Article

THE LOCUST *FORAGING* GENE

C. Lucas
Department of Biology, University of Toronto, Mississauga, Ontario, Canada; Department of Ecology and Evolution, University of Lausanne, Lausanne, CH-1015, Switzerland

R. Kornfein
Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

M. Chakaborty-Chatterjee and J. Schonfeld
Department of Biology, University of Toronto, Mississauga, Ontario, Canada

N. Geva
Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

M. B. Sokolowski
Department of Biology, University of Toronto, Mississauga, Ontario, Canada

A. Ayali
Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Our knowledge of how genes act on the nervous system in response to the environment to generate behavioral plasticity is limited. A number of recent advancements in this area concern food-related behaviors and a specific gene family called foraging (for), which encodes a cGMP-dependent protein kinase (PKG). The desert locust (Schistocerca gregaria) is notorious for its destructive feeding and long-term migratory behavior. Locust phase polyphenism is an extreme example of environmentally induced behavioral plasticity. In response to changes in population density, locusts dramatically alter their behavior, from solitary

Grant sponsors: Natural Science and Engineering Council of Canada; Canada Research Chairs Program; Fyssen Foundation; Canadian Institutes of Health Research.
Correspondence to: A. Ayali, Department of Zoology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, 69978, Israel. E-mail: ayali@post.tau.ac.il

ARCHIVES OF INSECT BIOCHEMISTRY AND PHYSIOLOGY, Vol. 74, No. 1, 52–66 (2010)
Published online in Wiley InterScience (www.interscience.wiley.com).
 DOI: 10.1002/arch.20363

and relatively sedentary behavior to active aggregation and swarming. Very little is known about the molecular and genetic basis of this striking behavioral phenomenon. Here we initiated studies into the locust for gene by identifying, cloning, and studying expression of the gene in the locust brain. We determined the phylogenetic relationships between the locust PKG and other known PKG proteins in insects. FOR expression was found to be confined to neurons of the anterior midline of the brain, the pars intercerebralis. Our results suggest that differences in PKG enzyme activity are correlated to well-established phase-related behavioral differences. These results lay the groundwork for functional studies of the locust for gene and its possible relations to locust phase polyphenism.

Keywords: *foraging* gene; locust; swarming behavior; kinase; density-dependent phase polyphenism

INTRODUCTION

Natural variation in behavior is influenced by interactions between many genes. These genes interact with the environment to generate behavioral plasticity. A remarkable example of environmentally induced behavioral plasticity can be found in locusts. In the desert locust (*Schistocerca gregaria*), solitary-phase locusts are cryptic in physical appearance and behavior (Rogers et al., 2003). They actively avoid contact with other locusts (Roessingh et al., 1993), and are relatively sedentary (Simpson et al., 1999). An increase in population density dramatically alters locust behavior; the gregarious locusts are generally more active, and march in huge bands of hoppers or form flying swarms as adults. The major behavioral characteristic of gregarious-phase locusts is their strong attraction to conspecifics, which translates into active aggregation behavior (Ellis, 1959, 1963; Uvarov, 1966). The phase transformation is thought to be a positive feedback process, with changes in behavior preceding and facilitating other phase-changes including biochemical, physiological, and morphological ones (Pener, 1991; Pener and Yerushalmi, 1998; Applebaum and Heifetz, 1999; Pener and Simpson, 2009). Recent findings have identified the primary sensory inputs inducing a density-dependent phase change in locusts (Rogers et al., 2004). Other neurobiology research has characterized differences in sensory interneurons that mediate behavioral changes between the locust phases (e.g., wind-sensitive interneurons, Fuchs et al., 2003; visual interneurons, Matheson et al., 2004). In marked contrast, little has been achieved in the study of the molecular and genetic contributions to this phenomenon (see recent reviews in Simpson et al., 2005; Simpson and Sword, 2008; Pener and Simpson, 2009). As a result, one is compelled to initiate an investigation into possible candidate genes from other organisms that could be involved in the locust phase-dependent behavioral polymorphism (Fitzpatrick et al., 2005).

In other species, there are a handful of well-studied examples that shed light on the genes and pathways that underlie natural behavioral variations and plasticity. In some cases, natural variation in a single gene can be identified that affects the regulation of complex yet distinct behavior patterns (e.g., de Bono and Bargmann, 1998; Krieger and Ross, 2002; Davies et al., 2004; Hammock, 2007). Interestingly, a number of recent achievements in this area involve food-related behaviors and the

foraging gene, which encodes a cGMP-dependent protein kinase (PKG) (Osborne et al., 1997; Ben-Shahar et al., 2002, 2003; Hirose et al., 2003; Fitzpatrick and Sokolowski, 2004; Ingram et al., 2005; Fussnecker and Grozinger, 2008; Garabagi et al., 2008; Heylen et al., 2008; Lucas et al., 2009; Lucas and Sokolowski, 2009).

