



Behavioral Genetics: Investigating the genes of a complex phenotype in fruit flies

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Synopsis:

This laboratory exercise uses both inquiry-based and active-learning approaches to uncover the genetic architecture of behavior in the model organism, *Drosophila melanogaster*. The exercise can be performed in either a single two-hour or two 60-minute lab periods and requires access to computers with an internet connection to help introduce students to modern genetic and genomic analysis. Students first will quantify behavioral interactions associated with mating in wildtype fruit flies. They will then connect these phenotypic ontologies to individual candidate genes using curated data from *Drosophila*'s model organism database, FlyBase. Students will explore known characteristics of chosen candidate genes including models of genic structure, genomic context, and known functional attributes including patterns of spatial and temporal gene expression.

Introduction:

Genomics has revolutionized the way we do biology. The availability of genome assemblies and expertly curated gene annotations across an ever-increasing number of species provides biologists with unprecedented data. Biologists routinely use these "big data" genomic resources to solve a diversity of problems from increasing food productivity (Morrell *et al.* 2012) to preserving our planet's diversity (Awise 2010) to understanding our past (Sankararaman *et al.* 2014; Lachance *et al.* 2012) to curing disease (Stearns 2012). These massive genomic data sets are primarily stored in publicly-funded online databases and databanks, and are available to anyone with internet access, thus, democratizing the process of discovery.

The genome also provides an ideal platform for students to learn the molecular basis of heredity. A hierarchical set of molecular elements—from base pairs to exons to genes to chromatin domains to chromosomes—are conveniently linked as a single string of letters that can be surveyed over different layers of resolution. Online genome browsers with associated genomic data provide interactive roadmaps to visualize and integrate such previously abstract concepts as the distance between genes, the conservation of regulatory domains, and the presence of alternative transcripts. Due to the universal nature of DNA and the genetic code, these concepts are easily transferable across taxa, thus, connecting disparate research communities and integrating data across different disciplines. Furthermore, today's students are increasingly computer-savvy and able to navigate the dynamic databases that host these genomes in addition to being able to quickly adapt to constantly updated data types and online resources. Databases also host many tutorials and demonstrations, providing a valuable, yet underutilized, educational resource.

A primary objective in genetics is to map phenotypic characters (e.g., weight, crop yield, disease susceptibility, etc.) to individual genes. This two-part laboratory exercise leverages the well-known model organism, *Drosophila melanogaster*, and its comprehensive phenotypic, genetic, and genomic knowledgebase, FlyBase, to study the genetics of a complex phenotype. Model organisms including *Saccharomyces cerevisiae* (budding yeast), *Caenorhabditis elegans* (nematode), *Danio rerio* (zebrafish), and *Drosophila melanogaster* (fruit fly) have traditionally paved the way towards understanding the genetic basis of many phenotypic traits. With the availability of fully sequenced and annotated genomes, we now have instant access to the precise genetic elements that encode complex characters as well as genomic tools to dissect their function.

Like humans, behavioral phenotypes in *Drosophila* are complex. However, unlike humans, much is known about the molecular, cellular, and evolutionary components that drive certain behaviors in fruit

flies (Sokolwski 2001). Fruit fly behaviors cover a large range including mechanosensation, optomotor, olfactory, and mating and over a century of fruit fly research has uncovered the underlying genes from screens of behavioral mutants and associated alleles from genetic variation found in natural populations. Surprisingly, many of the genes and genetic pathways that drive certain behaviors in *D. melanogaster* affect similar behavioral phenotypes in other organisms, including mammals. In fact, it has been estimated that nearly 70% of known genes in *D. melanogaster* have an ortholog in humans (Pfleger and Reiter 2008), making *Drosophila* an important genetic model to understand complex human phenotypes.

In the first part of this exercise, students will observe and track standardized courtship behaviors of fruitflies in a petri dish arena via online videos. Many of these complex behaviors have been previously categorized into distinct phenotypic ontologies, also known as controlled vocabularies (CVs), by experts in the fruit fly community. These CVs provide geneticists with a common dictionary of phenotypes that can be connected to individual candidate genes. In the second part of this exercise, students will query FlyBase, an extensive genetic and phenotypic database (www.flybase.org), for a behavioral phenotype that they previously measured. FlyBase demonstrates the power of using model organisms such as *Drosophila*, in terms of the volume and variety of data that they expertly curate for the scientific community. Students will choose two genes and compare their genic (exon/intron) structure, explore translated and untranslated regions (UTRs), and identify alternatively spliced transcripts as well as the experimental and computational evidence that supports them. Students will also explore the local genomic neighborhood that their genes of interest lie in. By understanding how a genome browser and its evidence and data tracks work, students will be able to further explore genes and genomes in many other organisms with a sequenced genome and a resource-rich community of active researchers.

This laboratory resource provides students with a systems genomics and bioinformatics framework to identify and characterize candidate genes of an observed complex phenotype. Upon completion of this exercise, students will have a better understanding of the power of utilizing model organisms and their characterized phenotypes, sequenced genomes, and attributed gene functions to discover and understand the genetic basis of complex traits.

