

Natural Variation in *Drosophila* Behavior:

The natural-variant approach in *Drosophila* behavioral genetics has been used to clarify the nature of the genes and allelic variants that affect normal individual differences in behavior, how they evolved and how they might differ from laboratory-generated mutants. Natural variants are useful tools for behavioral genetics analysis because they carry subtle alterations in genes, that allow them to survive in nature and may even be under positive selection. By comparison, many single-gene mutant studies in the lab have shown that the alleles are generated are often lethal or highly deleterious. While single-gene mutants might tell us what genes are necessary to produce a behavior they may not tell us what genetic variation underlies natural variation between individuals, populations or species.

There are multiple research strategies that can lead to understanding natural variation between individuals, populations or species. Some of these research strategies can be efficiently approached using wild populations of traditional lab organisms like *Drosophila*. First, insights into the genetic and molecular bases of natural variation can be gained by studying naturally occurring behavioral variants and using traditional genetic methods to identify the relevant loci (Sokolowski 1998). Second, one might look for naturally occurring genetic variation for genes that were first identified through mutagenesis (Sawyer et al 1997). Third, one can use Quantitative Trait Loci mapping techniques to analyze strain differences (Mackay 2001). Fourth, and more recently, one can breed isogenic (fully homozygous) lines from the wild that represent much of the initial diversity in the population (Pennisi, 2002). As genetic techniques and sequencing technology advance, more high throughput techniques will emerge.

In this lab we will take the first steps toward such an endeavor, we will collect wild stocks and quantify natural behavior among populations of *Drosophila*. Each student pair will collect wild flies from two different locations and culture them in the laboratory according to the general methods described in this handout. Students will select a behavioral assay from those listed on the following pages, or from the published literature, in order to construct their own testing apparatus. The second generation wild caught flies will be tested for behavioral differences between the population. A short write up (1-2 pages) will describe the collection site, behavioral assay methods, a graphical representation of the results with appropriate statistical analysis and a brief discussion including 3-4 references. The lab notebook will include all procedural detail and troubleshooting notes.

Timeline for this lab

- Wk 1** :: **Make fly culture media in at least 4 bottle**
 - :: **Put bottles out to collect flies for ~ 4 days.**
 - :: **Bring fly bottles into lab incubator**
- Wk 2** :: **Check fly bottles for proper moisture**
 - :: **Transfer wild flies to a new set of bottles (save initial collection bottles)**
- Wk 3** :: **Transfer F1 flies to new vials**
 - (be aware you must do this at a time other than regular lab as we will be at the zoo)**
 - :: **Trouble shoot your experimental paradigm using labstock flies.**
- Wk 4** :: **Transfer more F1 flies to new vials.**
- Wk 5** :: **Transfer more F1 flies to new vials.**
 - :: **If virgins are needed begin collecting these.**
 - :: **If special conditions are needed, establish these.**
- Wk 6** :: **Turn in Fly Lab Protocol Draft.**
- Wk 7** :: **Conduct fly behavioral assays.**
- Wk 7** :: **Turn in Fly Protocol WriteUp (include 1 data figure).**

Sokolowski, M. B. Genes for normal behavioral variation: recent clues from flies and worms. *Neuron* 21, 1–4 (1998).
Sawyer, L. A. Natural variation in the *Drosophila clock* gene and temperature compensation. *Science* 278, 2117–2120 (1997).
Mackay, T. F. C. Quantitative trait loci in *Drosophila*. *Nature Rev. Genet.* 2, 11–20 (2001).
Pennisi Abuzz about behavior. *Science* 320:1581 (2008)

BASIC PROTOCOLS FOR FLY CARE

Making fly culture media: Add 1 volume dry fly culture media to fly vial or bottle. Add one volume of water to fly vial or bottle. (volume depends on the size of the container, food should be ~ 0.5 - 1 cm deep). Add a pinch of dry yeast. Plug container with cotton plug. Wait 5 minutes before adding flies. Fly culture media can be refrigerated until needed.

Collecting Wild flies: Prepare 2 bottles of fly culture media. Take each bottle and place it near rotting food, damp mushrooms, indoors, outdoors, behind a restaurant etc. Choose two different locations that represent different fly habitats. You may also choose to supplement the media if it is your goal to attract flies with different preferences. Label the bottles and leave in situ for 4-5 days. Record as much information about the collection and conditions at the locations. Bring bottles into the lab and maintain in an incubator at 25 °C, 12:12 hour light:dark cycle.

