

Teaching Notes



The interchromosomal effect on recombination in *Drosophila melanogaster*.



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Genetic variation is one of the fundamental bases of all evolutionary change (Dawkins, 1995). Recombination plays a very important role in maintaining genetic variation by creating new gene combinations that can be selected (Kutschera and Niklas, 2004). Recombination has also often been linked with the evolution of sexual reproduction (Barton and Charlesworth, 1998; Burger, 1999; Charlesworth, 1993), and is an important mechanism for the elimination of deleterious mutations (Felsenstein, 1974). Since recombination is so important it is not surprising that it could be under genetic control and that a decrease in recombination in one chromosomal pair would be compensated for by an increase in activity in other pairs.

Arthur Steinberg was the first, we believe, to describe what later became known to some as the “Steinberg Effect.” The phenomenon he described (Steinberg, 1936, 1937) was that inversions increase crossingover on non-homologous chromosomes. In discussing the effect of autosomal inversions on crossingover in the X chromosome, he pointed out some of the characteristics of this phenomenon. In particular, increased crossingover is the result of an increase in multiple recombination. Working with the autosomal inversions Payne [In(3R)P], Curly [In(2L)Cy], and a combination of the two, he also showed that the effect of the combined inversions is greater in every region than is the effect of the inversions individually. But the effect of non-homologous inversions on crossingover varies among different regions of the chromosome. Later experiments confirmed the observation that the presence of one or more heterozygous inversions in one chromosomal pair of *D.*

melanogaster results in an increase in recombination in the other chromosomes (*e.g.*, Lucchesi, 1976). However, this effect is not uniform. On the X chromosome, for example, there is a marked increase in recombination frequency in both regions proximal and distal to the centromere, while the middle region remains largely unaffected (Portin and Rantanen, 1990; Chadov, 1999).

We have tested for these interchromosomal effects on recombination in *D. melanogaster* by measuring recombination of the X chromosome of sibling females in the presence and absence of multiple inversions that inhibit recombination of the second and third chromosomes. We observed that X chromosome recombination was significantly increased in females containing the inversions. However, this effect was more pronounced between genes located closer to the proximal end of the chromosome.

The interchromosomal effect on the X chromosome in *D. melanogaster* was demonstrated by using the following mating scheme (females are listed first).

P

$w\ m\ f\ /\ + ; T(2;3)Al-W, Cy\ L\ Ubx\ /\ + \times w\ m\ f\ /\ Y ; T(2;3)Al-W, Cy\ L\ Ubx\ /\ +$



F1

$w\ m\ f\ /\ + ; T(2;3)Al-W, Cy\ L\ Ubx\ /\ + \times w\ m\ f\ /\ Y$ $w\ m\ f\ /\ + ; + \times w\ m\ f\ /\ Y$

F2

Score F2 generation for recombination of the X chromosome in males

The recessive X chromosome alleles all produce distinctive phenotypes: white eyes (*w*, 1-0.1), miniature wings (*m*, 36.1), and forked bristles (*f*, 56.7). *T(2;3)Al-W* has multiple inversions superimposed on a translocation between the second and third chromosomes. This translocation is homozygous lethal; is marked with the dominant mutations Curly (*Cy*) wings, Lobe (*L*) eye, and Ultrabithorax (*Ubx*) enlarged halteres; segregates as a unit; and suppresses crossing over on both the second and third chromosomes by the elimination of recombinants that have extra or missing segments of the chromosome (see flybase@flybase.net). Initial crosses were between single pairs of flies to produce sibling females for the F1 cross. The F1 females were separated according to the presence or absence of lobed eyes (*L*). These F1 females were then crossed in single pairs with *w m f* / *Y* males so that recombination in the F1 females could be detected. The resulting F2 males were then scored for the presence or absence of X chromosome recombination.