Specifically, students will:

1. Observe, quantify, and classify a complex phenotype
2. Associate observed traits to curated phenotypic ontologies
3. Molecularly characterize potential candidate genes of a complex trait

Approach/Method (Instructor Guidelines):

1. Laboratory Preparation:

1.1. List of materials/supplies needed

1. Internet-accessible computers or tablets (one for each group of 2-3 students)
2. Student Handout on “Fruit Fly Ethomics & Genomics” (appended)

1.2 . Setting up the lab

This laboratory exercise is designed to function during two one-hour laboratory periods or a single two-hour period and can be divided into two parts: i) behavioral observations and classification into

phenotypic ontologies, ii) molecular characterization of genes and exploration of genomic tools/resources via FlyBase to address function.

Behavioral observations. In Part 1, pre-recorded videos of fruit fly behavior will be accessed via YouTube. Pairs of students will be assigned different videos by providing them with the URL links (email works best as they could simply cut-and-paste the link into a web browser). Each 15 minute video will display 5 males and 5 females in a small arena. The videos are found in the YouTube channel entitled, "Fruit Fly behavioral videos". You can use the YouTube search engine with the phrase, "ethomics and genomics" or the title of the lab, "Behavioral Genetics: Investigating the genes of a complex phenotype in fruit flies" to access these videos. Students will fill in their Worksheet (from their Student Handout) as they watch the video.

Using FlyBase to characterize candidate genes. In Part 2, the online biological knowledgebase for *Drosophila*, FlyBase, will be used to identify and characterize candidate genes involved in the behavioral phenotypes that the students observed in Part 1. It is recommended that the same student pair share the same computer and take turns navigating through FlyBase so that they can learn from each other. Alternatively, the instructor could demonstrate the exercise to the class via a large screen (an internet connection is still required), although this requires extra effort by the instructor to elicit full classroom engagement and severely limits the active-learning component of this exercise.

1.3. Concepts for pre-discussion

- Genotype vs. phenotype
- Using *Drosophila melanogaster* vs. other organisms as a genetic model
- Life cycle of a fruit fly, rearing flies in the lab, sexual differences (Student Handout, Figure 1; Additional Resources)
- Mutations, role of mutations with regards to gene function, mutants vs. mutations, phenotypes of mutant fruit flies
- Mating behavior, definition of "courting" as this term is used to identify candidate genes of interest in FlyBase. Show schematic diagram of fly mating behavior (Student Handout, Figure 2)
- Observing animal behavior: objectivity, measurements
- Graphing basics: labeling axes, bar vs. line
- Composition of DNA (nucleotides, chromosome), gene models, DNA>mRNA>protein (information flow), genomic annotations
- Biological databases, model organism databases (MODs), genome browsers

2. Running the Laboratory:

2.1. Part 1. Observing behavioral phenotypes: Courtship in fruit flies

Each student should possess a hardcopy of the Student Handout entitled, "Fruit Fly Ethomics & Genomics". Students should know how to "sex" fruit flies since they will differentiate males from females (Figure 1) to identify target behaviors. A number of short instructional videos are found in "Additional Resources" and should be viewed by students before/during the lab.

Via online videos, each student pair will observe five male and five female wildtype fruit flies in a petri dish for fifteen minutes and record behavioral patterns on the "Behavioral Observation Worksheet", found in their handout. Videos can be accessed through the YouTube Channel, "Fruit Fly Behavioral Videos": <https://www.youtube.com/channel/UCUOoMS6HVnqTQbIThcTEsLA>

Students will attempt to objectively categorize mating behaviors by constructing a basic “ethogram” based on the courtship ontologies defined in their handouts (Figure 2). This ethogram should be replicated on a blackboard so that each group can share their numerical observations with the class. Students will discuss the behavioral phenotypes they observed and will sum and average the frequencies of each behavior from each group.

2.2. Part 2. Characterizing behavioral candidate genes using FlyBase

Replay the video introduction to FlyBase at the beginning of the class (see YouTube link in Additional Resources section, below). The features of this online database can also be demonstrated on a large screen while students are navigating FlyBase on their computers. In particular, show students how to identify and functionally characterize genes associated with known phenotypes. It would be instructive to be familiar with FlyBase by spending a 1-2 hours navigating its many portals, prior to the demonstration. Students are to choose two genes from a courtship behavior using FlyBase’s “Vocabularies” tool, and then investigate each of their genes via their Gene pages and FlyBase’s genome browser, “GBrowse”. Students will explore known functional attributes of each gene by exploring FlyBase gene summaries including “Gene Ontology” found in each gene page, as well as the “Developmental stage subset” track found in GBrowse. Students will then formulate questions/hypotheses about the function of their genes of interest.

If there is not enough time during the class, all exercises in Part 2 (as well as Part 1) can be performed outside the lab period, assuming students have access to an internet connection.

