Published in final edited form as:

Science. 2008 March 21; 319(5870): 1679–1683. doi:10.1126/science.1151842.

***Drosophila* egg-laying site selection as a system to study simple decision-making processes**

Chung-hui Yang¹, Priyanka Belawat², Ernst Hafen², Lily Y. Jan¹, and Yuh-Nung Jan^{1,*}

¹Howard Hughes Medical Institute, Department of Physiology, Biochemistry, and Biophysics, University of California at San Francisco, San Francisco, CA 94143-0725, USA ²Institute for Molecular Systems Biology, ETH Zürich HPT E 72, Wolfgang-Pauli-Str.16, 8093 Zürich, Switzerland

The ability to select a better option from multiple acceptable ones is important for animals to optimize their resources. The mechanisms that underlie such decision-making processes are not well understood. We found that selection of egg-laying site in *Drosophila melanogaster* is a suitable system to probe the neural circuit that governs simple decision-making processes. First, *Drosophila* females pursue active probing of the environment prior to depositing each egg, apparently to evaluate site quality for every egg. Second, *Drosophila* females can either accept or reject a sucrose-containing medium depending on the context. Lastly, communication of the “acceptability” of the sucrose-containing medium as an egg-laying option to the reproductive system depends on the function of a group of Insulin-like peptide 7 (ILP7)-producing neurons. These findings suggest that selection of egg-laying site involves a simple decision-making process and provide an entry point towards a systematic dissection of this process.

Decision-making, in one view, is the process by which animals deliberate whether to invest in one action or not by taking into account the values and costs associated with available options. Selection of egg-laying site by *Drosophila* provides a plausible system to investigate such decision-making process; egg production is costly, thus the ability to weigh egg-laying options might have been selected to ensure better survival of offspring in uncertain environment. *Drosophila* females are known to be selective towards egg-laying sites and will withhold eggs when there are no appropriate sites (1-6). It is less clear, however, whether *Drosophila* females value a given egg-laying site differently according to the availability of other laying options. We examined the selection of egg-laying site of individual *Drosophila* females, in an attempt to find a genetically tractable system to study the molecular and cellular basis of simple decision-making processes.

The first indications that egg-laying site selection may employ a simple decisionbased process emerged from our observations of females as they lay eggs. For consistent viewing of egg-laying events, we deprived females of egg-laying for 24 hours before placing them in a chamber filled with grape-agar, an attractive egg-laying substrate (Fig. 1A, B). By reviewing hundreds of egg-laying events, we found that immediately prior to each physical egg-expulsion, females stereotypically bend their abdomen downward until it is nearly perpendicular to the substrate surface to extrude and insert the ovipositor into the substrate (Fig. 1C), before initiating a series of back-and-forth movements to expel and insert a single egg into the substrate (Movie S1). This behavioral component always accompanies the physical deposition of an egg and typically lasts about 6 seconds (Fig. 1I) on grape-agar. We called this behavioral component the

*To whom correspondence should be addressed. Email: yuhnung.jan@ucsf.edu

“ovipositor motor program” because it is reminiscent of the “oviposition motor program” of grasshopper egg-laying (7). We next found two more behavioral components that regularly follow the ovipositor program: an animal always grooms its ovipositor with its hind legs (Fig. 1D, Movie S1) for a few seconds and then stays immobile for a while as though it is resting (Fig. 1I). After the “clean and rest program” and before the initiation of the next ovipositor program, the animals presumably have the opportunity to locate an appropriate site for the next egg (Fig. 1H). Indeed, despite being placed in a relatively attractive grape-agar environment, the animals in virtually all cases display a “search-like” behavior by walking around and probing the substrates with their proboscis and ovipositor (Fig. 1E-G, Movie S1). Since the proboscis, legs, and ovipositor all contain sensory receptors (8), this “search-like” program should aid the animals in identifying appropriate egg-laying sites. In 199 out of 200 cases we observed (~20 egg-layings each by 10 animals), we detected the “search-like” behavior preceding the ovipositor program (Fig. 1J), though its duration varied. Thus, *Drosophila* females deposit their eggs one at a time and nearly always follow the cycle of search, oviposit, and clean and rest, with the search-like program lasting for from a few seconds to several minutes (Fig. 1J) while the other programs remain relatively constant in duration (Fig. 1I, fig. S1).

