Molecular Ecology (2005) **14**, 813–818 doi: 10.1111/j.1365-294X.2005.02450.x

Task-specific expression of the *foraging* gene in harvester ants

KRISTA K. INGRAM,* PETER OEFNER† and DEBORAH M. GORDON*
**Department of Biological Sciences, 371 Serra Mall, Stanford University, Stanford, CA 94305, †Stanford Genome Technology Center, 855 California Ave, Palo Alto, CA 94304*

Abstract

In social insects, groups of workers perform various tasks such as brood care and foraging. Transitions in workers from one task to another are important in the organization and ecological success of colonies. Regulation of genetic pathways can lead to plasticity in social insect task behaviour. The colony organization of advanced eusocial insects evolved independently in ants, bees, and wasps and it is not known whether the genetic mechanisms that influence behavioural plasticity are conserved across species. Here we show that a gene associated with foraging behaviour is conserved across social insect species, but the expression patterns of this gene are not. We cloned the red harvester ant (*Pogonomyrmex barbatus*) ortholog (*Pbfor*) to *foraging*, one of few genes implicated in social organization, and found that foraging behaviour in harvester ants is associated with the expression of this gene; young (callow) worker brains have significantly higher levels of *Pbfor* mRNA than foragers. Levels of *Pbfor* mRNA in other worker task groups vary among harvester ant colonies. However, foragers always have the lowest expression levels compared to other task groups. The association between foraging behaviour and the *foraging* gene is conserved across social insects but ants and bees have an inverse relationship between *foraging* expression and behaviour.

Keywords: colony organization, *foraging*, gene expression, qPCR, social insect, task behaviour

Received 29 September 2004; revision received 4 November 2004; accepted 29 November 2004

Introduction

Red harvester ants, *Pogonomyrmex barbatus*, live in large colonies of up to 10 000–12 000 workers in the southwestern deserts of the United States (Gordon 1992). All workers in a colony are morphologically similar, but on a given day, some individuals forage for seeds, while other individuals perform other colony tasks (Gordon 1989). As environmental conditions change, colonies adjust the numbers of workers allocated to specific tasks (Gordon 1996). Task decisions within a colony occur without central control, which leads to the question: what determines when workers forage?

In ants, younger workers tend to remain inside the nest while older workers perform tasks outside, such as foraging (Wilson 1971). In harvester ants, this progression of worker tasks occurs over the course of a year, the approximate lifespan of a worker (Gordon & Holldobler 1987; Fig. 1). It appears that young workers perform tasks related to brood care first inside the nest. They then progress to nest maintenance tasks, with brief trips out of the nest, then to patrolling tasks, with short morning forays from the nest, and finally to foraging tasks (Porter & Jorgensen 1981; Gordon 1989).

Foraging behaviour is associated with a cGMP-activated protein kinase gene (*foraging*) in several insect species (Osborne *et al*. 1997; Ben-Shahar *et al*. 2002). The function of *foraging* was initially described in the food-search behaviour of *Drosphila melanogaster* (Osborne *et al*. 1997). Differences in fruit fly foraging behaviour are linked to alternative alleles that result in changes in abundance of *foraging* mRNA and protein kinase activity. In the honeybee, *Apis mellifera*, the *foraging* gene is implicated in the behavioural division of labour (Ben-Shahar *et al*. 2002). Honeybee foragers have a higher expression of *foraging* than nurse bees, and treatment with cGMP causes precocious foraging in young bees. We cloned the ant ortholog (*Pbfor*) to *foraging* and studied the expression of *Pbfor* to determine whether this gene is associated with foraging behaviour in ants. In addition, we explored whether the expression pattern of *Pbfor* is specific to behavioural task across multiple task groups in field colonies.

Correspondence: Krista K. Ingram, Fax: 650-724-7073; E-mail: kingram@ants.stanford.edu

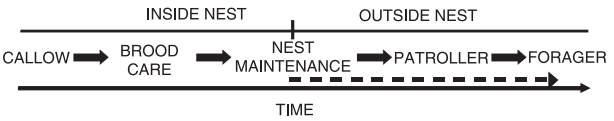

Fig. 1 Sequence of harvester ant tasks. Solid arrows depict hypothesized sequence of task behaviour during the lifetime of a worker. Nest maintenance workers may also become foragers without ever patrolling (dotted arrow).

