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The *foraging* Gene and Its Behavioral Effects: Pleiotropy and Plasticity

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Abstract

The *Drosophila melanogaster foraging* (*for*) gene is a well-established example of a gene with major effects on behavior and natural variation. This gene is best known for underlying the behavioral strategies of rover and sitter foraging larvae, having been mapped and named for this phenotype. Nevertheless, in the last three decades an extensive array of studies describing *for*'s role as a modifier of behavior in a wide range of phenotypes, in both *Drosophila* and other organisms, has emerged. Furthermore, recent work reveals new insights into the genetic and molecular underpinnings of how *for* affects these phenotypes. In this article, we discuss the history of the *for* gene and its role in natural variation in behavior, plasticity, and behavioral pleiotropy, with special attention to recent findings on the molecular structure and transcriptional regulation of this gene.

INTRODUCTION

Behavioral plasticity: an organism's ability to change its behavior in response to the experienced environment

Gene–environment interplay: a reciprocal relationship between gene and environment; arises from the idea that GxE interactions have proven insufficient for a complete understanding of behavior

Pleiotropy: the influence of a single gene on multiple independent phenotypes

Negative frequency-dependent selection: an evolutionary process that favors the rare allele in a mixed population; i.e., individuals with the rarer genotype have greater fitness advantage

To truly understand the relationship between genes and behavior, one must consider not only how genes are regulated throughout development of an organism to give rise to behavioral phenotypes, but also what circumstances can affect and change this regulation through the life course. Genes do not exercise their biological functions in isolation but operate in complex regulatory networks that are sensitive to environmental input. Thus, when studying behavioral phenotypes, the following must be considered: Individuals vary in their behavior in part because of genetic variability in the genes influencing behavior (behavioral genes). Many behavioral phenotypes are plastic, as individuals can adjust their behavior on the basis of present and past experiences (i.e., behavioral plasticity). Between individuals, differences in behavior originate not only in allelic variation at the genetic level but also in different levels of plasticity in a changing environment (i.e., gene–environment interplay) and variability in developmental processes (47). Behaviors are usually influenced not by single genes but rather by often complex interactions (epistasis) between a few (or many) genes. Last, one gene does not always have a single function; single genes can often influence multiple independent behaviors, a process known as pleiotropy.

The *Drosophila melanogaster foraging* (*for*) gene has become a well-established example of a behavioral gene in all these complex aspects of behavioral regulation. *for* encodes a cGMP-dependent protein kinase (PKG) (74), a type of signaling molecule that in the presence of its activator (cGMP) regulates other downstream target proteins primarily through phosphorylation. *for*'s behavioral variants and their plasticity and pleiotropy have provided an excellent model for studying how natural variation in a single gene influences behavior in diverse taxa, from perspectives as varied as ethology (15, 51, 63), evolution (37, 95, 101), genetics (1, 2), neuroscience (20, 21, 32, 85), and neuropharmacology (9, 19, 22, 23, 58).

In the 1980s and early 1990s, *for* received considerable attention for being a rare example of a gene with major effects on individual differences in behavior. At the time, it was thought that, according to the classic model of polygenic inheritance (33), normal differences in complex traits such as behavior were influenced by an infinite number of genes each with small, equal, and additive effects (104, 105). Consequently, it was assumed impossible to identify and map a gene that influenced normal individual differences in a behavioral trait. Time has shown that this was a technical issue, as polymerase chain reaction had not yet been invented and the idea of being able to sequence a genome was unfathomable. In the rare cases in which a single gene effect was isolated and identified, the gene was said to have a major effect on the trait. This definition of a major gene was not based on the calculation of its effect size (33) and did not discount minor (e.g., smaller) effects of other genes on the trait.

Our current understanding of the genetic architecture of behavioral traits is that many genes are involved, some of which have unequal effects on the phenotype (64). This idea fits with what is known about the structure of gene pathways in that some genes are master regulators of behavioral differences (e.g., transcription factors) (110). One such behavioral difference, regulated by a gene of major effect, is *D. melanogaster* larval foraging path length. Quantitative genetic analysis of this trait revealed a major effect on the autosomes and a smaller but significant effect on the X chromosomes (25). The autosomal factor was later mapped to the *for* gene, making this gene one of the first examples of a major regulator of behavior, underlying the rover–sitter behavioral polymorphism (1, 8, 51, 74). In 2007, the same behavioral polymorphism associated with *for* received renewed attention for being one of the first experimental studies of a behavioral gene under negative frequency-dependent selection (37, 101). Currently, *for* arguably provides one of the finest examples of molecular characterization of behavioral pleiotropy (1, 2, 5, 7). Furthermore, *for* is conserved across a wide range of species (**Figure 1**), with varied behavioral functions from insects to humans, making it a gene of broad interest.

