Introduction

The foraging gene (*for*) in *Drosophila melanogaster* provides a rare example of a single gene that underlies a naturally occurring complex behavior. This gene encodes cGMP-dependent protein kinase (PKG). Two variants of this gene have been isolated; individuals with the rover allele (for^R) move greater distances while feeding and have higher PKG levels than do individuals that are homozygous for the sitter allele (for^S). This difference is present in both larval (Sokolowski, 1980) and adult life stages (Pereira and Sokolowski, 1993). However, whether individual variation in food-search strategy is consistent from the larval to adult stage for a given individual has never been investigated. In order to address this question, the foraging behavior of individuals from populations of for^R and for^S flies was examined at both larval and adult developmental stages and then individuals were genotyped to verify the allele. While unable to confirm that individual foraging behavior is consistent between life stages, some preliminary data suggests that this is the case. Modifications to the adult foraging behavior protocol and repetition of the experiment would provide more conclusive results.

Methods

Adult flies from rover and sitter populations were moved to separate embryo collection chambers in order to collect developmentally synchronized larvae for behavioral assaying as described in Figure 1.

Embryo collection chamber

Developmentally synchronized (+/- 4 hours) larvae

24 hours

Move hatched larvae from apple juice collection plates to fly food plates

72 hours

Larvae behavioral assay
Move individual larvae into eppies containing fly food

8 days

Transfer individual adult flies into empty eppies

4 hours

Adult behavioral assay
Sacrifice flies for genotyping

Front view of adult testing arena

Top view of adult testing arena

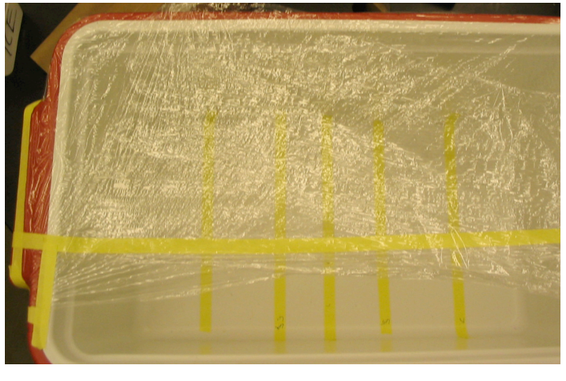

Figure 1. Flowchart depicting timeline of *Drosophila* larval and adult assays.

Results

Table 1. Foraging data for larval and adult fly behavioral assays.

Individual	Genotype[1]	Larval distance (cm)[2]	Adult distance (cm)[3]
B1	Rover	9	-
B2	Rover	11	-
B3	Rover	7	-
B6	Rover	17	4.75
O1	Rover	0	2.5
O2	Rover	11	-
O3	Rover	0	-
O4	Rover	11	-
O5	Rover	0	-
O6	Rover	10	-
L1	Rover	3	-
L2	Rover	7.5	-
L3	Rover	13	0
L4	Rover	0	-
L5	Rover	1	0
L6	Rover	13	0.5
G1	Sitter	8	0
G2	Sitter	1	0
G3	Sitter	3	-
G4	Sitter	2	0
G5	Sitter	2	-
G6	Sitter	2.5	-
R1	Sitter	1.5	-
R2	Sitter	5	-
R3	Sitter	0	0
R4	Sitter	5	-
R5	Sitter	4.5	2
R6	Sitter	3	-
W1	Sitter	0.5	-
W2	Sitter	6	-
W3	Sitter	6.5	0
W4	Sitter	10.5	0
W5	Sitter	1	-
W6	Sitter	1.5	0

1 Labeled genotype of adults used to obtain larvae for testing
2 Movement through yeast paste during five minute interval
3 Movement in arena after consumption of 0.25 M sucrose droplet

After behavioral assays, individual genotypes can be confirmed. A gross DNA preparation from each fly was used in a PCR protocol to amplify a portion of the foraging (*for*) gene. The two alleles are distinguishable by a restriction enzyme polymorphism based on a single amino acid change. At the nucleotide level, this creates a novel *Hin*P1 I site in the for^R allele (Renn, 2006). After PCR and digestion with *Hin*P1 I, products were run on a 2% agarose gel and visualized with EtBr staining. No PCR product was visible on the gel.

Discussion

As shown in Figure 2, *Drosophila* larval rover and sitter genotypes have distinct phenotypes, a confirmation of previous results (Sokolowski, 1980).