Materials and methods

Colony collections

Individual workers were collected from six field colonies and from two colonies maintained in the laboratory (laboratory conditions described in Gordon & Mehdiabadi 1999). All colonies were from a long-term study site in southeastern Arizona. In the field, the behaviour of individual workers was observed and recorded for each colony. Workers from five task groups (callows, interior workers, nest maintenance workers, patrollers and foragers) were collected from field colonies. Outside-nest tasks observed were foraging, collecting food; patrolling, scouting the nest and foraging area early in the morning; and nest maintenance, disposing of dirt from excavations of chambers inside the nest. Eight workers from each outside task group were immersed in liquid nitrogen. Nests were then excavated with shovels to collect callow workers (newly enclosed workers deep inside the nest) and interior workers (workers found in the lower brood chambers of excavated nests). In laboratory colonies, callow workers were collected from nest boxes and foragers were collected at the food sources in the foraging arena (Gordon & Mehdiabadi 1999). In order to preserve the actual gene activity under natural conditions, all workers were collected in liquid nitrogen and stored at −80 °C until brain dissection and analysis.

Cloning and sequencing of Pbfor

Conserved blocks of amino acid sequence from protein kinase genes (PKGs) in *Drosophila*, *Apis mellifera*, and mammals were identified with the CODEHOP program (Rose *et al*. 1998). Degenerate primers were designed to identify the ant homolog to *foraging*. The initial sequence of *Pbfor* was obtained by amplifying DNA extractions from harvester ants. Harvester ant-specific primers were designed from exon-coding regions and the transcribed regions of the gene were subsequently sequenced from harvester ant cDNA (complementary DNA) (ABI Big-Dye Sequencing technology on ABI 377). The nucleotide and amino acid sequences from the cGMP-binding and kinase domains and the 3′ end of *Pbfor* were aligned to orthologs found in GenBank using SEQUENCHER 3.1.1 (Gene Codes Corporation). A phylogeny was constructed using maximum parsimony methods (Branch and Bound search criteria; gaps were treated as missing data) in PAUP 4.0b10 (Swofford 2002). The heuristic search used 6012 base pairs, yielding 402 parsimony-informative sites. The tree was rooted with a designated outgroup sequence, *Drosophila melanogaster pkg-2*, a functionally distinct gene that arose from a historical duplication event. Tree robustness was assessed using bootstraps (1000 replicates). Amino acid similarity of *Pbfor* was calculated for each ortholog from the sequence alignments.

Northern blot of harvester ant RNA

Three major transcripts of *foraging* have been identified in *D. melanogaster* (Osborne *et al*. 1997), but only one has been found in *A. mellifera* (Ben-Shahar *et al*. 2002). To identify transcript number in *Pogonomyrmex barbatus*, total RNA was extracted from the heads of eight harvester ant workers and purified according to RNeasy Kit protocols (QIAGEN). Four μg of total RNA was loaded onto a formaldehyde-based agarose gel and ran at 5 V/cm. We performed a Northern blot analysis following Northern-Max protocols (Ambion). The membrane was hybridized with a 643 bp DIG-labelled harvester ant probe (Roche Industries), corresponding to the region of approximately 388–603 amino acids in the honeybee *foraging* protein (AAL93136).

Brain dissections and mRNA quantification

Individual worker brains were dissected from frozen heads in 100 μL of PBS and 10 μL of RNAlater (QIAGEN). Total RNA from individual brains was isolated with the RNeasy Mini Kit (QIAGEN). For analysis of *Pbfor* transcripts, 100 ng of total RNA was reverse transcribed with a oligo d(T)n primer according to TaqMan Reverse Transcription Reagents Kit protocols. Highly specific primers and probe were designed for *Pbfor* using PRIMER EXPRESS software (ABI). To normalize samples, 100 ng of total RNA was reverse transcribed with random oligonucleotide primers according to TaqMan Reverse Transcription Reagents Kit protocols for analysis of ribosomal transcripts. Levels of *18S rRNA* in individual brains were measured using specific primers from the 18SrRNA Kit (ABI).