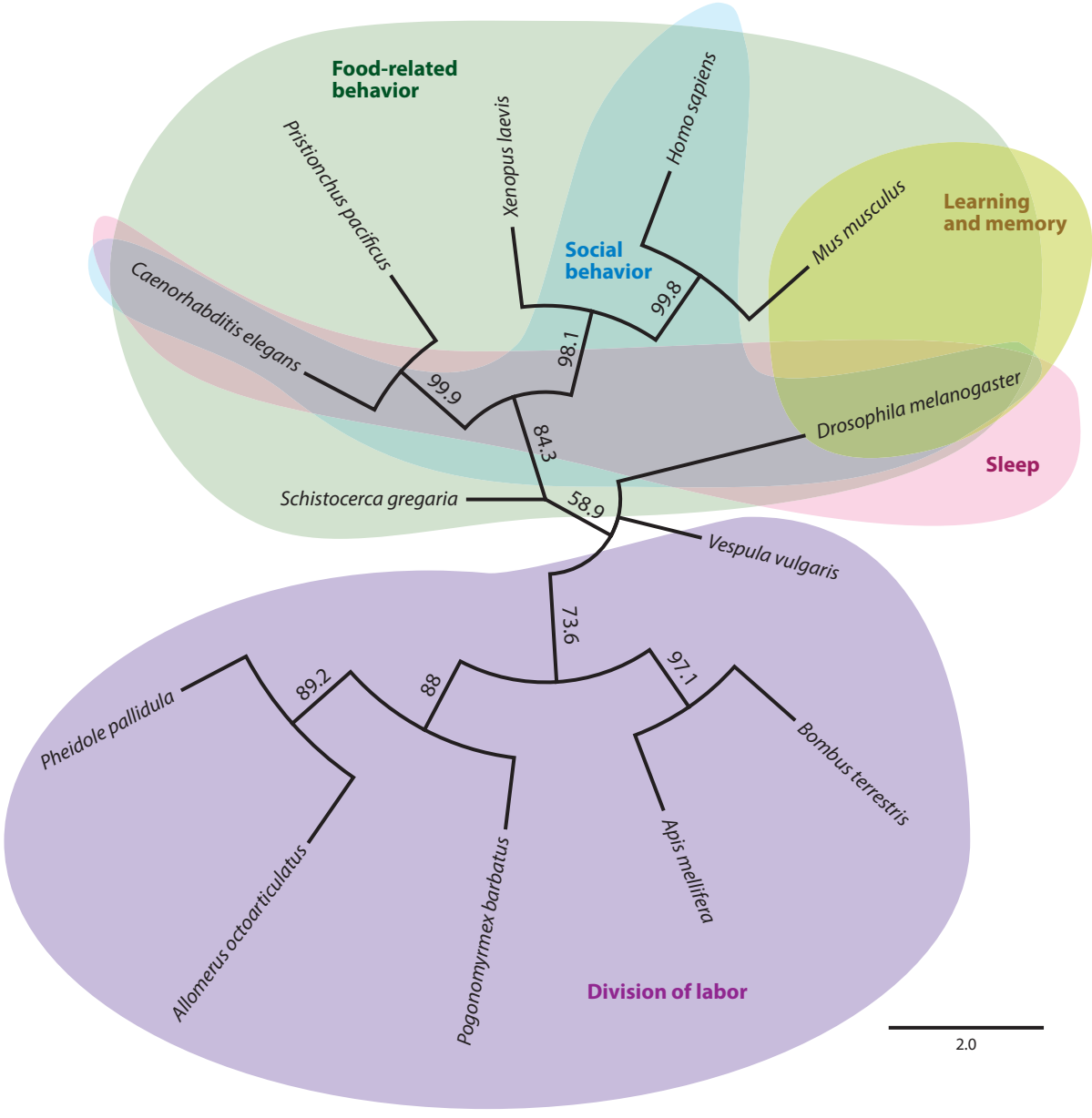

Figure 1

foraging-related behaviors are conserved across species. Behavior classes affected by the *foraging* gene in different species, as discussed in this article, are overlaid on a phylogenetic tree constructed from the alignment of *foraging* nucleotide sequences. Only known behavioral phenotypes are shown on the tree; for references, please refer to main text. The tree was made in RAxML (98) under a GTR+G model with 1,000 bootstrap replicates. The tree is a majority consensus tree. Alignment was done using the Geneious Prime 2019.0 (**https://www.geneious.com**) algorithm on *for* CDS sequences (GenBank accession number in **Table 2**). Owing to incomplete CDSs for some species, the alignment was trimmed to a common set of 1,310 sites. Abbreviation: CDS, coding sequence.

Density-dependent selection: an evolutionary process whereby the relative fitness of genotypes within a population is dependent on overall density of the population

In this article, we review what is known about *for* and its effects on behavior, addressing the many interconnected aspects of behavioral regulation, and then cover the molecular genetics of the gene. First, we provide an overview of *for* and natural variation in food-related behavior, learning and memory, sleep, and social behavior. Second, we discuss *for*'s role in behavioral plasticity and development and in social and eusocial behaviors. Finally, we discuss how recent findings on *for*'s molecular structure, regulation, and genetic interactions provide a model for understanding behavioral pleiotropy.

foraging AND NATURAL VARIATION IN BEHAVIOR

Larval Foraging Behavior

It was the discovery of a behavioral polymorphism in freely foraging *D. melanogaster* larvae in 1980 that laid the foundation for the work that would later lead to the discovery of the *for* gene and its many associated phenotypes (93). In this initial study, Sokolowski found that individual larvae differed in how far they traveled while foraging and accordingly could be classified into rover or sitter behavioral morphs, with rovers traveling significantly longer distances than sitters while on a feeding substrate (93). Quantitative genetic analysis mapped this behavioral difference to chromosome 2, with rover showing genetic dominance over sitter (25), and later localized the *for* gene to the cytological location 24A2–24A4 on the left arm of chromosome 2 (24). Precise genetic excision of the *for* gene, transgenic rescue, and overexpression proved that *for* affects larval foraging path length in a dosage-dependent manner, with higher *for* expression associated with longer path lengths (1). Although laboratory strains and wild populations tend to be either rover or sitter [for instance, the DGRP (*Drosophila melanogaster* Genetic Reference Panel) lines appear to be solely sitter, whereas CyO balancer larvae are rovers], some wild populations maintain both morphs (93, 95). The maintenance of the two morphs in a population is environmentally mediated by density-dependent selection, in which high density favors rovers and low density favors sitters (95), negative frequency-dependent selection, in which the frequency of one morph increases when it is rare relative to the other morph (37, 101), or more likely, a combination of these selection forces.

Akin to the rover and sitter behavior in fruit flies, the *Caenorhabditis elegans* homolog of *for*, *egl-4*, regulates the roaming and dwelling behavioral states and in this case, the same individual may switch between behavioral states (40).

Adult Foraging Behavior

Rovers and sitters also differ in their adult foraging strategies, with differences in complex movement patterns and feeding behavior. Reminiscent of the larval phenotype, after prompted to take a meal, adult rover females tend to move away from the food source, whereas sitters stay close to it (79). In accordance, rovers have significantly higher dispersal distances in the wild (30, 31). Nevertheless, if allowed to search for food after mild food deprivation, instead of being prompted to stay and feed, rovers are more exploratory and find and consume more food drops than sitters (5, 48). This difference in adult foraging behavior is determined by the expression of *for*'s promoter 4 transcripts and correlates with epigenetic modification of the same promoter (7).

Reminiscent of findings in *Drosophila*, a single nucleotide polymorphism (SNP) that affects the gene expression of the human homolog of *for*, *PRKG1*, significantly associates with the preferential adoption of patterns of goal pursuit in humans: the assessor (those who do the right thing) or locomotor (those who get on with it) behavioral regulatory modes (100). This genetic correlation holds true in a foraging task–based computer assay, with assessors assuming a more cautious search strategy, hugging the edge of the foraging environment, much like the search behavior of adult sitter fruit flies (100).

