Teaching Notes

Experimental observation of underdominance (heterozygous disadvantage) in *Drosophila melanogaster*.

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Many closely related species differ in their chromosome constitution (Bush *et al*., 1977; Bush, 1981; White, 1978; Bengtsson, 1980; Turner, 1983; Patton and Sherwood, 1983; Sites and Moritz, 1987; King, 1993; Nachman and Searle, 1995). White (1978) has stated: "..over 90 percent (and perhaps over 98 percent) of all speciation events are accompanied by karyotypic changes, and that in the majority of these cases the structural chromosomal rearrangements have played a primary role in initiating divergence."

The establishment of a new chromosomal variant (such as an inversion, fusion, or translocation), however, appears unlikely according to most population genetic models due to the lowered fitness of chromosomal heterokaryotypes (Wright, 1941; Bengtsson and Bodmer, 1976; Lande, 1979; Hedrick, 1981; Slatkin, 1981; Huai and Woodruff, 1998; Hoffmann and Rieseberg, 2008). Since all chromosomal mutations originate in the heterozygous condition, each of them is selected against efficiently when rare. Thus, almost all underdominant mutations, including chromosomal variants, should be eliminated almost immediately by natural selection and could seldom be established in a large panmictic population. For example, the second chromosome in humans was derived in our ancestors from a fusion of two chromosomes, which are both present in chimpanzees, gorillas, and orangutans (Figure 1).

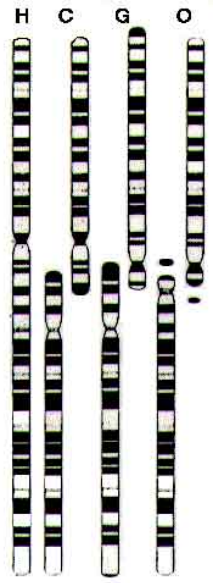


Figure 1. Chromosome two from humans (H) was formed from a fusion of two chromosomes that are now in chimpanzees (C), gorillas (G), and orangutans (O) (Yunis *et al.*, 1980).

How did this new chromosomal fusion event survive in our human ancestors when it would have been selected against in heterozygotes with the non-fused chromosomes? The expected inviable gametes that would be formed in our ancestor that were heterozygous for a fused chromosome would have extra or missing chromosome arms and, hence, thousands of extra and missing genes. This would lead to nonfunctional gametes or inviable embryos.

Many have assumed that new rearrangements, such as fusions, would only survive in very small populations where there is inbreeding and genetic drift (see Rieseberg, 2001; Hoffmann and Rieseberg, 2008 for reviews). In these populations, there would be the possibility of production of individuals that are homozygous for the rearrangement (Charlesworth, Lande and Slatkin, 1982; Futuyma and Mayer, 1980; Spirito *et al.*, 1983; Templeton, 1981). Another possibility is that some genetic changes leading to rearrangements occur in premeiotic germ cells of an individual leading to more than one sibling offspring with the same rearrangement. If these siblings mate some of their progeny could be homozygous for the fusion chromosome only two generations after the genetic change (Capanna *et al.*, 1997; Capanna and Redi, 1994; Huai and Woodruff, 1998).

These underdominant mutations can be modeled in the following one-locus, two-allele model of a population that is assumed to be in Hardy/Weinberg equilibrium except for selection. In this model:

1) s is the selection coefficient for the A_1A_1 genotype (fitness, $w = 1 + s$),
2) t is the selection coefficient for the A_2A_2 genotype (fitness, $w = 1 + t$),
3) the fitness of the A_1A_2 heterozygotes is set at one (fitness, $w = 1$),
4) the frequency of the A_1 allele is p,
5) the frequency of the A_2 allele is q,
6) the mean fitness, which is the sum of the relative contributions of all genotypes, is given the symbol ϖ,
7) the frequency of the A_1 allele at equilibrium is p_e,
8) the frequency of the A_2 allele at equilibrium is q_e.

The following is one possible derivation of the equations for the expected frequencies of the A_1 allele at equilibrium (p_e) and the A_2 allele at equilibrium (q_e), with underdominance: $q_e = s/(s + t)$ and $p_e = t/(s + t)$, as shown below.

