Teaching Notes

Response to selection in the presence and absence of genetic variation in *Drosophila melanogaster*.

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Genetic variation can be defined as alternative versions, or alleles, at some genes in natural populations or laboratory stocks. Without genetic variation within a population or species, evolution will not occur, or will be slow, because there must be time for rare mutations to give rise to the new variation needed for selection. Ewens (2003), for example, stated that "…with no genetic variation, natural selection cannot act".

Beginning with Darwin (1859), it has also been assumed that artificial selection (under human control) is an appropriate model for natural selection. Darwin began his arguments for evolution by natural selection by observing that breeders in a few generations could drastically change the form of plants and animals. These changes were especially evident in his famous domestic breeds of pigeons, such as the short-faced tumbler, the pouter and the turbit (see Chapter One of the Origin of Species by Means of Natural Selection by Charles Darwin, 1859). As another example, a farmer who selects for greater milk production will only be successful if the cows have some genetic variation. There would be no response to selection for increased milk production if the cows were highly inbred, with both copies of all genes having the same nucleotide sequences. The same lack of selection response will also occur for fitness traits in highly inbred organisms in nature, such as fig wasps and honeybee parasitic mites where brothers and sisters preferentially mate each generation

(http://www.figweb.org/Interaction/Life_cycle/index.htm; http://creatures.ifas.ufl.edu/misc/bees/varroa_mite.htm).

Hence, there should be no response to selection in a laboratory model organism that has no genetic variation, whereas selection response would be possible, and usually quick, if there is standing genetic variation. With this in mind, it is the objective of this *Drosophila* teaching exercise to measure in a few generations selection response for increased bristle numbers in an inbred line that is homozygous and lacks genetic variation and in a natural population line that contains genetic variation. The inbred line should show no response to selection, whereas the natural population line should respond to selection.

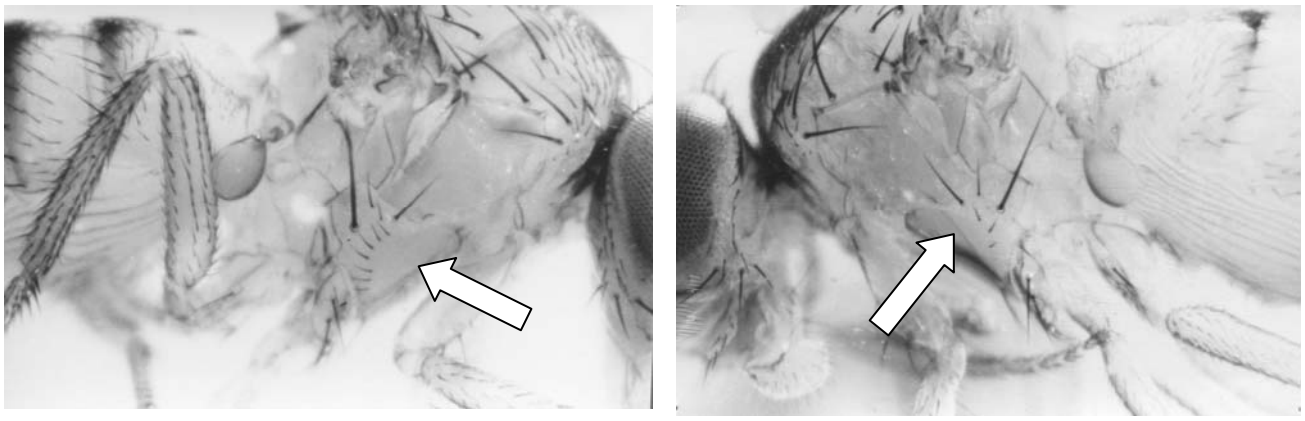

Figure 1. Sternopleural "heart-shaped" section of two *Drosophila* marked with white arrows. Sternopleural bristle numbers are: left, 11; right, 6. There is one isolated bristle near the ventral midline that cannot be seen here; it is invariant and is never counted.