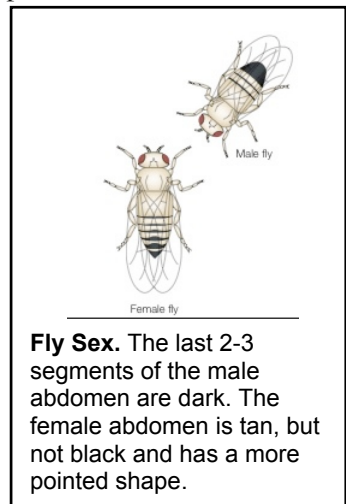
Culturing fly stocks: Stocks can be cultured by periodic mass transfer of adults to fresh food. Bottles or vials are tapped on counter top to shake flies away from the plug, the plug is rapidly removed and the old culture inverted over a fresh bottle or vial. Flies are tapped into the new vessel, and the two vials are rapidly separated and replugged. You will learn from experience, or you can knock flies out with CO₂ or cold to decrease escape rate. The frequency with which new subcultures need to be established depends on the health and fecundity of the genotype, the temperature at which it is raised, and the density of the cultures. Temperature has a large effect on the rate of *Drosophila* development. Generation time (from egg to adult) is approximately: 7 days at 29°C, 9 days at 25°C, 11 days at 22°C, 19 days at 18°C.

Aspirating flies: This technique allows transfer of flies without anesthesia. Use a 1 ml disposable syringe with a cotton plug. Connected the pipette to a piece of tygon tubing. Place pipette tip into fly vial at the edge of the foam plug, select fly and inhale to collect fly. Transfer fly and exhale.

Anesthetizing flies with CO₂: This technique allows for transfer of flies as well as sorting and counting. Open main valve on CO₂ tank and check the 1st stage regulator for adequate tank pressure. It should **not** be necessary to adjust second stage on the regulator. Use the stopcock to direct flow to the needle. Insert needle through cotton plug while holding vial on its side (watch that culture media stays in place). Gently tap the vial so flies fall. Remove the needle, being careful not to remove plug. Adjust stopcock to direct CO₂ to the flypad. Remove plug from vial and gently tap flies onto the fly pad. Use a paint brush to delicately move sort flies under the dissecting microscope.

Sexing flies: Anesthetize flies with CO₂ or cold. Under the dissecting microscope, gently role fly onto its back. If you are using cold to knock out the fly they must be kept on ice while under inspection. Consult diagram.

Virgin females: Most experimental schemes require virgin females. *D. melanogaster* adults do not mate for about 10 hours after eclosion, allowing virgins to be collected within 8-12 hours after the culture has been cleared of adults. Most females eclose in the morning. Clear the bottle of all adults in the evening and the next day most of the females should be virgins.



More detail is available in Cold Springs Harbor Protocols:

Maintenance of a *Drosophila* Laboratory: General Procedures

Michael Ashburner and John Roote Cold Spring Harb Protoc; 2007; doi:10.1101/pdb.ip35

Culture of *Drosophila*: The Laboratory Setup

Michael Ashburner and John Roote Cold Spring Harb Protoc; 2007; doi:10.1101/pdb.ip34

POTENTIAL IDEAS FOR SIMPLE BEHAVIOR MEASURES:

Pupation position: Pupation distances is tested by placing 10 first instar larvae into a glass vial containing 6mL culture media. The larvae are placed on the surface, and each vial is plugged with a cotton ball, leaving approximately 65 mm between the bottom of the cotton plug and the food surface. Cotton plugs should be marked to indicate the preparation day to facilitating identification and reducing the disturbance of younger cultures while the sexes of pupae set on earlier days are recorded. A row of vials containing only media is placed around the outer edge of the racks, ensuring that all test vials are surrounded by other vials containing media to reduce the exposure of the outermost test vials to direct light. The vials are left undisturbed in an incubator at 25 °C, 12:12 hour light:dark cycle, until pupation. Once all the larvae have pupated, the distances between the anterior pupal spiracles and the food surface is measured, and mean distances is calculated for each strain.

Riedl, C. A. L., Riedl, M., Mackay, T. F. C. & Sokolowski, M. B. 2007. Genetic and behavioral analysis of natural variation in *Drosophila melanogaster* pupation position. Fly 1: <http://www.landesbioscience.com/journals/fly/article/3830>

Locomotor reactivity: Single, 3-7 day old adult flies, collected under CO₂ exposure, are placed into vials containing 5 ml of standard culture media, and left overnight to acclimate to the new environment. To quantify locomotor reactivity, each fly is subjected to a mechanical disturbance by tapping the vial twice against a table, and recording the amount of time the fly is active in the 45 seconds immediately following the disturbance using a stopwatch to record movement, while a timer counts down the 45 s assay period. The measure of locomotor reactivity is a score ranging from 0 s to 45 s, denoting the total amount of activity during the assay period. Jordan KW, Morgan TJ, Mackay TFC: Quantitative trait loci for locomotor behavior in *Drosophila melanogaster*. *Genetics* 2006, 174:271-284.

Starvation resistance: Single sex groups of ten two-day-old flies are placed in vials containing non-nutritive medium (1.5% agar and 5 ml water). Survival is scored every 8 h until all flies are dead. This assay must be conducted with replicate measurements per line per sex.