Derivation of the expected underdominance equilibrium frequencies:

	A_1A_1	A_1A_2	A_2A_2
Freq. =	p^2	$2pq$	q^2
w =	$1 + s$	1	$1 + t$

After selection:

$$p^2(1+s)/\varpi \qquad 2pq(1)/\varpi \qquad q^2(1+t)/\varpi$$

with $\varpi = p^2(1+s) + 2pq(1) + q^2(1+t)$

$$\varpi = p^2 + sp^2 + 2pq + q^2 + tq^2$$

Since $p^2 + 2pq + q^2 = 1$,

$$\varpi = 1 + sp^2 + tq^2$$

What is q in the next generation (q^1 = ?):

$$q^1 = \frac{(pq)+q^2(1+t)}{\varpi}$$

$$q^1 = \frac{pq+q^2+tq^2}{\varpi}$$

$$q^1 = \frac{q(p+q+tq)}{\varpi}$$

$$q^1 = \frac{q(1+tq)}{\varpi}$$

$$q^1 = \frac{q+tq^2}{1+sp^2+tq^2}$$

The change in q = the new q^1 – previous q; $\Delta q = q^1 - q$:

$$\Delta q = \frac{q+tq^2}{1+sp^2+tq^2} - q$$

$$\Delta q = \frac{q+tq^2-q(1+sp^2+tq^2)}{\varpi}$$

$$\Delta q = \frac{q+tq^2-q-sp^2q-tq^3}{\varpi}$$

$$\Delta q = \frac{-sp^2q-tq^3+tq^2}{\varpi}$$

$$\Delta q = \frac{q(-sp^2-tq^2+tq)}{\varpi}$$

Since q = 1 – p,

$$\Delta q = \frac{q(-sp^2 - tq(1-p) + tq}{\varpi}$$

$$\Delta q = \frac{q(-sp^2 - tq + tpq + tq)}{\varpi}$$

$$\Delta q = \frac{q(-sp^2 + tpq)}{\varpi}$$

$$\Delta q = \frac{pq(-sp + tq)}{\varpi}$$

$$\Delta q = \frac{pq(-sp + tq)}{1 + sp^2 + tq^2}$$

Since at equilibrium neither the frequency of p or q is changing:

$$\Delta q = 0$$

$$\Delta q = \frac{pq(-sp + tq)}{1 + sp^2 + tq^2} = 0$$

This means that the numerator must be equal to zero as well. Hence,

$$-sp + tq = 0$$

$$-s(1 - q) = -tq$$

$$-s + sq = -tq$$

$$-s = -tq - sq$$

$$-s = q(-t - q)$$

$$q = \frac{-s}{-t - s}$$

$$q = \frac{s}{t + s}$$

Since q = 1-p, at equilibrium:

$$q_e = \frac{s}{s+t} \qquad p_e = \frac{t}{s+t}$$

Unlike heterozygous advantage (overdominance), with heterozygous disadvantage (underdominance) alleles are not usually maintained. Either p or q will go extinct dependent on their initial frequencies and the expected equilibrium values (see Figure 2). In Figure 2, p and q at equilibrium are equal to 0.5, and the fitness of A_1A_1, A_1A_2, and A_2A_2 genotypes are 1.33, 1, and 1.33, respectively. If the frequency of A_2 (q) goes above 0.5, q goes to fixation, whereas if the frequency of A_2 (q) goes below 0.5, q is lost (p goes to fixation). This can be seen by determining the change in q over time (Δq) from the following equation, where p = q = 0.5 and s = t = 1.33:

$$\Delta q = \frac{pq(-sp+tq)}{1+sp^2+tq^2}$$

$$\Delta q = \frac{(0.5*0.5)(-1.33*0.5+1.33*0.5)}{1+1.33*0.5^2+1.33*0.5^2}$$

$$\Delta q = \frac{(0.25)(0)}{1+1.33*0.5^2+1.33*0.5^2}$$

$$\Delta q = 0$$

Hence, q does not change over time.
If q goes up, say to 0.6, and s = t = 1.33,

$$\Delta q = \frac{pq(-sp+tq)}{1+sp^2+tq^2}$$

$$\Delta q = \frac{(0.4*0.6)(-1.33*0.4+1.33*0.6)}{1+1.33*0.4^2+1.33*0.6^2}$$

$$\Delta q = \frac{(0.24)(-0.532+0.798)}{1+0.2128+0.4788}$$

$$\Delta q = 0.0377$$

The frequency of q would then increase to 0.638. The frequency of q would go to 0.687 in the next generation, on to a final frequency of one (fixation). On the other hand, if q starts below 0.5, say at 0.4, the frequency will go down to 0.362 (change in q is -0.0378), then to 0.313, and to a final frequency of zero.