The homozygous inbred line, which contained essentially no genetic variation, was derived by 41 generations of single brother-sister matings of a sepia (*se*, dark eye color) laboratory stock. Since inbreeding decreases heterozygosity over time, after 41 generations of brother-sister matings the genes in the inbred (*se*) line should all have been homozygous (Hedrick, 2005). For a classroom exercise, almost any standard laboratory mutant strain will probably have been maintained for a long enough period of inbreeding sufficient to demonstrate the same phenomenon. The inclusion of the *se* marker in this inbred line insured that we did not get an accidental contamination of this line by another stock during the inbreeding matings. Such a contamination would give flies with wild-type (red) eyes. The natural population line was derived from six mated females collected in Perrysburg, Ohio. This Perrysburg line contained the genetic variation found in these six natural population females and their mates, for a total of 18 X chromosomes and 24 each of the three autosomes, since *D. melanogaster* has eight total chromosomes.

In the initial generation we counted the number of sternopleural bristles (see Figure 1) on each side of 30 females and 30 males of the inbred (*se*) and Perrysburg lines. We then selected and mated six females and six males with the highest bristle counts from each line for the next selection generation. We then repeated this selection scheme for eight generations.

The mean bristle number and standard deviation for females and males in each generation for the inbred (*se*) and Perrysburg lines are shown in the Table 1 and Figures 2 and 3. As predicted, the inbred (*se*) line, which did not contain standing genetic variation, did not respond to selection in eight generations. The slopes over eight generations for females and males are almost zero (= 0.021 and

0.001, respectively). Conversely, the Perrysburg line, which contained genetic variation, did respond to selection over the eight generations. The slope for females was 0.147 and for males was 0.269. These increases in bristles in the Perrysburg flies were significantly greater than in the inbred (*se*) flies ($P < 0.0001$ by an unpaired t-test, for females and males).

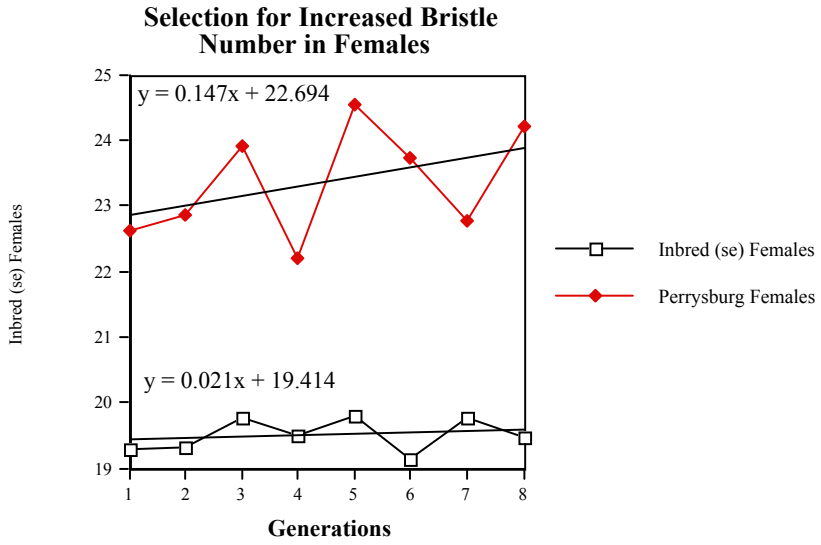

Figure 2. Changes in sterno-pleural bristle number in females.