The fruit fly, *Drosophila melanogaster,* exhibits a behavioral polymorphism in larval and adult-foraging behaviors, which arises from allelic variation in *for*. Larvae and adult flies with a rover allele (for^R) move greater distances while feeding than those with the sitter alleles (for^s) (Sokolowski, 1980; Pereira and Sokolowski, 1993). The *for* gene encodes a cGMP-dependent protein kinase (PKG) (Reaume and Sokolowski, 2009). Rover heads exhibit higher levels of *for* mRNA and higher PKG activity than sitter heads (Osborne et al., 1997). Interestingly, in flies, crowded populations select for animals with rover alleles whereas uncrowded ones select for those with sitter alleles (Sokolowski et al., 1997). The *for* gene also plays a role in behavioral plasticity in *D. melanogaster* in response to food deprivation (Kaun et al., 2007b). Moreover, the *for* gene was successfully used as a candidate gene to investigate the plasticity of food-related behaviors in other insect species including ants (Ingram et al., 2005; Lucas et al., 2009; Lucas and Sokolowski, 2009) and honey bees (Ben-Shahar et al., 2002).

In the present study, we cloned the locust *for* gene and placed it in an insect phylogeny of cGMP-dependent protein kinases (PKGs). We also examined FOR expression in locust brains and measured the PKG enzyme activities in female and male solitary and gregarious locusts. Our results lay the groundwork for functional studies of the locust *for* gene and its possible relations to locust phase polyphenism.

MATERIALS AND METHODS

Animals

Desert locusts, *Schistocerca gregaria* (Forskål), were reared for many consecutive generations under heavy crowding conditions, 100–160 animals in 60-liter metal cages. Cages were kept under controlled temperature and humidity conditions (30°C, 35–60%) under a 12:12 light-dark cycle (with lights on at 7 am). Direct radiant heat was supplied during daytime by incandescent electric bulbs to reach a final day temperature of 35–37°C. Locusts were fed daily with fresh grass and dry oats. In order to obtain locusts approaching the solitary phase, hatchlings from eggs laid by crowded-reared locusts were isolated within 4 h post-hatching and kept under isolation, one locust per 1.5-liter metal cage. Care was taken to keep locusts of the different phase groups under similar conditions (except density).

Migratory locusts, *Locusta migratoria*, were generously provided by Angela Lange from a colony housed at the University of Toronto (Mississauga, Canada). The locusts were raised under crowded conditions on a 12:12 light-dark cycle, at a temperature range of 30–34°C, and fed fresh wheat seedlings supplemented with bran and carrots.

Cloning the Locust for Gene

mRNA was extracted from 10 brains of *S. gregaria* (5 males and 5 females) or *L. migratoria* (females) dissected in saline PBS solution. All tissues were then quickly placed in RNA later solution until mRNA extraction using an Amersham Biosciences kit (GE Healthcare). Specific cDNA was amplified using a RACE kit from Clontech (Palo Alto, CA) with degenerate primers designed based on conservative regions of *for*

ortholog alignment of several species (sense primer: TGGGCCATYGANCGACARTG, antisense primer: AAGCCMTCRAACCAYTTGTGYTT). The primers were used for both locust species but with different annealing temperature (56°C for *S. gregaria* and 53°C for *L. migratoria*). Amplified bands were cloned with a TOPO TA Cloning from Invitrogen (Carlsbad, CA), using the pCR 2.1-TOPO vector in electrocompetent *E. coli* cells. Vectors from positive clones were extracted, purified, and sequenced. The cloned locust sequences were examined for the extent of their homology with known *for* genes using the NCBI database. After confirming that the newly cloned locust sequences matched known *for* gene sequences, nested specific primers were designed and used with newly extracted mRNA samples (*S. gregaria* sense primer: CCGGCAGGCAGAGTACACCGATTTC, antisense primer: CAACACGGCCAAAACCACCAACTCC; *L. migratoria* sense primer: TGTCAAACTGGTGGATTTTGGTTTTGC, antisense primer: TCGTTCTTGGGAAGTCAATAGCATCG) in order to extend and confirm the cloned locust *for* sequences.

Phylogeny

PKG protein sequences were obtained from the NCBI database using Blastx and nomenclature searches. As of November 2009, a total of 44 PKG proteins were found for over 30 insect species (Table 1) (variant sequences were discarded). Pairwise protein sequence alignments were made using the default settings of Muscle (SeaView; Galtier et al., 1996). Phylogenetic distances were used to build a neighbor joining tree using Phylowin (Galtier et al., 1996) on the conserved kinase domains and the remaining carboxyl terminal residues (around 300 aa) with 5,000 bootstrap replications. Bootstrapping is a resampling technique used to generate confidence estimates for the placement of nodes in phylogenetic trees (Page and Holmes, 1998). Bootstrap values range from 0 to 100 where higher values indicate low sampling error and, therefore, higher support for those nodes.