Food Responsiveness, Food Intake, and Fat Storage

for influences food-related phenotypes beyond foraging movement patterns in both larvae and adult fruit flies. Rover larvae have lower food intake and fat stores than sitter larvae, as well as higher glucose absorption and preferential allocation of glucose to lipids (1, 51). As with larval path length, larval food intake and fat levels are regulated by *for* in a dosage-dependent manner (1). The directionality of the differences found in rovers and sitters in these phenotypes does not always follow the directionality of the effects of gene dosage (1). For instance, while sitters and *for* null mutants have higher fat stores than rovers, rovers and *for* null mutants are more similar in food intake, having lower food intake scores than sitters (1). As discussed in detail in the section titled Molecular Causality, this suggests that the different food-related larval phenotypes are at least partially under independent regulation by *for*.

While sitter larvae have higher fat stores and food intake than rover larvae, adult sitters show lower sucrose responsiveness and food intake than adult rovers (5, 7, 48, 89). These differences between larval and adult rovers and sitters are likely due to adult sitters having higher fat storage (potentially due to their higher larval fat stores), making them less motivated to consume as much food and less sensitive to starvation stress than rovers (5, 27, 48).

Learning and Memory

Considering that rovers and sitters differ in so many behavioral phenotypes, it is certainly not surprising that this difference extends to several types of learning and memory, essential cognitive components of behavior. Rover larvae have higher learning indexes in olfactory-based appetitive learning tasks, a phenotype that can be manipulated by overexpressing *for* in the mushroom body (the fruit fly's learning and memory center in the brain) (50). Curiously, increased training results in reduced learning and memory in rovers, whereas it increases the performance of sitters (50). This strain difference might reflect different behavioral strategies, in which rovers (which disperse farther) place more importance on new situations, whereas sitters place more importance on repeated events.

Consistent with a possible evolutionary trade-off in how memories are allocated and stored, adult rovers have better short-term memory but poorer long-term memory than adult sitters (68). As in larvae, this difference can be manipulated by overexpressing *for* in the brain (68). The higher short-term memory performance of rovers is conserved across a variety of different learning paradigms (60, 84, 108). Rovers also show higher retroactive interference, a process in which older memories become less accessible with the gathering of new information (84). In contrast, adult rovers show less habituation in sucrose responsiveness (89), which might be more reflective of the rover–sitter difference in sensitivity to food deprivation than of a difference in nonassociative learning. Higher retroactive interference and poorer short-term memory might befit the rover strategy of roaming far in the search of food, which makes their environment more transitory (84).

To our knowledge, no associations between *for* and natural individual differences in learning and memory have been made for organisms other than the fruit fly. Nevertheless, there is evidence from mutants that *for* plays a role in learning and memory in mammals (34, 76), the details of which are discussed in the section titled Mutant Analysis.

Social Behavior

Beyond differing in how well they learn, rovers and sitters show a distinct difference in from whom they learn. Sitters learn significantly better when they are trained or tested in a group setting than when alone, while rovers seem indifferent to social context (58). Pharmacologically

activating PKG, the enzyme encoded by *for*, results in increased learning in sitters when they are alone and no effect on rovers under these conditions; in contrast, pharmacological inhibition of PKG results in no effect on sitters when they are alone and decreased learning on rovers under these conditions (58). This suggests that increasing or decreasing PKG influences social learning in fruit flies. Rovers and sitters also differ in how they aggregate when seeking refuge from harsh environments, although this difference has not been directly linked to *for* (38, 81). In addition to learning better from their peers and being more likely to aggregate in groups (38, 58, 81), sitter males are also less aggressive toward other males than rover males are (107). Sitters seem more sensitive not only to the social cues of other fruit flies, but also to eusocial signals from other insects (i.e., honey bee queen mandibular pheromone), even though fruit flies are not eusocial themselves (18).

A recent study of the mutualistic ant *Allomerus octoarticulatus* suggests that *for* affects not only intraspecies interactions but also interspecies ones. This study found that higher *for* expression and PKG enzyme activity are associated with higher aggression toward an intruder herbivore, a behavior that both protects the ant's plant host and provides alimentary protein for the ant colony. This effect seems to be moderated by the interaction of two PKG genes, one of which is *for* (*Aofor*) (67).

As with food search strategies, *for*'s role in natural variation in social behavior might be conserved in humans. Genetic variation in the human homolog of *for*, *PRKG1*, correlates with maternal sensitivity toward their infants and moderates the effects of early-life adversity on this trait (92). Furthermore, several genome-wide association studies have found weak associations between *PRKG1* and social-emotional behavioral syndromes (12, 70, 112) and susceptibility to substance abuse after trauma exposure (45, 82).

Olfactory Behavior

Although the abovementioned differences in olfactory learning in larvae are not due to a differential attraction to odors used in the learning assays (50, 68), adult rovers and sitters do differ in their attraction to yeast odors (91). This effect seems more likely to be a difference in olfactory perception rather than a higher-level response to food, as fed sitters are generally less motivated to search for and consume food (5, 48, 89). *for*'s role in natural variation in olfactory behavior is further enforced by a recent study that found a polymorphism in *for* to be one of the top polymorphisms associated with variation in olfactory behavior in the DGRP lines, a collection of inbred wild-derived lines with sequenced genomes (11).

Like in social behavior, the role of *for* in olfactory behavior might be conserved in other species. In *C. elegans*, the regulation of the alternate behavioral states (roamers and dwellers) requires *egl-4* in the olfactory sensory neurons, indicating that *egl-4*-linked regulation of locomotory behavior is associated with sensory perception (40). Furthermore, *egl-4* is required for adaptation of these sensory neurons to prolonged stimulation (52, 61).

Sleep

Higher resilience to food deprivation in sitter adults has been proposed to underlie an evolutionary trade-off that makes sitters more vulnerable to sleep deprivation than rovers (27). Rovers perform better at learning tasks after 24 h of sleep deprivation than sitters, and overexpressing *for* in the mushroom bodies of sitters recapitulates this rover phenotype (27). This finding suggests that while the lower tolerance of rovers to food stress would seem like an evolutionary disadvantage, it might be counterbalanced by their higher resilience to other stresses, such as sleep deprivation. This could contribute to selection on the rover–sitter polymorphism (37, 95, 101), as a community

or ecological system is unlikely to experience a constant single stressor but rather alternating, varied stressors (39). Further evidence that natural variation in *for* is involved in sleep comes from an artificial selection experiment on an outbred population constructed with the DGRP lines. By selecting for low and high sleep duration, this study identified two polymorphisms in *for* as candidates for regulating night sleep duration (44). *for*'s function in sleep might also be evolutionarily conserved, as *egl-4* also regulates sleep in *C. elegans*, albeit this was discovered with the use of mutants and not natural alleles (83).

