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Job switching in ants
Role of a kinase

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Abbreviations: *for*, *foraging* gene; PKG, cGMP dependent protein kinase; *ppfor*, *Pheidole pallidula foraging* gene

Reproductive division of labor is a defining characteristic of eusociality in insect societies. The task of reproduction is performed by the fertile males and queens of the colony, while the non-fertile female worker caste performs all other tasks related to colony upkeep, foraging and nest defence. Division of labor, or polyethism, within the worker caste is organized such that specific tasks are performed by discrete groups of individuals. Ordinarily, workers of one group will not participate in the tasks of other groups making the groups of workers behaviorally distinct. In some eusocial species, this has led to the evolution of a remarkable diversity of subcaste morphologies within the worker caste, and a division of labor amongst the subcastes. This caste polyethism is best represented in many species of ants where a smaller-bodied minor subcaste typically performs foraging duties while larger individuals of the major subcaste are tasked with nest defence. Recent work suggests that polyethism in the worker caste is influenced by an evolutionarily conserved, yet diversely regulated, gene called *foraging (for),* which encodes a cGMP-dependent protein kinase (PKG). Additionally, flexibility in the activity of this enzyme allows for workers from one task group to assist the workers of other task groups in times of need during the colony's life.

In a recent article, Lucas and Sokolowski[1] report that PKG mediates behavioral flexibility in the minor and major worker subcastes of the ant *Pheidole pallidula*. By changing the task-specific stimulus (a mealworm to induce foraging or alien intruders to induce defensive behavior) or pharmacologically manipulating PKG activity, they are able to alter the behavior of both subcastes. They also show differences in the spatial localization of the FOR protein in minor and major brains. Furthermore, manipulation of *ppfor* activity levels in the brain alters the behavior of both *P. pallidula* subcastes. The *foraging* gene is thus emerging as a major player in regulating the flexibility of responses to environmental change.

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Evolutionary Conservation of the *foraging* Gene

To date the *foraging* gene is found in 42 species and in several taxa including bacteria, insects, nematode, fish, birds and mammals.[1-12] In several species *for* is associated with natural behavioral variations linked to foraging activities. This link was first described in *Drosophila melanogaster* where *for* allelic variants (rover, *for*^{R}, and sitter, *for*^{s}) produce a natural polymorphism in foraging behavior.[6] Rover larvae have longer foraging trails, higher *for* mRNA and PKG activity levels as compared to sitters. These traits continue into adulthood where adult rovers display greater post-feeding locomotion.[13] Drosophila *for* is implicated in metabolic plasticity permitting the adoption of alternative metabolic strategies in nutritionally stressful environments.[14] In the honey bee *Apis mellifera*, *Amfor* plays a role in the long term behavioral transition from nursing to foraging that occurs as workers mature. In the worm *Caenorhabditis elegans*, mutating *for*'s counterpart *egl-4* alters foraging and olfactory adaptation, in addition to body size and lifespan.[2,3,15] In flies and honey bees, increases in PKG lead to more foraging whereas in *C. elegans* and species of ants investigated to date (*Pheidole pallidula*, *Pogonomyrmex barbatus* and *Solenopsis invicta*) decreases in PKG lead to foraging. These findings suggest that although the regulatory pathways underlying this gene may differ, the behavioral function of *for* may be evolutionarily conserved across species.

foraging Gene and Behavioral Polyethism

In *D. melanogaster* and *C. elegans*, allelic variation of the *for* gene produces distinct behavioral phenotypes. Alternatively, in the eusocial honey bee, members of the worker caste display an age-related transition in behavioral repertoires, known as temporal polyethism, where young workers perform tasks such as nursing inside the colony and move outside the nest to fill the role of foragers only later in life.[10,16] Temporal polyethism in *A. mellifera* is correlated with *for* mRNA expression.[10,17]

Nearly all species of eusocial insects display temporal polyethism, but as mentioned above, in some species the division of labor within the worker caste has led to the evolution of morphologically distinct groups, or subcastes, of workers. The most highly derived form in the evolution of physical castes, complete dimorphism, is displayed by the ant *Pheidole pallidula*, where the worker caste is subdivided into two morphologically distinct size groups; members of the two subcastes ordinarily display distinct behavioral repertoires in the same environment.[16] The subcaste of major workers (soldiers) have enlarged heads that house hypertrophied mandibular muscles and large mandibles, and are primarily involved in colony defence and patrolling. The subcaste of minor workers (foragers) are smaller and perform foraging tasks. We have shown that the behavioral differences between subcastes are linked to different levels of PKG activity by manipulating foraging and defence stimuli. Interestingly, pharmacological manipulation of PKG activity is enough to stimulate foraging behavior in majors. This implies a causal relationship between PKG activity, behavioral flexibility and the environmental stimulus. Although many genes are involved in the development and regulation of caste polyethism in *P. pallidula*, altering *ppfor* activity alone changes foraging and defence behavior in a predictable manner. Thus, *ppfor* plays a role in behavioral flexibility in ants.