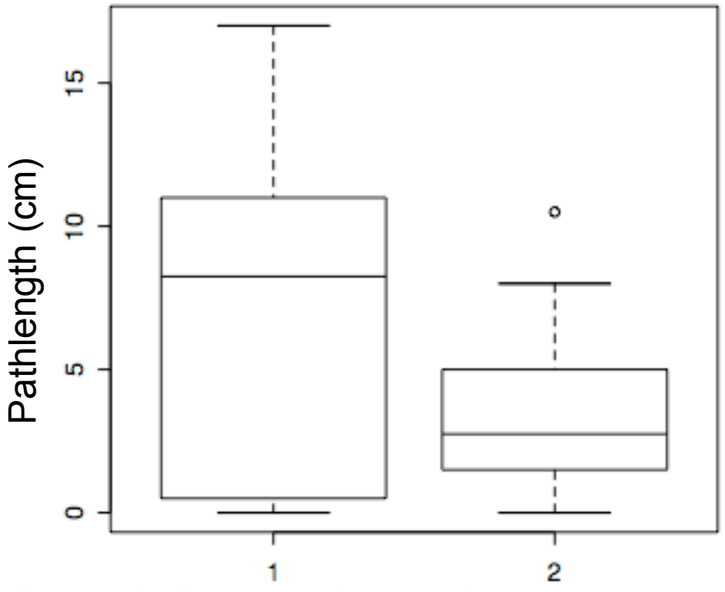

Figure 2. Boxplot of larval foraging distance. 1 = rover; 2 = sitter. Mean distance (rover) = 7.09 cm; mean distance (sitter) = 3.53 cm. T-test p-value = 0.03.

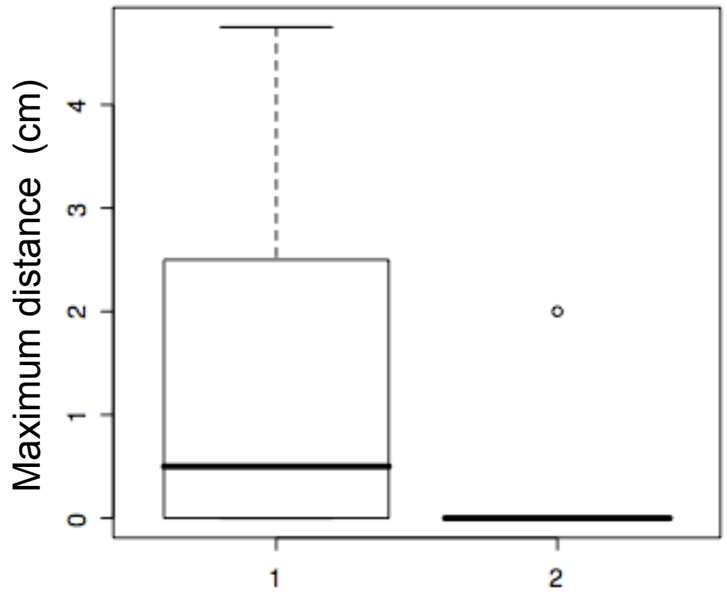

Figure 3. Boxplot of adult foraging distance. 1 = rover; 2 = sitter. Mean distance (rover) = 1.55 cm; mean distance (sitter) = 0.25 cm. T-test p-value = 0.24.

We could not confirm the same phenotypic difference in adult flies (as previously shown by Pereira and Sokolowski, 1993) (Figure 3). We hypothesize this is because of the few number of flies that survived to undergo the adult behavioral assay (a factor that made us unable to definitively answer the question of foraging behavior consistency through life stages, see Table 1). Additionally, those that did survive underwent gassing (a change from the original protocol) that may have confounded the results by making the flies less responsive. Confirmation of individual fly genotype by PCR and restriction enzyme digest yielded no results. This could be due to problems adapting the larval crude DNA prep protocol to adults. PCR conditions may also need to be optimized. Due to time constraints, these adaptations could not be adopted.

References

Pereira HS and Sokolowski MB. 1993. PNAS USA 90:5044.
Renn, S. 2006. B342 Week 6 Lab Protocol.
Sokolowski MB. 1980. Behav Genetics 10:291.

Acknowledgments

The authors would like to thank Suzy Renn for her generous gift of rover and sitter *Drosophila melanogaster* and guidance with protocols, and the stockroom for resources.