Changes in linkage disequilibrium over time in *Drosophila melanogaster* for two sex-linked loci.

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1. Linkage Disequilibrium Definition and Theory:

If a diploid population has two linked genes with two alleles each (*A*, *a* and *B*, *b*), the genes can occur as four possible haplotypes AB, ab, Ab and aB, with haplotypes defined as multilocus genotypes of a chromosome or chromosome region and here underlined. If this population also fits the Hardy-Weinberg assumptions (large population size, no mutations, no selection for or against any of the four haplotypes, no migration into or out of the population, and random matings among individuals with the four haplotypes) the frequencies of the four haplotypes can be estimated from the expected frequencies of alleles at the two loci and from random matings of all combinations of alleles as shown below. Note that *p* is the frequency of the *A* allele, *q* is the frequency of the *a* allele, *s* is the frequency of the *B* allele, and *t* is the frequency of the *b* allele.

	A(p)	*a(q)*
B(s)	AB(ps)	aB(qs)
b(t)	Ab(pt)	ab(qt)

With the Hardy-Weinberg assumptions, it is then expected that

$$(AB)(ab) - (aB)(Ab) = 0 \qquad (1)$$

This is called being in linkage equilibrium, because transmission of alleles at the *A* locus are independent of the alleles at the *B* locus and the fitness of all haplotypes are equal (Hedrick, 2005).

As an example, if the frequencies of alleles are $p(A) = 0.3$, $q(a) = 0.7$, $s(B) = 0.4$ and $t(b) = 0.6$ and the frequencies of haplotypes are AB = 0.12, ab = 0.42, aB = 0.28, and Ab = 0.18 in a population of bullfrogs in Lake of the Pines in East Texas, and this population fits the Hardy-Weinberg assumptions, then (AB)(ab) - (aB)(Ab) should be equal to 0. Is this true?

$$\{[(0.3)(0.4)] \times [(0.7)(0.6)]\} - \{[(0.7)(0.4)] \times [(0.3)(0.6)]\} = 0.0504 - 0.0504 = 0$$

Hence this population is at linkage equilibrium, with the frequencies of haplotypes being equal to the frequencies of the two alleles in each haplotype (for example, AB = [(0.3)(0.4)] = 0.12, etc.).

There are genetic and demographic possibilities, however, where the haplotypes are not associated randomly. This can be due to: 1) some combination of alleles at the two loci give haplotypes that are advantageous and increase the fitness of individuals (average number of offspring); 2) some haplotypes have randomly drifted to a higher or lower frequency than the Hardy-Weinberg expectation (genetic drift); 3) frequencies of some haplotypes have increased due to migration from another population or migration out of the population; 4) a haplotype is closely linked to a beneficial gene; 5) new recurrent mutations, or premeiotic clusters of mutations (Woodruff and Thompson, 2002) have increased the frequency of a haplotype(s); or 5) an interaction of one or more of 1-4.

In these population situations, where one or more of the Hardy-Weinberg assumptions are not valid, the excess of some haplotypes above the Hardy-Weinberg expectation, and concomitant decrease of some haplotypes below the Hardy-Weinberg expectation, is called linkage disequilibrium (the nonrandom association of alleles at different loci into gametes) (Lewontin and Kojima, 1960). A

more inclusive term, which includes linked and unlinked loci, is gametic disequilibrium (see discussions of this topic in Halliburton, 2004; Hedrick, 2005; Hartl and Clark, 2006). With linkage disequilibrium:

$$(\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab}) \neq 0 \qquad (2)$$

Historically the amount of linkage disequilibrium is called D, because this is the amount of deviation from a random association of alleles at different loci, and

$$D = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab}) \qquad (3)$$

Using this equation for D, in our previous example where $\underline{AB}$ = (0.3)(0.4) = 0.12, $\underline{ab}$ = (0.7)(0.6) = 0.42, $\underline{aB}$ = (0.7)(0.4) = 0.28 and $\underline{Ab}$ = (0.3)(0.6) = 0.18:

$$D = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab})$$
$$D = (0.12)(0.42) - (0.28)(0.18)$$
$$D = 0.0504 - 0.0504$$
$$D = 0$$

This bullfrog population in East Texas has no linkage disequilibrium for the four haplotypes.