Immunocytochemistry and Neuronal Staining

Immunohistochemistry was as in Belay et al. (2007). Briefly, whole brains were dissected in PBS (0.1 M, pH 7.4), fixed in 4% paraformaldehyde, and blocked in 4% normal goat serum (Jackson ImmunoResearch, West Grove, PA) in 0.5% Triton X-100/PBS. The specific guinea pig antibody called anti-FOR, described in Belay et al. (2007), was used at 1:150; the neuropile marker mouse mAb nc82 was used at 1:20 (22, 23). Incubation was for 48 h at 4°C. After incubation, brains were washed several times in 0.5% Triton X-100/PBS before adding a goat Cy2-conjugated anti-mouse and a Cy5-conjugated anti-guinea pig Ig (1:100, Jackson ImmunoResearch) for 24 h at 4°C. For negative controls, brains were incubated in only secondary antibody, in the absence of primary antibody or in pre-absorbed anti-FOR serum. The specificity of the primary antibody, anti-FOR antibody generated in guinea pig, was measured by using Western blot immunodetection.

For neurobiotin staining, stumps of the cut nerves (NCC1) were isolated in Vaseline vessels and exposed to distilled water for 5 min. Incubation was in neurobiotin (5% in distilled water, Vector Laboratories, Burlingame, CA) at 4°C or for 2–3 days. After overnight fixation in paraformaldehyde (4% in distilled water), preparations were rinsed in several changes of PBS containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO). Subsequently, tissues were incubated overnight in Cy3-conjugated streptavidine (Amersham Biosciences, Piscataway, NJ) in PBS

Table 1. GenBank Accession Number and Detailed Informations About the Insect Species Used for the Phylogenetic Analysis

Scientific name	*Common name*	*PKG type*	*GenBank*	*Unigene*
Acyrthosiphon pisum	Pea aphid	I	XM_001952056.1	Aps.15600
Acyrthosiphon pisum	Pea aphid	II	XM_001947008.1	
Aedes aegypti	Yellow fever mosquito	I	XM_001652896.1	Aae.12121
Aedes aegypti	Yellow fever mosquito	II	XM_001651359.1	Aae.709
Anopheles gambiae	Mosquito	I	XM_319605.4	
Anopheles gambiae	Mosquito	II	XM_314690.4	
Apis mellifera	Honey bee	I	AF469010.1	
Bombus ignitus	Bees	I	dbj_AB491725.1	
Bombus terrestris	Bees	I	gb_FJ816699.1	
Bombyx mori	Silkworm moth	I	AF465601.1	Bmo.749
Diabrotica virgifera virgifera	Western corn rootworm beetle	I	gb_DQ913742.1	
Drosophila ananassae	Flies	I	XM_001961954.1	
Drosophila ananassae	Flies	II	XM_001961529.1	
Drosophila erecta	Flies	I	XM_001968555.1	
Drosophila erecta	Flies	II	XM_001968175.1	
Drosophila grimshawi	Flies	I	XM_001993194.1	
Drosophila grimshawi	Flies	II	XM_001988546.1	
Drosophila melanogaster	Fruit fly	I	NM_058139.3	Dm.6584
Drosophila melanogaster	Fruit fly	II	NM_057865.3	Dm.4323
Drosophila mojavensis	Flies	I	XM_002003128.1	
Drosophila mojavensis	Flies	II	XM_002003588.1	
Drosophila persimilis	Flies	I	XM_002014941.1	
Drosophila persimilis	Flies	II	XM_002020546.1	
Drosophila pseudoobscura pseudoobscura	Flies	I	XM_001356655.2	
Drosophila sechellia	Flies	II	XM_002041587.1	
Drosophila simulans	Flies	I	XM_002078022.1	
Drosophila simulans	Flies	II	XM_002077639.1	
Drosophila virilis	Flies	II	XM_002052831.1	
Drosophila willistoni	Flies	I	XM_002065449.1	
Drosophila willistoni	Flies	II	XM_002066659.1	
Drosophila yakuba	Flies	I	XM_002087818.1	
Drosophila yakuba	Flies	II	XM_002087431.1	
Lobesia botrana	Moth	I	gb_DQ666642.1	
Locusta migratoria	Migratory locust	I	FJ214984	
Mythimna separata	Northern armyworm moth	I	gb_GQ844298.1	
Nasonia vitripennis	Jewel wasp	I	XM_001603499.1	
Nasonia vitripennis	Jewel wasp	II	XM_001599276.1	Nvi.4830
Pediculus humanus corporis	Human body louse	II	XM_002432638.1	
Pheidole pallidula	Ant	I	EF999975	
Pogonomyrmex barbatus	Red harvester ant	I	AY800387.1	
Schistocerca gregaria	Desert locust	I	FJ214985	
Tribolium castaneum	Red flour beetle	I	XM_968614.2	Tca.5335
Tribolium castaneum	Red flour beetle	II	XM_963625.1	Tca.3123
Vespula vulgaris	Wasp	I	gb_EF136648.1	

containing 0.3% Triton X-100 at room temperature. After rinsing with PBS, preparations were dehydrated in a graded series of ethanol, cleared in 60% glycerol at 4°C overnight and mounted onto polylysine-coated glass slides under Fluorescent Mounting Medium (Golden Bridge Life Science, WA) and cove-slipped.

