To provide insight into mechanisms underlying this social facilitation effect, it would be interesting to investigate the changes in phermonal profiles of individual and groups of flies in this paradigm. From an evolutionary perspective, investigations of natural variation in social facilitation and whether it exists in aversive and appetitive learning paradigms would be revealing.

Learning and memory may also affect male-male fighting in *Drosophila* and the establishment of relatively stable hierarchical relationships (Yurkovic et al., 2006). Surprisingly, flies will fight for 5 hr or more when presented with food and a female "resource," in this case, the smell of a female provided by a headless female fly. During this fighting period, some flies progressively lunge more and retreat less, whereas others retreat more and lunge less, allowing the researchers to categorize flies as winners and losers, respectively. Subsequent pairing of these flies with familiar and unfamiliar competitors shows that the flies' previous experience affects their subsequent aggressive behavior. Former losers fight differently when they were paired with unfamiliar flies compared to familiar winner flies. This suggests that male flies may form dominance hierarchies or networks when they are in groups. The exciting possibility that flies have the ability to recognize individuals could be addressed in studies of this type.

The relationship between group and isolated rearing and sleep and memory is reviewed in Donlea and Shaw (2009). Flies increase their sleep need after being housed in groups or after LTM induction. Flies mutant in the learning gene *rutabaga* and the circadian gene *period* do not exhibit increases in sleep when exposed to group rearing. Expressing these genes in the ventral lateral neurons, part of the output pathway of the circadian circuitry, restores the effect of social interaction on sleep. An increase in the number of synaptic terminals in the ventral lateral neuron projections into the medulla correlates with the effect of social experience on sleep (Donlea et al., 2009). These studies set the stage to uncover the brain circuitry associated with the relationship between sleep, social experience, learning, and memory.

Together, these studies demonstrate that *Drosophila* possesses a rich behavioral repertoire allowing for many paradigms to study social learning. This species is ripe for neurogenetic analyses of the mechanisms underlying social learning in a variety of contexts.

## Eusocial Insects

### *Honey Bee: Apis mellifera*

*Division of Labor and Gene Expression.* In the field of sociobiology, social behavior is defined from the perspective of eusocial insects (honey bees, ants, and termites) that live in structured societies with division of labor between reproductives and workers, overlapping generations, and cooperation between caste members (Wilson, 1971, 1975). Social insects behave according to the needs of their colony. The colony is their social context, and their behavior is considered social because it is a response to colony needs. Social insect researchers are interested in group behavior and do not study the behavior of an individual. They study the highly stereotyped behavior patterns of types of individuals in the colony. For example, they might compare the workers who nurse the larvae to the workers who forage within a colony. In recent years, honey bee researchers have shown that changes in gene expression underlie social behavior (Honeybee Genome Sequencing Consortium, 2006). Eusocial insects such as the honey bee are advantageous for these studies because of their well-known stereotyped patterns of social behavior that change through the lifetime of the individual bee.

Eusocial insects exhibit a reproductive division of labor between queen bees and worker bees. Queen bees are essentially egg-laying machines who control the behaviors of their worker bee daughters through chemical means. These sterile worker bees cooperatively care for the offspring in the nest. When a worker bee is born, it spends the first 3 weeks of its life as a nurse working in the honey bee hive tending the eggs and developing larvae. It then transitions from nurse to middle-age jobs such as food storage and then to forager for collection of nectar and pollen (Robinson, 1992). Differences in gene expression in the brains of nurse and forager bees have been studied intensively under various conditions. One early study of the genomics of social behavior showed that RNA profiles in the brain were associated with differences in task, whether a bee was a nurse or a forager (Whitfield et al., 2003). Age-related changes in mRNA associated with the transition from nurse to forager were associated with almost 40% of the more than 5000 genes assayed. When age was uncoupled from the behavioral task, it was found that gene expression patterns correctly predicted the task performed in 57 out of 60 bees. Interestingly, there are also social influences on the ontogeny of circadian activity rhythms in forager honey bees (Bloch et al., 2001; Meshi and Bloch, 2007). Manipulation of genes whose RNA expression level is associated with behavioral task reveals that, for certain genes, manipulation of the level of expression of a single gene is sufficient to change the task of workers (Ben-Shahar et al., 2002, 2004; Amdam et al., 2004; Ament et al., 2008).