Now let us look at a population of water snakes on the South Bass Island of Lake Erie in Ohio, where $\underline{AB}$ = 0.47, $\underline{ab}$ = 0.47, $\underline{aB}$ = 0.03 and $\underline{Ab}$ = 0.03. Does this population show linkage disequilibrium for the $\underline{AB}$, $\underline{ab}$, $\underline{aB}$ and $\underline{Ab}$ haplotypes?

$$D = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab})$$
$$D = (0.47)(0.47) - (0.03)(0.03)$$
$$D = (0.2209) - (0.0009)$$
$$D = 0.22$$

In addition, since AB = (p)(s) + D; ab = (q)(t) + D; Ab = (p)(t) – D; and aB = (q)(s) – D, the D value can also be determined from:

$$D = AB - (p)(s) \qquad (4)$$

In the above example, D = 0.47 – (0.5)(0.5) = 0.22.

Hence, this water-snake population is in linkage disequilibrium for these four haplotypes. Since water snakes have been observed to swim between Ohio and South Bass Island, one possibility is that linkage disequilibrium was caused by migration.

It should be noted that D can range from + 0.25 to - 0.25. This can be seen from the two possible extremes of haplotype frequencies:

AB = 0.5, ab = 0.5, aB = 0 and Ab = 0 or AB = 0, ab = 0, aB = 0.5 and Ab = 0.5

$$D = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab}) \qquad D = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab})$$
$$D = (\underline{0.5})(\underline{0.5}) - (\underline{0})(\underline{0}) \qquad D = (\underline{0})(\underline{0}) - (\underline{0.5})(\underline{0.5})$$
$$D = + 0.25 \qquad D = - 0.25$$

See Hedrick (1987, 2005) for other measures of linkage disequilibrium.

2. Expected Changes in Linkage Disequilibrium (D) Over Time if a Population is in Hardy-Weinberg Equilibrium:

If it is assumed that the water-snake population of South Bass Island no longer has migration of snakes from the mainland of Ohio, that the four haplotypes ($\underline{AB}$, $\underline{ab}$, $\underline{Ab}$ and $\underline{aB}$) are neutral in affecting fitness, and that the population fits the other Hardy-Weinberg assumptions, what happens to the previously discussed D value of 0.22 over time? What is the rate of change in D per generation? How many generations would it take for D to be reduced by one-half or to a low level such as 0.01?

Since we previously assumed that the genes of the *A* locus and the *B* locus are linked to the same chromosome in this water-snake population, the reduction in D from 0.22 (call it D_o) to zero (linkage equilibrium) will occur over generations (t) based on the recombination rate (r) between the two genes *A* and *B*. In one generation, D_o will be reduced by a value of 1 – r, such that,

$$D_1 = (1 - r)^1 D_0 \quad (5)$$

And for any generation t: $$D_t = (1 - r)^t D_o \quad (6)$$

For example, the value of D_{50} can be determined after 50 generations (t = 50) if the beginning D_o value is known and the recombination frequency (r) between loci *A* and *B* is known. Figure 1 shows that the reduction in D from 0.22 is faster for higher frequencies of recombination (r) between genes. Note that an r value of 0.5 would imply that the two loci are unlinked. The D values in Figure 1, and in Table 1 and Figure 2, are from the Mathematica program.

