

Laboratory Exercises To Examine Recombination & Aneuploidy in *Drosophila*

• DENNIS R. VENEMA



Chromosomal aneuploidy, a deviation from an exact multiple of an organism's haploid chromosome number, is a difficult concept for students to master. Aneuploidy arising from chromosomal non-disjunction (NDJ) is particularly problematic for students, since it arises in the context of meiosis, itself a challenging subject. Students learning NDJ are forced to actively apply their knowledge of normal meiosis, often revealing previously unapparent deficits in their understanding. Teaching NDJ is thus an important opportunity for students to review and apply their skills in modeling meiosis. Despite this opportunity, standard methods for teaching NDJ are passive. While experiments illustrating recombination mapping, sex-linkage, and other core genetics concepts are readily available for high school and undergraduate genetics courses, simple exercises demonstrating NDJ are not. This is regrettable, for non-disjunction is not only medically important but also historically significant as the original proof for the chromosomal theory of inheritance (Bridges, 1916). Presented here is a straightforward method to detect NDJ of the sex chromosomes in *Drosophila* in the context of laboratory exercises to examine recombination of sex-linked loci. The exercises presented here allow for detecting maternal and paternal NDJ events within a single cross, an advantage since the use of standard markers obscures paternal NDJ events (Bridges, 1916). An additional advantage of this method is that it easily allows for recombination mapping with recessive alleles *in trans* as well as *in cis*. This method allows students to simultaneously investigate recombination and aneuploidy with live organisms, and as such is complementary to theoretical methods of teaching recombination and NDJ.

The exercises presented here are based on a *Drosophila* virgining system that has recently been developed as a method to perform teaching crosses without the need for collecting virgins

manually (Venema, 2006). This system employs a modified Y chromosome that harbors a conditional, dominant lethal mutation: a regulatory sequence that is activated in response to heat connected to a protein called *head involution defective*, or "hid" that activates programmed cell death. Since this modified Y chromosome (abbreviated as Y{hs-hid}) is present only in males, temporarily raising the temperature of a Y{hs-hid} *Drosophila* culture during larval stages will cause all males to undergo systemic apoptosis and die. Female larvae, however, survive the heat shock and remain virgin after hatching since there are no surviving males to mate with (Venema, 2006). The use of this system is of great advantage for educators in that large numbers of virgins can be obtained without scoring or segregating young female flies. This ability to use large numbers of flies in pedagogical crosses also facilitates finding the results of rare genetic events in the progeny.¹

Venema (2006) describes a scheme for recombination mapping between autosomal loci using this system; however, recombination mapping of sex-linked loci in *Drosophila* is more convenient for high school or introductory-level university courses. Additionally, examining recombination between sex-linked loci allows for the simultaneous detection of chromosomal NDJ events in the progeny, since some *Drosophila* sex chromosome aneuploids are viable (Table 1). Four possible exercises are presented: two versions of a dihybrid testcross and two versions

Table 1. Phenotypes for various *Drosophila* sex-chromosome euploid and aneuploid karyotypes.

Karyotype	Phenotype
XX	normal female, fertile
XY	normal male, fertile
XO	male, sterile
XXY	female, fertile
OY	non-viable

¹ The Y{hs-hid} *Drosophila* strains described here are available from the author at minimal cost, however, the U.S. Department of Agriculture requires a permit to import *Drosophila* into the USA. Heat-treated cultures of selected lines described in this paper are available from Carolina Biological Supply Co. (www.carolina.com) for educators in the USA.

of a three-point testcross. The dihybrid testcross exercises map sex-linked loci with the recessive mutant alleles either together as a parental genotype (i.e., recessive alleles *in cis*) or with recessive mutations inherited from both parents (recessive alleles *in trans*). Similarly, the options for the three-point testcross are mapping the three loci with mutant alleles all *in cis*, or with two *in cis*, and the third *in trans*. The dihybrid or three-point testcross options allow for instructors to choose the exercises of appropriate complexity for their particular grade level and learning objectives. Within each option, there are two reasons for providing alternate versions. First, given the ease of collecting virgins with the heat-shock method, it is possible to have student pairs map using both the *in cis* exercise as well as the *in trans* version and then compare the data obtained. Standard sex-linked mapping exercises using *Drosophila* map only with mutant alleles *in cis* for convenience. Comparing maps based on *in cis* and *in trans* mapping reinforces the concept that “recombinant” and “parental” genotypes are dependent on the parental strains used. Secondly, the two versions of each exercise allow for detecting different non-disjunction events in the F1 progeny of the cross that generates the dihybrid or trihybrid females for testcrossing.

○ *Drosophila* Strains

Dihybrid Testcross Exercises

$w^1 cv^{18}$
 $w^1 cv^{18} Y\{hs-hid\}$
 $w^+ cv^+$
 $w^1 cv^+ Y\{hs-hid\}$
 $w^+ cv^{18} Y\{hs-hid\}$

Three-Point Testcross Exercises

$y^1 w^1 cv^{18}$
 $y^1 w^1 cv^{18} Y\{hs-hid\}$
 $y^1 w^1 cv^+ Y\{hs-hid\}$
 $y^+ w^+ cv^+$
 $y^+ w^+ cv^{18} Y\{hs-hid\}$

○ Methods

All crosses should be reared at 25 °C in an environment with approximately 60-80% relative humidity to prevent desiccation, if possible. The generation time of *Drosophila* is temperature-dependent; the times described for these exercises will not be accurate if other temperatures are used. All stock cultures and student crosses should be seeded with live yeast sprinkled onto the media surface to encourage egg laying. Note that crosses should employ males with the Y{hs-hid} chromosome only when the intention is to kill all F1 male larvae. Attempting to use the Y{hs-hid} chromosome in place of a normal Y when the intention is to score male and female progeny will skew the male: female ratio severely (Venema, 2006). The exercises employ two or three recessive mutations linked on the X chromosome (Figure 1): All are commonly used markers in teaching crosses and are easy to score (Flybase Consortium, 2003; Carolina, 2005). The *white*¹ allele (w^1) produces white eyes in contrast to wild-type red (w^+); the *crossveinless*¹⁸ allele (cv^{18}) produces wings lacking cross veins in contrast to cross-veined wings (cv^+); and the *yellow*¹ allele (y^1) produces a yellow body color in contrast to wild-type tan (y^+). Photographs of these phenotypes are included in the *Carolina Drosophila Manual* (Carolina, 2005). An additional useful resource is the “Learning to Fly” poster published by the developmental journal *genesis* (www.wiley.com/genesis). This poster has large, clear photographs of *white* and *yellow* mutants, as well as photographs to aid students in sexing flies.