We next examined what the females seek by presenting them with different egg-laying options in the behavioral chamber. We placed in the chamber a sweet (sucrose-containing) and a bitter (lobeline-containing) (9) soft agarose medium that are separated by a region of hard agarose that deters egg-laying and prevents simultaneous detection of the two soft media. Surprisingly, animals consistently laid more eggs on the lobeline side of the chamber (Fig. 2A-C, G, and fig. S2). This bias against sucrose medium was not caused by intrinsic attraction to the lobeline medium for egg-laying, however, because animals tended to avoid lobeline when the other option was a plain medium (Fig. 2E, G), consistent with previous findings on quinine avoidance in egg-laying (10). Furthermore, females avoided laying eggs on the sucrose-containing medium in our chambers no matter whether the alternative was lobeline-containing, plain, or a substrate containing sodium chloride at the same concentration as the sucrose (Fig. 2A-D and G).

To determine whether females actually encountered both options before laying an egg on the lobeline medium, we next tracked individual animals for two hours and found that for each egg deposited on the lobeline side, the animals paid an average of 1.5 prior visits to the sucrose option (Fig. 2I). Animals often probed the sucrose substrate actively but rarely activated the ovipositor program while they were still in contact with this medium (Movie S2); in contrast, activation of the ovipositor program was frequently seen when the animals were probing the lobeline medium (Movie S2). Thus, lack of significant egg-laying on the sucrose medium is not due to animals' not making regular contacts with this medium. General repulsion to the sucrose-containing medium in our chamber is not the cause either--animals were often seen actively feeding on the sucrose medium by extending onto it their proboscis (Fig. 2F, Movie S3). Moreover, the avoidance of egg-laying on the sucrose-medium (when it is paired with a plain one) is concentration dependent: it attenuates and can even turn into mild attraction as the concentration of sucrose decreases (Fig. 2H).

The reliance on neuronal activities of the sweet taste receptor (*Gr5a*) neurons (11) is evident in the experiments employing the *GAL4-UAS* method to express in these *Gr5a*-expressing neurons a hyperpolarizing Kir2.1 potassium channel (12). This manipulation significantly increased egg-laying on the sucrose-containing medium (Fig. 2J, K), suggesting that sucrose detection through the *Gr5a* neurons is important for the low egg-laying on this medium in the two-choice chamber.

To determine if the desirability, or “value”, of the sucrose-containing medium for egg-laying might change according to context, we examined egg-laying in single-choice chambers that contain either only the sucrose-containing medium or the lobeline-containing medium. Interestingly, females lay comparable numbers of eggs in the two single-choice chambers (Fig. 3A-C); thus, in the absence of other options, females readily accept the sucrose-containing medium for egg-laying. Furthermore, the same sucrose-containing medium can even become a *preferred* option for egg-laying if it is paired with a more repulsive medium that contains twice the amount of sucrose (Fig. 3D, E).

To explore further how context impacts the valuation of sucrose medium, we varied the physical separation between the sucrose- and lobeline-containing media in the two-choice chamber. We found that when the distance between the two choices was increased to 3- or 10-fold, egg-laying on the sucrose medium progressively increased (Fig. 3F), and some of the individual animals tested actually laid more eggs on the sucrose-containing medium than on the lobeline one (Fig. 3F). This result suggests that the preference of sucrose medium over lobeline is not absolute and animals can weigh the “desirability” of egg-laying sites versus the “effort it takes to locate them” in selecting sites for egg-laying. Interestingly, this distance-dependent response to sucrose-containing medium was not altered by food deprivation prior to assays (Fig. 3F), suggesting that egg-laying and foraging are distinctive tasks with distinctive substrate preferences.