All labeled preparations were analyzed and photographed using an Olympus inverted system microscope (IX70, Olympus, Tokyo, Japan) equipped with a digital camera or alternatively were examined using a LSM 510 META confocal microscope (Carl Zeiss, Jena, Germany).

PKG Assays

PKG enzyme assays (modified from Kaun et al. 2007b) were performed on dissected brain tissues of *S. gregaria* solitary and gregarious locusts one week after adult emergence. Locusts were briefly anesthetized in CO_2, their brain dissected out and stored at −80°C. Individual brains were homogenized on ice, for males and females of both phases separately, in 25 mM Tris (pH 7.4), 1 mM EDTA, 2 mM EGTA, 0.05% Triton X-100, 5 mM β-mercaptoethanol (Sigma Aldrich), and protease inhibitor cocktail (Roche Diagnostics, Laval, QC, Canada). Samples were sonicated 4 times for 5 sec on ice in a Branson Sonifier 250 (Branson, CT) at the lowest setting (duty cycle 20 and output control 4). Samples were then centrifuged at 10,000 RPM for 5 min at 4°C. The supernatant of each sample was then removed and used to determine the total protein amount using a protein assay kit (Bio-Rad Laboratories, Hercules, CA) prior to the cGMP-dependent protein kinase activity assays. The final concentration of the PKG activity mixture contained 40 mM Tris-HCl (pH 7.4), 20 mM magnesium acetate, 0.2 mM [γ^{32}P]ATP (500–1,000 cpm/pmol) (Amersham, Pharmacia Biotech, Baie D'Urfe, QC, Canada), 13 μg/ml of a heptapeptide substrate highly specific to the PKG (RKRSRAE) (Promega, Burlington, ON, Canada), 3 μM c-GMP (Promega), and 4.6 nM of a highly specific c-AMP-dependent protein kinase inhibitor (Ki50% = 2.3 nM) (5–24 from Calbiochem, San Diego, CA). As a control, reactions were performed in the presence of 468 nM of a PKG inhibitor K-5823 (Calbiochem). The reaction mixtures were incubated at 30°C for 10 min. The reaction was terminated by spotting 70 μl of the reaction mixture onto Whatman P-81 filters, which were then soaked with 75 mM H_3PO_4 for 5 min and washed three times with 75 mM H_3PO_4 to remove any unbound [^{32}P]ATP. Finally, filters were rinsed with 100% ethanol and air dried before quantification. For quantification of PKG activity, counts were taken in a Wallac 1409 Liquid Scintillation Counter (Perkin Elmer, Woodbridge, ON, Canada) using a universal scintillation cocktail (ICN). The specific PKG activity was expressed as pmol of ^{32}P incorporated of the PKG substrate per min per mg of protein. Data were analyzed using two-way ANOVA and presented as means ± S.E. In all figures, $P < 0.001$, $P < 0.01$, and $P < 0.05$ are represented as ***, **, and *.

RESULTS

Laboratory-Reared Locusts Approach the Two Extreme Density-Dependent Phases

The desert locust colony at Tel Aviv University shows clear and very consistent phase differences in response to the distinct rearing conditions used. These included all reported differences in morphometric ratios, coloration and pattern, and development. Further, recently reported neurophysiological differences (Fuchs et al., 2003) and behavioral differences (Geva et al., 2010) are also very robust and consistent.

The Locust Foraging Gene

We isolated a 1,258-bp fragment of the *S. gregaria for* ortholog, which we call *sgfor* (GenBank accession number FJ214985). It had typical PKG domains (Hofmann et al., 2006) including a serine/threonine kinase domain (with a catalytic site) and two cGMP-binding domains. The same degenerate primers and techniques were used to clone the *L. migratoria* for ortholog *lmfor* (GenBank accession number FJ214984). The *lmfor* fragment was 560 bp long and did not differ from a fragment of *sgfor*'s kinase domain, which contained the catalytic site. The *S. gregaria sgfor* DNA sequence has 91% pairwise identity with *L. migratoria*'s *lmfor* sequence. Both locust DNA sequences are around 70% pairwise identical to the *for* gene sequence of *D. melanogaster.* A comparison of the two locusts *for* amino acid sequences and the corresponding region of the fly FOR protein are shown in Figure 1. The kinase domain is well conserved in these species and the catalytic sites are identical. We studied the phylogenetic relationships of the newly-cloned locust *for* sequences as compared to all currently known insect PKG-encoding genes (Fig. 2). Two types of PKG (I and II) are represented on the tree. PKG type I is known to play a role in food-related behaviors and plasticity (reviewed in Lohmann et al., 1997). As shown in Figure 2, all the PKG type I protein sequences cluster together and appear to be derived from PKG type II sequences. Within the type I cluster, we can distinguish a group formed by the dipterans and a group with all the social insects except the wasp *Vespula vulgaris*. The two locust sequences are the locust *foraging* genes and are part of the PKG type I cluster.