The behavior of social insects can be environmentally manipulated. For example, by initiating a hive with a thousand or so same-age young nurses, honey bee age can be uncoupled from task, generating precocious foragers that are as young as nurses. Thus, it is possible to manipulate the social environment and measure the affect of this manipulation on individual members of the honey bee colony.

Although recent research has shown that genetic variation exists even within the honey bee hive (Smith et al., 2008), traditional genetic analyses are difficult to perform on honey bees because it is a massive undertaking to maintain large numbers of selected lines or mutant individuals. A mutant line of *Drosophila* can be maintained in a vial, whereas each mutant line of honey bee requires a hive! Also, some eusocial insects are very difficult to rear and breed in the lab. However, gene expression can be manipulated with pharmacological approaches (Ben-Shahar et al., 2002) and through RNAi techniques (Amdam et al., 2003). Finally, natural genetic variation in many of the traits described above can be studied using the many subspecies of the honey bee whose migration patterns and degree of genetic similarity at the molecular level are becoming increasingly well understood (Zayed and Whitfield, 2008).

*Epigenetics in Social Insects.* The sequencing of the honey bee genome revealed that they have a fully functional methylation system, making it possible to study the link between epigenetics and social behavior (Wang et al., 2006). The overall levels of DNA methylation in the honey bee are lower than in vertebrates, and the location of the CpG islands are more commonly found in coding rather than in 5′ and 3′ regulatory regions (Maleszka, 2008). Early results on methylation in honey bees showed that the development of workers correlated with increased methylation. Knockdown of DNA methyltransferase 3 increased the probability of a larva developing into a queen bee (Kucharski et al., 2008), which correlated with altered expression of genes involved in growth and metabolism (for example, insulin-related genes). The honey bee provides an excellent model to study developmental plasticity in response to environmental cues, which is also a central topic in mammalian research (Gluckman et al., 2007).

*Social Learning and Decision Making.* The functioning of animal social groups can tell us much about the evolution of group decision making. Recently, swarms of honey bees have been used to study how animal groups make decisions. Individual preferences for a honey bee nesting site get molded into a single choice for the group by a remarkable process (reviewed in Seeley et al., 2006; Seeley, 2010). Nest site choice is made by a swarm of some ten thousand honey bees that work together to find nesting sites. Individual members of the swarm search out a dozen or more possible nesting sites, and then the group makes a collective choice of their new home. How is this accomplished? Through a number of studies performed over more than a decade, Seeley and colleagues observed and experimentally manipulated honey bee swarms in the field. These elegant experiments coupled with the development of decision models predict and explain exactly how a collective decision is made. The decision for a specific nest site begins with only a few hundred bees in the swarm that fly out and independently search for potential nest sites. These scout bees then return to the swarm and share the news of their finds using the honey bee dance language: the waggle dance (Figure 4). An individual scout's dance tells other scouts how far and in what direction a particular potential nest site is located. The waggle dances also vary in strength according to site quality. The dancing scout bees recruit uncommitted scouts to sites, and the better the site, the greater the number of recruits. If a recruit, after inspecting a site, is ''convinced'' about the goodness of a site, then she too will dance for this site, thus creating positive feedback in the interest for a site. In this way, strong interest develops only for very good sites, and ultimately, only the best site remains in the contest. This work has revealed that the honey bee group functions by ''structuring each deliberation as an open competition of ideas, promoting diversity of knowledge and independence of opinions among a group's members and aggregating the opinions in a way that meets time constraints yet wisely exploits the breadth of knowledge within the group'' (Seeley et al., 2006). Studying group decision making in honey bees offers lessons for how human groups can achieve ''collective intelligence.''

Almost nothing is known about the molecules and neural circuitry involved in changes in the waggle dance. Candidate genes for dance language have been suggested by comparing the gene expression profiles of species of bees that dance differently (Sen Sarma et al., 2009). The identification of genes and neural substrates for honey bee dance language is a fascinating area for future study that has much promise for integrating mechanistic and evolutionary investigations into the ''social brain.''