GxE interaction: a statistical interaction wherein a given phenotype is best explained by the interaction between genes and environment rather than by the effects of genes or environment alone or the net sum of the two

BEHAVIORAL PLASTICITY AND GENE–ENVIRONMENT EFFECTS

In addition to underlying natural variation in behavior in a range of phenotypes, *for* has also been widely implicated in regulating the behavioral response to environmental conditions (e.g., behavioral plasticity). Often interactions between genetic background and the environment modulate this behavioral plasticity, giving rise to gene–environment (GxE) effects and individual differences in behavior (6). Rovers and sitters are a good example of this, as their *for*-associated phenotypes are often plastic, with rovers and sitters differing in their plasticity. Furthermore, GxE interactions implicating *for* have also been found in humans (45, 82).

Food-Related Phenotypes

Not surprisingly, the expression of food-related phenotypes associated with *for* is sensitive to food conditions in the environment. For instance, the difference in larval path length depends on the availability of food, since rovers and sitters have similar (long) path lengths on a nonnutritive substrate (51, 94). Larval path length also depends on food availability and quality and varies day to day (presumably because of other environmental effects), albeit no GxE interactions are observed in this case, with both rovers and sitters reducing their path lengths to a similar extent when less or lower-quality food is available (8, 41). Conversely, demonstrative of a GxE interaction, the reduction of quantity or quality of available food results in a rise in larval food intake in both rovers and sitters such that the rover–sitter differences in this trait are lost under these conditions. Nevertheless, rover larvae maintain higher carbohydrate absorption efficiency, develop faster, and have higher survivorship compared with sitter larvae when food is limited (51).

Like rover and sitter larvae, adult rovers and sitters respond to food deprivation by increasing their foraging behavior and sucrose responsiveness, with 24-h-food-deprived sitters essentially phenocopying fed rovers (48). Nevertheless, this is likely a consequence of different homeostatic starting points and not a GxE interaction, as both rovers and sitters increase foraging behavior to similar extents in response to acute food deprivation (48). In contrast, a clear GxE effect can be seen in rover and sitter responses to food deprivation in other adult food-related phenotypes (53). A complex GxE interaction is also observed with early (larval) nutritional adversity and adult exploratory behavior. Sitters are more sensitive to early-life (larva) rearing conditions, adjusting their adult behavior more in response to early food deprivation than rovers (17). Furthermore, the effect of balancing selection on rovers and sitters in a population is also dependent on food, as frequency-dependent selection can be observed in a low-nutrient environment but not in higher-nutrient conditions (37, 101).

At the intersection of food-related and social behavior traits lies the findings of GxE interactions on *PRKG1* and alcohol abuse in humans. The human rs1729578 SNP in an intron of *PRKG1* has been associated with increased risk for alcohol abuse, but only in individuals who have experienced lifetime trauma (45, 82). In fact, in people who did not experience trauma, this SNP was negatively associated with alcohol misuse (45). These findings were replicated in three independent study cohorts (45, 82).

Hypoxia and Thermotolerance

Further support that *for* underlies differential susceptibility to the environment comes from the stress/neurophysiology literature. Sitter larvae are more resistant to high temperatures and anoxia, maintaining normal synaptic transmission and feeding movements at significantly higher temperatures and lower oxygen, than rover larvae (19, 22, 23). Pharmacological manipulations showed that this was directly related to the PKG pathway and K^+ channel activity (19, 22, 23). The effect of *for* on thermotolerance and anoxia tolerance might be linked to spreading depolarization, an electrophysiological response to stress that results in a loss of ion gradients and a surge of extracellular K^+ (10, 97), and thus could explain synaptic failure under stress (19). Although sitter neurons have naturally lower K^+ currents than rover neurons, they also show increased spontaneous activity and excitability (85), which suggests that the rover–sitter difference in hypoxia and thermotolerance arises from a more complex interaction than from a direct effect of *for* on neuronal excitability due to K^+ channel activity. One caveat of pharmacological manipulation with PKG activators or inhibitors is that it does not allow the uncoupling of *for*-specific effects from other PKGs (there are two other PKGs in *D. melanogaster*). Either way, the function of PKGs in thermotolerance and spreading depolarization seems to be conserved in locusts (*Locusta migratoria*) (10, 22), frogs (*Xenopus laevis*) (86), and mice (9).

Together, the differences in food-related behaviors and stress tolerance support the idea that the different behavioral strategies of rovers and sitters might be affected by natural selection in a changing environment. For instance, the higher tolerance of sitters to thermal and anoxic stress might be offset by their higher sensitivity to poor larval feeding environments.

Social and Eusocial Behaviors

for's extensive role in regulating behavior and plasticity in fruit flies made it a likely candidate behavioral gene in other species (36). These studies of candidate genes have revealed some astonishing associations between *for* expression and social and eusocial behaviors in a variety of species (**Figure 1**). In both bees and ants, *for* is associated with environment- and age-dependent division of labor, but the directionality and nature of this relationship vary from species to species, a possible indication of evolutionary divergence in the underlying mechanism.

In honey bees (*Apis mellifera*), a strictly eusocial species, the *for* homolog *Amfor* is associated with caste differences in behavior (14, 15). Honey bees have a strict age-associated division of labor, in which young adult bees perform in-hive duties such as nursing and cleaning and older bees forage outside the hive. The age at which bees switch from nursing to foraging depends on the colony's needs, and as such, this behavioral switch underlies plastic regulation (87). Foraging bees have higher *Amfor* expression (specifically of the *Amforα* splice variant), higher PKG activity, and higher sensitivity to dietary sugar than nurse bees (15, 103). Increasing PKG activity pharmacologically in nurses causes precocious foraging and increases gustatory sensitivity, showing a direct causal link between PKG activity and foraging behavior (103). This effect of *for* on the change in behavioral task from nurse to forager might be mediated through a PKG-dependent increase in positive phototaxis in forager bees (14), although others have not found this association (103) perhaps because of methodological differences. Similar patterns between *for* expression and transition from nursing to foraging are observed in the eusocial bumble bee *Bombus terrestris*, with foragers having higher *for* (*Btfor*) than nurses (106). In the bumble bee *Bombus ignitus* and in the wasp *Vespula vulgaris*, however, *for* (*Bifor* and *Vvfor*, respectively) expression levels were higher in nurses than in foragers (57, 109). This contradictory relationship might be a function of age-dependent decreases in *for* after the transition to foraging (46, 106), or it may indicate different regulation of the pathways by which *for* influences division of labor in different eusocial species.