Sequential placement of the same animals first in a two-choice chamber and then in a sucrose medium-only single choice chamber (Fig. 3G) revealed speedy recovery of egg-laying interests on the sucrose-containing medium (Fig. 3H), suggesting that the substrate evaluation process is efficient and performed predominantly on an egg by egg (search by search) basis. However, compared to the number of eggs deposited in a lobeline medium-only chamber, there were fewer eggs in the sucrose medium-only chamber (S2 vs. L2, Fig. 3H), while no difference was observed when naïve flies were introduced to the single-choice chambers (Fig. 3C); it thus appears that prior experience may exert some influence in egg-laying decision. Taken together, our results suggest that *Drosophila* females possess some neural process that assigns “acceptability” or “value” to a given egg-laying substrate by taking readily into account the availability of other options. Such “value” can then be used by the motor output systems to decode whether a particular option is appropriate to trigger the physical egg-laying action in the given context.

To begin discerning the neural circuitry that underlies egg-laying site selection, we first sought to identify specific neurons that regulate egg-laying rate as they might be engaged by the “value system” to control egg-laying on a given substrate. Neurons that express an insulin-like neuropeptide, ILP7, are of interests since ILP7 shares some homology with Relaxin, an important reproductive hormone in mammals (13,14). Our antibodies revealed that ILP7 is present in only very few cells in the larval and adult CNS (Fig. 4A, B, fig. S6) with some cells sending projections to the sub-esophageal ganglion (SOG) and distinct positions in the ventral nerve cord (Fig 4A, B), which are all sites where gustatory information relay might occur. Moreover, projection of ILP7 to the female internal reproductive tract can also be found (Fig. 4C), in support of the notion that ILP7 may be the *Drosophila* Relaxin. Interestingly, many of the ILP7 neurons are also positive for *fruitless* expression in adult males (fig. S7). This result suggests a potential role of these neurons in male reproductive behavior since *fruitless* has been shown to be a master regulator of male courtship behavior in *Drosophila* (15).

We next created an *ILP7-GAL4* to specifically alter *ILP7*-neurons' function (Fig. 4F-H, fig. S4). Using the *GAL4-UAS* approach, we found that females with hyperpolarized *ILP7*-neurons showed no discernable developmental defects but displayed virtually no ovipositor motor programs and are thus sterile (Fig. 4I). To distinguish between the possibility that *ILP7*-neurons

are required merely for house-keeping processes to push the egg through the internal reproductive tract, which could account for the fully penetrant egg-jamming phenotype due to silencing *ILP7*-neurons (fig. S5), and the possibility that *ILP7*-neurons also participate in conveying the “acceptability” of potential laying options to the reproductive system (e.g., the reproductive tract and the ovipositor motor program), we examined the effect of elevated *ILP7* level--in *ILP7*-neurons or ubiquitously--on egg-laying choice (Fig. 4J, fig. S6). In both cases, the elevation of *ILP7* level caused the animals to be more receptive to laying eggs on the sucrose medium in the “regular” two-choice chambers (Fig. 4J). These results are consistent with the idea *ILP7* might participate in the relay of the “appropriateness” of a given option to the reproductive systems to execute egg-laying on that option.