Once you have R/R (homozygous for a rearrangement) and +/+ (homozygous for the non-rearranged chromosomes) organisms in a population, they will tend to keep apart and not share genes, because their heterozygous hybrids (R/+) will have a reduced fitness. Their hybrid progeny will be selected against. Hence, this is a possible mechanism for the evolution of reproductive isolation that could lead to speciation. This is a controversial model, however, because chromosomal rearrangement differences between species may arise after speciation and have nothing to do with the initial reproductive isolation events (Carson, 1982). Others have proposed that heterozygotes for chromosome rearrangements, such as inversions, serve as a trap for the alleles of genes that are involved in reproductive isolation (Noor *et al.*, 2001; Navarro and Barton, 2003; Hoffmann and Rieseberg, 2008); recombination events within inversion heterozygotes do not lead to functional gametes (Hedrick, 2005).

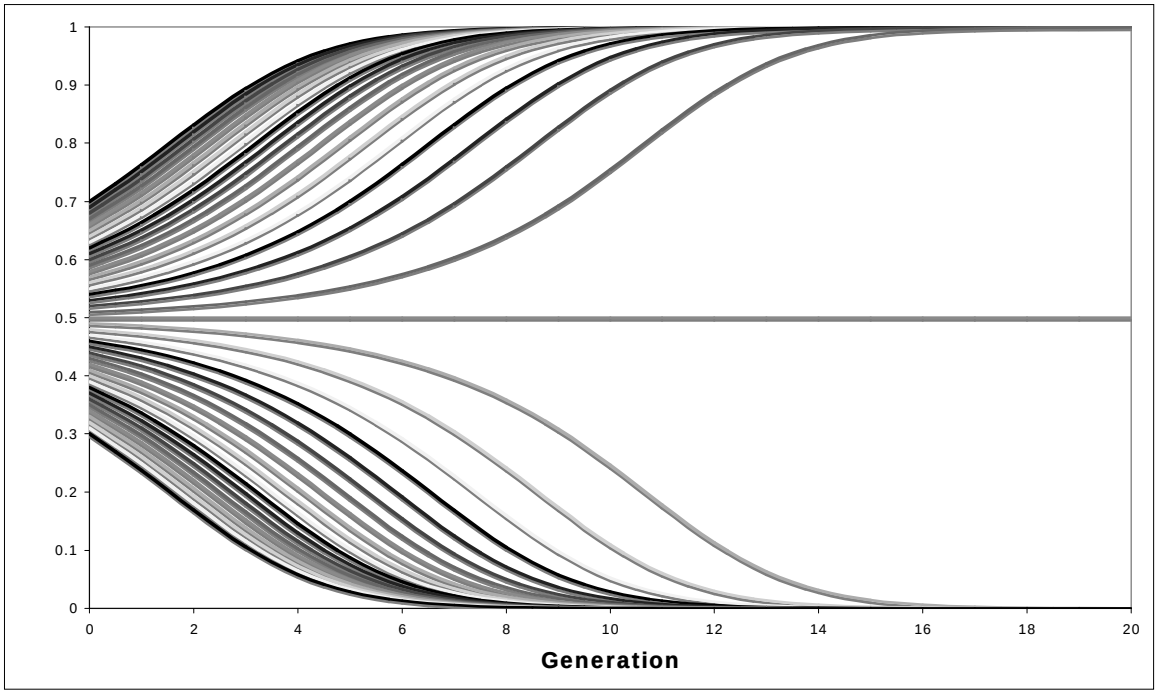


Figure 2. The frequency of $A_2(q)$ over time expected for underdominance, if s = t = 1.33 and there are different starting frequencies for $A_2(q)$ from 0.3 to 0.7.

We attempted to observe underdominance in *Drosophila melanogaster* by mating two stocks with different karyotypes and phenotypes: 1) C(2L)RM, dp^{ov1}/F(2R), *cn c bw*, which has rearranged chromosomes, white eyes (due to the interaction of *cn* and *bw*), dumpy/curved wings, and is discussed in Grell (1970) and Ashburner (1989); 2) *al* dp^{ov1} *b pr cn c px sp*, which has normal chromosomes, dumpy/curved wings, black body color, reddish/purple eyes, and extra wing veins. In Figures 3, the second chromosomes of the C(2L)RM, dp^{ov1}/F(2R), *cn c bw* stock are shown in comparison to the *al* dp^{ov1} *b pr cn c px sp* stock that has two metacentric, 2L-2R chromosomes. Two metacentric 3L-3R chromosomes, two X chromosomes in females, one X and one Y chromosome in males, and two very small fourth chromosomes (2N = 8) are also part of the normal karyotype of *D. melanogaster*. In Figure 3, only the *dp*, *cn* and *bw* markers are shown.