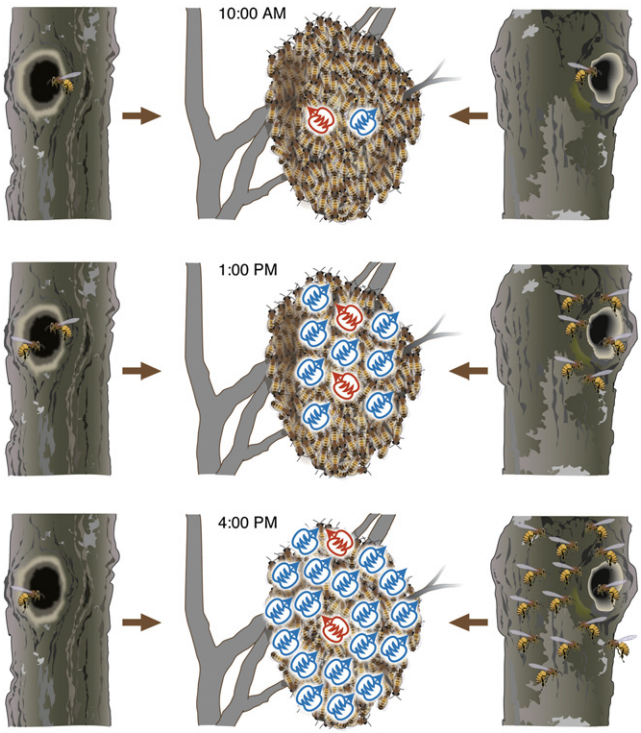


**Figure 4. Scout Bees Tune the Strength of Their Waggle Dancing in Relation to Site Value, which Builds a Consensus of Dancing Bees for the Best Site**

Here, two scouts simultaneously discover two potential nest sites, one with a large entrance opening (left) and one with a more desirable small opening (right). Each scout then returns to the swarm and performs a waggle dance for her site, but the scout from the right tree performs three times as many waggle dance circuits (blue symbol) as the scout from the left tree (red symbol). The result is that, 3 hr later, the number of bees committed to the right tree has increased 6-fold, whereas support for the left tree has increased only 2-fold, and the majority of dancing bees favor the right tree. After 3 more hours, the number of scouts at the right tree has ballooned, and the numerous dances in support of this site have nearly excluded the left-tree site from the debate (figure provided by Tom Seeley, from Seeley et al., 2006).

### *Ants*

The amazingly diverse life histories of the many species of ants are beautifully described in Holldobler and Wilson's book, *The Ants* (Holldobler and Wilson, 1990). Relatively little is known about the genetic and neural mechanisms underlying social behavior in ants. An exception that comes to mind is research on the regulation of queen number in colonies of the fire ant *Solenopsis invicta* (Ross and Keller, 1998, 2002; Keller, 2009). This species exhibits a social polymorphism with single queen (monogyne form) and multiple queen (polygyne form) colonies. The probability of single as compared to multiple queen colonies is associated with variation at the *Gp-9* gene, which is thought to encode a putative odorant-binding protein that may affect pheromone production or perception (but see Leal and Ishida, 2008). The genotypes of the workers and the queens are associated with a suite of behaviors that distinguish the colonies. For example, queens with two B alleles have higher body fat reserves that help them fly independently and start a new colony by feeding the progeny from their own body reserves and raising the first cohort of workers alone. The multiple phenotypic differences between ants from single and multiple queen colonies could arise through pleiotropic effects of *Gp-9* or as a result of association with a suite of genes closely linked to *Gp-9*. Interestingly, phylogenetic analysis suggests that single-queen colonies preceded multiple-queen colonies in the *Solenopsis* genus.

*The foraging Gene Affects Behavior in Multiple Species.* Finding and consuming food is critical for growth and reproduction, and a fascinating variety of foraging behaviors have evolved in response to the environmental pressures of food finding (Shettleworth, 2010). The *foraging* (*for*) gene regulates food-related behaviors in a variety of simple animals (for review see Reaume and Sokolowski, 2009). It encodes a cGMP-dependent protein kinase (PKG) common to almost all animals (Osborne et al., 1997). In *D. melanogaster*, natural allelic variation in *for* results in rover or sitter larval and adult food-related behaviors (de Belle and Sokolowski, 1987; de Belle et al., 1989; Pereira and Sokolowski, 1993). Rover animals move more while foraging for food than do sitters, but in the absence of food their locomotion does not differ, suggesting that the feeding environment acts as a stimulus for the expression of rover/sitter behavioral differences. Rover and sitter variants exist in nature in the Toronto area in stable frequencies (70:30 rover to sitter) (Sokolowski, 1980; Sokolowski et al., 1997), and recent evidence suggests that the polymorphism may be maintained by balancing selection through negative frequency-dependent mechanisms that occur during larval competition (Fitzpatrick et al., 2007). This means that during larval competition, each type does better when it is the rare form. Whether these larval interactions have a social component remains to be determined.