Heat Shock

Care must be taken when preparing cultures for heat shock to ensure complete killing of male larvae without harming

females. Cultures must not contain eggs younger than 24 hours old, nor must they contain pupae about to hatch. Young eggs will not yet be able to express the *hs-hid* transgene, and mature male pupae may survive if shocked too late in pupal development. Additionally, cultures must be cleared of adults before heat shocked to remove adult males before virgins hatch (Venema, 2006). These issues are addressed if the following schedule is followed:

1. Breeding stock is transferred to a new vial or bottle and allowed to lay eggs for three to four days.
2. The adults are transferred to a new vial or bottle on Day 4.

Figure 1. Sex-linked recessive mutations to study recombination and non-disjunction in *Drosophila*.

- A) Wild-type *Drosophila* eyes (red)
- B) The *white* mutation
- C) Genetically *white* (w^1) males carrying the {hs-hid}Y chromosome exhibit pale orange eyes.
- D) A wild-type *Drosophila* wing has two crossveins perpendicular to the wing axis (arrowheads).
- E) The *crossveinless* (cv^{18}) mutant, lacking both crossveins
- F) Wild-type *Drosophila* pigmentation (tan)
- G) The *yellow* (y^1) mutant
- H) Sex-combs on the front legs of male flies (arrows) are the most reliable way to distinguish males from females.
- I) A recombination map of the X chromosome showing the relative positions of *yellow* (y), *white* (w), and *crossveinless* (cv). Not to scale; centromere position is arbitrary.

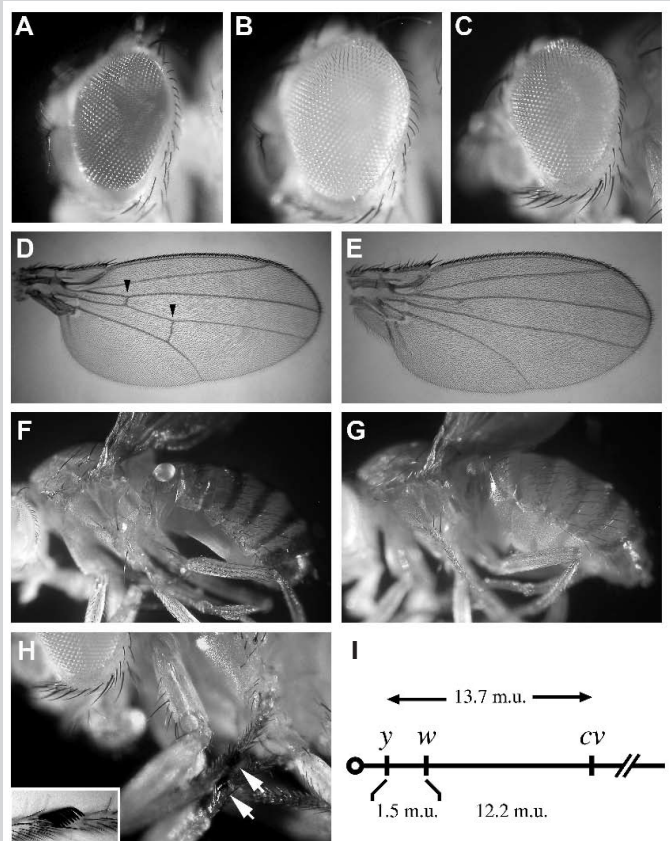
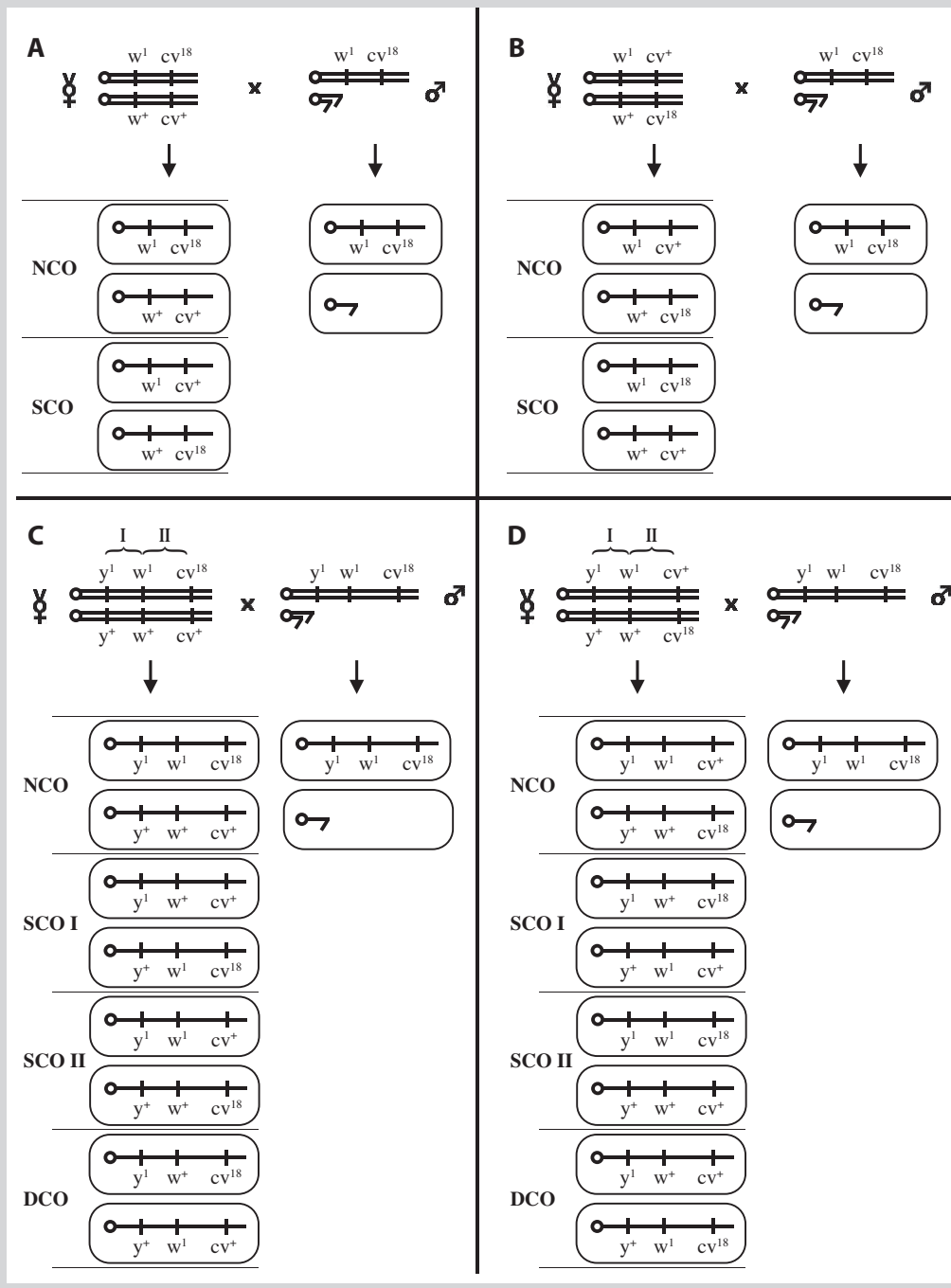