The number of flies showing recombination between white and miniature in flies heterozygous for the *T(2;3)Al-W* inversions was 1138 out of 2619 (43.45%). In contrast, the number of flies showing recombination between white and miniature in flies without the inversions was 494 out of 1723 (28.67%, $P < 0.05$). The number of flies showing recombination between miniature and forked with the inversions present was 658 out of 2619 flies (25.12%). In contrast, the number of flies without the inversions showing recombination between miniature and forked was 395 out of 1723 (22.96%, $P < 0.05$) (Table 1). The reported map distances are: *w* to *m* = 34.6 and *m* to *f* = 20.6 (Lindsley and Zimm, 1992). Since frequency of recombination is used to calculate the map points along chromosomes (1% recombination = 1 map unit), these results would translate into an increase in the map distances between white/minature and miniature/forked in females with heterozygous autosomal inversions, compared to the females with structurally normal second and third chromosomes, by 14.78 and 2.16 map units, respectively (Figure 1).

Table 1. Results from recombination.

Recombinant genes	Number of flies exhibiting recombination	Total flies	% of flies exhibiting recombination
<i>w/m</i> with inversion	1138	2619	43.45
<i>w/m</i> without inversion	494	1723	28.67
<i>m/f</i> with inversion	658	2619	25.12
<i>m/f</i> without inversion	395	1723	22.96

In a separate set of experiments carried out by one of us (JNT) as an undergraduate many years ago, recombination was measured for second chromosome loci:

echinoid (*ed*, 2-11.0), dumpy (*dp*, 2-13.0), and clot (*cl*, 2-16.5) with X chromosome inversions In(1)AM and In(1)AB (Lindsley and Zimm, 1992). For In(1)AM, crossingover increased about 71% between *ed* and *dp* (control: 1.10 cM; experimental: 2.63 cM), but the estimates for *dp* to *cl* were only slight higher (control: 3.01 cM; experimental: 3.76 cM). For In(1)AB, crossingover increased about 44% between *ed* and *dp* (experimental: 1.82 cM) and 42% between *dp* and *cl* (experimental: 5.18 cM).

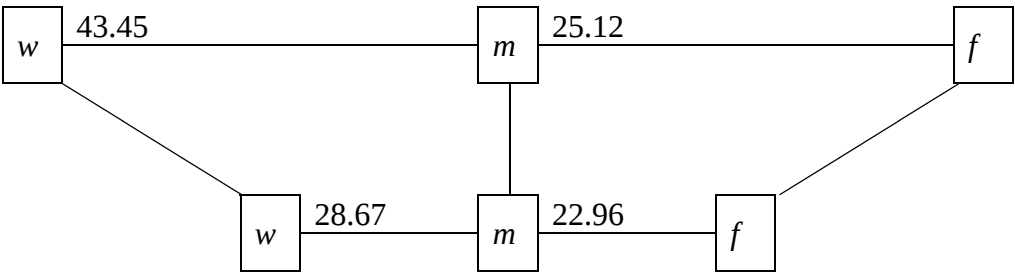


Figure 1. Map of distances for inversion (top) vs. non-inversion (bottom) flies.

In summary, the crosses shown above are an effective method to demonstrate the interchromosomal effect on recombination in *D. melanogaster*. Topics of discussion based on this exercise might include: 1) the role of recombination in increasing the genetic variation available for selection and evolution, 2) the role that recombination may have played in the evolution of sexual reproduction, 3) the discrepancy between the observed map distances recorded in the control and those recorded in the literature, and 4) why recombination may be more prevalent in both the distal and proximal ends of the chromosome but reduced in the middle. A related question is whether there might be a comparable effect for heterozygous translocations (*cf.*, Hinton, 1965) or other chromosomal rearrangements. This could be the basis for an in-depth discussion of experimental design.