To measure mRNA levels of *Pbfor* in individual ant brains, real-time quantitative reverse transcription–polymerase chain reaction (qRT–PCR) techniques were used on an ABI 7700 with TaqMan PCR reagents and protocols. Levels of *Pbfor* mRNA and *18S rRNA* were quantified based on the number of PCR cycles (Ct) at which samples crossed a threshold of flourescence intensity using the 2^{-DDCt} method (ABI User Bulletin 2). Each individual brain was analysed in triplicate and Ct measurements for both genes were averaged over the three replicates per individual (if one of the Ct values differed by > 0.5 cycles from the average of

the other two replicates, it was excluded). The total number of discarded data points in this study represented an average of 5% of the data (6% of target gene values and 4% of *18S rRNA* values).

Expression levels of *Pbfor* were normalized relative to 18S ribosomal RNA expression levels for each individual. In each colony, normalized estimates of *Pbfor* expression were averaged across individuals in a particular task group and these means were converted to mRNA abundance values using an arbitrary scale.

The use of *18S rRNA* as an endogenous control can be problematic if the copy number of the control gene differs dramatically (> 5–10 cycles) from the target gene as the measurement of *18S rRNA* copy number may be limited during cDNA synthesis or because of the dynamic range. The average copy numbers (Ct) of *18S rRNA* per task group ranged from 25.0 to 27.2 cycles. The average copy numbers of target gene mRNA per task group ranged from 30.5 to 31.8 cycles. The average difference in copy number between *18S rRNA* and target gene (among individuals within task groups) is 7.3 cycles with a range from four to 14 cycles. Only four out of 26 task group (per colony) averages had Ct differences between target and control gene greater than 10 cycles.

Using two different protocols for cDNA synthesis of target genes and endogenous control genes can introduce systematic biases in results. In this study, there was more variation in *18S rRNA* Ct values than in target gene Ct values. However, there was no systematic difference in the *18S rRNA* yield across task groups, the most important site of potential error in estimating the relative differences between task groups ($F = 0.487$; $P = 7.45$). One additional concern in using *18S rRNA* as an endogenous control is the possibility of overall differences in the rate of transcription between treatment groups. For example, if foragers had a higher cellular transcription rate than callows, then the relative expression of foraging would be lower in foragers than in callows. The similarity in *18S rRNA* Ct values across task groups suggests that task groups do not differ in *18S rRNA* activity. There were small differences in average *18S rRNA* yields between colonies. These differences could represent a real colony difference (some colony are more or less active than others) or a methodological bias in sample processing. For example, colony 7 had higher *18S rRNA* Ct values than other colonies, but this is not reflected in the relative expression values between task groups.

Analysis of expression patterns

Individual (per colony) ANOVA tests were used to test expression differences between callow workers and foragers in field and laboratory colonies. A two-way ANOVA was used to test for overall expression differences between task groups and between colonies. Individual ANOVA tests and Fisher's PLSD (Protected Least Significant Difference) were used to test for differences in expression patterns across multiple task groups in field colonies and for significant differences between foragers and other colony task groups.

Results

We find significant differences in the expression of *Pbfor* in red harvester ant workers of different tasks. In field and laboratory colonies, callow worker brains have significantly higher levels of *Pbfor* mRNA than forager brains (Fig. 3; $N = 8$ brains per task group/colony). The two-way ANOVA showed an overall difference in task group ($F = 79.3$, $P < 0.0001$) and colony ($F = 8.8$, $P < 0.005$) with significant task by colony interaction ($P < 0.05$). There were no significant differences in *18S rRNA* levels between callows and foragers. The abundance of mRNA transcripts in callow brains is approximately twice of that found in forager brains.

In all four field colonies where workers were measured from multiple behavioural tasks, foragers had lower expression levels than ants of other task groups (Fig. 4; $N = 4$ brains per task group/colony). There is considerable variation among field colonies in the expression pattern of *Pbfor* across tasks. We find no clear pattern of expression differences among tasks other than foraging. For example, the expression levels increase across these tasks in colony 5, but no such trends was observed in other colonies.