Figure 2. Four exercises to examine sex-linked recombination and NDJ in *Drosophila*.

The testcross employed for sex-linked recombination mapping in each of the four exercises is shown. Virgin females (designated by a Venus symbol combined with a “V”) may be either dihybrid (A or B) or trihybrid (C or D). For the dihybrid exercises, recessive alleles of *white* (w^1) and *crossveinless* (cv^{18}) are used. The recessive alleles may be present on the same chromosome homolog (i.e., recessive alleles *in cis*, as in A) or on opposite homologs (i.e., recessive alleles *in trans*, as in B). In both cases, the dihybrid virgin females are mated to ($w^1 cv^{18}$) tester males. The gametes resulting from the dihybrid females in both crosses are designated as resulting from no crossover events between the *white* and *crossveinless* loci (NCO) or from a single crossover between them (SCO). For the trihybrid exercises, a recessive allele of *yellow* (y^1) is added. The *in cis* trihybrid exercise has all three recessive alleles on one homolog (C), whereas the *in trans* trihybrid exercise retains y^1 and w^1 together *in cis* and places cv^{18} *in trans* on the opposite homolog (D). In both cases, the trihybrid virgin females are testcrossed to ($y^1 w^1 cv^{18}$) tester males. The interval between the *yellow* and *white* loci is designated as “I”; the interval between *white* and *crossveinless* is designated as “II.” Gametes produced by trihybrid females in both crosses fall into four general categories: those resulting from no crossovers between the loci under study (NCO); those resulting from a single crossover in interval I (SCO I); those resulting from a single crossover in interval II (SCO II); or those resulting from a double crossover event (DCO).



3. The culture is re-checked for adults on Day 5 (i.e., for adults that may have freed themselves from being stuck in the media).
4. The culture is heat shocked on Day 6 or 7.
5. Virgins are collected from the culture starting on Day 10.

The heat shock must be performed in a 37+/-1 °C water bath for two hours (an air incubator is not effective). The culture vial or bottle must be submerged below the level of the bottom of the plug to prevent larvae from crawling above the waterline during the heat shock. Vials can be held at the correct level by attaching them to a large beaker with rubber bands; the beaker should be filled with water and allowed to equilibrate at 37 °C before attaching the vials. A circulating water bath is not needed. Note that all larvae will die if the culture is shocked longer than two hours or at a higher temperature. After the heat shock, cultures should be returned to a 25 °C incubator until virgin collection.

○ **Testcross Options**

Four possible exercises are presented: two dihybrid testcrosses (Figure 2A,B) and two trihybrid testcrosses (Figure 2C,D). The dihybrid exercises employ the w^1 and cv^{18} mutations inherited together on the same chromosome homolog (*in cis*, Figure 2A) or on separate homologs (*in trans*, Figure 2B). The trihybrid exercises add the y^1 mutation. The trihybrid *in cis* exercise has all three recessive mutations on one homolog (Figure 2C), whereas the trihybrid *in trans* exercise places cv^{18} *in trans* to y^1 and w^1 (Figure 2D).

○ **Dihybrid Testcrosses: An Overview**

Note that the dihybrid exercises are identical to their trihybrid counterparts except for the

omission of the y^1 mutation. As such, the detailed discussion on the trihybrid exercises (see below) is also relevant and should be consulted. While the core crosses of the dihybrid exercises are the two testcrosses (Figure 2A,B), several crosses are required to produce the dihybrid virgins for testcrossing. A mating scheme for the *in cis* dihybrid testcross is shown in Figure 3; the equivalent scheme for the *in trans* dihybrid testcross is shown in Figure 4. For the *in cis* exercise, the P-1 cross is merely heat-shocking a $w^1 cv^{18} Y\{hs-hid\}$ stock to provide virgin females, which are then crossed to wild-type males (P generation) to produce the F1 (Figure 3). At a low frequency, exceptional F1 individuals may arise through NDJ, some of which will be apparent. The F1 generation is the testcross diagrammed in Figure 2A.