There are a number of similarities between the roles of the *for* gene orthologs in simple animals. In *C. elegans*, the *for* ortholog *egl-4* has many pleiotropic functions and affects the roaming and dwelling behavior of the worms, reminiscent of rover and sitter larval behavior (Fujiwara et al., 2002). As in *Drosophila*, the *C. elegans* behaviors affected by *egl-4* are not considered social. In social insects, *for* affects the change from nursing to foraging in the honey bee *Apis mellifera* (Ben-Shahar et al., 2002) and the ant harvester ant *Pogonomyrmex barbatus* (Ingram et al., 2005), as well as the switch between foraging and defending the nest in the ant *Pheidole pallidula* (Lucas and Sokolowski, 2009). Due to its conserved functions in behavior across a broad phylogenic range, research on the *foraging* gene provides fascinating cross-species comparisons. Given its role in mediating multiple behaviors, *for* is a candidate gene for social interactions.

Studies from *Drosophila* suggest that *for* has many of the elements that might be important for building more complex insect societies. First, *for* has pleiotropic effects on a variety of behaviors and physiologies. In *Drosophila*, *for* affects food intake, absorption of carbohydrates, lipid storage, learning and memory, and response to stress (Kaun et al., 2007a, 2008; Kaun et al., 2007b; Mery et al., 2009; Scheiner et al., 2004; Dawson-Scully et al., 2007; Kent et al., 2009). In adult flies, *for* is expressed in some neurons in the brain, the mushroom bodies, parts of the central complex and the visual system, as well as outside the nervous system in parts of the digestive system and the fat bodies (Belay et al., 2007; Belay et al., unpublished data). Second, *for* is responsive to the environment. Several behaviors exhibit plasticity directly applicable to changes in *for*

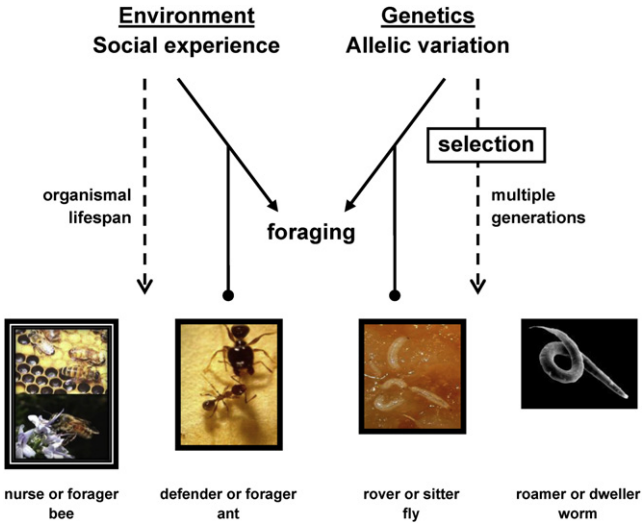


**Figure 5. A Candidate Genes for Social Behavior**

The *foraging* gene and cGMP-dependent protein kinases in worms, flies, honey bees, and ants (model modified from Wang et al., 2008). The *foraging* gene is influenced by an interaction between genes and the environment. In honey bees and ants, the social environment affects *foraging* gene activity and/or gene expression. These environmental influences can act during the lifetime of the individual in short or longer time frames. In *Drosophila*, natural selection has given rise to rover and sitter genetic variants with differences in suites of food-related behaviors. Genetic variation has arisen over longer evolutionary timescales. *foraging* is also involved in plastic changes in behavior within the lifetime of the individual in *Drosophila*. In *C. elegans*, genetic mutants in the *foraging* ortholog exhibit alterations in suites of phenotypes, some of which are food related. The *foraging* gene is a common molecular target of environmental, social, and genetic influences in a wide range of simple animals.

expression. For example, well-fed rovers have higher levels of RNA expression and PKG activity in their heads than sitters (Osborne et al., 1997). Food-deprived rovers behave like sitters, moving less and eating more, and there is a corresponding decrease in PKG enzyme activity under conditions of food deprivation (Kaun et al., 2007a, 2008). A short period of refeeding restores both rover levels of behavior and PKG enzyme activity. A second example of *for*'s involvement in plasticity involves learning and memory (Kaun et al., 2007b; Mery et al., 2009). In olfactory avoidance paradigms, rover flies exhibit better STM whereas sitters exhibit better LTM. When *for* is targeted to the mushroom bodies of sitter flies, the levels of STM and LTM are restored to a rover state. It may be that rovers' repeated food patch leaving has different cognitive requirements than sitters, who feed more locally, leading to selection for better STM in rovers and LTM in sitters. The combined functions of *for* in food-related behaviors and physiology along with learning and memory may have been important for its conserved role in social insect behaviors, where many of the interactions in the colony focus on nutritional needs and foraging decisions involving cognitive functions. Note that the mammalian *for* ortholog is also pleiotropic, affecting many phenotypes (reviewed in Reaume and Sokolowski, 2009).