FOR Expression in the Locust Brain

The FOR protein was found to be expressed in a very distinct group of neuronal cell bodies of the anterior midline of the brain (Fig. 3A, B). The examples shown in Figure 3C demonstrate a very robust and consistent expression pattern of FOR in the locust

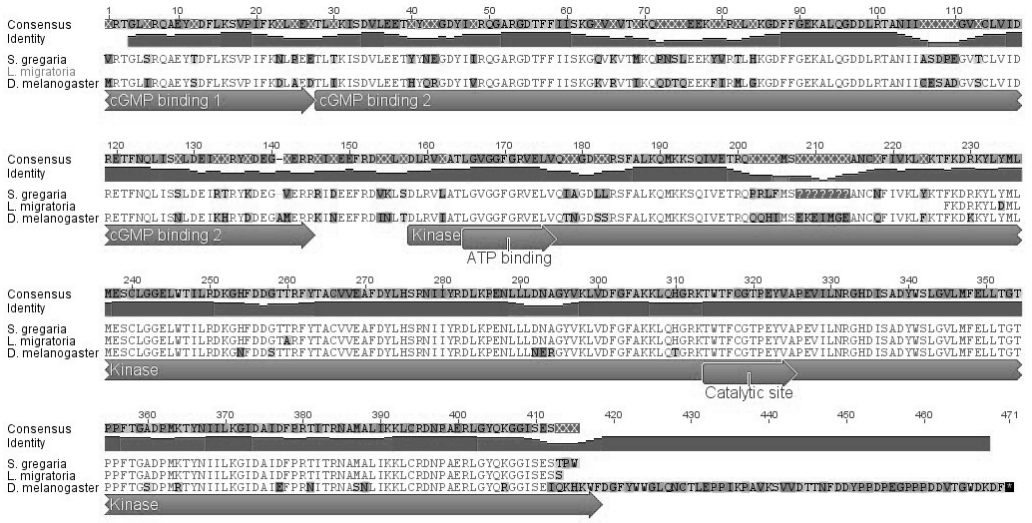

Figure 1. Alignments of amino acid sequence of *S. gregaria*, *L. migratoria,* and *D. melanogaster* using Muscle (SeaView, Galtier et al., 1996). Identity and consensus sequence is shown using Geneious software. The different identified domains of the PKG are shown for *D. melanogaster.*