The Northern blot analysis shows only one transcript of *Pbfor*, at a size of approximately 3 kb. The protein sequence of the *Pbfor* transcript (AY800387) contains the cGMP binding and kinase domains of a PKG and is 68% similar to PKGs from mammals, 85% similar to the fruit fly ortholog, *for*, and 93% similar to the honeybee ortholog, *Amfor*. The maximum parsimony phylogeny (Fig. 2; CI = 0.93, RI = 0.44, HI = 0.07) shows high similarity between

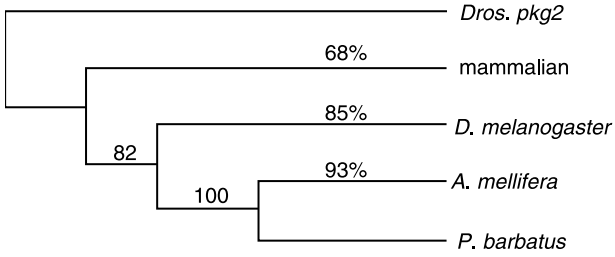

Fig. 2 Maximum parsimony tree of nucleotide similarity in cGMP-activated protein kinase genes (CI = 0.93, RI = 0.44, HI = 0.07). Values in bold type represent amino acid similarity of *Pbfor* (AY800387) to *foraging* orthologs at each branch *Apis mellifera Amfor* (AF469010), *Drosophila melanogaster for* (*dg2*) (NP_477490); and mammalian *pkg1* (077676). Bootstrap values (1000 replicates) are given in small type at the nodes. The outgroup for the phylogeny was a *D. melanogaster pkg2* sequence (NG_000569), a functionally distinct gene that arose from a historical duplication event.

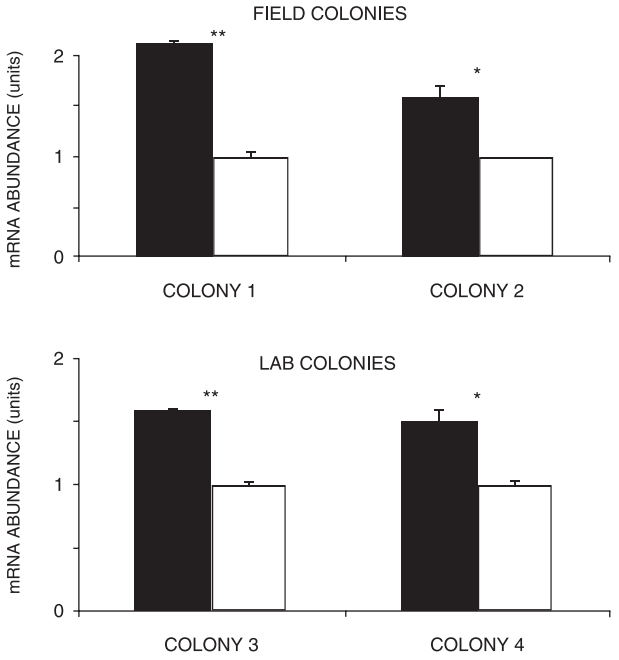

Fig. 3 qRT-PCR analysis of *Pbfor* expression in individual brains of callow workers and foragers from four unrelated colonies. Ants were collected from two colonies in the field and two colonies maintained in the laboratory. All colonies were from a long-term study site in southeastern Arizona. Bars represent mean level of *Pbfor* mRNA relative to *18S rRNA* levels [± SE (converted to same arbitrary scale as the means); $N = 8$ brains per group]. Shaded bars represent callow workers; clear bars represent foragers. A two-way ANOVA shows an overall difference in task group ($F = 79.3$, $P < 0.0001$) and colony ($F = 8.8$, $P < 0.005$) with significant task by colony interaction ($P < 0.05$). Results of individual colony ANOVAs show significant differences between task groups in all four colonies (*$P < 0.05$, ** $P < 0.005$).

ant and bee *foraging* nucleotide sequences. As expected, the social insect *foraging* orthologs (*Amfor* and *Pbfor*) are more closely related to *Drosophila foraging* than to mammalian PKG orthologs.