References: Barton, N., and B. Charlesworth 1998, *Science* 281: 1989-1990; Burger, R., 1999, *Genetics* 153: 1055-1069; Chadov, B., E. Chadova, S. Kopyl, and G. Anan'ina 1999, *Genetika* 35(4): 459-466; Charlesworth, B., 1993, *J. Heredity* 84(5): 345-350; Dawkins, R., 1995, *River Out of Eden*, New York: BasicBooks; Felsenstein, J., 1974, *Genetics* 78: 737-756; Kutschera, U., and K. Niklas 2004, *Naturwissenschaft* 91(6): 255-276; Hinton, C.W., 1965, *Genetics* 51: 971-982; Lindsley, D., and G. Zimm 1992, *The Genome of Drosophila melanogaster*, Academic Press, Inc., NY; Lucchesi, J., 1976, In: *The Genetics and Biology of Drosophila*. (Ashburner, M., and E. Novitski, eds.), Vol. 1a, Academic Press, NY; Portin, P., and M. Rantanen 1990, *Genetica* 82(3): 203-208; Steinberg, A., 1936, *Genetics* 21: 615-624; Steinberg, A., 1937, *Proc. Nat. Acad. Sci. USA* 23: 54-56.



A genetic analysis of unknown eye and body color mutations in *Drosophila melanogaster*; a multi-week laboratory exercise for undergraduates.

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Most students first learn about Mendelian inheritance as a predictable pattern for a trait to be expressed in offspring where the genotypes, or at least the phenotypes, of the parents are known. For example, given two individuals heterozygous for a dominant trait, the offspring are expected to be produced in a 3:1 ratio, where 75% have the phenotype of the parents, and 25% express the recessive trait because they will be homozygous recessive. In research, whether the question is of a medical disease or about an interesting characteristic found in a natural population, the underlying genetic basis is typically unknown. Instead, the real-life problem is to understand the inheritance of an interesting unknown phenotype rather than to simply verify inheritance of a known genotype. Here we present a laboratory protocol to teach these skills that can be applied in a 1-credit weekly course accompanying a genetics or evolution lecture. The concept for this laboratory was derived and original strains were obtained from a genetics laboratory course still taught at Cornell (MacIntyre, 1999; Debra Nero, personal communication).

Available from the authors at r.krebs@csuohio.edu is the complete 8-week laboratory protocol and the strains of flies where students will investigate the genetic basis of two phenotypes, white eyes and dark body. Many different genes can affect eye color, and the strains used each have two different eye color mutations. Body color is affected by one of two mutations, either the gene black or ebony. While all of these mutations follow Mendelian inheritance, the goal of the student is to make a series of crosses and use the phenotypes of the progeny to interpret which mutations their strain possesses. Crosses using a chromosomal marker strain enable the student to discriminate between chromosomes. *Drosophila* have four chromosomes. Two are large autosomes, chromosome 2 and chromosome 3. Chromosome 1 is the sex chromosome, and genes on this chromosome produce a sex-linked inheritance pattern. The small fourth chromosome is ignored in this laboratory assignment, or it may be considered as a "none-of-the-above" option. Linkage relationships among loci also can be identified thereby giving a fairly complete introduction of the underlying organization of the genes producing the dark-bodied white-eyed phenotype at a chromosome level.

At the end of the laboratory, students are expected to be able to answer the following questions and to produce results to defend their conclusions:

- 1) How many genes are involved?
- 2) How are these genes inherited (dominant, recessive, etc.)?
- 3) Where is each gene located? On which chromosome does each of these genes occur?
- 4) Are any of your genes genetically linked; do they reside on the same chromosome?

Background

The normal brick-red eye color of *Drosophila* is due to the presence of two separate pigments in the eyes, a brown pigment and a red pigment. The two pigments are made by separate biochemical pathways, and mutations can prevent the formation of either pigment without affecting the other pigment at all. If a mutation prevents the synthesis of the red pigment, only the brown pigment is

made; this mutant fly has brown eyes. Similarly, if the brown pigment pathway is blocked, only the red pigment is made and the fly has bright red eyes. If both pigment pathways are blocked, neither pigment is made, and the fly has white eyes. White eyed flies are completely blind.

Table 1. Potential mutations present in your *Drosophila* unknowns, their chromosomal location and phenotype.