al dp^{ov1} *b pr cn c px sp*	C(2L)RM, dp^{ov1}/F(2R), *cn c bw*
2L-------*dp*-----o---*cn*-----*bw*---2R	2L---*dp*----------o----------*dp*----2L
2L-------*dp*-----o---*cn*-----*bw*---2R	2R--*bw*----*cn*---o
	2R--*bw*----*cn*---o

Figure 3. Second chromosomes of the *al* dp^{ov1} *b pr cn c px sp* stock, which has a normal karyotype, and the C(2L)RM, dp^{ov1}/F(2R), *cn c bw* stock, which has rearranged second chromosomes (o = centromeres).

The inviable progeny expected from crosses between the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* and *al dp*^{ov1} *b pr cn c px sp* are shown in Figure 4.

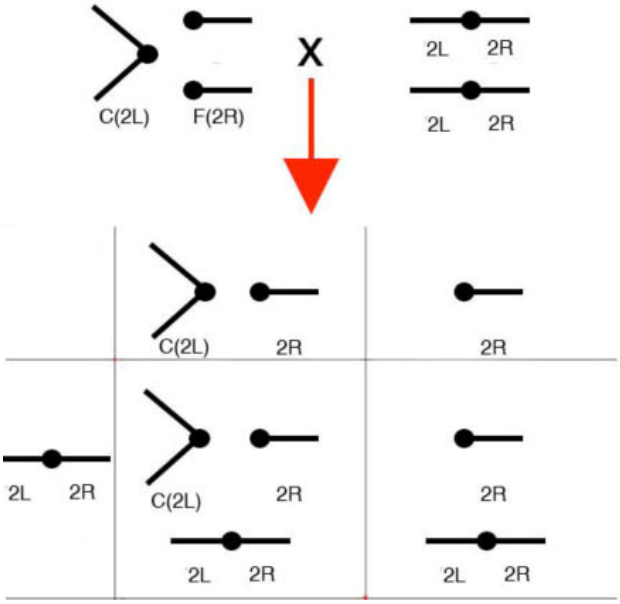


Figure 4. Crosses between the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* stock, which has rearranged second chromosomes, and the *al dp*^{ov1} *b pr cn c px sp* stock, which has a normal karyotype. All progeny are expected to be lethal because of aneuploidy for chromosome arms (three 2L or one 2L).

The *al dp*^{ov1} *b pr cn c px sp* stock, which has multiple visible markers that each reduces fitness, was selected for use, because it was assumed to have a low fitness, as does the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* stock (Ashburner, 1989). One-half of the progeny from crosses between C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* flies are expected to be inviable, because they have extra or missing 2L arms as shown in Figure 5. The aneuploidy imbalance of the approximately 2,600 genes on the left arm of the second chromosome is expected to cause early embryo death.

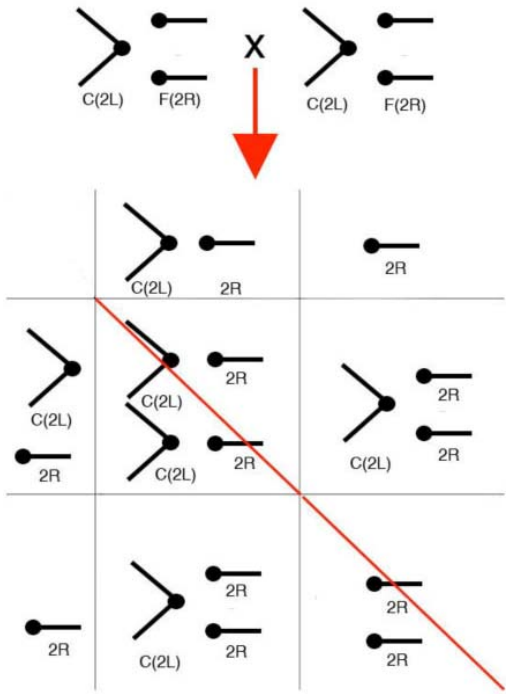


Figure 5. Crosses between C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* flies gives one-half of progeny that are lethal, due to aneuploidy of chromosome arms (four 2L or no 2L).

