Teaching Notes

A simple lab exercise using inbred *Drosophila* strains for introducing quantitative genetics to undergraduates.

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**Abstract**

Students compare mean values between two inbred strains of *Drosophila* for a simple behavioral or anatomical trait. This allows detection of genetic differences between strains affecting the trait, direct partitioning of phenotypic variation into genetic and non-genetic components, and an estimate of heritability. Mean, variance, standard error of the mean, and t-test are introduced and applied to the analysis. The lab is suitable for non-major or major level undergraduate genetics courses as a complement to the more common laboratory exercises for Mendelian genetic analysis. The lab employs simple methods for observing behavioral or morphological traits, uses statistical methods that are simple enough to calculate by hand with pencil and paper to optimize comprehension, and requires no crosses other than maintenance of true-breeding inbred stocks with wild-type phenotypes.

**Concept**

Labs designed to illustrate Mendelian patterns of heredity are often conducted in genetics courses, frequently using *Drosophila* crosses to illustrate patterns of heredity for one or several discrete traits. More complex traits and patterns of heredity, for instance estimation of heritability for quantitative genetic traits, are rarely analyzed in an undergraduate genetics course, in spite of the prevalence and importance of polygenic quantitative traits in natural populations and in human phenotypes (Mackay and Falconer, 1996; Mackay and Anholt, 2006). Impediments to the incorporation of lab instruction for quantitative genetic traits include the difficulty of experimental designs required to partition genetic variation among relatives, and the complexity of associated statistical analyses (cf., Mackay and Falconer, 1996). Quantitative genetic analysis, however, is simplified by complete inbreeding which creates a uniformly homozygous and isogenic strain (Mackay and Falconer, 1996).

Measuring phenotypic variation among flies from just two inbred strains allows direct and simple estimation of genetic vs. non-genetic components of phenotypic variance (Mackay and Falconer, 1996; Hegmann and Possidente, 1981). Since completely inbred strains are isogenic, phenotypic variation among individuals within the same strain estimates only non-genetic effects. Since inbred strains are also homozygous there are no dominance interactions so genetic differences among them estimate only additive genetic effects. Heritability estimates derived from inbred strain comparisons are, therefore, narrow sense heritabilities (Mackay and Falconer, 1996). The only statistical computations required are the mean and variance for a sample of flies from each strain. These, in turn, allow estimation of the heritability for the trait, defined as the proportion of phenotypic variation caused by genetic variation (Mackay and Falconer, 1996) which is otherwise difficult to obtain without comparing relatives across generations. Recently, a large set of inbred lines constituting the *Drosophila melanogaster* Reference Panel (Mackay et al., 2012) has become available from the *Drosophila* stock center in Bloomington, Indiana (USA) (http://flystocks.bio.indiana.edu/, *e.g.* inbred line RAL-21, stock number 28122, through inbred line RAL-913, stock number 28265). These inbred lines were derived from wild type flies, and the genome of each line has been sequenced. Any two of
these lines, or other inbred strains, can be used for the basic quantitative genetic analysis described here, and larger sets of strains permit more sophisticated genetic analyses (cf., Mackay and Falconer, 1996; Possidente and Hegmann, 1981) in more advanced courses and in research.

Methods

Students can breed or be given vials with flies from any two different inbred strains that have common environmental histories. Students are either assigned a trait to assay on a sample of individual flies from each strain, or design their own assay. Designing their own assay affords the opportunity for students to practice observational skills and research design but requires additional lab time. Limiting the subjects to a single sex is optimal to simplify the statistical analysis and minimize within-strain variation. Some simple traits we have assayed range, for example, from body size and sternopleural bristle number, to time required to awake from anesthesia, frequency of grooming, maximum height reached during one minute in a vertical pipette, latency to mating, distance moved per unit time in a horizontal pipette, location in a horizontal pipette after five minutes of acclimation after a sound occurs or an odor is applied to a cotton plug on one end and a blank cotton plug or different odor or sound is presented on the opposite end.

The trait mean, variance, and standard error of the mean are calculated for each strain. We assume a simple additive model where $V_p = V_g + V_e$. $V_p$ represents phenotypic variance, $V_g$ represents genetic variance, and $V_e$ represents non-genetic variance. $V_p$ can be estimated directly as the variance of all the flies from both strains, or as the sum of $(V_g + V_e)$, which can be estimated independently (below). $V_e$ is estimated directly for each strain as the variance among flies within a strain and can be pooled, or averaged for estimates based on the same sample size for a best estimate of $V_e$. Once $V_p$ and $V_e$ are obtained, $V_g$ can be estimated by subtraction, or from the difference between the strain mean values (below). Since inbreeding doubles the additive genetic variance relative to an outbred population (cf., Mackay and Falconer, 1996; Hegmann and Possidente, 1981), estimating $V_g$ by subtraction of $V_e$ from $V_p$ estimates $2V_g$, so this estimate of $V_g$ should be halved to obtain the final $V_g$.