Mutant	abbreviation	location	phenotype
brown	<i>bw</i>	II	brown eye color
scarlet	<i>st</i>	III	bright red eyes
cinnabar	<i>cn</i>	II	bright red eyes
white	<i>w</i>	I	white eyes
claret	<i>ca</i>	III	brown eye color
white-brownex	<i>w^{Bwx}</i>	I	brown eye color
vermillion	<i>v</i>	I	bright red eyes

responsible for creating white eyes and dark bodies in your flies. The possible mutations in the lines provided are presented below.

In this experiment, all of the mutations are recessive; wild-type (+) is dominant. The wild type stock has **brick** red eyes.

- Brown (*bw*) is a mutation that completely prevents synthesis of the red pigment. Flies that do not make this red pigment have brown eyes; *bw* is on the second (II) chromosome.

- Claret (*ca*) is a mutation that prevents synthesis of the red pigment later in the pathway than does brown. Flies that do not make this red pigment have brown eyes; *ca* is on the third (III) chromosome.

- Vermillion (*v*) is a mutation that completely prevents synthesis of the brown pigment. Flies that do not make this brown pigment have bright red eyes. *v* is on the X chromosome.

- Scarlet (*st*) is a mutations that completely prevents synthesis of the brown pigment. Flies that do not make this red pigment have bright red eyes; *st* is on the III chromosome.

- Likewise, Cinnabar (*cn*) is a mutation that completely prevents synthesis of the brown pigment, giving bright red eyes; *cn* is on the II chromosome.

- White (*w*) is a mutation that prevents the fly from placing these pigments in the eyes, creating white-eyed flies by a single mutation; *w* is on the X chromosome.

- white-brownex (*w^{Bx}*) is a second mutant allele of the white gene, and therefore also is a mutation that prevents the fly from placing pigments in the eyes properly. However, some brown pigments are laid down, creating brown-eyed flies; *w^{Bx}* therefore is on the X chromosome, as it is an alternative mutation of "white".

Body color: ebony (III) or black (II): These two independent mutations are on different chromosomes.

Fewer mutations are known that affect body color. The two used here are ebony and black. Because of these names, get in the habit of referring to mutant flies as "dark bodied" rather than using the descriptor "black" as that can cause confusion when discussing the genotype.

Outline of the multi-week laboratory

Over nine weeks, each student, while working in groups of three, will carry out genetic crosses on *Drosophila* flies. Together, they will interpret results and will test the hypotheses they develop to explain genetic inheritance of eye and body color traits. Each student starts with a white-

eyed, dark-bodied *Drosophila* strain. After collecting data on progeny of genetic crosses, students will produce a formal laboratory report that defends their conclusions.

Lab 1. Introduction to *Drosophila* and its genetics

Goal: To introduce fly anatomy and the procedures required to successfully handle live flies and to perform successful genetic crosses on them. At first, this lab introduction may seem trivial, but students that fail to master these techniques will struggle throughout the assignment.

Lab 2. The F₁ cross

Goal: To begin the genetic crosses that will enable you to determine underlying patterns of inheritance of the dark-body and white-eye traits. An F₁ cross begins by mating a mutant fly that is homozygous for one or more recessive traits to one that is wild-type (normal in expression for all traits and homozygous for the wild-type allele). Each cross requires two weeks for offspring to emerge.

Lab 3. Chromosome mapping

Goal: To use genetically designed strains that control patterns of inheritance, and therefore can be used for chromosomal mapping of *Drosophila*. Two aspects of these strains make them useful in genetics: first, the strains harbor inversions of the second and third chromosome. Inversions are chromosome constructs that suppress crossing-over (recombination). Second, within the inversion is a "dominant-lethal" genetic marker. These markers are so named because they express a phenotype that occurs when present in one copy (showing dominance genetically), but when homozygous, they kill the developing embryo. By constructing crosses with these strains, you can easily follow the inheritance of a chromosome because the dominant marker allows you to follow inheritance of this chromosome across generations, and the absence of the dominant trait indicates that the fly received the alternative chromosome carrying the recessive mutation from its parent. Mutations therefore can be mapped quickly to a specific chromosome.