To determine if the expected inviable progeny were produced from the crosses in Figure 4, virgin C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* females were mated with males of the *al dp*^{ov1} *b pr cn c px sp* stock, and vice versa; no progeny were recovered. This confirmed the cytology of the two stocks and confirmed that imbalanced second chromosomes caused embryo lethality. This also means that the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw*/*al dp*^{ov1} *b pr cn c px sp* heterozygotes have a fitness of zero.

Next we added different combinations of frequencies of the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* females and males and *al dp*^{ov1} *b pr cn c px sp* females and males to half-pint bottles of *Drosophila* food at 25°C, removed the parents after seven days, scored the progeny for seven days, and transferred all of the progeny to new bottles. This protocol was repeated until either the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw*, white eyed flies, or the *al dp*^{ov1} *b pr cn c px sp*, reddish/purple eyed, flies went to fixation (100%).

The results from these crosses are shown in Figure 6. In the legend the first number in parenthesis is the proportion of C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* flies and the second number is the proportion of *al dp*^{ov1} *b pr cn c px sp* flies. For example, 20:80 means that 10 females and 10 males of the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* stock were placed into a bottle with 40 females and 40 males of the *al dp*^{ov1} *b pr cn c px sp* stock. The frequency of the first progeny of each cross is given as generation one (Foster *et al*., 1972).

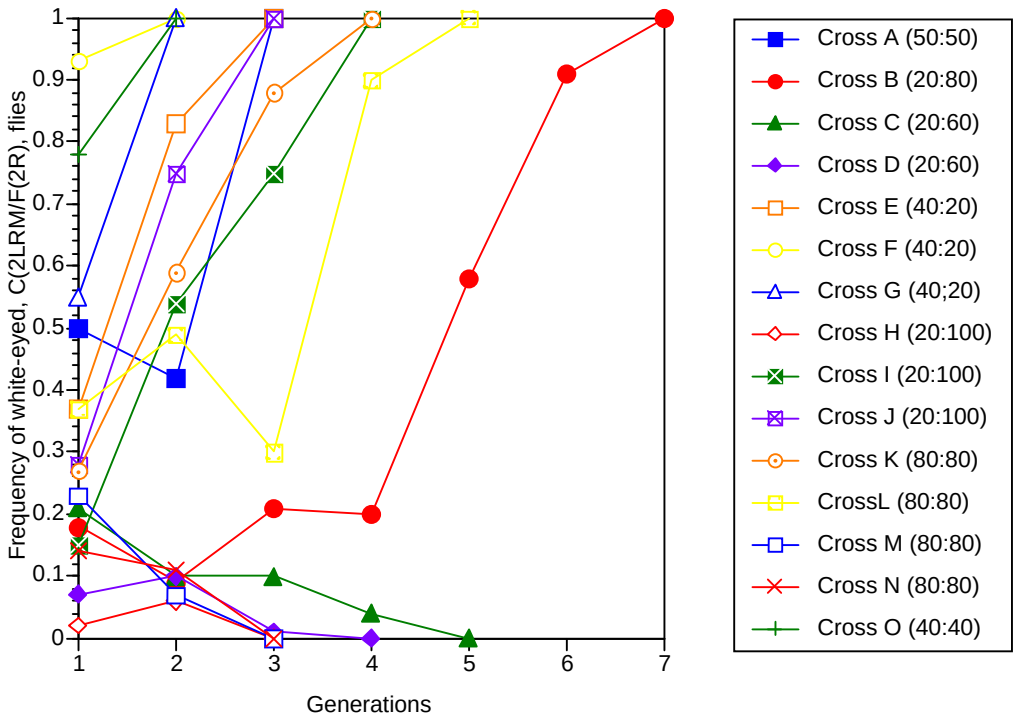
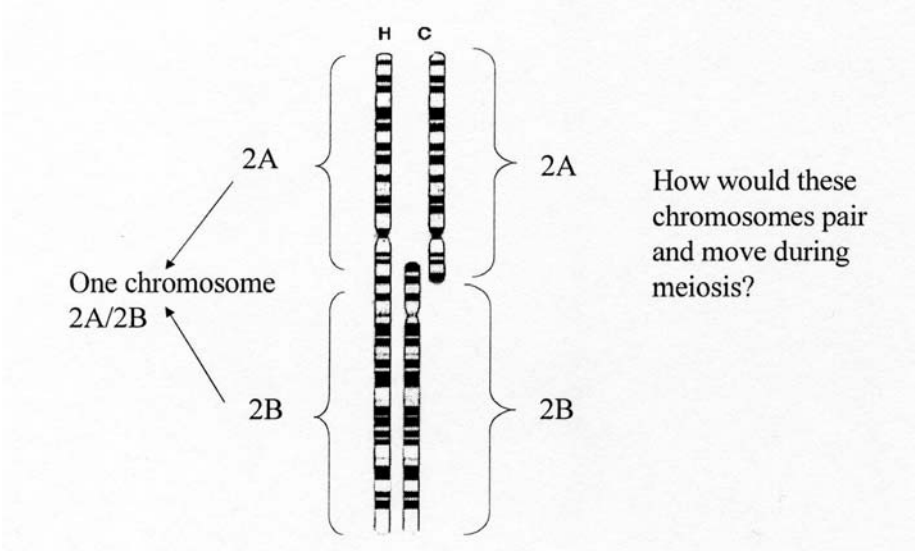