Harbison ST, Yamamoto AH, Fanara JJ, Norga KK, Mackay TFC: Quantitative trait loci affecting starvation resistance in *Drosophila melanogaster*. *Genetics* 2004, 166:1807-1823.

Chill-coma recovery: Twenty-five 3- to 7-day-old flies per line per sex per generation are transferred without anesthesia into an empty vial and placed on ice for 3 h. The flies are then transferred to room temperature, and the recovery time is recorded as the length of time necessary for an individual to right itself and stand on its legs. (Similar assay can be done for heat tolerance using a water bath to warm vials to 42 C)

Morgan TJ, Mackay TFC: Quantitative trait loci for thermotolerance phenotypes in *Drosophila melanogaster*. *Heredity* 2006, 96:232-242.

Ethanol sensitivity: 50 – 60 same-sex flies are aspirated into a glass column with mesh partitions (an inebriometer), which is filled with saturated ethanol vapors. The flies lose postural control due to ethanol exposure and fall down the partitions to the bottom of the column, where they were collected at one minute intervals. The elution time is recorded as the measure of ethanol sensitivity.

Inebriometer construction: <http://anatomy.ucsf.edu/heberlein/Protocols/Inebriometer%20instructions.pdf>
Weber, K.E. 1988. An apparatus for measurement of resistance to gas-phase reagents. *Drosophila Information Service* 67: 91-93.

Copulation latency: For each strain, 20 pairs of 3- to 7-day- old virgin flies are aspirated into vials containing approximately 3 ml standard culture medium. The score recorded for a pair is the number of minutes from introduction to the vial until initiation of copulation.

Mackay TFC, Heinsohn SL, Lyman RF, Moehring AJ, Morgan TJ, Rollmann SM: Genetics and genomics of *Drosophila* mating behavior. *Proc Natl Acad Sci USA* 2005, 102:6622-6629.

Locomotor senescence: Approximately 200 2- to 4-day-old same-sex flies from each strain are placed in separate bottles. The flies are transferred to fresh bottles every 2-3 days, and each week (on days 14, 21, 28, 35, and 42), 25 flies of each line and sex were scored for locomotor reactivity (see above).

Aggressive Behavior. Socially experienced, 3–7-d-old males, are used for this assay. Aggression of single individuals is quantified by placing one experimental male, with wild-type eye color, with three reference white-eyed males. The flies are placed in a vial without food for 90 min, after which they are transferred (without anesthesia) to a test arena containing a droplet of food. After a 2 minute acclimation period, the flies are observed for 2 min. The following behaviors are scored as aggressive encounters: kick—leg extension from one fly to another resulting in physical contact; chase; charge—rapid approach leading to head-to-head orientation; wing-raise—extension of wings in response to proximity/approach of another fly; and box—high impact interaction involving front legs of both flies. The score of the experimental fly is the number of encounters in which it exhibited an aggressive behavior, including interactions initiated by the experimental fly and those in which he responds aggressively to a reference fly.

Chen S, Lee AY, Bowens NM, Huber R, Kravitz EA (2002) Fighting fruit flies: A model system for the study of aggression. *Proc Natl Acad Sci U S A* 99: 5664–5668.

Food preference:

Extract plant, spice or food extract by grinding in acetone. Mix with 1 ml extract with 0.5 ml corn oil. Pour into fly vial and swirl until acetone evaporates. Add dry fly culture media to vial and moisten with water. Using 1ml disposable pipettes, connect two different food sources to a neutral third vial by inserting into a whole in the side. Seal connections well with parafilm. Add flies to neutral chamber and house in an incubator at 25 °C, 12:12 hour light:dark cycle for 4 days. Remove connections and plug holes. Test the progeny with the same food source options.

Climbing ability: Ten flies are selected from a population and placed in a 250-ml glass graduated cylinder that is sealed at the top with parafilm to prevent escape. A fiber-optic lamp illuminates the cylinder from the top. The flies are gently knocked to the bottom of the cylinder and the time required for 50% of the flies to cross the 150-ml line (17.5 cm) is recorded. Four trials should be completed for each population. Times for each population are averaged for statistical analysis using Student’s t-test.

Flight assay: Flight assays were performed essentially as in Benzer (1973) and Elkins et al. (1986). Groups of 10 flies are dropped into the top of a 500-ml glass graduated cylinder through a glass funnel the end of which reaches the 500-ml mark. The inside surface of the cylinder is coated with paraffin oil, causing flies to become stuck where they strike the wall. The strongest fliers initiate flight immediately and become stuck near the top of the cylinder. The weaker fliers fall farther and become stuck near the bottom of the cylinder. The vertical distribution of each group of flies over the length of the cylinder measures flying ability.