As in bees, *for* is involved in caste-specific foraging behavior in a variety of eusocial ant species. Nest workers and patrollers of both field and laboratory colonies of red harvester ants, *Pogonomyrmex barbatus*, have higher *for* (*Pbfor*) expression than foragers (49). Similarly, pharmacological and behavioral manipulations show that foraging behavior directly correlates with PKG activity, and nest defenders of the ant *Pheidole pallidula* appear to express *for* (*Ppfor*) in more brain cells compared with foragers (63). In the ant *Cardiocondyla obscurior*, however, differences in *for* (*Cofor*) expression between nurses and foragers (i.e., higher *Cofor* expression in nurses) seem to be an artifact of age rather than correlating with behavioral task (72).

One of the most striking examples of social behavioral plasticity in noneusocial insects is the transition from solitary to gregarious in the desert locust *Schistocerca gregaria* (96). At low population densities, desert locusts are solitary and sedentary, avoiding conspecifics. When population density increases, these locusts switch drastically to gregarious behavior, forming large migratory swarms. This switch is correlated with an increase of PKG activity in the brain, which shows *for* (*Sgfor*) staining in the pars intercerebralis (62). Nevertheless, the contribution of other PKGs to this phenotype cannot be ruled out at this point, and so far only the cAMP-dependent protein kinase A (PKA) pathway has been causally related to this behavioral switch (75).

MOLECULAR CAUSALITY

In 1989, *for* was genetically localized using a technique called lethal-tagging that associated the change in larval behavior from rover to sitter after mutagenesis with nearby induced lethal mutations that could be more readily mapped (24). Three cytologically visible, independently generated pupal lethal mutations, all of which did not complement for lethality and behavior, played an important role in the later localization of *for* (24). At that time, no gene involved in natural variation in behavior had been localized in any organism, likely owing to the lack of a sequenced genome, precise mutants, and transgenic tools. Among the first tools available to link the *for* gene causally to rover and sitter behavioral differences was the mutant line S2 (24, 79) and later a cloned complementary DNA (cDNA) construct (24, 74, 79). S2 is a rover line carrying an induced mutation that confers it sitter behavior in many of the *for*-related phenotypes. However, the S2 mutation was never mapped to a specific alteration in DNA sequence and has been found to affect some *for*-related phenotypes and not others (e.g., 18, 53). The *foraging* gene cDNA, although proven useful in phenocopying rover behavior in sitters for some phenotypes, has the inherent caveat that overexpression might not recapitulate endogenous expression patterns and as such makes a clear definition of causality difficult.

The molecular characterization of the *for* gene (1) and the generation of a large variety of tools that have helped researchers investigate the causality of *for*-related phenotypes have progressed enormously in the last few years (**Table 1**). Some of these tools include precise null mutants (1, 78), genomic rescues and drivable overexpressors (1, 2), promoter-specific *for*-GAL4 lines (2), drivable and constitutive tagged *for* lines (20, 78), and general and transcript-specific RNA interference (RNAi) lines (5, 21). Furthermore, the genomes of the rover and sitter strains have been sequenced (5). The availability of these tools has prompted new avenues of research, addressing the molecular underpinnings of how *for* regulates its many associated behavioral phenotypes.

Mutant Analysis

The rover and sitter strains present a valuable system to study natural variation in behavior, as they are certainly more representative of behavioral differences occurring in nature than engineered mutants and transgenic fruit flies. Nevertheless, the rover and sitter strains differ not only at the *for*

Table 1 Verified transgenic tools for the *Drosophila melanogaster foraging* gene published in functional/behavioral studies

Tool	Symbol	Reference	Availability	Notes
for null mutant	for^{0}	1	BDSC #76119	Precise excision of the *for* locus by recombineering
for null mutant	for^{20-29}	78	Liu laboratory	Point mutation in the *for* kinase domain, resulting in a frameshift
for duplication	for^{dup}	1	Sokolowski laboratory	Duplication of the *for* locus at its genomic location
for insertion on chromosome 3	for^{BAC}	1	Sokolowski laboratory	BAC with *for* genomic sequence inserted on chromosome 3, can be used for rescue or overexpression in combination with for^{0} or wild type
Constitutive *for* insertion with flag-4c tag	$for^{BAC:flag\text{-}4c}$	20	Sokolowski laboratory	BAC with *for* fused to a flag and a tetracysteine (4c) tag; inserted on chromosome 3 Allows for acute protein inactivation with light, and immunodetection with FLAG antibody
for-promoter 1 driver	for^{pr1}-*Gal4*	2	Sokolowski laboratory	*for* promoter 1 sequence fused to GAL4, inserted on chromosome 3
for-promoter 2 driver	for^{pr2}-*Gal4*	2	Sokolowski laboratory	*for* promoter 2 sequence fused to GAL4, inserted on chromosome 3
for-promoter 3 driver	for^{pr3}-*Gal4*	2	Sokolowski laboratory	*for* promoter 3 sequence fused to GAL4, inserted on chromosome 3
for-promoter 4 driver	for^{pr4}-*Gal4*	2	Sokolowski laboratory	*for* promoter 4 sequence fused to GAL4, inserted on chromosome 3
Drivable *for* cDNA	*UAS-*for^{T1}	2	Sokolowski laboratory	*for* P1 isoform CDS under UAS control, inserted on chromosome 3 Can be used for tissue-specific rescue or overexpression in combination with for^{0} or wild type
Drivable *for* cDNA with flag-4c tag	*UAS-*$for^{flag\text{-}4c}$	20	Sokolowski laboratory	*for* P1 isoform CDS fused to a flag and a tetracysteine (4c) tag under UAS control, inserted on chromosome 3 Allows for acute for protein inactivation with light, and immunodetection with FLAG antibody
V5 tag inserted in the *for* locus	for^{v5}	78	Liu laboratory	V5 tag inserted 3′ of the genomic *for* sequence by CRISPR
for common-coding RNAi	*UAS-for RNAi*	21	Sokolowski laboratory	*for* RNAi under UAS control targeting all transcripts, inserted on chromosome 3
for common-coding RNAi	*UAS-for RNAi v38320*	78	VDRC	*for* RNAi under UAS control targeting all transcripts, inserted on chromosome 3
for-promoter 4 RNAi	*UAS-*for^{pr4} *RNAi*	5	Sokolowski laboratory	*for* RNAi under UAS control targeting promoter 4 transcripts, inserted on chromosome 3