The F1, when allowed to interbreed, produces an F2 with four phenotypic categories allowing for standard recombination mapping. An equivalent mating scheme for the *in trans* dihybrid exercise is shown in Figure 4. The P-1 cross requires heat shocking a $w^1 Y\{hs-hid\}$ stock to provide virgin females for crossing to $cv^{18}/Y\{hs-hid\}$ males. The offspring of this cross are heat-shocked as larvae to eliminate all male progeny and produce $\{w^1 cv^+/w^+ cv^{18}\}$ virgin females for testcrossing. Rare XO flies will survive the heat shock and appear as exceptional males in the F1. The F1 $\{w^1 cv^+/w^+ cv^{18}\}$ virgin females are then crossed to $w^1 cv^{18} / Y$ males to produce the testcross diagrammed in Figure 2B. This testcross will produce four phenotypic categories and provide data for recombination mapping.

Figure 3. Dihybrid mapping of white to crossveinless with recessive alleles in cis.

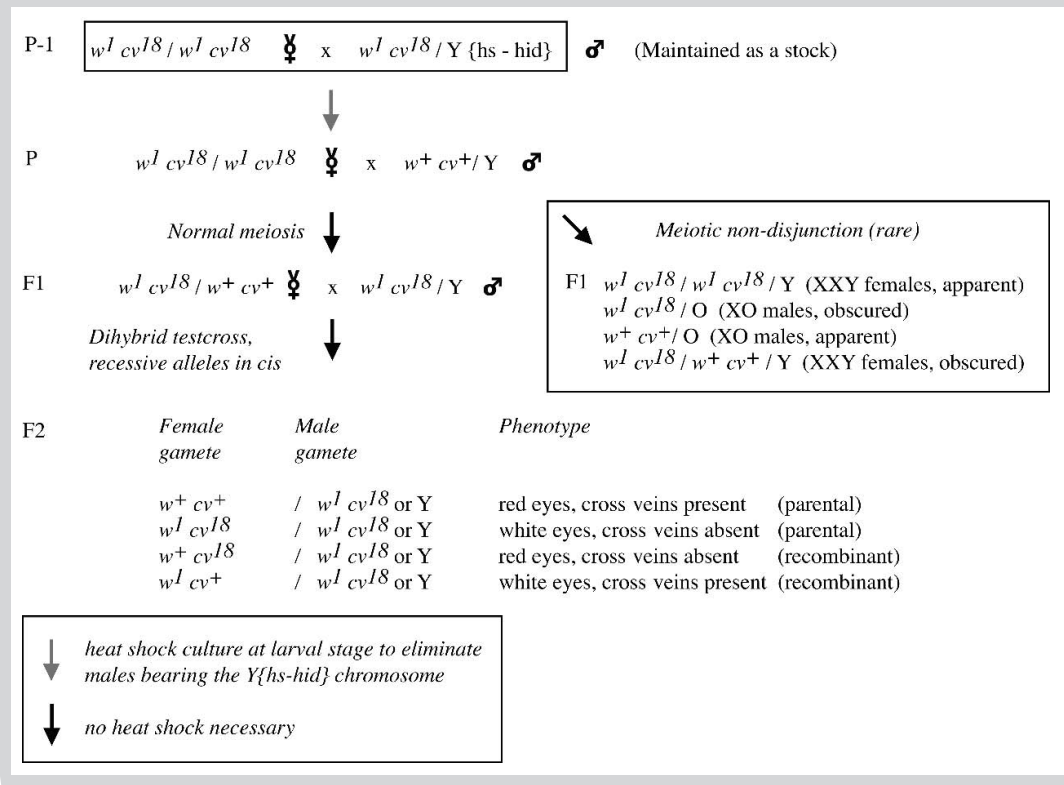
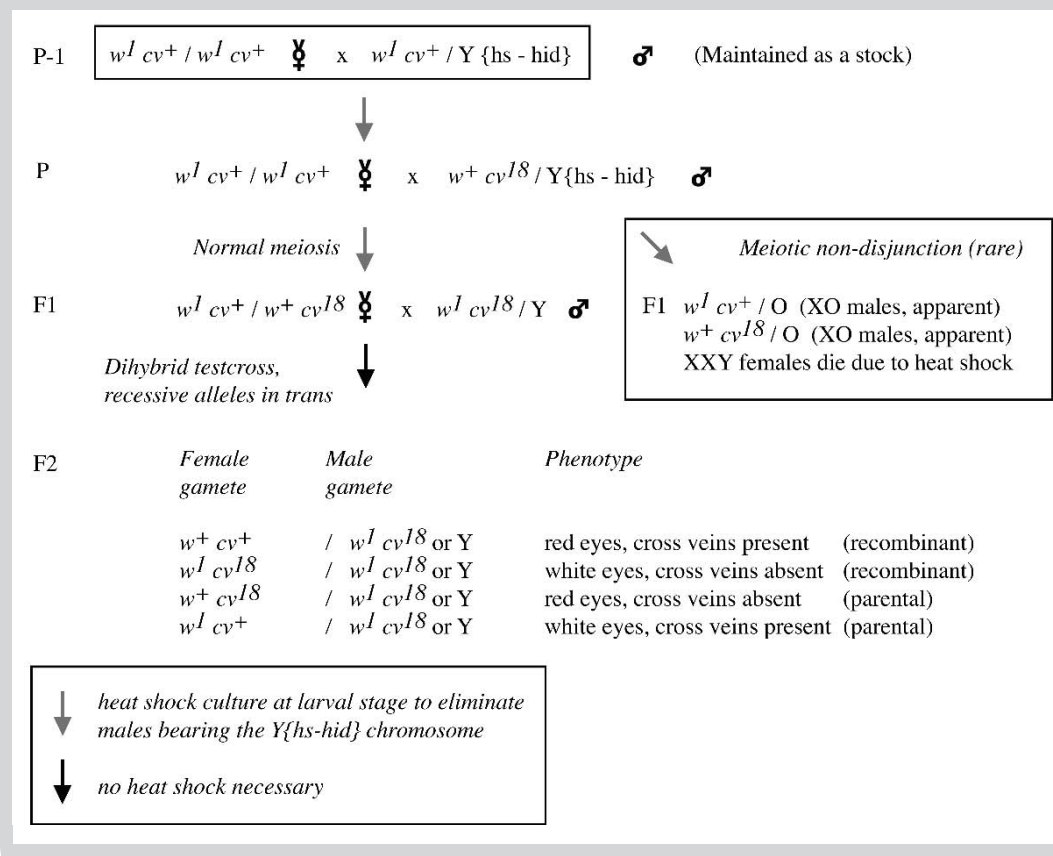


Figure 4. Dihybrid mapping of white to crossveinless with recessive alleles in trans.