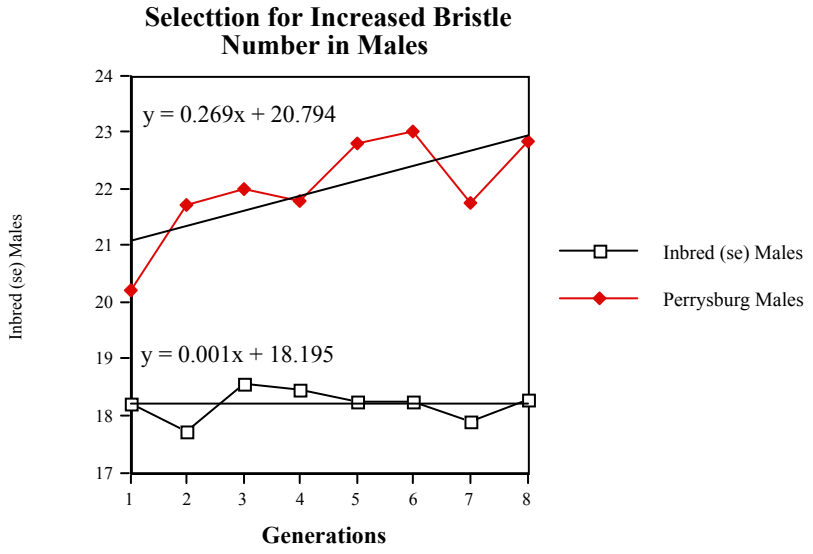

Figure 3. Changes in sterno-pleural bristle number in males.

Hence in only eight generations or less, since there were no delays in the response to selection in the Perrysburg line, it was shown that standing genetic variation is required for rapid response to selection in *Drosophila*, supporting the hypothesis that genetic variation is needed for response to natural selection and for evolution in nature.

A class discussion of the results of this experiment could include: 1) How many generations would it take for a response to selection in the inbred line? Remind students that this would depend on the occurrence of rare, new mutations. Significant responses in long-term selection experiments, sometimes with irradiated lines, have been reported by Clayton and Robertson (1955, 1964) and others (see Lynch and Walsh, 1998), and Mackay *et al*. (2005) got such a response in about 15-20 generations. 2) What is the heritability for bristle number increase in the Perrysburg line? Heritability (h^2), the relative importance of heredity in determining phenotypic values, can be estimated in one generation from a selection experiment, with heritability = R/S, where R is the response to selection and S in the selection differential (Falconer and Mackay, 1996). As an example from Table 1, for the Perrysburg females in the first generation R = 0.24 (the average number of

Table 1. Selection for sternopleural bristle numbers in an Inbred (*se*) line with no genetic variation and in a Perrysburg natural population line that had standing genetic variation.

Gen	Inbred (*se*) Females	Sel[b]	R[c]	Perrysburg Females	Sel	R	Inbred (*se*) Males	Sel	R	Perrysburg Males	Sel	R
1	19.30[a] (1.15)	21.17 (1.87)	0.03	22.63 (2.41)	26.33 (3.70)	0.24	18.20 (1.32)	20.33 (2.13)	-0.47	20.20 (1.75)	22.33 (2.13)	1.50
2	19.33 (1.06)	21.00 (1.67)	0.44	22.87 (2.08)	25.83 (2.96)	1.03	17.73 (1.46)	19.83 (2.10)	0.84	21.70 (1.66)	24.17 (2.47)	0.30
3	19.77 (1.31)	21.83 (2.06)	-0.27	23.90 (2.01)	26.33 (2.43)	-1.70	18.57 (0.86)	19.50 (0.93)	-0.10	22.00 (1.95)	24.50 (2.50)	-0.23
4	19.50 (1.11)	21.17 (1.67)	0.30	22.20 (2.28)	25.67 (3.47)	2.33	18.47 (1.04)	19.83 (1.36)	-0.24	21.77 (1.20)	24.50 (2.73)	1.03
5	19.80 (1.24)	21.50 (1.70)	-0.66	24.53 (1.66)	26.83 (2.30)	-0.80	18.23 (1.19)	19.83 (1.60)	0.01	22.80 (1.54)	25.17 (2.37)	0.20
6	19.14 (1.38)	21.17 (2.03)	0.63	23.73 (2.36)	27.50 (3.77)	-0.96	18.24 (1.19)	19.83 (1.59)	-0.34	23.00 (1.55)	25.17 (2.17)	-1.27
7	19.77 (1.25)	21.33 (1.56)	-0.30	22.77 (1.87)	25.50 (2.73)	1.43	17.90 (1.40)	19.83 (1.93)	0.37	21.73 (1.98)	24.83 (3.10)	1.10
8	19.47 (1.11)			24.20 (2.19)			18.27 (1.41)			22.83 (2.18)		
Avg		1.79	0.02		3.05	0.22		1.66	0.01		2.50	0.38
		h^2 = 0.01			h^2 = 0.11			h^2 = 0.01			h^2 = 0.15	

[a]Mean (Standard Deviation).