Abbreviations: BAC, bacterial artificial chromosome; BDSC, Bloomington Drosophila Stock Center; CDS, coding sequence; CRISPR, clustered regularly interspaced short palindromic repeats; RNAi, RNA interference; UAS, upstream activation sequence; VDRC, Vienna Drosophila Resource Center.

locus but in their whole chromosome 2; thus, mutant analysis provides an important complement to this study system. Studies using mutants of *for* (and its homologs in other species) have not only unequivocally proven that *for* modulates many of the rover- and sitter-associated phenotypes but have also shown that many of *for*'s functions are conserved. In *D. melanogaster*, the regulation of larval path length, food intake, and fat storage was shown using a *for* null mutant, genomic

duplication alleles, transgenic rescues, and overexpressors linearly regulated by gene dosage (1). Similarly, the *for* null mutant in conjunction with tissue-specific rescues and knockdowns was used to causally link *for* to larval nociception, nerve terminal growth, neurotransmission, and synaptic vesicle recycling (20, 21). Furthermore, P-element insertions that affect *for* expression were used to show that the *for* protein P1 isoform regulates habituation, a simple form of learning (29).

In nematodes, *egl-4* mutant alleles affect almost the whole repertoire of the *Drosophila for*-related phenotypes. These phenotypes include the alternate roamer and dweller behavioral states, food-related movement patterns, fat storage, food intake, sleep, and olfactory adaptation (40, 52, 59, 61, 83). Similarly, mouse mutants of *for*'s homolog *Prkg1* (*Pkg1*) differ from wild type in many of the same phenotypes, including food-seeking behavior (28), sleep (35), learning and memory (35, 76, 77), neuronal excitability (34, 56), and fat storage (43). Curiously, although *for* affects many similar phenotypes across species, the mode of action by which *for* regulates these phenotypes likely differs. For instance, whereas *for* mutants in fruit flies are fatter (1), in mice and nematodes they are leaner (43, 59). This finding suggests that *for* may act in distinct regulatory pathways to regulate the same phenotype in different species. Animal mutant models are also useful in the functional interpretation of human studies. For instance, whereas the correlative studies of genetic variation in *for*'s human homolog *PRKG1* and substance abuse do not functionally implicate *PRKG1* in this GxE interaction (45, 82), studies of *Prkg1* mutant mice confirm a functional role for *Prkg1* in reward and addiction (28).

Gene Structure and Regulation

for's diversity in function and its intricate relationship to behavior in diverse species are perhaps not surprising if one considers the complexity of this gene in terms of its gene products and their spatiotemporal regulation. *for*'s gene structure and complexity vary across species (**Table 2**). The most complex versions of the gene are found in *D. melanogaster* and *C. elegans*, with 21 and 17 transcripts and 12 and 8 potential protein isoforms, respectively. In humans, mice, and eusocial insects, on the other hand, only 3–6 transcripts and 2–4 known protein isoforms have been reported. Nevertheless, it is important to note that the gene structure and function have been best characterized in fruit flies, and more transcripts, protein isoforms, or both might be discovered in other species in time.

In *D. melanogaster*, *for* has 4 promoters that give rise to 21 different transcripts, encoding 12 potential protein isoforms (1). These promoters are independently regulated, expressing in different tissues at different times and affecting different phenotypes (2, 5, 21) (**Figure 2**). In the last two years, some striking examples of this functional independence have been characterized. The first of these studies found that the rover–sitter differences in expression from promoter 4 (but not the other three promoters) are regulated by the epigenetic regulator G9a (5). This histone methyltransferase, one of the many epigenetic regulators involved in *Drosophila* behavior (4), methylates histones in the promoter 4 region to a higher degree in rovers, which have lower expression of this promoter in their central nervous system (CNS) and ovaries than sitters. This difference in methylation/expression was shown to underlie the rover–sitter differences in adult foraging behavior, which could be transgenically rescued by promoter 4–specific RNAi knockdown in sitters (5). Promoter 1 (but not the other promoters) was recently shown to regulate larval nociception (21). Activating promoter 1–positive cells optogenetically results in escape-like curling and rolling behavior, a phenotype that is impaired in *for* null mutants and can be rescued by reintroducing *for* in a promoter 1 expression pattern. This correlates with findings that rovers have increased expression of promoter 1 in their larval CNS and are more sensitive to nociception (21). Like *for* null mutant fruit flies, *Prkg1*-deficient mice have impaired nociceptive reflexes owing to

Table 2 ***foraging*** **gene structure and products in species with known behavioral effects**

Species (common name)	Gene name	Gene size (kb) genomic/CDS	Promoters/ TSS	Transcripts/splice variants	Protein isoforms	GenBank accession no.
Drosophila melanogaster (fruit fly)	*for*	29.74/0.76–3.27	4	21	12	NT_033779
Homo sapiens (human)	*PRKG1*	1307.2/0.85–2.02	4	6	2	NC_000010
Mus musculus (mouse)	*Prkg1*, *cGKI*	1200.55/2.02–2.06	2	6	2	NC_000085
Caenorhabditis elegans (nematode)	*egl-4*	3.1/0.59–2.35	7	17	8	NC_003282
Pristionchus pacificus (nematode)	*Ppa-egl-4*	ND/2.58	NT	NT	NT	EU375876
Apis mellifera (honey bee)	*Amfor*	79.87/2.03–2.22	3	4	1	NC_037650.1
Bombus terrestris (buff-tailed bumble bee)	*Btfor*	114.39/1.70–2.03	4	4	4	NC_015774
Bombus ignitus (bumble bee)	*Bifor*	ND/2.09	NT	NT	NT	dbj_AB491725.1
Vespula vulgaris (common wasp)	*Vvfor*	ND/2.04	NT	NT	NT	EF136648.1
Pogonomyrmex barbatus (red harvester ant)	*Pbfor*	53.73/2.03–2.33	3	3	3	NW_011933670
Pheidole pallidula (ant)	*Ppfor*	ND/1.84–3.92	NT	2	2	EF999976, EF999975
Cardiocondyla obscurior (ant)	*Cofor*	ND	NT	NT	NT	N/A
Allomerus octoarticulatus (ant)	*Aofor*	22.19/2.03	NT	NT	NT	KX809576
Locusta migratoria (migratory locust)	*Lmfor*	ND/0.56	NT	1	1	FJ214985
Schistocerca gregaria (desert locust)	*Sgfor*	ND/0.56	NT	NT	NT	FJ214985
Xenopus laevis (African clawed frog)	*prkg1.S*	413.44/2.02–2.06	2	2	2	NC_030737.1

Abbreviations: CDS, coding sequence; N/A, not available; ND, not determined; NT, not tested; TSS, transcription start site.

developmental defects in axonal growth (90, 102). Last, in another recent study, *for*'s four promoters were shown to have tissue-specific expression patterns in larvae, regulating different larval food-related phenotypes (2). Promoters 1, 3, and 4 regulate larval path length, fat storage, and food intake, respectively (2).