Figure 6. Frequencies of C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* (white eyed flies), over time.

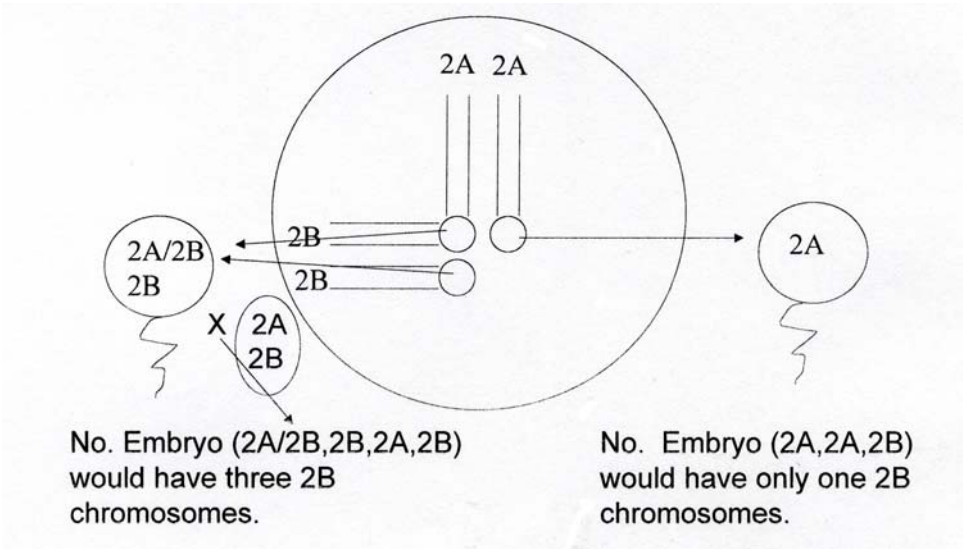
The results in Figure 6 show that the equilibrium (flex) point above which C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* goes to fixation and below it is lost is about 0.2. Cross B and Cross I seem to be exceptions, but notice that although these crosses began with frequencies of C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* below 0.2, Cross I quickly moved above 0.2, possibly by drift, and then went to fixation, whereas Cross B moved above 0.2 in five generations and then quickly went to fixation. This equilibrium point (0.2) suggests that the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* stock is about four-times as fit as the *al dp*^{ov1} *b pr cn c px sp* stock, which has normal chromosomes. It would be of interest to