PKG and Adaptive Foraging

Optimal foraging theory[18,19] predicts that animals weigh the costs and benefits associated with different foraging strategies in order to maximize their fitness. Differences between majors and minors can be seen as an adaptive response of the colony used to moderate the conflict between performing foraging or defensive behavior. Where a solitary animal has evolved to weigh the costs and benefits of performing a given behavior, the worker population of the eusocial colony divides the workforce and thus shares the costs and benefits of the tasks performed by the specialized subcastes. The presence of morphologically distinct worker subcastes in the colony, such as in *P. pallidula* where majors are built for defence and minors for foraging, should confer an advantage upon eusocial species. In natural populations of *P. pallidula* the majors comprise around 10% of the colony population and at this abundance prevent the production of more majors, but in colonies exposed to intraspecific competition the ratio of majors produced by the colony increases.[16,20]

PKG Activity Levels: Predetermination and Flexibility

Flexibility in caste polyethism also permits rapid responses to changing environmental and social stimuli. In ants, it is inefficient for behaviors such as foraging and defence to be performed simultaneously by an individual. One hypothesis emerging from Lucas and Sokolowski[1] is that PKG acts as a molecular switch between foraging and defence. In stable colonies, majors and minors exhibit PKG activity levels characteristic of their subcaste. This "baseline" PKG activity level is likely established early in life concurrently with subcaste determination, perhaps during larval development when the decision point that determines subcaste development is reached.[16] Lucas and Sokolowski[1] show that the different "baseline" PKG activity levels of majors and minors can increase or decrease in response to colony stress (introduction of intruder ants or a live prey item). In a social insect colony, the recruitment of workers to foraging or defence activities fluctuates according to the colony's needs, and the regulation of these behaviors within a subcaste may be most efficiently accomplished by changing the level of a single molecule like PKG. The need to recruit greater numbers of workers to a task than are present within a single subcaste could produce a selection pressure to exploit the pre-existing behavioral regulatory system and lead to the evolution of a molecular switch.

PKG activity was higher in majors than minors in all test conditions. The finding that majors have more FOR-immunoreactive brain cell clusters than minors suggests a neuroanatomical basis for the reported major-minor behavioral differences. Analysis of PPFOR expression in *P. pallidula* worker brain show several clusters of immunoreactivity, some of which are near the central complex, a region known to be involved in spatial perception and navigation. Given the role of this centre in the integration and coordination of sensory inputs with motor output, it is tempting to investigate the potential of the central complex to act as a switch for moving from one behavioral state to another.[21-23] Whether PKG functions in this manner requires more subtle manipulations of PKG enzyme activities and measures of possible thresholds involved in switches between foraging and defence under stressful conditions. The rapid behavioral response (less than 5 min) of *P. pallidula* workers to the foraging and defence stimuli suggests that the PKG protein acts directly on the nervous system, allowing socially-induced behavioral flexibility. This is in contrast to *Amfor*'s role in the more long term transcriptional changes associated with temporal polyethism in honey bees.[10]

Few genes and molecules have been definitively shown to influence social behavior in eusocial insects. We demonstrate that the activity of the foraging gene which encodes a cGMP-dependent protein kinase (PKG) determines whether a worker ant will adopt the role of forager or defender. Majors have more cells in their brains that express PPFOR and higher PKG activity than minors. Environmental stimulation or pharmacological manipulation of PKG activity alters the tendency of both subcastes to forage and defend, demonstrating the pivotal role played by this enzyme in social interactions.

Our study is one of the first to investigate the molecular basis of ant social behavior and provides a framework for future mechanistic and evolutionary studies. It also provides a means to characterize the role of PKG in the evolution of physical castes, and has identified candidate regions of the CNS to be targeted in future investigations. Localization of PKG in the brain also suggests a role for this molecule in the integration and interpretation of context-specific sensory input that is required for the generation of appropriate behavioral output. PKG is known to mediate flexibility in an array of disparate behaviors, such as feeding, food search behavior, stress tolerance, learning and memory, and now defence.[24] This recurring theme across such a wide variety of organisms suggests that the foraging gene and the PKG molecule that it encodes may have evolved as a general behavioral modifier.

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