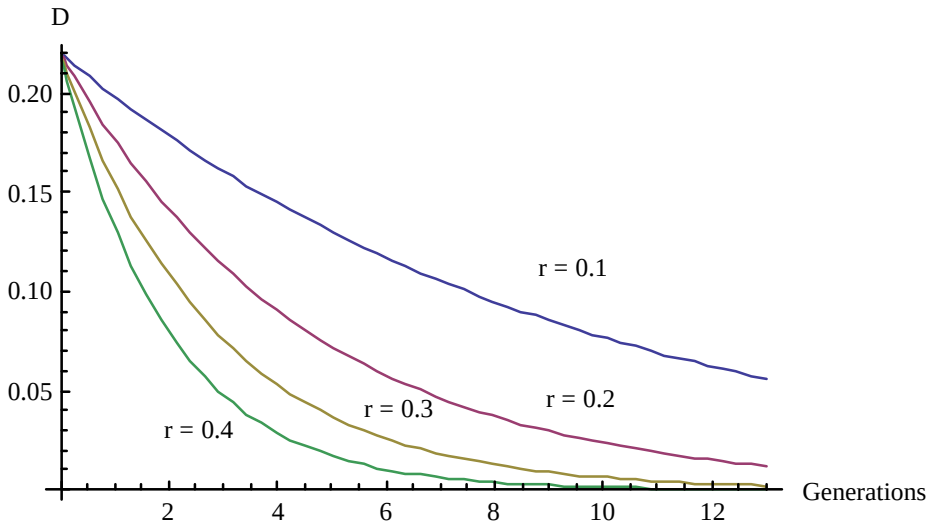

Figure 1. Expected rate of decay of linkage dis-equilibrium (D) over generations with different recombination (r) frequent-cies between two auto-somal linked genes in an organism where recombi-nation occurs in females and males.

One way to show that equation (5) is correct (D drops by 1 – r each generation) is to determine what happens to D in one generation in a population of known haplotype frequencies and recombination frequency, D_o and r, and to compare this D value with the result from equation (4). For example, let us assume that the water-snake population of South Bass Island has haplotype frequencies of AB = 0.47, ab = 0.47, aB = 0.03 and Ab = 0.03 and, hence, $D_o = 0.22$. In addition, it will be assumed that the recombination frequency between the *A* and *B* loci is 20% (r = 0.2). This means that in A B/a b snakes, because of recombination at meiosis, the expected gametes are 47% A B, 47% a b, 3% A b, and 3% a B. If this water-snake population fits the Hardy-Weinberg assumptions, including no migration, the expected frequencies of haplotypes in progeny can be determined from random matings between males and females as shown below. In this table the proportion of possible diploid progeny are shown for each possible union of gametes (eggs and sperm) and are numbered from 1 to 16.

gametes	AB (0.47)	ab (0.47)	Ab (0.03)	aB (0.03)
AB (0.47)	1 AB/AB (0.2209)	2 AB/ab (0.2209)	3 AB/Ab (0.0141)	4 AB/aB (0.0141)
ab (0.47)	5 ab/AB (0.2209)	6 ab/ab (0.2209)	7 ab/Ab (0.0141)	8 ab/aB (0.0141)
Ab (0.03)	9 Ab/AB (0.0141)	10 Ab/ab (0.0141)	11 Ab/Ab (0.0009)	12 Ab/aB (0.0009)
aB (0.03)	13 aB/AB (0.0141)	14 aB/ab (0.0141)	15 aB/Ab (0.0009)	16 aB/aB (0.0009)

Based on r = 0.2 for the *A* and *B* loci, one can now determine the expected frequency of AB gametes among each of the 16 possible diploid progeny and the total AB frequency from all possible progeny. Note that recombination is assumed to occur in both sexes. Since AB = ab, one can also determine the frequency of ab, and, since (Ab + aB) = 1 – (AB + ab) and Ab = aB, one can then determine the frequencies of Ab and aB.

From cross results in the above table, the expected frequencies of AB gametes (haplotypes) in the next generation are as follows (details of how frequencies were determined are also given):

Cross results 1 = 0.2209 (all gametes are AB)
Cross results 2 = 0.08836 (0.4 × 0.2209 or ½ nonrecombinants × 0.2209)
Cross results 3 = 0.00705 [(0.4 × 0.0141) + (0.1 × 0.0141)] or [(½ nonrecombinants × 0.0141) + (½ recombinants × 0.0141)]
Cross results 4 = 0.00705 [(0.4 × 0.0141) + (0.1 × 0.0141)]
Cross results 5 = 0.08836 (0.4 × 0.2209)
Cross results 6 = 0 (no AB gametes expected)
Cross results 7 = 0
Cross results 8 = 0
Cross results 9 = 0.00705 [(0.4 × 0.0141) + (0.1 × 0.0141)]
Cross results 10 = 0
Cross results 11 = 0
Cross results 12 = 0.00009 (0.1 × 0.0009)
Cross results 13 = 0.00705 [(0.4 × 0.0141) + (0.1 × 0.0141)]
Cross results 14 = 0
Cross results 15 = 0.00009 (0.1 × 0.0009)
Cross results 16 = 0
Total frequency of AB (1 - 16) = 0.426

Hence, the frequency of AB = 0.426, ab = 0.426, Ab = 0.074, and aB = 0.074. Using these values and equation (3):

$D_1 = (AB)(ab) - (aB)(Ab)$
$D_1 = (0.426)(0.426) - (0.074)(0.074)$
$D_1 = (0.181476) - (0.005476)$
$D_1 = 0.176$

Is this the same D_1 value as obtained from equation (5): $D_1 = (1 - r)D_o$? Yes.