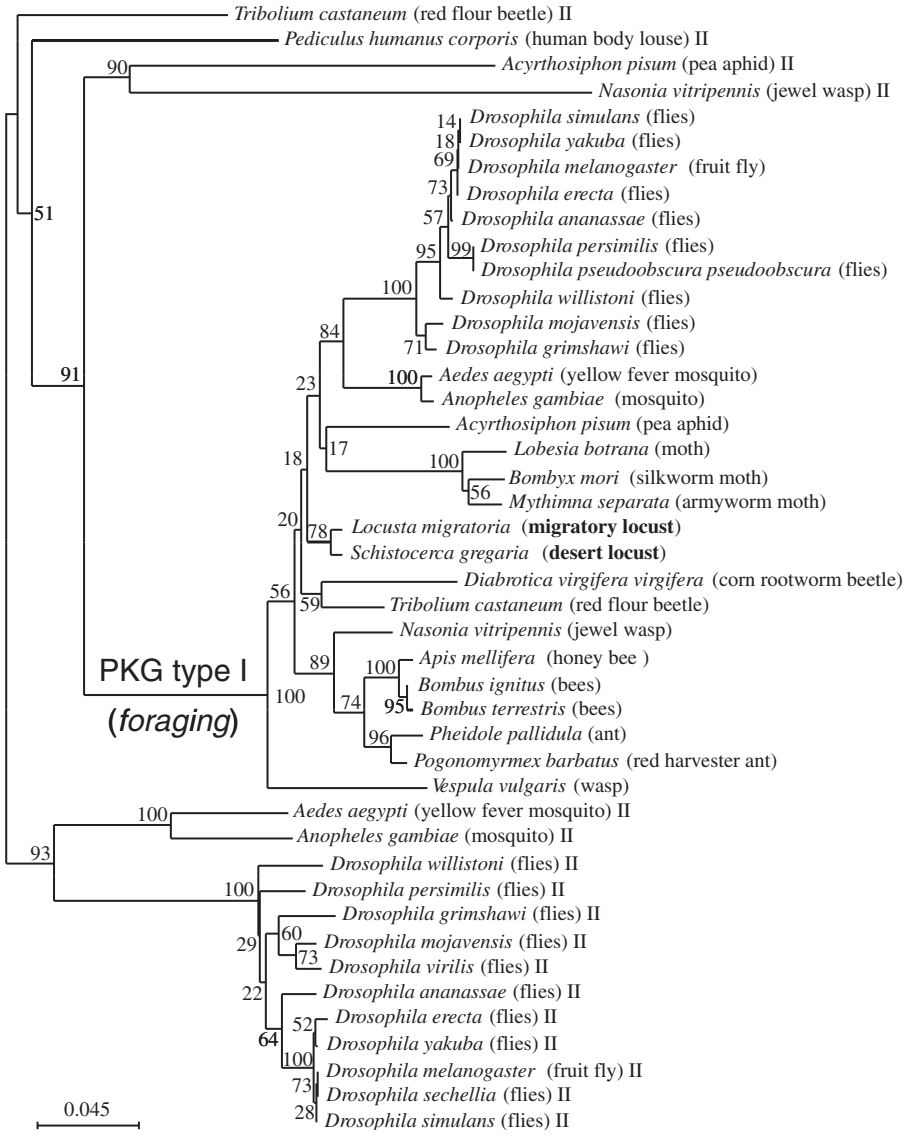

Figure 2. Phylogenetic analysis (Neighbour joining method) of the relationship of 44 PKG sequences spanning 30 insect species (variant sequences were discarded). Pairwise comparisons using 5,000 bootstrap replications were used to build the tree. The 2 locust species are in bold. Further detailed information about the insect species used can be found in Table 1.

brain. Similar staining patterns were observed in adult male and female locusts, as well as in gregarious and solitary-reared animals and also in larvae. The brain area stained is known as the pars intercerebralis (PI) of the protocerebrum. It contains the largest collection of neurosecretory cells in the central nervous system of the locust, most of which have fibers that run directly to the corpora cardiaca (CC) via nervus corporis cardiaci I (NCCI, Burrows, 1996). Hence, in order to confirm the identity of the FOR-positive cells, neurons of the PI were back-filled through the NCCI nerve leading from the PI to the CC. As can be seen in Figure 4A, the area of the brain stained by

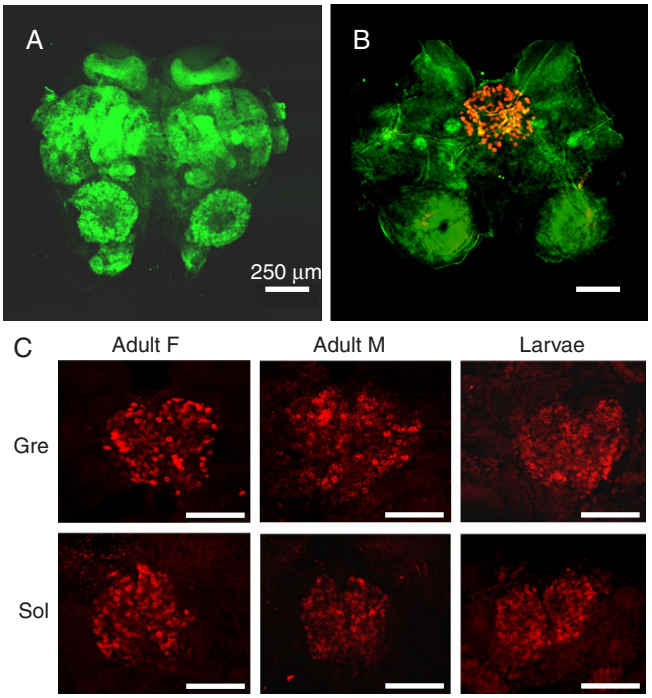

Figure 3. FOR expression patterns in the locust brain. **A:** Merged confocal image of frontal 3-µm optical sections of a locust brain showing the major brain neuropils (in green, mouse antibody, nc82). **B**: A distinct PKG-IR region is seen as a cluster of cell bodies in the anterior midline of the brain (double staining with anti-FOR in red and nc82 in green). **C:** Similar FOR expression patterns can be observed in gregarious (Gre) or solitary-reared animals (Sol), in adult male (M), female (F), and larvae. Scale bars = 250 µm.

backfilling NCCI was very similar to the area expressing FOR. Indeed, when we employed double staining of FOR together with neurobiotin backfilling of the NCCI, many cells within this area showed double labeling (Fig. 4B).

Phase-Related Differences in PKG Activity

The PKG activity of gregarious locusts was found to be significantly higher than that of solitary locusts ($F_{(1,28)} = 13.29$, $P < 0.001$) (Fig. 5). Differences in PKG activity are sex specific ($F_{(1,28)} = 23.16$, $P < 0.001$), with higher PKG activity found in males than in females. No interaction was found between sex and phases ($F_{(1,28)} = 0.17$, $P = 0.68$). This sex difference in PKG activity was found for both solitary ($P < 0.01$) and gregarious locusts ($P < 0.001$).