These results suggest that, in flies, *for* is genetically variable but also changes its expression in response to the environment (Figure 5). The combination of allelic variation, pleiotropy, and the gene's environmental responsiveness in both the feeding and learning arenas may have set the stage for its co-opted functions in social insects where it plays a role in switching from one task to another according to the needs of the colony. Little is known about whether *for* plays a role in social behavior in *Drosophila*. However, social feeding paradigms are being generated and could be used to address *for*'s role in social interactions in flies.

*C. elegans* and *D. melanogaster* genes that affect behavior can be used as candidate social genes in other species whose genetics are not well characterized (Fitzpatrick et al., 2005). This candidate gene approach was used to identify a role for the *for* gene orthologs in honey bees and ants. The relative number of nurses and foragers in a honey bee colony arises from the social requirements of the colony. In the honey bee, *for* plays a role in the switch from workers nursing in the hive to foraging outside the hive. Like *Drosophila* rovers, forager bees have higher levels of FOR in their brains than do nurse bees, and the expression level of the honey bee *for* gene in individual bees is upregulated during the switch from nursing to foraging (Ben-Shahar et al., 2002). A similar relationship was found between ant nurses and food gatherers in *P. barbatus* (Ingram et al., 2005). A causal relationship between task and PKG was shown in experiments demonstrating that increasing PKG levels in honey bee nurses results in a switch of more bees to foragers (Ben-Shahar et al., 2002). The honey bee *for* gene plays a role in the social workings of the colony, as it is involved in the decision to change from nurse to forager. What molecules act upstream of *for* in honey bees and how it exerts its effect downstream is for the most part not known. Interestingly, microarray analyses show that the *for* and insulin signaling pathways are involved in rover/sitter foraging (Kent et al., 2009) and the transition from nurse to forager in *A. mellifera* and that both are nutritionally regulated (Ament et al., 2008).

Studies of the *for* ortholog in harvester ants showed that ants who work in the nest differ in their *for* RNA levels from ants that forage for food outside the nest (Ingram et al., 2005). However, the direction of the differences in ants is opposite that found in flies and honey bees, with ants in the nest having higher levels than those outside the nest. This directional difference in PKG enzyme activity was also found in another ant species, *Pheidole pallidula* (Lucas and Sokolowski, 2009). Sister worker ants of *Pheidole pallidua* have morphologically different worker subcastes; one specializes in defense and is large with powerful mandibles, whereas the other is small and specializes in foraging. The worker who specializes in defense is able to modify its behavior depending on the needs of the ant colony. When foragers need defenders to use their mandibles to cut up large prey items, the defenders oblige, and their PKG activity becomes more like that of the foragers (Lucas and Sokolowski, 2009). When alien intruders enter the colony, the differences between the PKG enzyme activities of the defenders and foragers become more pronounced. Together these results show that *for* plays a role in a worker ant's switch from defending the nest to foraging and that these changes in behavior proceed according to the needs of the ant colony and are thus social.

The example of the *foraging* gene and its functions across species shows how natural selection has resulted in the "reuse"

of genes involved in individual behavior for more complex social behaviors (Figure 5). Although the *for* gene plays important roles in food-related behaviors in worms, flies, honey bees, and ants, important differences are emerging. In flies and honey bees, higher levels of the gene are associated with rover/forager, whereas in *C. elegans* and ants lower levels of the gene are associated with roamer/foragers, suggesting important differences in the regulation of *for* in these organisms. So far, differences in the spatial localization of FOR protein has only been studied in the ant brain. Future work involving the manipulation of *for* expression will help determine the spatial and temporal requirements for different behaviors in the different species. How the physical environment (e.g., food quality and distribution), the social environment (colony needs), and the expression of FOR in peripheral tissues interact within the animal to affect plasticity in social behavior is an important subject for future study.