[b]Sel = Number of bristles in selected flies (Selection differential = S)

[c]R = Selection response (change in the number of bristles from one generation to the next).

h^2 = narrow sense heritability = R/S.

bristles went from 22.63 to 22.87) and S = 3.70 (the average bristle number in the base population was 22.63, whereas the number was 26.33 in the selected flies). Hence, heritability = 0.24/3.70 = 0.07. The heritability for the first generation of Perrysburg males was 0.70, for the inbred (*se*) females was 0.02, and for the inbred (*se*) males was zero (the response to selection was negative, *i.e.*, the number of bristles went down). The average heritability over the eight generations is shown in Table 1. Note that the average heritability is 11 times higher in Perrysburg females versus inbred (*se*) females and 15 times higher in Perrysburg males than in inbred (*se*) males. 3) Why were there any responses to selection in the inbred (*se*) females or males? It may be that 41 generations of brother/sister inbreeding does not completely remove all standing genetic variation in the base *se* stock, or that some new mutations arose in the inbred *se* stock during this experiment. Since a number of genes contribute to bristle number, the overall mutation rate for this trait may be as high as 0.1 or more (Lynch and Walsh, 1998). 4) How many genes control bristle number in *D. melanogaster*? Gurganus *et al*. (1999) have estimated that about eight quantitative trait loci (polygenes) control bristle number in *D. melanogaster*.

References: Clayton, G. A., and A. Robertson 1955, Am. Nat. 89: 151-158; Clayton, G. A., and A. Robertson 1964, Genet. Res. 5: 410-422; Darwin, C., 1859, *The Origin of Species by Means*

of Natural Selection. John Murray, London; Ewens, W.J., 2003, Population genetics. Nature Encyclopedia of the Human Genome 4: 623-625; Falconer, D.S., and T.F.C. Mackay 1996, *Quantitative Genetics.* Longman Group Ltd., Essex, England; Gurganus, M.C., S.V. Nuzhdin, J.W. Leips and T.F.C. Mackay 1999, Genetics 152: 1585-1604; Hedrick, P.W., 2005, *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, Massachusetts; Lynch, M., and B. Walsh 1998, Genetics and Analysis of Quantitative Traits. Sinauer Assoc., Inc., Sunderland, MA; Mackay, T.F.C., R.F. Lyman, and F. Lawrence 2005, Genetics 170: 1723-1735.

Adaptation of standard *Drosophila* whole-mount *in situ* hybridization protocols for use in the undergraduate teaching laboratory.

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Introduction

The process of *in situ* hybridization allows for examination of a gene's transcription pattern. Analysis of expression patterns can indicate when and where the product of that gene might function. The following protocol is an adaptation of standard *Drosophila* whole-mount *in situ* hybridization protocols (Tautz and Pfeifle, 1989; Patel, 1996) for use in the undergraduate teaching laboratory. It can be completed over the course of two to three laboratory sessions and involves little work for students outside of the normal laboratory meeting time. This exercise is suitable for a number of upper-level undergraduate courses, such as genetics, molecular biology, cell biology, or developmental biology, and could also likely be used at the introductory level. It involves only standard molecular biology equipment, including micropipetters, a water bath (shaking is helpful, although not necessary), and a rotating shaker. Although initial purchase of the chemicals required for this procedure can be a bit expensive, most of the chemicals can be stored over time and used in subsequent years.

During the process of *in situ* hybridization, probes for particular genes are hybridized to mRNA molecules within tissues. In short, gene-specific probes that are chemically modified through the incorporation of digoxigenin-UTPs are bound to tissues that have been fixed and then permeablized. The probe, which is complementary to mRNA molecules of a particular gene, is allowed to hybridize to mRNA in the tissue. Excess unbound probe is removed in a series of wash steps. Alkaline phosphatase-conjugated antibodies against digoxigenin are then bound and used to visualize where probe has hybridized. Substrate for alkaline-phosphatase is added, and the product of this substrate's reaction with alkaline-phosphatase produces a bluish-purple color that corresponds to where the gene of interest is expressed (Tautz and Pfeifle, 1989; Patel, 1996).