Discussion

Foraging *in harvester ants*

The expression of *Pbfor* (the ant homolog to the *foraging* gene) in harvester ant workers differs significantly according to task, suggesting that this gene plays a role in task behaviour in ants. The association of this gene with foraging behaviour appears to be conserved across Hymenoptera. Many genes and biochemical pathways are expected to be associated with a behaviour as complex as social foraging. The key to understanding the role of *foraging* in food-related behaviour is to determine what the *foraging* phenotypes in insect species have in common.

Observations from field colonies support the association between *Pbfor* expression and foraging behaviour in red harvester ants. It appears that adult workers first perform tasks inside the nest and only become foragers as older workers. The progression of workers through different task behaviours can be quite variable (Gordon 1989) and we did not uncouple worker age and task in this study. If the expression of *foraging* in ants is entirely age-dependent, this gene may regulate developmental processes rather than foraging behaviour. In honeybees, experimental manipulations that uncoupled age and task revealed that the expression of *foraging* is specific to behaviour (Ben-Shahar *et al*. 2002). The expression pattern of *Pbfor* shows a significant down-regulation only in foragers, not in workers of other outside tasks, suggesting that the gene is associated with the behaviour, rather than the age of a worker ant.

Task switching in harvester ants occurs in response to environmental conditions. Studies of marked individuals in field colonies have shown only some transitions are possible, and most are irreversible (Gordon 1989). For example, a worker may switch from patrolling to foraging, but once a worker switches to foraging, it never switches back

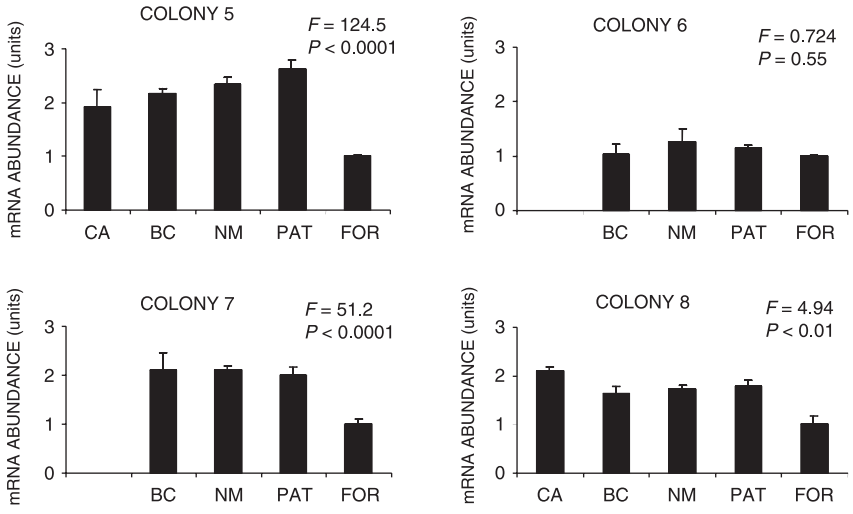

Fig. 4 qRT-PCR analysis of *Pbfor* expression in individual brains of workers from different task behaviour groups in four unrelated field colonies. Inside nest tasks include callow workers (CA) and interior workers (IN); outside nest tasks include nest maintenance (NM), patrolling (PAT) and foraging (FOR). [Callow ants (CA) were not collected from colonies #6 & 7]. Bars represent mean level of *Pbfor* mRNA relative to *18S rRNA* levels [± SE (converted to same arbitrary scale as the means); $N = 4$ brains per group]. Results of individual colony ANOVAs are given for each colony. Foragers have significantly lower levels of mRNA (Fisher's PLSD, $P < 0.05$) than all other tasks in every colony except colony 6.

to other tasks. Thus, the transition to foraging behaviour in ants may be associated with additional genetic and physiological changes that reduce the likelihood of the worker performing other behavioural tasks.