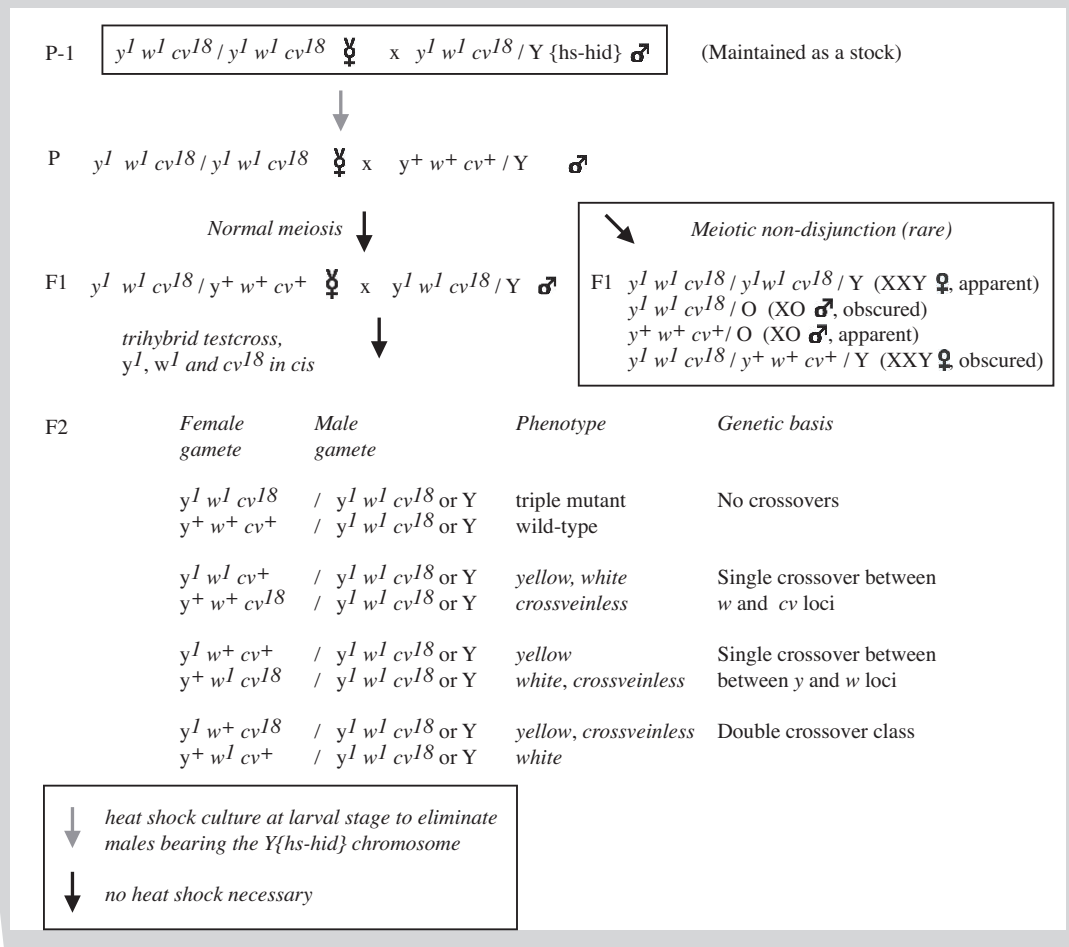


Three-Point Testcrosses: Getting Started

Mating schemes for the trihybrid exercises are shown in Figures 5 and 6. The following will describe the three point testcross exercises in detail; however, the dihybrid testcrosses (see above) are identical except for replacing y^1 with y^+ in all stocks. (As such, ignoring the y locus in what follows effectively converts this section into a detailed description of the dihybrid exercises.) Approximately two-and-a-half weeks prior to the first lab, transfer cultures of the $y^1 w^1 cv^{18} Y\{hs-hid\}$ and $y^1 w^1 cv^+ Y\{hs-hid\}$ stocks into fresh bottles. Allow the adult flies to lay eggs for three to four days, and then clear the adults from the cultures. Be sure to remove all adults from the bottles. Two to three days after clearing, heat shock the cultures (see Methods) to kill male larvae. Continue to culture the bottles at 25 °C until an adequate number of virgins emerge. It is possible that flies obtained in this manner will also have rare XO flies (that is, aneuploid flies with only one X chromosome) that survive the heat shock since they lack the $Y\{hs-hid\}$ chromosome (see below). While XO flies are phenotypically male, they are sterile and will not compromise the virgins obtained (Venema, 2006).