$V_g$ may also be estimated directly by squaring half the difference between the strain means for the trait and dividing by two (McKay and Falconer, 1996; Hegmann and Possidente, 1981), which estimates $V_g$ for the special case of two inbred strains. The strain difference between the mean values for a trait estimates twice the average additive genetic effect of allelic differences between the strains ($2a$). Dividing the mean strain difference by two estimates “$a$”, squaring “$a$” estimates twice the additive genetic component of variance, and halving this value estimates $V_g$.

Finally, heritability (the proportion of phenotypic variance caused by genetic variance: McKay and Falconer, 1996) can be estimated as $V_g/V_p$. We compare the two strain means using Student’s t-test and assume that a significant difference represents genetic variation caused by genetic differences between the strains.

Sample Calculation

The set of data below contains anatomical measurements on ten individual flies of the same sex from each of two inbred strains for body size defined as the distance in mm from the posterior tip of the scutellum to the anterior edge of the pronotum (measurement method adapted from Lefranc et al., 2000). The data represent extreme strain values from a set of 65 inbred strains analyzed (unpublished), in order to illustrate the case of two inbred strains with a significant strain difference.

Strain 1 Values from ten flies: (4.4, 4.2, 4.2, 4.2, 4.1, 4.5, 4.3, 4.3, 4.3, 4.3);
Mean$_1$ = 4.28, Variance$_1$ = Ve$_1$ = 0.013;
Strain 2 Values from 10 flies: (2.3, 2.5, 2.3, 2.5, 2.8, 2.7, 2.6, 2.7, 2.8, 2.7);
Mean$_2$ = 2.59, Variance$_2$ = Ve$_2$ = 0.034;
Ve = (Ve$_1$ + Ve$_2$) × 0.5 = 0.024;
Total Variance = Vp = 0.77;
Vg from inbred strain mean difference = 0.5[((Mean$_1$ - Mean$_2$)/2)$^2$] = 0.357;
Vg from (Vp-Ve)/2 = 0.373;
Heritability ($h^2$) = Vg/Vp from estimating Vg as 0.5[((Mean$_1$ - Mean$_2$)/2)$^2$] = 0.94;
Heritability ($h^2$) = Vg/Vp from estimating Vg as (Vp-Ve)/2= 0.94.

Discussion

The lab permits an introduction to the analysis and genetic basis of quantitative traits, with no more investment of time or resources than that required to teach Mendelian genetic lab exercises using *Drosophila*. It is inexpensive, concept-rich and investigative, permits the introduction of parametric statistical methods for genetic analysis, and lends itself to collaborative work within groups, class presentations and formal lab reports. The availability of multiple inbred strains for class use will increase the likelihood that some students will select two strains with different mean values for the assayed trait. The lab can also be made incrementally more complex at the discretion of the instructor with respect to genetic concepts, experimental designs, and statistical methods. Limitations of the methods can also be examined, such as inability to identify specific genetic loci, the absence of dominance effects, the absence of epistasis other than additive by additive interactions (Mackay and Falconer, 1996), the limited number of genotypes represented, and potential for confounding genetic differences with maternal effects or epigenetic mechanisms. We present this exercise to facilitate the inclusion of quantitative genetic analysis in undergraduate courses so that students at this level may acquire a more comprehensive and realistic understanding of genetic concepts and analysis.

References:

Can selection alter the frequency of recombination of *Drosophila melanogaster*?

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It has been observed that recombination can increase the amount of new multi-gene genetic variation, which in turn can influence adaptive evolution (Muller, 1964; Maynard Smith, 1978, 1988; Michod, 1995). What is not completely understood is how selection influences frequencies of recombination. In *Drosophila melanogaster* there are three reports that recombination is increased for the autosomes in selection experiments for DDT resistance (Flexon and Rodell, 1982), resistance to daily temperature fluctuations (Zuchenko *et al*., 1985), and geotaxis (Korol and Liliad, 1994). The influence of selection on the frequency of recombination, however, has only been estimated for the sex chromosomes in one *Drosophila melanogaster* experiment (Korol and Liliad, 1994), and no study has combined selection for bristle number with recombination.

To measure the influence of natural selection on the rate of recombination, we selected for increased sternopleural bristle numbers on the thorax of *Drosophila melanogaster* and determined if there was a concomitant change in the frequency of recombination for two X-linked genes (white eyes, $w$, and singed bristles, $sn^3$). It is our hypothesis that selection will increase the frequency of recombination for X-linked genes.

As a non-selection control, we measured the frequency of recombination between $w$ and $sn^3$ in the Canton-S wild-type stock background before selecting for increased bristle numbers by the following crosses.