The procedure described below is modified from Patel (1996), Patel *et al.* (2001), and Duman-Scheel *et al.* (2002). Although this protocol was written for use of *Drosophila* embryos or imaginal discs, comparable protocols have been used on embryos of many different species (Patel *et al.*, 2001; Duman-Scheel *et al.*, 2002). I generally choose to use flies in the teaching lab, because the procedure can be completed very rapidly. Please refer to Patel (1996) for more details, including instructions for making solutions. Critical modifications that make the following protocol suitable for undergraduate labs include: elimination of the xylene treatment (Patel *et al.*, 2001), replacement of the tricky proteinase-K treatment with a detergent treatment step and addition of SDS to the

hybridization solution (Patel *et al.*, 2001; Duman-Scheel *et al.*, 2002), modified blocking and wash steps that are more appropriate for time constraints found in the teaching laboratory, and delay of the color reaction such that students can perform the reactions themselves in lab during week two. Although this protocol typically works quite well for students, you will probably want to refrain from using this streamlined procedure in your research experiments, which are likely more sensitive.

When preparing for this laboratory, I typically fix the embryos (according to Patel, 1996) or imaginal discs (in PEM with 4% formaldehyde) myself and then let the students use these tissues. However, if desired, students could fix tissues themselves during the laboratory period preceding the sequence described below. Since probe quality is of the utmost importance for the success of this protocol, I synthesize my own probes prior to the lab. Riboprobes are typically necessary when the Proteinase K step is eliminated. I highly recommend using riboprobes that you have made and used successfully in past experiments. Detailed steps for probe synthesis can be found in Patel (1996). Below, you will find the protocol that I give students with a few notes added to help the lab instructor.

General Suggestions

1. In order to cut down on potential RNAse contamination and to protect yourself from noxious chemicals, *wear gloves throughout the entire procedure.*
2. You will be working with micro-volumes of reagents, so be sure to pipette carefully and accurately.
3. Embryos/discs are extremely small and do not always sink very quickly. Be careful not to lose them during the course of the experiment.
4. Take care not to let the tissues stick to the sides of the tube, and make sure that they are covered by solution constantly so that they will not become desiccated.
5. Unless otherwise specified, use a 1 ml volume for all washes.
6. Students typically work in groups of four. Different groups may be using different probes/flies with different genotypes, so it is important to follow the instructions given to your group. The class can pool all of the data at the end of the experiment in a "show-and-tell" session.

Procedure

Lab Period 1

1. Obtain embryos/discs that have been previously fixed (they can be stored in methanol at –20^{o} C). The embryos/discs can be kept in microfuge tubes for the duration of the experiment. I typically use approximately 50 µl volumes of embryos/discs per tube. Rehydrate the tissue with phosphate buffered saline (PBS) with one five-minute rinse. Proceed with 2× 15 minute rinses in PTw (PBS + 0.1% Tween-20).

2. For embryos only (do not do this for imaginal discs): Remove the PTw, and add 1 ml of Detergent/Tween-20 solution (1.0% SDS, 0.5% Tween-20, 50 mM Tris-HCl at pH 7.5, 1.0 mM EDTA at pH 8, 150 mM NaCl). Incubate embryos in the Detergent/Tween 20 solution for 30 minutes with shaking. Steps 1 and 2 can be completed by the lab instructor/teaching assistant prior to class if time is an issue.

3. Rinse 2× 10 minutes in PTw.

4. Remove the PTw and rinse 1× 5 minutes with 500 µl of 50% PTw/50% Hyb solution. Hyb solution contains: 50% formamide, 5× saline sodium citrate (SSC), 0.1% Tween-20, and 50.0 µg/ml heparin (adjust pH of Hyb solution to 5 for use with riboprobes). Replace the 50% PTw/50% Hyb with 500 µl of Hyb solution for 10 minutes. While the tissues are incubating in Hyb, move on to step 5.

5. Heat-denature the Hyb+DNA solution (Hyb solution + 100.0 µg/ml sonicated salmon sperm DNA) by boiling it for 10 minutes. *For embryos only,* add 0.3% sodium dodecyl sulfate (SDS) to the Hyb+DNA solution. The boiling step denatures double-stranded DNA, which is the main blocking reagent in the Hyb+DNA solution. Store the denatured Hyb+DNA on ice until you are ready to use it.