Considering the findings above, the questions that come to mind are, How is *for* regulated to give rise to these spatiotemporal expression patterns, and how does this factor into the regulation of the rover–sitter phenotypes? Unsurprisingly, there does not seem to be a simple answer. The rover and sitter strains show marked differences in expression from the different promoters (1, 5, 21) as well as the different protein isoforms. Also, unsurprisingly, these differences are tissue dependent (5, 21). What underlies these differences in expression is likely differential binding of transcription factors and transcriptional regulators due to the many SNPs found between the

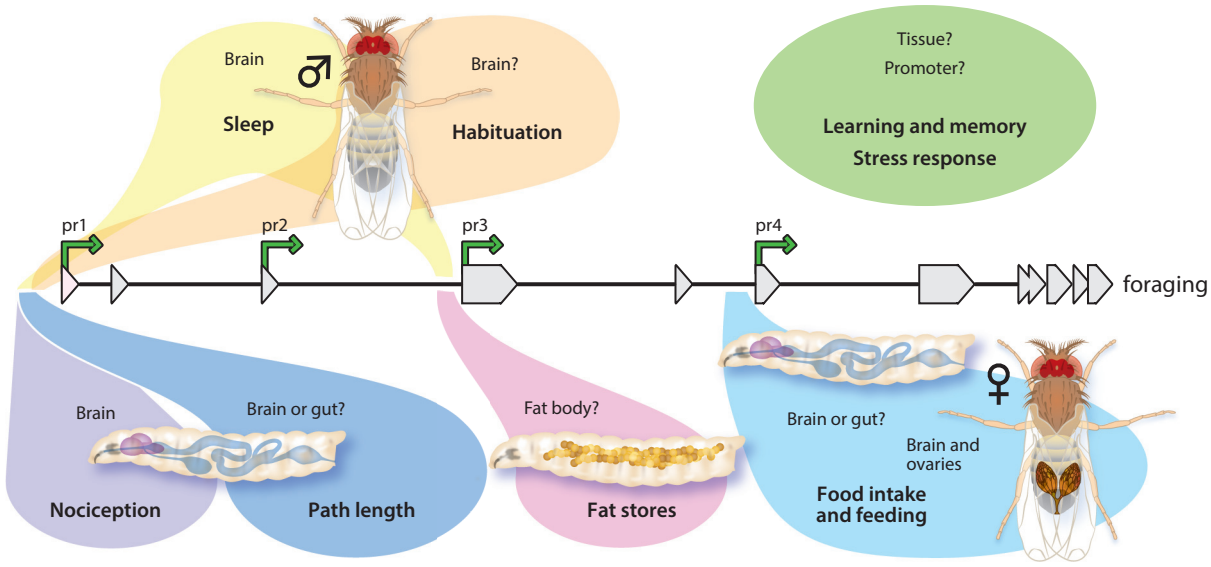

Figure 2

Pleiotropic effects of *foraging*'s promoters. In *Drosophila*, each of the *foraging* gene's promoters regulates distinct behavioral phenotypes in a variety of tissues. Promoter 1 regulates larval nociception (via expression in a neuronal circuit) (21), larval path length (tissue uncertain) (2), and adult sleep (mushroom bodies) (27). Promoter 3 affects fat stores in larvae (tissue uncertain) (2). Habituation is regulated by promoter 1, 3, or both (in olfactory receptor neurons and mushroom bodies) (29). Promoter 4 regulates feeding behavior (adult female brain or ovaries) (5) and in larvae (tissue uncertain) (2). Promoter 2 has so far not been associated with a phenotype; different forms of learning and memory and stress response regulated by *foraging* have not yet been associated with specific promoters or their associated tissues. Abbreviation: pr, promoter.

rover and sitter *for* DNA sequences (5, 7). Which SNP is responsible for rover–sitter differences in larval foraging path length is still not known, likely because the differences might originate from a collection of SNPs rather than from a single SNP. However, recent advances in elucidating the expression differences that underlie some of *for*'s phenotypes have provided starting points for studies of candidate SNPs (2, 5, 21).

While it was originally thought that rover–sitter differences were due to the higher PKG activity in rover heads (65, 74), it is now clear that the relationship is more complicated. For instance, the rover–sitter difference in adult foraging behavior arose from expression levels of promoter 4, which are higher in sitters than in rovers (5). In contrast, the rover and sitter larval path-length phenotypes are associated with promoter 1 expression differences, with rover having higher expression than sitters, at least in dissected CNSs. The transcriptional regulation underlying *for*'s behavioral phenotypes, including rover–sitter differences, thus must be investigated case by case. Furthermore, different promoters and phenotypes are likely regulated by different transcriptional regulators. G9a, for instance, regulates promoter 4 expression in rovers and sitters to affect adult foraging behavior, but so far other promoters and phenotypes do not seem to be regulated by G9a (5, 7).

PLEIOTROPY

for has long been known for its behavioral pleiotropy. Considering the current knowledge of the gene's structure across species, its complexity might correlate with the number of phenotypes that it regulates. In species in which *for* regulates many phenotypes (e.g., fruit flies, nematodes), the

gene structure is more complex, with more promoters, transcripts, and protein isoforms, than in species in which *for* has been associated with fewer behaviors (e.g., eusocial insects) (**Table 2**). Future research will determine whether the relationship between the species-specific complexity of *for*'s gene structure and the extent of pleiotropy holds in other species.