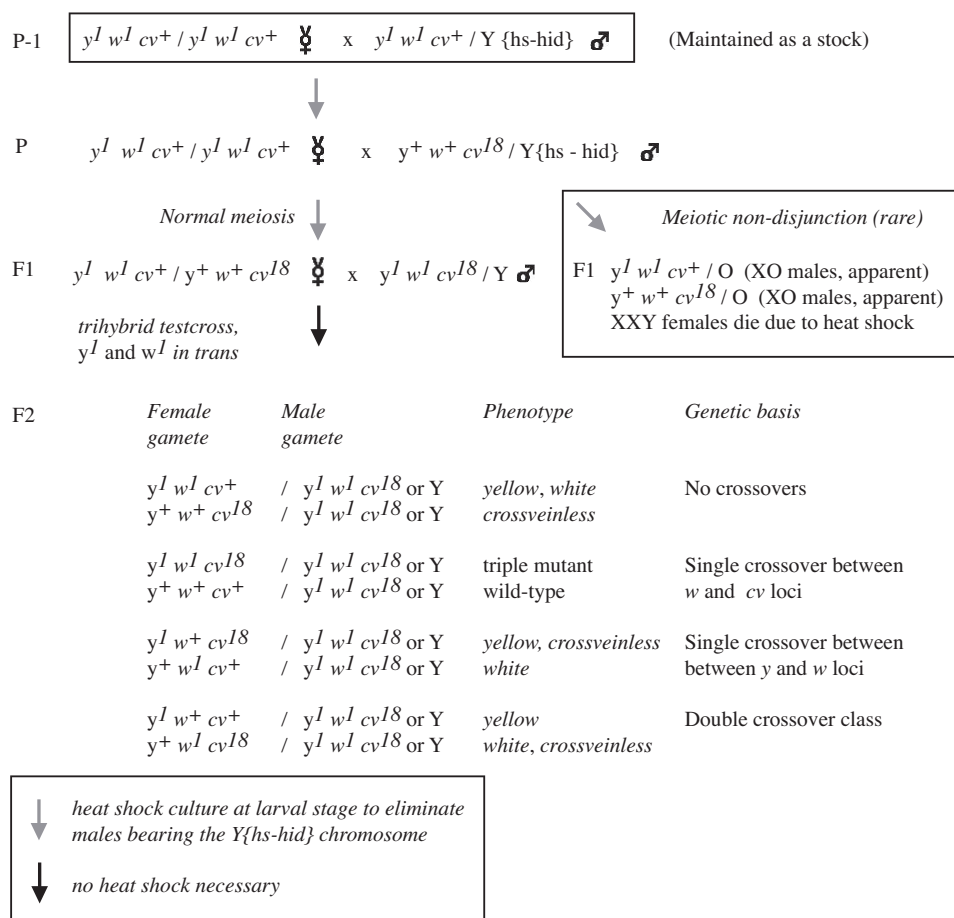
In the first lab (Week 1), provide students with $y^1 w^1 cv^{18} / y^1 w^1 cv^{18}$ virgins and have them mate these females to $y^+ w^+ cv^+ / Y$ males to commence the *in cis* exercise. Similarly, students will mate $y^1 w^1 cv^+ / y^1 w^1 cv^+$ virgins to $y^+ w^+ cv^{18} / Y\{hs-hid\}$ males to start the *in trans* exercise. A good starting point is 20 virgin females crossed to 2-3 males per vial: Using more flies may overcrowd the vial with progeny. This lab period should also allow for students to examine cultures of wild-type flies and the various mutant lines to familiarize them with scoring the mutant phenotypes as well as sexing flies (Figure 1). Working in pairs or groups, students should record their observations in a laboratory notebook, as well as the details of the crosses they performed. Students can also be required to predict the outcome of their particular cross assuming different modes of inheritance. Venema (2006) provides pre-lab questions for a similar exercise that could be easily adapted for these crosses. Having students predict the outcome of their crosses heightens the inquiry level of the exercise and more closely models an actual experiment.

Figure 5. A three-point testcross to map *yellow*, *white*, and *crossveinless* and examine NDJ: mapping with all recessive alleles *in cis*.



Both sets of crosses should be cleared of adult flies three to four days after mating. The mating for the *in cis* exercise should be left to hatch without further manipulation, since the F1 progeny will form the desired three-point testcross automatically (Figure 5, refer also to Figure 2C). Indeed, this is the reason for the popularity of mapping *in cis* on the X chromosome: Collecting F1 virgin females is not necessary. The *in trans* mating should be heat shocked two to three days after clearing to kill the F1 $y^1 w^1 cv^+ / Y\{hs-hid\}$ males and thus ensure that the F1 $y^1 w^1 cv^+ / y^+ w^+ cv^{18}$ females remain virgin (Figure 6). The heat shock can be done in the intervening lab period for the students to observe. In the next lab period (two weeks after the first), students need to determine the mode of inheritance for the three mutations by examining the *in cis* F1 progeny, identify any unexpected aneuploid progeny that arose through NDJ (see below), and transfer the expected progeny to a fresh culture to form the F2. For the *in trans* exercise, the students need to examine their F1 flies (which will be only virgin females except for rare XO aneuploids resulting from NDJ) and mate the $y^1 w^1 cv^+ / y^+ w^+ cv^{18}$ virgin females to $y^1 w^1 cv^{18} / Y$ tester males to form the desired testcross (Figure 6, refer also to Figure 2D). These tester males can be obtained from a stock culture or from excess F1 males from the *in cis* cross. As before, have students set up crosses between 20 virgins and 2-3 males in vials. Although the number of F1 progeny obtained will vary from vial to vial (Tables 2, 3) students should have enough F1 flies for at

Figure 6. A three-point testcross to map *yellow*, *white*, and *crossveinless* and examine NDJ: mapping with *crossveinless* in trans to *yellow* and *white*.



The *in cis* exercise thus allows for detection of NDJ only in the female parent.

In contrast, the F1 of the *in trans* exercise allows for detection of NDJ events in both parents. In this exercise, all F1 XXY females die due to the $Y\{hs-hid\}$ chromosome; thus only XO males can be observed. Phenotypically yellow, white XO males result from the fusion of (n-1) sperm lacking a sex chromosome with normal oocytes (Figure 6). There are three possible NDJ events that can produce such sperm: NDJ of the X-Y pair at Meiosis I, NDJ of the X at Meiosis II, or NDJ of the Y at Meiosis II (Figure 7E-G). These events are not distinguishable in this experiment. The $y^l w^l cv^+ / O$ category would be obscured if this cross were performed with a normal Y chromosome, as noted by Bridges (1916). These exceptional XO males can also be readily distinguished from any $y^l w^l cv^+ / Y\{hs-hid\}$ F1 males that may survive due to improper timing of the heat shock (Venema, 2006); such males will have a pale orange eye color (Figure 1C) that is easily distinguished from fully white-eyed $y^l w^l cv^+ / O$ males. The second observable aneuploid in the F1 of this cross results from NDJ of the X to a (n-1) oocyte in the female parent that then fuses with a normal X-bearing sperm

least two vials per exercise. Students needing extra flies can also obtain them from other groups with an excess amount.