In summary, *Drosophila* females can accept or avoid a given sucrose-containing medium for egg-laying depending on context. Whereas such context-dependent avoidance of high sucrose medium shows little discernable advantage in the laboratory setting (embryo hatching rate on sucrose-containing and lobeline-containing media are comparable, fig. S3), it could have been selected for by virtue of predation avoidance and larval dietary balance (protein/carbohydrate ratio). Our finding that *Drosophila* employs a simple decision-making process in selecting egg-laying site raises the possibility that fruit fly has the capacity to compare and assess available options by performing integrations and amplifications in its nervous system. Dopamine and octopamine are both candidates for mediating such amplification/integration processes: the former is important for decision-making in primates and flies (16-18) and is used to signal the unconditional stimulus of “punishment” during learning tasks in *Drosophila* (19,20); the latter is a reinforcing signal for appetitive conditioning in both *Drosophila* and honeybees (19-21) and an important regulator of egg-laying in *Drosophila* (22). In addition, our work suggests that the *ILP7*-expressing neurons are important for proper execution of egg-laying decision, thus providing an additional anatomical and molecular entry point into dissecting the decision-making processes during egg-laying site selection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We wish to dedicate this paper to Seymour Benzer who pioneered the field of *Drosophila* neurogenetics. We are very grateful to K. Scott, G. Davis, B. Baker, J. Carlson, B. Dickson, and the Bloomington Stock Center for reagents. We thank Q. Yuan and W. Song for suggestions on the behavioral set-up and experiments. This work is supported by an NIH grant (RO1 NS40929) to Y.-N. J. and a Swiss National Foundation grant to E. H. C.-H. Y. was supported by a Jane Coffin Childs fellowship. Y.-N. J. and L. Y. J. are investigators of the Howard Hughes Medical Institute.

References and notes

1. van Delden W, Kamping A. Behav Genet Sep;1990 20:661. [PubMed: 2126926]
2. Takamura T, Fuyama Y. Behav Genet Jan;1980 10:105. [PubMed: 6775626]
3. Allemand R, Bouletreau-Merle J. Experientia Dec 1;1989 45:1147. [PubMed: 2513224]
4. Spradling, A. The development of *Drosophila melanogaster*. In: Michael Bate, AM., editor. Developmental genetics of oogenesis. Cold Spring Harbor Laboratory Press; 1993. p. 1-70.
5. Richmond RC, Gerking JL. Behav Genet May;1979 9:233. [PubMed: 115458]
6. Eisses KT. Behav Genet May;1997 27:171. [PubMed: 9210788]
7. Thompson KJ. J Exp Biol May;1986 122:387. [PubMed: 3723074]
8. Hallem EA, Dahanukar A, Carlson JR. Annu Rev Entomol 2006;51:113. [PubMed: 16332206]
9. Marella S, et al. Neuron Jan 19;2006 49:285. [PubMed: 16423701]
10. Mery F, Kawecki TJ. Proc Natl Acad Sci U S A Oct 29;2002 99:14274. [PubMed: 12391295]

11. Chyb S, Dahanukar A, Wickens A, Carlson JR. *Proc Natl Acad Sci U S A* Nov 25;2003 100(Suppl 2):14526. [PubMed: 14523229]
12. Baines RA, Uhler JP, Thompson A, Sweeney ST, Bate M. *J Neurosci* Mar 1;2001 21:1523. [PubMed: 11222642]
13. Brogiolo W, et al. *Curr Biol* Feb 20;2001 11:213. [PubMed: 11250149]
14. Hudson P, Haley J, Cronk M, Shine J, Niall H. *Nature* May 14;1981 291:127. [PubMed: 7231533]
15. Ryner LC, et al. *Cell* Dec 13;1996 87:1079. [PubMed: 8978612]
16. Sugrue LP, Corrado GS, Newsome WT. *Nat Rev Neurosci* May;2005 6:363. [PubMed: 15832198]
17. Schultz W, Dayan P, Montague PR. *Science* Mar 14;1997 275:1593. [PubMed: 9054347]
18. Zhang K, Guo JZ, Peng Y, Xi W, Guo A. *Science* Jun 29;2007 316:1901. [PubMed: 17600217]
19. Schwaerzel M, et al. *J Neurosci* Nov 19;2003 23:10495. [PubMed: 14627633]
20. Schroll C, et al. *Curr Biol* Sep 5;2006 16:1741. [PubMed: 16950113]
21. Hammer M, Menzel R. *Learn Mem* May-Jun;1998 5:146. [PubMed: 10454379]
22. Monastirioti M, Linn CE Jr. White K. *J Neurosci* Jun 15;1996 16:3900. [PubMed: 8656284]

Fig 1. The egg-laying behavioral sequence of *Drosophila* females

(A) The grape-agar chamber used for observing egg-laying behavior.