DISCUSSION

The *foraging* gene functions in plastic responses to environmental change in a variety of animals. In *D. melanogaster*, a previous history of food deprivation changes rovers into sitters and decreases PKG levels (Kaun et al., 2007b, 2008) and *for* plays a role in learning and memory in larval and adult flies (Kaun et al., 2007a; Mery et al., 2007). In *P. pallidula*, the plastic behavior of workers is related to PKG enzyme activity and worker ants who work as guards have five more FOR immunoreactive clusters of cells in their

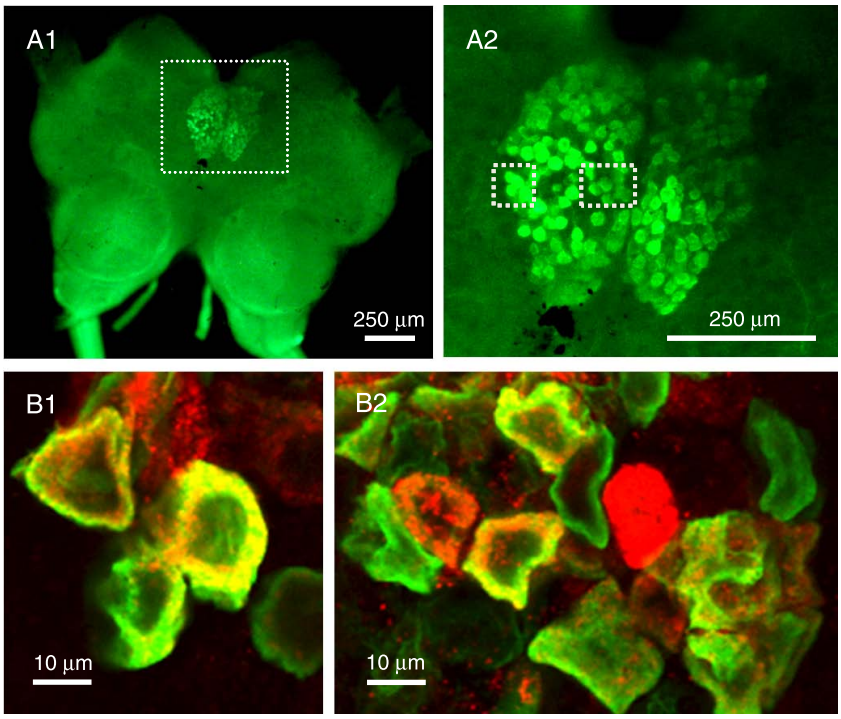

Figure 4. **A:** Merged confocal image of frontal 3-µm optical sections of a locust brain following neurobiotin backfilling from both left and right NCC1 nerves. The cell bodies stained are in the brain area known as the pars intercerebralis (PI) of the protocerebrum and largely overlap the area immunostained by anti-FOR (compare to Fig. 3). The area marked in A1 is enlarged in A2. Scale bars = 250 µm. **B:** Many cell bodies in this area were yellow after double staining with anti-FOR (red) and neurobiotin backfill (green). Cells shown in B correspond to the areas marked in A2. Scale bar = 10 µm.

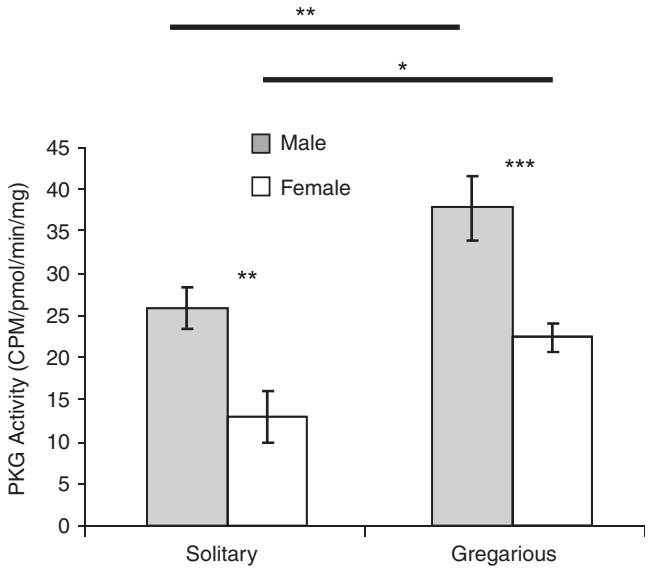

Figure 5. PKG activity (mean ± S.E.) of solitary ($n = 24$) and gregarious ($n = 20$), and male ($n = 18$) and female ($n = 26$) *S. gregaria*. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

brains than do their smaller-sized sister worker ants who work as foragers (Lucas and Sokolowski, 2009). Ants become either defenders or foragers depending on the food and social cues they receive as larvae (Passera and Suzzoni, 1991; Bloch et al., 2002). Defenders have higher PKG enzyme activity than foragers; however, defenders can exhibit flexibility in behavior and are able to engage in foraging activities, depending on the needs of the colony (Lucas et al., 2009). This plastic behavior is correlated to PKG enzyme activity and pharmacological manipulations of PKG activity change this behavioral plasticity (Lucas and Sokolowski, 2009). In *C. elegans*, PKG plays a role in olfactory adaptation as well as other plastic phenotypes (Fujiwara et al., 2002; L'Etoile et al., 2002; Hirose et al., 2003; Raizen et al., 2008). In the honey bee, *for* plays a role in the long-term plasticity changes involved in the switch from nursing to foraging (Ben-Shahar et al., 2002, 2003). No differences in spatial localization of the *for* transcripts were found in nurse and forager honey bees (Ben-Shahar et al., 2002) or in rover and sitter flies (Belay et al., 2007). Finally, PKG is known to function in learning and memory in mammals (Reaume and Sokolowski, 2009).