Expression pattern of foraging *differs in ants and bees*

Interestingly, our results show that the relationship between *foraging* expression and foraging behaviour differs in ants and bees. Expression of *foraging* is up-regulated in honeybee foragers and down-regulated in red harvester ant foragers. An important implication of this result is that while similar behaviours and genetic pathways may be conserved across species, the regulation of these pathways may evolve. During the independent evolution of social behaviour in ants and bees, different regulatory mechanisms were used to harness the same genetic pathways for the same function, foraging behaviour.

Differences in the expression of *foraging* in ants and bees may reflect both evolutionary history and ecological pressures. Evidence suggests that physiological correlates of age-related polyethism evolved independently in advanced species of Apidae and Vespidae. In primitively eusocial bees and wasps (including *Polistes*), foragers tend to have low JH (juvenile hormone) titres relative to other task/age groups (O'Donnell & Jeanne 1993). In honeybees, JH titres increase with age with foragers having the highest JH titres (Jassim *et al*. 2000). Similarly, in *Polybia occidentalis*, a highly eusocial wasp, topical application of a JH analogue accelerates the rate of age polyethism (O'Donnell & Jeanne 1993). Understanding the role of JH and associated neuroendocrine pathways in red harvester ants may aid in interpreting how gene regulation and physiology interact to mediate task behaviours across different social insect species.

The natural history of foraging behaviour also differs in ants and bees. Forager bees externally collect pollen and ingest nectar for storage. Harvester ant foragers gather food for storage but do not ingest the seeds they carry. In this species, it is the larvae and young workers that consume most of the food (MacKay 1985). Thus, if *for* is related to feeding behaviour in harvester ants, we would expect the observed pattern. However, previous studies of honeybees have hypothesized that *for* is related to the development of visual processing systems and positive phototaxis (Ben-Shahar *et al*. 2002, 2003). Expression differences may be linked to the processing of visual information which is extremely important for honeybee foraging (Ben-Shahar *et al*. 2003) but is much less so for many ant species, including harvester ants. Comparisons of *foraging* expression patterns in species of ants that use visual landmarks in navigation and foraging may help elucidate the connection between visual processing and *for* gene expression in social insects.

The association of *foraging* with visual information processing suggests that expression of this gene may depend on daily activity rhythms. This study did not measure fluctuations of *Pbfor* expression in red harvester ants because workers from separate task groups were collected simultaneously. Ben-Shahar *et al*. (2003) found that cGMP treatment had no effect on the period of rhythmicity or the onset of circadian rhythms in honeybees. The association between circadian rhythmicity and behavioural development in social insects remains an intriguing direction for future research.

An interesting alternative function for PKGs comes from studies of learning in mice. Knockout mice lacking cGMP-dependent protein kinase I in Purkinje cells in the cerebellum exhibited impaired motor learning skills, particularly for the vestibulo-ocular reflex (Feil *et al*. 2003). c-GMP PKGs may be involved in visually based motor learning, a critical component of the dance language and orientation trips involved in honeybee foraging, but perhaps less necessary for ants that rely on chemical communication.

It is also possible that differences in gene expression patterns between species may not represent separate molecular pathways or functions. Gene expression patterns do not always reflect gene activity patterns. For example, it is possible that *foraging* in red harvester ants is involved in a negative feedback loop in which ants with low gene expression actually have higher protein kinase enzyme activity. If this were the case, the enzyme activity patterns would be similar across ants and bees. We are currently analysing the PKG enzyme activity in task groups of harvester ants.

cGMP signalling and food-related behaviours

The association of *foraging* gene expression and foraging behaviour in ants suggests that the influence of cGMP-activated protein kinase pathways on foraging behaviours is conserved across some insects, although the mechanism of regulation differs in flies, bees and ants. However, the association between cGMP signalling pathways and food-related behaviours is not limited to insects (Sokolowski 2002). A cGMP-activated kinase pathway influences the feeding behaviours of the nematode, *Caenorhabditis elegans* (Fujiwara *et al*. 2002), and social feeding aggregations of *C. elegans* involve a cGMP-gated ion channel (Coates & de Bono 2002). It is interesting to note that the mechanism for high locomotor feeding activity in *C. elegans* involves a decrease in the PKG protein, similar to the down-regulation of *Pbfor* in the red harvester ant, while high locomotor feeding activity in *Drosophila* and foraging behaviour in honeybees involve an increase in PKG protein. This suggests that while genetic pathways involved in similar behaviours may be conserved across a broad range of species, the mechanisms underlying the regulation of behaviours can evolve.