Three-Point Testcrosses: Examining & Explaining F1 Aneuploids

Examining the F1 progeny of both crosses allows for the opportunity to identify rare, unexpected F1 progeny and explain their origin. In the F1 of the *in cis* exercise, two categories of exceptional progeny may be observed. Phenotypically female flies carrying two $y^l w^l cv^{18}$ X chromosomes plus a Y (Figure 5) arise from NDJ of the X in the female parent to produce an (n+1) oocyte that fuses with a normal Y-bearing sperm (Figure 7A). The NDJ event in the female parent may occur at either the Meiosis I (Figure 7C) or Meiosis II (Figure 7D) division; however, it is not possible to distinguish these events in this experiment. The converse outcome of these non-disjunction events in the female parent are (n-1) oocytes lacking an X chromosome; again either through NDJ at Meiosis I or II (Figure 7C,D). Fusion of these (n-1) oocytes with normal, X-bearing sperm (Figure 7A) results in phenotypically wild-type XO males carrying only an $y^+ w^+ cv^+$ X chromosome (Figure 5). Two other aneuploid progeny categories that result from NDJ in the male parent may be present in the F1, however, these individuals cannot be phenotypically distinguished from the expected euploid F1 progeny (Figure 5).

from the male. This sequence of events produces a phenotypically crossveinless XO male bearing a single $y^+ w^+ cv^{18}$ X chromosome (Figure 6). Thus the *in trans* exercise provides an unprecedented opportunity to detect the results of NDJ in either parent within a single cross. As students examine the F1 progeny of both exercises and compare their obtained results to their predictions, some will encounter these unexpected categories at a low frequency. As these flies are discovered, groups with unexpected flies can report their findings to the class such that all students can attempt to explain their origin with meiosis diagrams (Figure 7). Sample F1 data (including the proportion of exceptional progeny obtained for both exercises) is given in Tables 2 and 3. The average number of F1 females obtained was the same in both exercises, and more than adequate for students to set up two testcross vials per exercise. As expected, the proportion of aneuploid progeny observed in both exercises was very low.

Three-Point Testcrosses: Mapping

The remainder of both exercises is standard recombination mapping using the F2 progeny, with the added detail of comparing mapping data between the *in cis* and *in trans* experiments. The F1 testcrosses should be cleared of adults three to four days after mating to avoid overcrowding the cultures. The F2 flies

Table 2. Sample F1 data for the *in cis* exercise. Replicate crosses at 25 °C between 20 virgin females and 2-3 males were set in vials and allowed to breed for four days before clearing. Progeny were scored two weeks after mating.

Vial	Normal meiosis		Aneuploid progeny (non-disjunction)	
	$y^l w^l cv^{I8} / y^+ w^+ cv^+$ females	$y^l w^l cv^{I8} / Y$ males	$y^+ w^+ cv^+ / O$ males	$y^l w^l cv^{I8} / y^l w^l cv^{I8} / Y$ females
1	73	47	0	0
2	77	29	0	0
3	65	15	0	0
4	61	23	0	1
5	56	14	0	0
6	58	11	0	0
7	46	28	0	0
8	58	30	0	0
9	82	25	0	0
10	109	38	0	0
total	685	260	0	1
average	68.5	26	0	0.1

Table 3. Sample F1 data for the *in trans* exercise. Replicate crosses at 25 °C between 20 virgin females and 2-3 males were set in vials and allowed to breed for four days before clearing. Vials were heat shocked on Day 7 after mating and scored on Day 14.

Vial	Normal meiosis	Non-disjunction	
	$y^l w^l cv^+ / y^+ w^+ cv^{I8}$ virgin females	$y^l w^l cv^+ / O$ males	$y^+ w^+ cv^{I8} / O$ males
1	82	0	2
2	59	0	0
3	24	0	0
4	134	2	0
5	81	0	0
6	18	0	1
7	60	0	0
8	71	0	1
9	90	0	1
10	65	1	0
total	684	3	5
average	68.4	0.3	0.5

will be ready for scoring in lab on Week 4. Students should carefully score their flies, record their data, and map the loci with both data sets. Students should map each interval separately, use these data to assemble two chromosome maps, and revise their maps to account for the double crossover class (if observed). The data from the various student groups can be kept separate to illustrate sampling variation, combined to increase the sample size, or both. Students should then compare their maps obtained with the *in cis* and *in trans* exercises to each other as well as to the published map (Figure 11) and explain any differences.