(B) Top view of the chamber through the camcorder.

(C) Animal performing the ovipositor motor program. Note the downward-bending of the abdomen. Red arrow: ovipositor.

(D) Animal cleaning the ovipositor (red arrow) with its hind legs.

(E) Animal probing the substrate with its proboscis (red arrow).

(F, G) Animal probing the substrate with its ovipositor (red arrow).

(H) Egg-laying temporal sequence. The physical deposition of an egg starts from **A** and continues to **B**. In between the end of **B** and the beginning of the next **A** is period **C**, in which

animals are consistently seen displaying the probing actions described in (E, F, G). Ovi: ovipositor motor program.

(I) Time spent on each of the programs described above. **A**: 6.7 ± 0.25 sec, **B**: 101 ± 4.7 sec, and **C**: $113.2 \text{ sec} \pm 18.9$ sec. For programs **A** and **B**, $N=50$ egg-layings displayed by 4 animals from 4 chamber. For the program **C**, $N=77$ egg-layings displayed by 4 animals from 4 chambers. Error bars indicate S.E.M. Note that a comparison of behavioral durations between *CantonS* and *W1118* is shown in fig. S1.

(J) Distribution of time spent on the search-like program. $N=77$ individual egg-layings displayed by 4 animals from 4 chambers. Unless otherwise mentioned, all quantifications in this work were conducted by using *CantonS* animals or transgenic animals in *CantonS* background.

Fig 2. *Drosophila* avoid laying eggs on media containing high level of sucrose

(A-E) Representative outcomes of various two-choice tests. Single-female assay was used in (C), the rest were 3-female assays. S: sucrose, L: lobeline, P: plain. The same designations will be used throughout the figure legend.

(F) Animals extending their proboscis onto the sucrose medium (arrows) for foraging.

(G) Preference index (PI) of various two-choice conditions (see methods for derivation). N: NaCl. l: agar with 1/3 grape juice. h: agar with 1X grape juice. "Single" indicates that single animals were used in this assay; no difference was found between PI from single- vs. 3-female assay ($p > 0.5$, Mann-Whitney Test). Significant deviations from 0 were found for all conditions tested ($p = 0.01$ for L/P, $p < 0.0001$ for the rest, Wilcoxon Signed Rank Test). Note that all box-

whisker plots in this work display median, 25/75%, and 10/90% quartiles of each data set. The last entry shows that females prefer agar with less grape juice for egg-laying. The number of assay used, the mean PI are: (29, -0.82), (26, -0.79), (10, -0.89), (34, -0.27), (50, -0.83), (34, -0.62).

(H) Females avoid sucrose medium less as its concentration drops in the *sucrose vs. plain* two-choice assays and even showed a slight preference at 0.5mM setting ($p=0.0116$, *Wilcoxon Signed Rank Test*). No significant avoidance can be found at 1mM ($p=0.095$, *Wilcoxon Signed Rank Test*). The rest of entries all show significant avoidance of sucrose medium ($p<0.0001$, *Wilcoxon Signed Rank Test*). The number of assay used, the mean PI are: (49, 0.18), (34, -0.102), (36, -0.33), (34, -0.56), (34, -0.83), (35, -0.90).

(I) For each egg deposited on the lobeline medium in two-choice chambers, animals pay 1.51 ± 0.17 and 1.61 ± 0.15 respective prior visits to each substrate. N=185 egg-depositions by 8 females.

(J, K) Hyperpolarizing *Gr5a* neurons decreases egg-laying bias against the sucrose-containing medium. The difference between animals carrying *Gr5a-GAL4* with or without *UAS-Kir2.1* is significant (*Mann-Whitney Test*). Number of assay used and mean PI: (38, -0.52), (40, -0.79), (41, -0.09). **** $p<0.001$. The exact cause(s) for the reduction of PI of *Gr5a-GAL4/+* animals is unclear, however, these animals contain two insertions of *Gr5a-GAL4* transgenes and thus excess of GAL4 proteins in these neurons alone could potentially contribute to reduction of neuronal function.