$D_1 = (1 - 0.2)0.22$
$D_1 = 0.176$

Hence, the two equations (3) and (5) give the same D results. This means that equation (6) is also correct for determining the value of D for any number of generations.

$$D_t = (1 - r)^t D_o$$

One can also determine how many generations for D to be reduced by one-half or to 0.01 by rearranging equation (6) as follows:

$$t = \frac{\ln \frac{D_t}{D_o}}{\ln(1 - r)} \qquad (7)$$

Hence, for the water-snake population, where D_o is 0.22 and r is 0.2, the number of generations to reduce D by one-half and to 0.01 is:

$$t = \frac{\ln \frac{0.11}{0.22}}{\ln(1 - 0.2)} = 3.11 \quad \text{and} \quad t = \frac{\ln \frac{0.01}{0.22}}{\ln(1 - 0.2)} = 13.85$$

3. Using Two Sex-Linked Loci to Model Changes in Linkage Disequilibrium (D) Over Time in *Drosophila melanogaster*:

The experimental objective of this exercise is to model the change in linkage disequilibrium (D) over time using two visible mutations in *D. melanogaster*. One problem with using recessive autosomal or dominant autosomal alleles of two linked genes is that it is not possible to identify all alleles and, therefore, one cannot determine the frequency of haplotypes each generation. For example, using the wild-type alleles a^+ and b^+ and their mutant alleles *a* and *b*, one cannot differentiate between the genotypes $\underline{a^+\ b}$ / $\underline{a\ b}$ and $\underline{a^+\ b}$ / $\underline{a^+\ b}$; they both are a^+ and *b* in phenotypes, but in the former there is one a^+ allele and in the latter there are two a^+ alleles. The same problem also occurs for dominant mutations (for example, $\underline{A\ B^+}$ / $\underline{A^+\ B^+}$ has the same phenotype as $\underline{A\ B^+}$ / $\underline{A\ B^+}$). One way to get around this problem is to use protein electrophoresis of allozymes, where one can distinguish among homozygotes and heterozygotes. For example, for the alcohol dehydrogenase locus of *D. melanogaster*, Adh^F / Adh^F, Adh^S / Adh^S, and Adh^F / Adh^S flies give different banding patterns on starch or cellulose actetate electrophoretic gels (see Thompson *et al*., 2000). This electrophoretic technique has been used to follow changes in linkage disequilibrium (D) over time in *D. melanogaster* for the allozyme loci isocitrate dehydrogenase and phosphoglucomutase (Clegg *et al*., 1980).

In this exercise two sex-linked recessive visible loci (*white* eyes and *singed* bristles) will be used to measure linkage disequilibrium changes over time. This allows for the determination of the frequency of haplotypes in hemizygous males where the X-linked loci are carried against a Y chromosome that does not contain copies of the two genes. Hence, the frequencies of the four haplotypes ($\underline{+\ +}$, $\underline{a\ b}$, $\underline{a\ +}$, and $\underline{+\ b}$) can be determined directly from $\underline{+\ +}$ / Y, $\underline{a\ b}$ / Y, $\underline{a\ +}$ / Y, and $\underline{+\ b}$ / Y males each generation. Hedrick (1976) performed a similar set of experiments using the closely linked (r = 0.015) mutations *y* (yellow body color, 1-0.0) and *w* (white eyes, 1-1.5) of *Drosophila melanogaster*.

The rate of change in linkage disequilibrium (D) over time for two sex-linked loci is different from autosomal loci, however, because there is no recombination for sex-linked genes that are paired against Y chromosomes in males. In fact, the same problem occurs for autosomal loci in *D. melanogaster*, because males do not undergo recombination for any chromosome. The lack of recombination in males for sex-linked genes leads to an expected reduction in the rate of change of D over time as compared with autosomal genes in organisms where recombination occurs in females and males (see discussions in Bennett, 1963; Bennett and Oertel, 1965; Hedrick, 2005). Bennett and Oertel (1965) concluded that for two sex-linked loci the expected reduction in linkage disequilibrium (D) each generation is:

$$\frac{1}{4}\left\{(1-r)+\left(\sqrt{(1-r)(9-r)}\right)\right\}$$

which is about $(1 - r)^{2/3}$

Hence:

$$D_1 = \left[\frac{1}{4}\left\{(1-r)+\left(\sqrt{(1-r)(9-r)}\right)\right\}\right]D_o \qquad (8)$$

and for any generation t:

$$D_t = \left[\frac{1}{4}\left\{(1-r)+\left(\sqrt{(1-r)(9-r)}\right)\right\}\right]^t D_o \qquad (9)$$