6. Remove the Hyb solution from the tissues and add 100 µl of denatured Hyb+DNA solution. Place these tubes in a floating rack located in the 60^{o} C water bath. This step, which is referred to as pre-hybridization, is useful for removing non-specific background. Keep the tubes in the water bath for 30 minutes.

7. Toward the end of the pre-hybridization step, heat-denature the riboprobe resuspended in Hyb+DNA solution (add 0.3% SDS only if the probe will be used on embryos) by boiling it for five minutes. This helps to remove secondary structure in the RNA probe, which makes it more likely to bind to mRNA.

8. Take your tubes from the water bath. Remove the Hyb solution, and add 100 µl of the denatured probe. Mix the embryos with the probe by taking your pipette tip and stirring the solution gently.

9. Place your tubes in the 60^{o} water bath, where they will remain overnight (note that hybridization temperatures can be optimized for specific riboprobes, but do not go above this temperature if you are using discs, which are more fragile).

10. Consult with your laboratory partners and devise a plan for performing the wash steps on day two (see below). In a group of four, each student will need to do one wash.

Day Two

11. Remove and save your probe (it can be reused) in a labeled microfuge tube. Then complete the following series of washes *at 60^{o} C* with pre-warmed solutions*:*

1× 30 minutes with 1 ml of Hyb solution (typically done by the instructor/TA)
4× 30 minutes with PTw (washes can go longer; one wash done per student)

At room temperature:
1× 30 minutes with PT (PBS + 0.1% Triton-X; typically performed by the instructor)

12. Remove the wash solution, and add 300 µl of anti-Dig antibody at a dilution of 1:2000 in PT. Mix gently with your pipette tip. Leave the antibody on overnight at 4^{o} C or at room temperature for two hours.

13. Remove the antibody. Fill the tube with PT. The tubes can be stored at 4^{o} C for at least one week (until the next lab period).

Lab Period 2

14. Rinse the tissue 4× 5 minutes with PT at room temperature.

15. Rinse the tissue 3× 5 minutes in alkaline phosphatase (AP) buffer (it is critical that this is done properly to ensure quicker reaction times).

16. Add 300 µl of BCIP/NBT solution, the substrate for alkaline phosphatase that turns a purple color. Carry out the majority of this reaction in the dark. Occasionally, transfer a few embryos/discs (using a cut tip) to a depression slide in order to observe the progress of the reaction; you need not worry about briefly exposing the reaction to light when you are checking its progress. This reaction can take anywhere from several minutes to several hours to complete.

17. Seeking the opinion of your instructor, stop the reaction by removing the NBT/BCIP and rinsing 3× 5 minutes with PT and 1× 5 minutes with PBS. Remove the PBS, and add 500 µl of 70% glycerol solution. Leave your embryos at room temperature so that the tissues will clear.

18. Mount the embryos on slides. Analyze and photograph the results. You may wish to dedicate another lab period to data analysis.

References: Duman-Scheel, M., N. Pirkl, and N.H. Patel 2002, Dev. Genes Evol. 212: 114-123; Patel, N.H., 1996, *In situ* hybridization to whole mount *Drosophila* embryos. In: *A Laboratory Guide to RNA: Isolation, Analysis and Synthesis* (Kreig, P.A., ed.), pp. 357-369. Wiley Liss, New York; Patel, N.H., D. Hayward, S. Lall, N. Pirkl, D. DiPietro, and E. Ball 2001, Development 128: 3459-3472; Tautz, D., and C. Pfeifle 1989, Chromosoma 98: 81-85.

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by 15 December to insure their inclusion in the regular annual issue. Submissions in Microsoft Word, which is now the program we use for our page setup, are especially helpful. Submissions by email are also possible, but if they are sent as attached files, we have greatest success using MS Word or Rich Text Format. Pictures and line drawings should be as sharp and high contrast as possible. Where tables are concerned, it is useful to have a paper copy to facilitate accurate formatting. Details are given in the Guide to Authors.