Density-dependent phase polyphenism in the desert locust is another example of plastic changes in response to environmental change. Previous pioneering work has provided some evidence for molecularly based phase differences (e.g., differences in the number and amount of peptides present in the corpora cardiaca and the hemolymph, Clynen et al., 2002; An unidentified solitary-specific gene and a SPARC-like gregarious-specific gene, Rahman et al., 2003). However, a full understanding of the molecular basis of this phenomenon is still lacking.

How might PKG act to affect locust behavior? Our findings of FOR expression in the brain PI cells, in close association with the major locust neurosecretory centers, opens multiple routes for PKG to affect locust physiology and behavior. In respect to locust phases, gregarious locusts are generally more active than solitary ones and show increased propensity for long-range marching or migratory flight (Pener and Simpson, 2009). This could be correlated to the higher PKG activity in gregarious locusts, similar to rover flies and forager honey bees. Rovers and sitters differ in levels of adipokinetic hormone and in acquisition, allocation, and storage of energy (Kaun et al., 2008). In locusts, in addition to the high PKG activity reported here, the gregarious phase is characterized by high levels of lipid reserves, higher hiperlipaemic response to flight, and increased adipokinetic response (mediated by CC neurohormones; Ayali and Pener, 1992, 1995; Ayali et al., 1996; Pener et al., 1997). PKG signaling also plays a role in modulating environmental stresses, such as thermal stress (in *D. melanogaster*, Dawson-Scully et al., 2007). Phase differences are also related to thermotolerance in locusts, including, for example, expression of heat shock proteins (Wang et al., 2007) and response to pathogens (Elliot et al., 2003, 2005). PKG is also involved in phototaxis behavior in bees (Ben-Shahar et al., 2003) and in circadian clock-related behaviors such as quiescence or sleep in both *C. elegans* and *D. melanogaster* (e.g., Raizen et al., 2008). In locusts, gregarious animals are characterized by diurnal flight behavior, while solitary locusts fly at night. Fuchs et al. (2003) describe an identified flight-related inhibitory interneuron, which is sensitive to illumination level, and show a remarkable and highly significant increase in activity during the dark but only when locusts are crowded locusts. Lastly, serotonin (5-hydroxtryptamine, 5HT) is both necessary and sufficient for locust phase transformation, with increasing levels of 5HT accompanying (and inducing) gregariousness (Anstey et al., 2009). A connection between the 5HT transporter (SERT), present in all animals from flatworm to human (Caveney et al., 2006) and

cGMP/PKG signaling is also known; activation of cGMP/PKG-linked pathways increases SERT activity and rapidly alters both 5HT uptake and clearance rates (e.g., Miller and Hoffman, 1994; Zhu et al., 2004a,b). Specifically, Zhang et al. (2007) reported that PKG phosphorylates human SERT (at Thr-276) and thus increases its activity. These reports are not fully consistent with our own results, as higher PKG activity in gregarious locusts compared to solitary ones, implies a down-regulation of 5HT in the gregarious phase (in contrast to Anstey et al., 2009). Reported differences in 5HT action and its regulation in vertebrates as compared to invertebrates (e.g., 5HT and aggression; Edwards and Kravitz, 1997), and even within the same animal depending on the social context, suggest that the relationships between PKG and 5HT require further study.

Our study lays a foundation for investigations into the functional role of the *for* gene in locust behavior, and specifically density-dependent phase polyphenism. A comparison of studies across species confirms a general role for the *foraging* gene and the PKG molecule in behavioral plasticity with an emphasis on food-related behaviors. Interestingly, the phylogeny suggests that species within a cluster with their closely related protein sequences can have an inverse relationship between *foraging* gene levels and behavior. Further studies are needed to understand the species-specific mechanisms underlying the *foraging* gene's function in suites of plastic behaviours.

ACKNOWLEDGMENTS

We thank A. Lange for providing migratory locusts, A. Hofbauer for the nc82 antibody, H. Belay for her help with brain dissections, A. Belay for protocols and advice on the immunohistochemical work, and M. Nicolas for help on phylogeny. We are grateful to U. Homberg for constructive advice regarding brain structures and neuronal identity and J. Meunier for discussions and comments on the manuscript. Research was supported by the Natural Science and Engineering Council of Canada and the Canada Research Chairs Program grants to M.B.S. C.L. was supported by the Fyssen Foundation and a Gene by Environment training grant from the Canadian Institutes of Health Research.

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