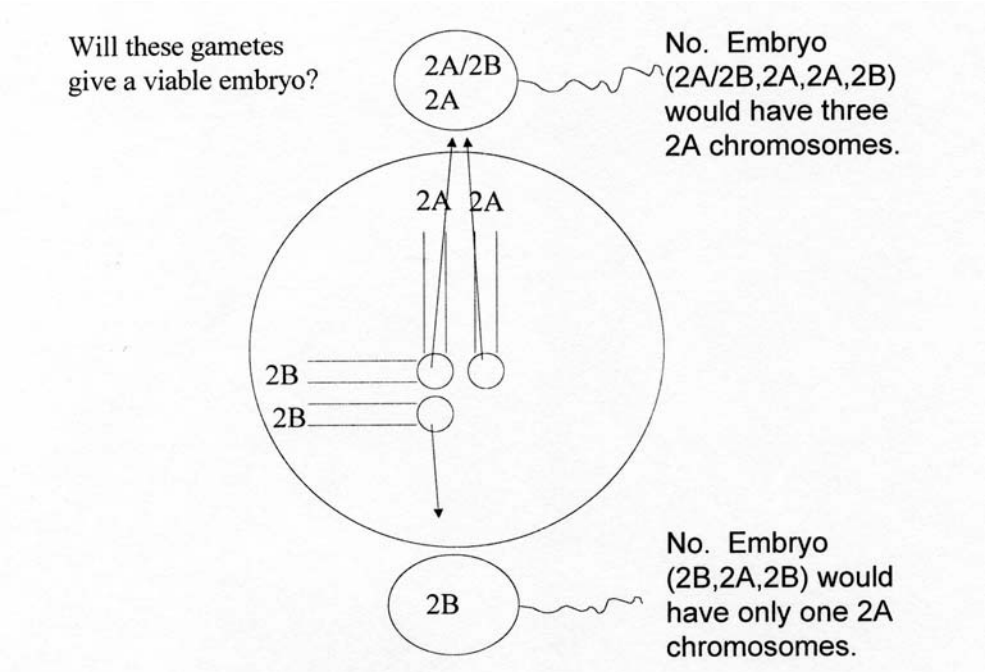
compete the C(2L)RM, *dp*^{ov1}/F(2R), *cn c bw* stock with a stock that has a higher fitness than the *al dp*^{ov1} *b pr cn c px sp* stock to determine if the equilibrium point goes above 0.2, as observed in this teaching exercise.

A class discussion of the results of this teaching exercise could include estimating the expected reduction in fitness, based on functional and nonfunctional gametes, in the early humans that were heterozygous for the fused second chromosome and two unfused chromosomes. Students might begin by drawing these chromosomes paired at metaphase I of meiosis, and then asked what proportion of gametes would be nonfunctional or would lead to lethal embryos, because of missing or extra segments of chromosomes. The answer, as shown below, is that two-thirds of the gametes would be aneuploid for chromosome arms. Let the current, fused, human chromosome number two be labeled with a 2A arm and a 2B arm, which correspond to the original chromosomes that are called 2A and 2B.

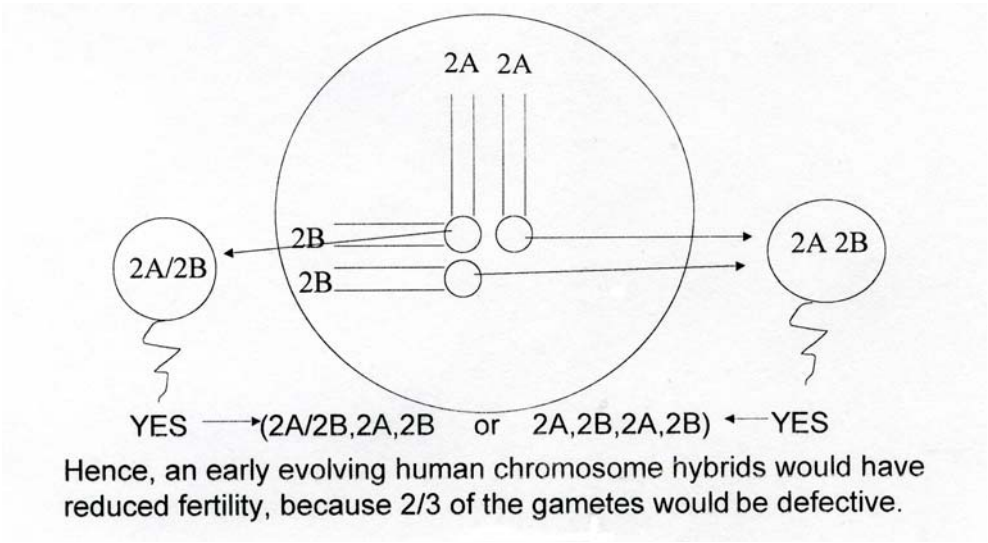


Will these gametes give a viable embryo?





Will these gametes give a viable embryo? YES



Students could also be asked to search PubMed at NCBI (National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/pubmed/) for articles that show reduced fitness for organisms with chromosome rearrangement heterozygotes. They might start with Hauffe and Searle (1998), where translocation heterozygotes in mice have about a two-fold reduction in litter size compared to mice with normal chromosomes.

Finally, students could see Britton-Davidian *et al.* (2000) for an example of the recent evolution of chromosome races of mice on the island of Madeira.