As defined above, behavioral pleiotropy involves different behaviors regulated independently by a single gene. This can be accomplished by a gene's multiple gene products, spatiotemporal expression patterns, or different interacting partners and molecular pathways. In the case of *for*, a combination of all these options is likely involved.

In fruit flies, independent *for* phenotypes are regulated by different promoters (**Figure 2**). Larval path length, nociception, and habituation are regulated by promoter 1 (2, 21, 29), larval fat storage is regulated by promoter 3 (2), and larval food intake and adult feeding behavior are regulated by promoter 4 (2, 5).

All the *for* transcripts encode a cGMP-dependent-binding and protein kinase; however, their predicted proteins vary in their substrate recognition, cGMP binding, and dimerization domains (1). Thus, functional complexity can be accomplished by these different proteins having different interacting partners in different cells. The expression patterns of *for*'s transcripts vary both by tissue (2) and by developmental stage (16, 42, 71). Even within the same phenotype or the same promoter, the regulation of *for*'s function is nuanced. Different types of learning and memory, for instance, require *for* expression in distinct brain areas (68, 108). Similarly, promoter 1 regulates both nociception and habituation by acting in distinct brain circuits (21, 29).

In mammals, for which *prkg1* has only two known transcripts, functional pleiotropy seems to be accomplished by development and tissue-specific functions. *prkg1* regulates fear memory and long-term potentiation in adult mice (76) through cAMP response element (CRE)-binding protein (CREB)/CRE-mediated gene expression in neurons of the lateral amygdala (77). *prkg1* also regulates long-term potentiation in the hippocampus, but only in juvenile mice and not in adults (55, 56). *prkg1*'s role in fat regulation is also tissue specific. Overexpression of *prkg1* results in lean mice that are protected against diet-induced weight gain (69). This effect is mediated by muscle mitochondrial biogenesis, which increases energy consumption and thus reduces weight gain (69). Conversely, genetic or diet-induced obesity diminishes *prkg1* signaling in gonadal or visceral white adipose tissue, but not in inguinal or subcutaneous white adipose tissue (88). Similarly, *PRKG1* expression is reduced in visceral adipose tissue but not in subcutaneous adipose tissue of obese humans (88). Furthermore, *prkg1* mouse mutants were deficient in brown fat cell differentiation and normal thermogenesis (43). Taken together, these studies show that *prkg1* plays a role in regulating fat and metabolism in muscle and different types of adipose tissue, but the effects and mode of action of *prkg1* seems to be tissue specific. Although less work has been done on tissue-specific functions of *for* in fruit flies, it is likely that many such functions will be discovered in the future. For instance, *for* plays a triple role in the *Drosophila* larval neuromuscular junction (NMJ), regulating synaptic growth in glia and synaptic vesicle exocytosis and endocytosis in neurons (20).

DISCUSSION

In this article, we focused on *for*'s role in the regulation of behavior. Nevertheless, *for* also regulates phenotypes that are not strictly behavioral. The most blatant of these phenotypes is the lethality of the homozygous *for* null mutants. While *for* null mutant fruit flies are pupal lethal (1), *Prkg1* mutant mice die as juveniles (80), but which function of *for* underlies this lethality is currently not known. Such a vital role for a complex behavioral gene was first exemplified with the *fruitless* (*fru*) gene, which shares remarkable parallels with *for*. *fru* is also highly pleiotropic, has four promoters, and is pupal lethal, and distinct transcripts regulate its sex-specific behavior and lethal functions

(3, 13). As suggested by recent Nobel Laureate Jeffrey C. Hall, this multifunctionality might well be a common theme among genes that specify behaviors (13).

Behavior is intimately linked to development and physiology, and *for* plays a role in these processes as well. At the *Drosophila* NMJ, *for* affects neuronal signaling by regulating synaptic vesicle exocytosis and endocytosis (20). This effect is acute rather than developmental, as the phenotype of the *for* null mutant can be replicated by acutely inactivating FOR protein at the NMJ (20). Independent of regulating synaptic vesicle exocytosis and endocytosis, *for* also developmentally affects axonal growth at the NMJ, an effect likely driven by promoter 3 expression in glia (20), and axon guidance in the embryo (78). Similar to this neurodevelopmental role in fruit flies, *prkg1* affects neuronal migration (26) and axonal growth (90) in mice. Furthermore, *for* pleiotropically affects *D. melanogaster* Malpighian tubule (i.e., the *Drosophila* kidney/liver) physiology (65, 66). Different *for* transcripts are expressed in distinct cells of the Malpighian tubule membrane and this differentially affects fluid transport in response to stimulants (66). Rovers and sitters also differ in this phenotype: Sitters are more sensitive to fluid transport stimulation and have slightly less PKG activity as well as reduced cGMP levels in Malpighian tubules, perhaps due to higher cGMP–phosphodiesterase (an enzyme that breaks down cGMP) activity (65).

The downstream and upstream interactors of *for* likely play a central role in mediating the gene's pleiotropic effects. Although some interactors have been identified in different contexts, to date only the histone methyltransferase G9a has been shown to regulate behavior through an interaction with *for* (5). Nevertheless, other putative interactors are worth mentioning. The gene *lilliputian* (*lilli*) lies close to *for* on the 2L chromosome. The transposable element 189Y, which lies in *lilli* (108), was originally thought be inserted in the *for* gene (74) and has been associated with a variety of *for*-regulated phenotypes. Among these are larval path length (74), FOR protein expression (74), habituation (32), and neurotransmission (85). *PRKG1*'s association with maternal sensitivity might arise through its role of phosphorylating the serotonin transporter (99, 111). Other known downstream targets of *for* are CREB, through which *for* might act in learning and memory (77); PP2A, involved in *for*'s function in thermotolerance (22); and the transcription factor *lola*, which might mediate *for*'s effects on axon guidance (78). In bees both *lilli* and *CREB* are master regulators of transcription in the brain; their expression predicts the expression of more than 2,000 genes regulating transcriptional networks in the brain that correlate with behavioral states (110).

As discussed in this article, *for* is a prime example of a gene that contributes to behavioral pleiotropy and behavioral variation. With recent advances in molecular analysis, *for* promises to transition to be an equally effective model for understanding the molecular mechanisms underlying behavioral pleiotropy. The gene regulatory mechanisms underpinning individual differences in behavior are of wide interest, with several exciting examples in humans (54), voles (73), and fruit flies (5) emerging in recent years. We are confident that in future years many more such examples will emerge and that genes like *foraging* will provide a base for such investigations.

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