Lab 4. The F₂ cross and backcrosses

Goal: Students will collect offspring from their F₁ crosses, score their phenotypes for males and females, and pair these flies together to produce a second generation of offspring, the F₂ flies. Concurrently, F₁ males will be mated to individuals that are homozygous for the recessive mutant traits, using females provided from the original unknown strains. This procedure is called a backcross in genetics or a testcross by animal breeders. The offspring of these crosses will enable you to identify what color mutations are present, *i.e.*, brown eyes, bright red eyes or white eyes, in the line. Note that the reciprocal backcross using female offspring is not used unless a protocol that enables the students to obtain virgin females is included.

Lab 5. Chromosome mapping II

Goal: Students will collect offspring from the cross of the dominant marker strains, and set up males in backcrosses to their original unknown lines. Co-occurrence of unknown and marker traits (or lack of co-occurrence of phenotypes) will enable students to define the chromosome on which the unknown gene lies. This determination is done as a process of elimination. A recessive trait expressed along with a dominant-lethal cannot rest on the same chromosome as that particular marker trait. The reason is that a recessive trait must occur on both chromosomes to be expressed, and therefore, for it to occur in an individual that also expresses a dominant marker trait within an inversion, the unknown mutation must reside on a different chromosome than the marker.

Lab 6. Genetic analysis I.

Goal: Students will collect and score offspring from Lab 4, and test their hypotheses as to the underlying genetic basis of the traits, *i.e.*, sex linkage and/or physical linkage. In the report, students will carry out a statistical test of their predictions to examine whether or not their results can be explained simply by chance variation.

Lab 7. Genetic analysis II.

Goal: Students will score offspring from the chromosome mapping experiments. Special attention should be paid to females, as these flies will not express any sex-linked traits. Additional data can be collected from the F₂ flies and backcrosses made in Lab 4. Each group should finish clarifying hypotheses, and make sure their data are of sufficient quantity to formally test them. By the end of class, students should divide duties in their group relevant to preparing the report.

Lab 8. Students complete all data collection. Each student should possess a complete copy of all data. Each group will schedule an appointment with the instructor, but they should work together to analyze and interpret their results prior to this meeting. Reviewing analyses with the instructor is critical to the successful and rewarding completion of the laboratory. Our experience is that students require help to understand how their data can lead them to a correct solution. Subsequently, students can correct their analyses, produce a thought-out laboratory report, and gain a real appreciation of research protocols and hypothesis testing.

Specific protocols

(1) Set up of initial crosses between flies from the unknown strain and wild type flies.

In Labs 2, each group of students will prepare separate crosses of unknown flies (Table 2), which will produce the offspring they need to have available in two weeks to conduct the second generation of crosses. Students should get in the habit of referring to their strain as an unknown as it contains three separate mutations, two producing the white-eye color and one the dark-body color.

Table 2. Monohybrid and dihybrid crosses for body and eye color.

Replicates	Cross ID	Flies to cross
2	1A	unknown (mutant) females X wild type males
2	1B	wild type females X unknown (mutant) males

To make a new stock culture bottle or cross, first add a few grains of dried yeast, put the culture bottle on its side and carefully place the anaesthetized flies on the side of the bottle, and cap the bottle. Leave the capped bottles on their sides until the flies have recovered from the anesthetic. Knocked-out flies can become stuck and die when placed directly on the moist surface of the food. Be sure,

however, to reset the bottle upright before larvae have had a chance to "work" the food to any great extent.

Only a few flies of each sex are necessary; 3-5 females with 3-5 males are best for setting up cultures or crosses within vials. If glass half-pint bottles are used, then use 10-12 of each sex per bottle.

In Lab 4, after offspring from these first crosses emerge, several additional crosses are made. First, males and females are paired to produce an F₂ cross. This step is important as these are the only crosses in which recombination of linked loci can be observed, as all other crosses use male

offspring (where recombination does not occur). Second, male offspring are crossed back to females from the unknown strain (these flies can be provided by the instructor). This cross will give a different predicted frequency of phenotypes than will the F_2 cross. Suggested is that students make these predictions and later test them by a χ^2 analysis separating the analysis as a dihybrid cross for eye color and as a monohybrid cross for body color. Generally not enough offspring are scored to consider all possible phenotypes for three genes in a single analysis.