## Discussion

To understand the relationships between genes, environment, and social behavior, both the individual's phenotype and the phenotypes of its social partners must be understood at the genetic, molecular, and neurobiological level (Keller, 2009). There has been a flurry of recent publications that use simple model organisms to study mechanisms underlying social interactions. Although this research is in its early stages, common themes are emerging that will help form a conceptual framework for this research. Many of these behaviors are relevant to investigations of social behavior in more complex animals, including social interactions around sex, aggression, learning and memory, parenting, and foraging. As in more complex animals, all of these are affected by social context, including isolated versus group rearing, group composition, learning from experienced animals, and response to information about social dominance.

Communication is vital to social interactions. Chemical communication through pheromones is a key element of communication used across species. In several species, the same pheromone or class of pheromones is used in different social interactions. How they are differentially regulated and communicated to affect a number of specific social interactions within species is not known. Touch and visual communication are also important in social interactions, and while auditory communication is also likely involved, it has been less studied in the models discussed here (but see auditory communication in *Drosophila* courtship, reviewed in Villella and Hall, 2008).

Even for simple animals, multiple sensory inputs from social signals need to be integrated in the brain to produce effective responses to the social situation. Changes in the brain are likely to happen at different timescales. Longer timeframes of response suggest transcriptional changes in gene expression and have been measured using microarrays. In some cases, causation can be shown using pharmacological, mutant, and/or transgenic approaches. Shorter timescales suggest physiological responses resulting from changes in gene activity. It is unclear what brain regions are involved in integrating information from social interactions, although the mushroom bodies and central complex are good candidate regions in insects. Identification of the spatiotemporal pattern of expression of genes that play a role in social behavior has in some cases identified brain regions and in others only a few neurons important for normal social interactions. Further localization and manipulation of gene products will help define a map of the brain regions responsible for integrating social behavior. Of course, this is a challenging task because most genes that affect social behavior have pleiotropic effects on other phenotypes, making it necessary to precisely manipulate the temporal and spatial expression requirements of these gene products. Another less causal approach is to assess genome-wide expression patterns in selected brain regions and how they respond to social interactions. Cross-species comparisons using both approaches will help us determine whether there is a "social brain."

From the hundreds of genes whose expression changes on microarrays during a social interaction, it is possible to identify single causative genes that can significantly affect social behavior in certain contexts (e.g., *npr-1*, *foraging*, *octopamine*). These genes may have larger effects than others because of their position at a hub in the gene network and/or their responsiveness to the social environment. Indeed, natural allelic variation in a gene can affect hundreds of downstream genes, which can help identify pathways of interest (*foraging*; Kent et al., 2009). Traditional genetic dissection approaches can identify single-gene effects on social behavior through mutagenesis, analysis of natural genetic variants, and analysis of epistatic interactions between small groups of genes. Investigations have shown that there are both single and multigene effects on social behavior. Analysis of epigenetic modification of the genome in response to social interactions is an exciting new field of investigation in simple model animals, which will undoubtedly lead to new insights in the coming years.

Analysis of the phenotypes of social behavior mutants can help us understand what is required for normal social behavior. Given the conservation of gene function for many phenotypes, including behavioral ones, simple animal research may inform us of what genes are involved in generating normal social behavior in other more complex organisms. Clock genes are a perfect example of this, as clocks are found in all organisms and basic clock mechanisms are conserved (Benca et al., 2009). Circadian timing mechanisms are important for social behavior in flies and may play a role in bipolar disorder in humans (reviewed in Flint and Shifman, 2008).

Mutants that disrupt many of the social interactions described here might provide good candidate genes for the abnormal reciprocal social interactions in autism. Aggressive interactions that repeatedly end in defeat could be used to model chronic defeat syndrome found during depression and identify candidate genes for this disorder. But one needs to be cautious because comparisons between animal models and human social disorders should be based on similar genetic and physiological mechanisms, not just whether the behaviors appear similar. This leap from simple models to humans not only relies on the conservation of DNA sequence and function across a broad phylogenetic range but also on the idea that complex behavior in mammals derives from simpler modules of behavior in simpler organisms. The idea of a "molecular toolkit" for social behavior common to all organisms has been discussed (Toth and Robinson, 2007).

Understanding gene-environment interdependencies in social behavior is a difficult challenge. Genetic, genomic, and epigenetic analyses in simple model organisms are beginning to uncover genes and pathways involved in these interactions. However, addressing the environmental components is also challenging because many abiotic and biotic factors relevant to social interactions vary temporally and spatially. The conceptual framework for studying social behavior combined with new tools in genetics, molecular biology, genomics, neurobiology, animal tracking, and imaging are bound to aid in these investigations.

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