Fig 3. Avoidance of sucrose-containing medium is context-dependent

(A-C) Comparable numbers of eggs were laid in the two single-choice (sucrose vs. lobeline) chambers, $p > 0.5$, *Mann-Whitney Test*. Number of assay used and sample mean are: (39, 67.6), (38, 67.6).

(D, E) Pairing the same sucrose medium (100mM, S) with different options alters the number of eggs deposited on (D), and the PI against this medium (E). 2S: 200mM sucrose. Assay number and sample mean for (D): (29, 7.3), (35, 4.9), (39, 33.26), (36, 40.89). For (E): (29, -0.82), (35, -0.82), (39, -0.01), (36, 0.37). **** $p < 0.0001$, ** $p < 0.005$, *Mann-Whitney Test*.

(F) Comparison of the PI shows that animals avoided the sucrose-containing medium less when distance between the sucrose and lobeline media increases. Single females were used. Black bars: without food deprivation, grey bars: 24 hours food deprivation prior to experiments. In both treatments, there were significant differences between 1X and 3X and between 3X and 10X. Food deprivation for did not significantly impact PI for all three distances tested. Assay number and mean PI are: (52, -0.84), (23, -0.84), (47, -0.5), (55, -0.46), (31, -0.002), and (35, -0.08). ** $p < 0.005$, *** $p < 0.001$, *Mann-Whitney Test*.

(G) Exposure assay set-up: animals were placed in two-choice “exposure” chambers for 1 hour before being transferred into sucrose or lobeline only single-choice chambers.

(H) Despite robust preference for lobeline medium in the exposure chambers (S1 vs. L1), the difference in egg numbers between the two single-choice chambers (S2(a+b) vs. L2(a+b)) is less although still significant. Number of assay used and the sample mean are: (69, 2.15), (69, 25.54), (34, 30.09), (35, 36.16). * $p = 0.05$, **** $p < 0.0001$, *Mann-Whitney Test*.

Fig 4. Adequate level of ILP7 is important for normal egg-laying and proper bias in egg-laying site selection

(A-C) ILP7 antibody staining in adults. (A) ILP7 neurons send projection into SOG (red arrow) in the brain, (B) ILP7 is present in very few cells (blue bracket) with distinctive projection in the thoracic segments in VNC (red arrow), (C) ILP7 is also present on the female internal reproductive tract.

(D, E) Specificity of ILP7 antibodies was confirmed by the reduced signal in animals that over-express an *ILP7-RNAi* construct (E). (D) Staining control.

(F-H) *ILP7-GAL4* specificity was confirmed by the co-localization of signals from ILP7 staining (F) and *UAS-nuclear-RFP* expressed by *ILP7-GAL4* (G) in the same cells (H). (I)

Reducing neuronal function of *ILP7*-neurons via *Kir2.1* over-expression causes a loss of ovipositor program. For the two control groups, number of eggs laid was used to substitute for the number of ovipositor program. The number of assays and the sample mean are: (20, 59.7), (20, 67.2), (10, 0). **** $p < 0.0001$, *Mann-Whitney Test*.

(J) Animals carrying an *HS-ILP7* construct or over-expressing *UAS-ILP7* by *ILP7-GAL4* avoided sucrose medium significantly less than control animals in *sucrose vs. lobeline* two-choice assays. Number of assay used and sample mean are: (21, -0.87), (23, -0.51), (15, -0.73), (20, -0.86±0.03), (20, -0.11). **** $p < 0.0001$, *Mann-Whitney Test*. Note that *HS-ILP7* animals that were raised at 18 degree show comparable sucrose avoidance to controls animals in *sucrose vs. plain* two-choice assays ($p > 0.5$, *Mann-Whitney Test*). Number of assay used and sample mean are: (14, -0.88) vs. (34, -0.9).