References: Ashburner, M., 1989, Drosophila: *A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, pp. 775-780; Bengtsson, B.O., and W.F. Bodmer 1976, Theor. Popul. Biol. 9: 260-281; Britton-Davidian, J., J. Catalan, M. da Graca Ramalhinho, G. Ganem, J.-C. Auffray, R. Capela, M. Biscoitos, J.B. Searle, and M. da Luz Mathias 2000, Nature 403: 158; Bush,

G.L., 1981, Stasipatric speciation and rapid evolution in animals. In: *Evolution and Speciation: Essays in Honor of M.J.D. White*. (Atchley, W.R., and D.S. Woodruff, eds). Cambridge University Press, pp. 201-218; Bush, G.L., S.M. Case, A.C. Wilson, and J.L. Patton 1977, Proc. Natl. Acad. Sci. USA 74: 3942-3946; Capanna, E., M.V. Civitelli, and M. Cristaldi 1977, Bollettino di Zollogia 44: 213-246; Capanna, E., and C.A. Redi 1994, Bollettino di Zollogia 61: 285-294; Carson, H.L., 1982, Speciation as a major reorganization of polygenic balances. In: *Mechanisms of Speciation* (Barigozzi, C., ed.). Alan R. Liss, Inc., pp. 411-433; Charlesworth, B., R. Lande, and M. Slatkin 1982, Evolution 36: 474-498; Grell, E.H., 1970, Genetics 65: 65-74; Foster, G.G., M.J. Whitten, T. Prout, and G. Gill 1972, Science 176: 875-880; Futuyma, D.J., and G.C. Mayer 1980, Syst. Zool. 29: 254-271; Hauffe, H.E., and J.B. Searle 1998, Genetics 150: 1143-1154; Hedrick, P.W., 1981, Evolution 35: 322-332; Hedrick, P.W., 2005, *Genetics of Populations*: Jones and Barlett Publishers, Sudbury, MA; Hoffmann, A.A., and L.H. Rieseberg 2008, Annu. Rev. Ecol. Evol. Syst. 39: 21-42; Huai, H., and R.C. Woodruff 1998, Genetica 102/103: 489-505; King, M., 1993, *Species Evolution: The Role of Chromosome Change.* Cambridge University Press; Lande, R., 1979, Evolution 33: 234-251; Nachman, M.W., and J.B. Searle 1995, Trends in Ecology and Evolution 10: 397-402; Navarro, A., and N.H. Barton 2003, Science 300: 321-324; Noor, M.A., K.L. Grams, L.A. Bertucci, and J. Reiland 2001, Proc. Natl. Acad. Sci. USA 98: 12084-12088; Patton, I.L., and S.W. Sherwood 1983, Annu. Rev. Ecol. Syst. 14: 139-158; Rieseberg, L.H., 2001, Trends in Ecology and Evolution 16: 351-358; Sites, J.W., and B. Moritz 1987, Syst. Zool. 36: 153-174; Slatkin, M., 1981, Evolution 35: 477-488; Spirito, F., C. Rossi, and M. Rizzoui 1983, Evolution 37: 785-797; Templeton, A.R., 1981, Annu. Rev. Ecol. Syst. 12: 23-48; Turner, B.I., 1983, Am. Nat. 122: 153-154; White, M.J.D., 1978, *Modes of Speciation*. San Francisco, Freeman; Wright, S., 1941, Am. Nat. 75: 513-522; Yunis, J.J., J.R. Sawyer, and K. Dunham 1980, Science 208: 1145-1148.

Fruit fly as a model for alcoholism: Integration of laboratory pedagogy and student-directed research.

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Overview

Drosophila melanogaster is a good model system for examining genetic predisposition to alcoholism. Flies exhibit a characteristic behavior upon alcohol exposure that includes hyperactivity and then sedation (Wolf *et al.*, 2002; Heberlein, 2000). Flies also become tolerant to drug exposure, with single or multiple exposure (Berger *et al.*, 2004). The alcohol-based behaviors are robust and easy to illicit and analyze, ideal for introducing behavioral analysis to undergraduates. The fly model system also has many tools amenable to genetic analysis that are easily manipulated by students. Pedagogies using the model and tools can be used to engage students in active learning in order to achieve student outcomes related to model systems, genomic analysis, use of computer-based tools, and data analysis.

We developed a laboratory module for our General Biology in which insertion mutations available from the Bloomington Stock Center have been screened for alterations in ethanol sedation behavior and tolerance (http://flystocks.bio.indiana.edu/). These inserts are available through the