3. Student Evaluation:

This exercise is meant to demonstrate, firsthand, that a complex phenotypic trait such as mating behavior is encoded by multiple genes that can modulate their expression across space (tissue) and time (development stage). Students should be evaluated on the answers from their two worksheets (Behavioral Observation and Candidate Gene Worksheets):

- 1) The quality and precision of their behavioral observations and answers to the questions
- 2) The quality of their bar graphs
- 3) The student’s ability to identify candidate genes of interest
- 4) Understanding the molecular differences between their candidate genes
- 5) The student’s ability to address new functional hypotheses about how their genes of interest may interact to express a complex phenotype

4. Additional Resources:

Wikipedia entry for *D. melanogaster*: http://en.wikipedia.org/wiki/Drosophila_melanogaster

D. melanogaster sexing quiz: <http://www.unc.edu/~abcook18/sexing.html>

Mutant courtship videos (*D. melanogaster*; found in Supplemental Data):
<http://www.sciencedirect.com/science/article/pii/S0092867405004071>

Paper on behavioral observation strategies: Altmann, J., 1974. Observational study of behavior: Sampling methods. *Behaviour* 49:227-267

Introduction to biological databases:

http://papadantonakis.com/images/7/79/Introduction_to_Biological_Databases.pdf

Introduction to FlyBase: <https://www.youtube.com/watch?v=9GrgWPAp1oM>

5. Answer Key (to Part 2 of Student Handout):

Remember: FlyBase is constantly updating, so answers may not reflect the current release version.

- (a) “developmental process”, “reproduction”
- (b) 5,086 bp
- (c) abdominal A, big bang, transformer, loquacious, scarface (and 5000+ others)
- (d) ken[02970]: all flies have abnormal terminalia
- (e) 23
- (f) female reproductive system
- (g) lack of “certain” anatomical features
- (h) protein-coding
- (i) 7,169 bp
- (j) draw a diagram of “ken-RA” from the Transcript track
- (k) embryos_14-16, embryos_16-18, embryos_18-20 (hrs after egg laying)
- (l) Since *ken*’s phenotype is the absence of female genitalia, we may expect that the gene is normally expressed in adult females (e.g., Adf_Ecl_1days) as well as in late developmental stages (e.g., WPP_4days).
- (m) *TM4SF* is directly 3’ to *ken*, and appears to possess three alternative transcripts.
- (n) *D. melanogaster* is an example of a model genetic organism (http://genome.wellcome.ac.uk/doc_WTD020803.html). With their short generation time and easy care, the biology of fruit flies has helped us study many aspects of biology. Even though they are invertebrates, they share many common traits and genes with humans and have helped to shed light on how our own genetic architecture works. It is amazing how much we know about these little flies, but it is also amazing how much of this information pertains to our own development and well-being.

6. Optional Supplement for Live *Drosophila* Observations:

In Part 1, online behavioral videos are provided for students to observe and quantify mating behavior. For those intrepid instructors who would rather have their students observe live fruit flies, we provide the following OPTIONAL instruction.

6.1 List of materials/supplies needed:

1. Wildtype *Drosophila melanogaster* (wild-caught flies from nature are not recommended due to their high activity levels)
2. Glass vials/jars and cotton plugs/tops
3. *Drosophila* food (http://flystocks.bio.indiana.edu/Fly_Work/culturing.htm)
4. FlyNap (to anesthetize the flies)
5. Other fly rearing/handling tools such as fine paint brushes

6. Petri dish (snugly sealed to ensure that flies cannot escape)
7. Hand lens or magnifying glass (a microscope is preferable)
8. Timer (a watch or phone would suffice)

6.2 Setting up the lab

Ordering fruit flies. Flies from a *D. melanogaster* wildtype stock (e.g., Oregon-R, Canton-S) are needed for behavioral observations. In North America, fruit fly stocks can be ordered from Ward's Science (<https://www.wardsci.com>) or Carolina Biological Supply Company (<http://www.carolina.com>). Stocks usually take at least 2-3 weeks to receive and at least a few weeks to expand the population size so order well ahead of time.

Fruit fly rearing, maintenance, and handling. *Drosophila* can be maintained using minimal effort and space (http://flystocks.bio.indiana.edu/Fly_Work/culturing.htm). Flies should be sexed and sorted into individual petri dishes before the students arrive. FlyNap anesthetizes the flies for easy handling and transferring: <http://www.carolina.com/drosophila-fruit-fly-genetics/flynap-anesthetic-kit/173010.pr>. Refrigerating the flies at 4C for 5-10 minutes also provides a quick and easy way to reduce fly activity before handling, but is not as effective.

6.3 Potential problems

1. A major challenge is to time the observation period for students so that flies are maximally active but have not had a chance to begin their mating rituals. Males and females could initially be separated by rigging a thin vertical piece of paper across the petri dish: this separator could then be pulled out just before the students commence their observations.
2. While in the petri dish, fruit flies should have adequate ventilation and not be overheated by direct light sources. A few grains of yeast in each arena will also provide the fruit flies with nourishment.
3. During the observation period, students should not distract the flies (e.g., no tapping or shaking the petri dish).

More information on how to handle, anesthetize, and sex fruit flies:

<http://www.carolina.com/teacher-resources/Video/how-to-observe-phenotypes-with-drosophila-melanogaster/tr11212.tr>

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