Acknowledgements

We would like to thank S. Palumbi, M. Feldman, R. Kendall, and J. Kendall for comments on a previous version. We are grateful to A. Filipowicz and M. Greene for assistance in the field collections and to D. Petrov for lab space. This work was supported by NIH-NIGMS NRSA (KKI) and by funding from the Stanford Genome Technology Center.

References

Ben-Shahar Y, Robichon A, Sokolowski MB, Robinson GE (2002) Influence of gene action across different time scales on behavior. *Science*, **296**, 741–744.

Ben-Shahar Y, Leung HT, Pak WL, Sokolowski MB, Robinson GE (2003) cGMP-dependent changes in phototaxis: a possible role for the *foraging* gene in honeybee division of labor. *Journal of Experimental Biology*, **206**, 2507–2515.

Coates JC, de Bono M (2002) Antagonistic pathways in neurons exposed to body fluid regulate social feeding in *Caenorhabditis elegans*. *Nature*, **419**, 925–929.

Feil R, Hartmann J, Luo C *et al*. (2003) Impairment of LTD and cerebellar learning by Purkinje cell-specific ablation of cGMP-dependent protein kinase I. *Journal of Cell Biology*, **163**, 295–302.

Fujiwara M, Sengupta P, McIntire SL (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron*, **36**, 1091–1102.

Gordon DM (1989) Dynamics of task switching in harvester ants. *Animal Behaviour*, **38**, 194–204.

Gordon DM (1992) How colony growth affects forager intrusion in neighboring harvester ant colonies. *Behavioral Ecology and Sociobiology*, **31**, 417–427.

Gordon DM (1996) The organization of work in social insect colonies. *Nature*, **380**, 121–124.

Gordon DM, Holldobler B (1987) Worker longevity in harvester ants. *Psyche*, **94**, 341–346.

Gordon DM, Mehdiabadi N (1999) Encounter rate and task allocation in harvester ants. *Behavioral Ecology and Sociobiology*, **45**, 370–377.

Jassim O, Huang Z-Y, Robinson GE (2000) Juvenile hormone profiles of worker honeybees, *Apis mellifera*, during normal and accelerated behavioral development. *Journal of Insect Physiology*, **46**, 243–249.

MacKay WP (1985) A comparison of the energy budgets of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Oecologia*, **66**, 484–494.

O'Donnell S, Jeanne RL (1993) Methoprene accelerates age polyethism in workers of a social wasp (*Polybia occidentalis*). *Physiological Entomology*, **18**, 189–194.

Osborne KA, Robichon A, Burgess E *et al*. (1997) Natural behavioral polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science*, **277**, 834–836.

Porter SD, Jorgensen CD (1981) Foragers of the harvester ant, *Pogonomyrmex owyheei*: a disposable caste? *Behavioral Ecology and Sociobiology*, **9**, 247–256.

Rose TM, Schultz ER, Henikoff JG, Pietrokovski S, McCallum CM, Henikoff S (1998) Consensus-degenerate hybrid oligonucleotide primers for amplification of distantly-related sequences. *Nucleic Acids Research*, **26**, 1628–1635.

Sokolowski M (2002) Social eating for stress. *Nature*, **419**, 893–894.

Swofford DL (2002) PAUP*. *Phylogenetic Analysis Using Parsimony (*and other methods), 4.0 edn*. Sinaeur, Sunderland, MA.

Wilson EO (1971) *The Insect Societies*. Harvard University Press, Cambridge, MA.

This work comprises part of K.K. Ingram's postdoctoral research in the laboratories of P. Oefner and D.M. Gordon. The Oefner lab specializes in developing genome technology to study the behaviour and evolution of natural populations. The Gordon lab investigates the behaviour, ecology, and population genetics of ants, including a long-term study on a population of harvester ants.