Remind students that at each step they should observe their unknown flies and compare them with the wild type flies. They should note eye color, body color, wing and bristle differences (which are important for the chromosome marker crosses).

(2) Set up of initial marker crosses in Lab 3 to determine which chromosomes carry the mutations in the unknown strains (Table 3).

Table 3. The necessary crosses between a marker strain and the unknown strain.

Replicates	Chromosome marked	Flies to cross
2	II	Cy/Pm females X unknown (mutant) males
2	III	Sb/D females X unknown (mutant) males

Marker flies have plum eyes (*Pm*) and curly wings (*Cy*) on the second chromosome, and two different mutations that shorten the bristles on the third chromosome, stubble (*Sb*, bristles short and thick) and dichaete (*D*), which refers to the thoracic bristle phenotype of two macrochaete instead of four. Dichaete can be harder to observe in an individual that also possesses stubble. Remember,

for these marker flies, each copy of the chromosome only has one of the two mutations, and therefore progeny will receive either Curly or Plum AND either Stubble or Dichaete. Also note that "unknown" stocks all have wild type alleles at all the marker loci.

Pm *Plum* – second chromosome (associated with an inversion) – Eyes have a dark purple color. Avoid using this to score other eye color effects. **Homozygous lethal.**

Cy *Curly* – second chromosome (associated with an inversion) – wings curled up strongly, rarely overlapping wild type at 25°C, frequently overlapping at 19°C. Wing texture slightly thinner, with wrinkles in upper surface. Usually **homozygous lethal**, but may emerge as dwarf with more extreme wing character.

D *Dichaete* – third chromosome – refers to the thoracic bristle phenotype of two macrochaete instead of four. **Homozygous lethal.**

Sb *Stubble* – third chromosome – 58.2 – bristles condensed to less than 1/2 normal length, the blunt stubble being somewhat thicker than the base of wild type bristle. **Homozygous lethal.**

Because the goal is simply to determine on what chromosome mutations reside, students do not need to make all possible crosses. They only need to cross males of their unknown strain to virgin females of the marker strains.

When offspring emerge, students should check the emerging flies to verify that the dominant marker genes are segregating properly, and then collect the heterozygous males as instructed – all offspring are heterozygous for the mutations present in the unknown strains and for the dominant-

lethal markers they carry. Each fly that is collected will have only one set of the markers (refer to Table 3). For chromosome II, students should collect Curly males for the eye color analysis. The Plum mutation would interfere with the scoring of eye color in the next generation. Males with either mutation may be used for the chromosome III analysis, but students often have an easier time scoring stubble.

These heterozygous males are crossed to virgin females from the unknown strain that again are provided by the instructor. Each student should predict what offspring types are expected in the second generation. For example, where a male is chosen that is Curly and Stubble, and he is mated to a female homozygous for the unknown traits, the following offspring can be expected: traits present on the second chromosome will only appear in offspring that do not receive the Curly allele; traits present on the third chromosome will only appear in offspring that do not receive the Stubble allele. Thus a bright red-eyed phenotype present in a Curly female must possess the scarlet mutation, because the trait cannot be on chromosome II due to the presence of curly, and it cannot be on the X chromosome, because the marker male was wild-type for all genes on that chromosome.

Conclusions

By combining the results from the three types of crosses, back-crosses, F2 crosses, and the chromosome marker analyses, each group should be able to explain how their strain develops white eyes and a dark body. A rubric on how students can prepare their report, as well as a suggested grading scheme, is present in the complete laboratory document that is available from the authors.

We recommend that the actual writing of the report remains a group project. Each student is responsible for co-writing the report, and each receives the same grade, modifiable only if necessary by the group's individual assessment. All group members should be satisfied with the write-up before turning in the report. Dissatisfaction can be described in a separate page signed only by the concerned individuals.

References: MacIntyre, R., 1999, Dros. Inf. Serv. 82: 130.