Benzer, S., 1973 Genetic dissection of behavior. *Sci. Am.* 229: 24–37.

Elkins, T., B. Ganetzky and C. F. Wu, 1986 A *Drosophila* mutation that eliminates a calcium-dependent potassium current. *Proc. Natl. Acad. Sci. USA* 83: 8415–8419.

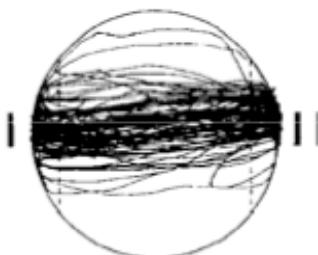
Buridan’s paradigm: A fly will walk spontaneously between two opposing inaccessible landmarks, vertical black stripes) in an otherwise uniformly white surround. With their wings shortened (to prevent flight), one fly at a time is placed in the centre on an 8.5 cm elevated circular white disk that is surrounded by a water-filled moat. The fly is allowed to walk undisturbed for 10 minutes. The number of complete arena crossings is counted.

Bultooft H, Gotz KG, Herre M (1982) Recurrent inversion of visual orientation in the walking, *Drosophila melanogaster*. *J Comp Physiol A* 148: 471-481

<http://www.youtube.com/watch?v=YwGUlgiGcg4>

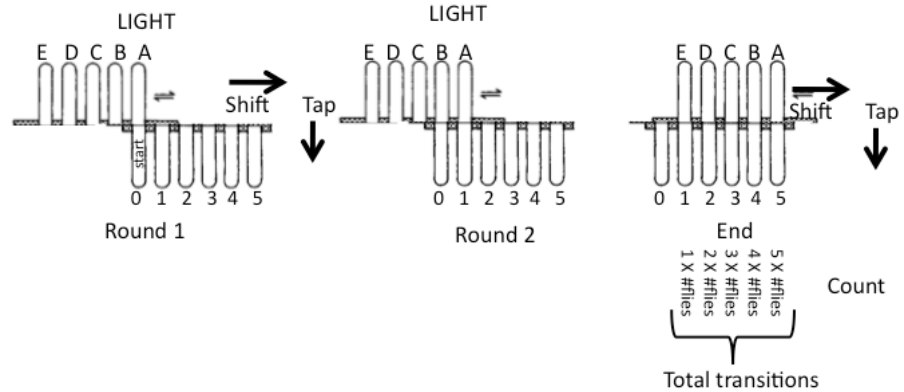
Software is available to conduct automated tracking experiments:

<http://buridan.sourceforge.net/>



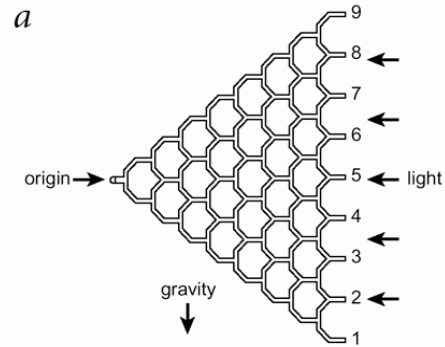
WT(B)

Fast phototaxis: A countercurrent apparatus is used to measure fast phototaxis. In these experiments, a population of ~100 flies is placed in the first of six clear tubes (0), fixed in a rack and laid on a horizontal surface, and transferred through the tubes at 30 (can be changed) sec intervals as they moved toward a light source. Following each shift flies are tapped back to the original side of the apparatus. This can be used to quantify total transitions for a population or to select high and low phototaxis individuals.



Geotaxis: A single run consists of group of 75 same sex adults (females or males) aged 2–6 d post eclosion. The geotaxis maze is built of Tygon tubing with one entry, repeatedly bifurcating through 7 levels with Y connectors. The 9 outlets are fit with collection vials. The maze is mounted to a vertical board with a fluorescent light mounted on the outlet side. At 10 AM, the vial containing test flies is attached to the single opening and Flies start move through the maze toward a light source throughout the day. The test is ended at 3:00 PM by collecting and counting flies in the 9 collection vials. All tests are run at room temperature. The maze can be cleaned between each trial by washing it with dilute Alconox and rinsing it extensively with deionized water.

See Pitman, JL, DasGupta, S., Krashes, MJ, Leun, B., Perrat, PN and Waddell S., (2009) There are many ways to train a fly. Fly 3:art#3
<http://www.landesbioscience.com/journals/fly/article/7726/>



Larval Assays:

Many other assays are available for larval behavior such as phototaxis, food preference, foraging path length etc. Please consult the literature for such assays.

Independent project ideas:

Students may use artificial selection in order to determine whether observed differences in behavior are heritable. After consulting the literature to identify candidate genes, students may use qPCR to determine whether gene expression differences underlie behavioral differences. An ambitious group might even use microarrays to study gene expression (consult with me ahead of time).