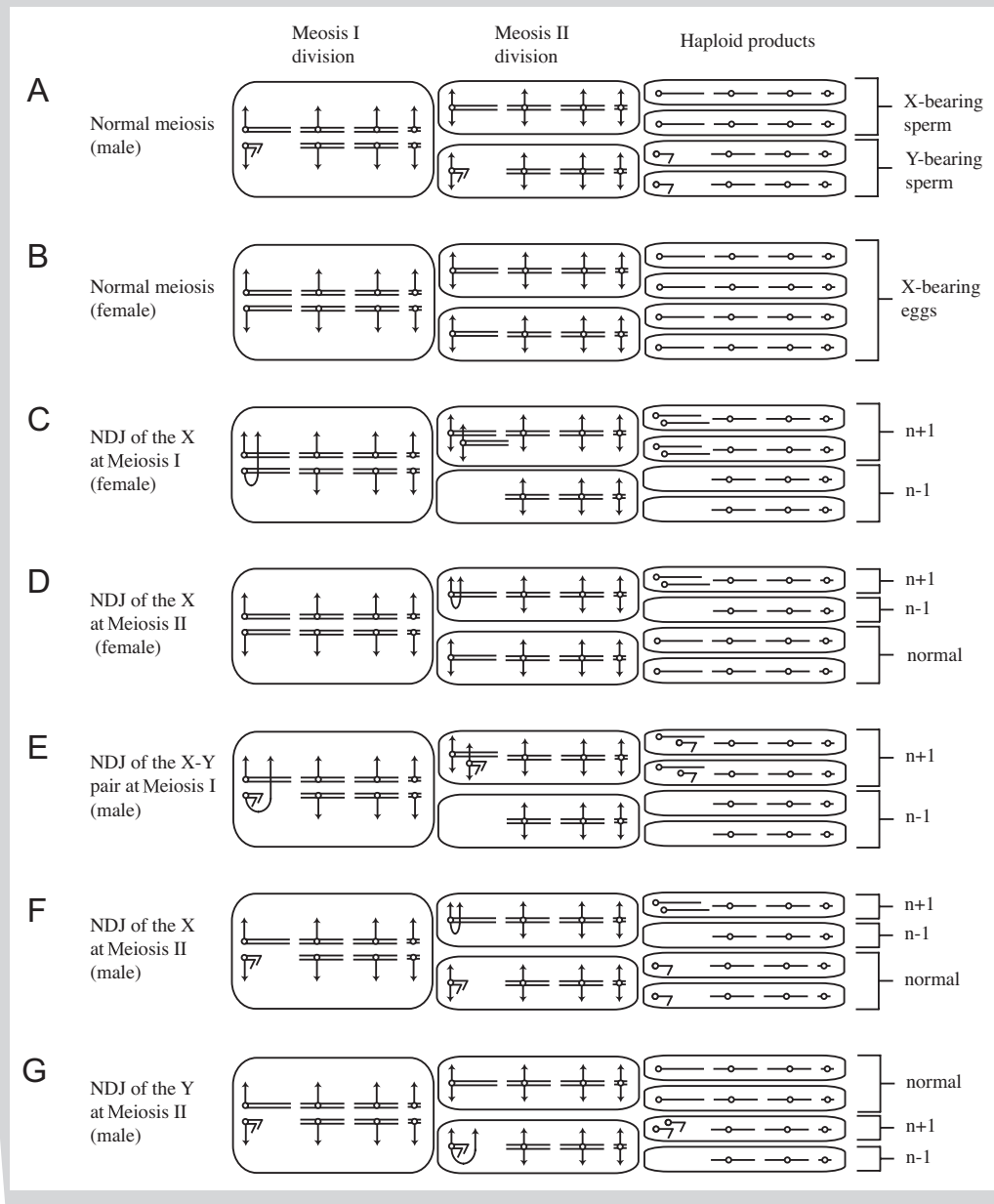
If flies resulting from double crossover events are present, students may also calculate an interference value if desired. Interference assesses the independence of single crossover events by comparing the observed frequency of the double crossover class to the value predicted from the two single crossover classes. A detailed discussion of interference can be found in Griffiths et al. (2008).

Students may inquire if flies resulting from NDJ events are present in the F2. While the frequency of NDJ events in the F1 parents should approximate the frequency observed in the parental crosses, the varied phenotypes of the F2 (eight categories for both genders) obscure the aneuploid progeny present.

○ Adjustments for Varied Levels of Instruction

These exercises present a range of possible classroom activities that may be tailored to different instructional levels. The options range from performing only the simplest testcross (the dihybrid *in cis* exercise) up to the paired three-point *in cis* and *in trans* exercises. The emphasis placed on explaining NDJ events is also scalable to different levels. The simplest approach is to simply explain unexpected F1 progeny for the students, while more senior students can be required to

Figure 7. Meiosis in *Drosophila*. Flies have one sex chromosome pair (XX or XY) and three autosomal pairs ($2n = 4$). Normal disjunction of the sex and autosomal chromosomes in males (A) and females (B) produces (n) gametes. Non-disjunction of the X or Y leads to (n+1) or (n-1) eggs and sperm (C-G).



explain their origin based on their knowledge of meiosis and an introduction to viable sex-chromosome aneuploids in *Drosophila*.•

References

- Bridges, C.B. (1916). Non-disjunction as proof of the chromosome theory of heredity. *Genetics*, 1, 1-52.
- Carolina *Drosophila* Manual. (2005). Burlington, NC: Carolina Biological Supply.
- FlyBase Consortium. (2003). The FlyBase database of the *Drosophila* genome projects and community literature. *Nucleic Acids Research*, 31, 172-175.
- Griffiths, A.J.F., Wessler, S. R., Lewontin, R. C. & Carrol, S. B. (2008). *Introduction to Genetic Analysis (9E)*. New York: W. H. Freeman.
- Venema, D. R. (2006). Enhancing undergraduate teaching and research with a *Drosophila* virginizing system. *CBE-Life Sciences Education*, 5, 353-360.

BIO

DENNIS R. VENEMA is Associate Professor and Chair of the Biology Department, Faculty of Natural and Applied Sciences, Trinity Western University, Langley, B.C., V2Y 1Y1, Canada; e-mail: dennis.venema@twu.ca.

THANK YOU SUSTAINING MEMBERS!

American Institute of Biological Sciences,
Washington, DC

BSCS, Colorado Springs, CO

Carolina Biological Supply, Burlington, NC

Connecticut Valley Biological,
Southampton, MA

Fotodyne, Inc., Hartland, WI

Holbrook Travel, Gainesville, FL

Kendall/Hunt Publishing Co., Dubuque, IA

Nasco, Inc., Fort Atkinson, WI

PASCO Scientific, Roseville, CA

SimBiotic Software, Ithaca, NY

Vernier Software & Technology,
Beaverton, OR

Ward's Natural Science, Rochester, NY

Interested in becoming a Sustaining Member? Call NABT at (800) 406-0775.