For example, for the South Bass Island population of water snakes, where D_o is 0.22 and r is 0.2:

$$D_1 = \left[\frac{1}{4}\left\{\left(1-0.2\right)+\left(\sqrt{(1-0.2)(9-0.2)}\right)\right\}\right]^1 0.22$$

$$D_1 = (0.86332)(0.22)$$

$$D_1 = 0.1899304$$

Notice that this D_1 value is higher than the D_1 value (0.176) previously obtained for autosomal loci in organisms where recombination occurs in both sexes. The expected reductions in D each generation for two sex-linked genes, as compared to reductions in D for two autosomal genes, where recombination occurs in both sexes, is shown in Table 1 and Figure 2. Note, for example, that at generation 13 the D_{13} value for autosomal loci with recombination in both sexes is 0.0151182, whereas for two sex-linked loci with recombination only in females D_{13} is 0.0377124, a 2.5-fold difference. Linkage disequilibrium is clearly expected to go down slower over time for two sex-linked loci with recombination only in one sex as compared to that for two loci with the same r values, but with recombination in both sexes.

Table 1. Expected changes in linkage disequilibrium (D) over time for two sex-linked loci (*w* and *sn*^{3}), where recombination only occurs in females, and for two autosomal loci, where recombination occurs in both sexes. In both cases, r = 0.2 and D_o is 0.22.

	D Values	
Generations	Sex-Linked Loci (*w* and *sn*^{3}); Recombination Only Occurs in Females[a]	Autosomal Loci; Recombination Occurs in Both Sexes[b]
G1	0.22	0.22
G2	0.1899304	0.176
G3	0.1639707	0.1408
G4	0.1415592	0.11264
G5	0.1222109	0.090112
G6	0.1055071	0.0720896
G7	0.0910866	0.0576168
G8	0.0786367	0.0461373
G9	0.0678886	0.0369098
G10	0.0586096	0.0295279
G11	0.0505989	0.0236223
G12	0.0436830	0.0188978
G13	0.0377124	0.0151182

[a]D values are derived from equation (9) [b]D value are derived from equation (6)

4. Materials and Methods:

In this exercise, the reduction in linkage disequilibrium (D) over time will be determined for the two sex-linked genes *w* (white eyes; map position 1.5) and *sn*^{3} (singed-3, very short bristles; map position 21.0), with there being about 20% expected recombination in females each generation between *w* and *sn*^{3}, *i.e.*, r = 0.2 (Lindsley and Zimm, 1992).

This exercise was started by mating 60 $\underline{+\ +}$ / $\underline{w\ sn^3}$ virgin females and 60 males of the proportions 24 $\underline{+\ +}$ / Y, 24 $\underline{w\ sn^3}$ / Y, 6 $\underline{w\ +}$ / Y and 6 $\underline{+\ sn^3}$ / Y in each of four bottles. This gives the beginning frequencies of the four haplotypes in females and males as: $\underline{+\ +}$ = 0.47, $\underline{w\ sn^3}$ = 0.47, $\underline{w\ +}$ =

0.03, and $\underline{+\ sn^3}$ = 0.03, with the frequency of the *w* allele and the sn^3 alleles being 0.5 in females and males. In addition, the beginning linkage disequilibrium (D_o) value, using equation (3) was:

$D_o = (\underline{AB})(\underline{ab}) - (\underline{aB})(\underline{Ab})$

$D_o = (0.47)(0.47) - (0.03)(0.03)$

$D_o = 0.22$ (same as the water-snake population)

Since D_o is 0.22 and r is 0.2, the expected reductions in D over time for the $w\ sn^3$ crosses are shown in Table 1 and Figure 2 for sex-linked loci. Will the D values that are observed over generations using the *w* and sn^3 loci in this exercise be similar to the theoretical expectation?

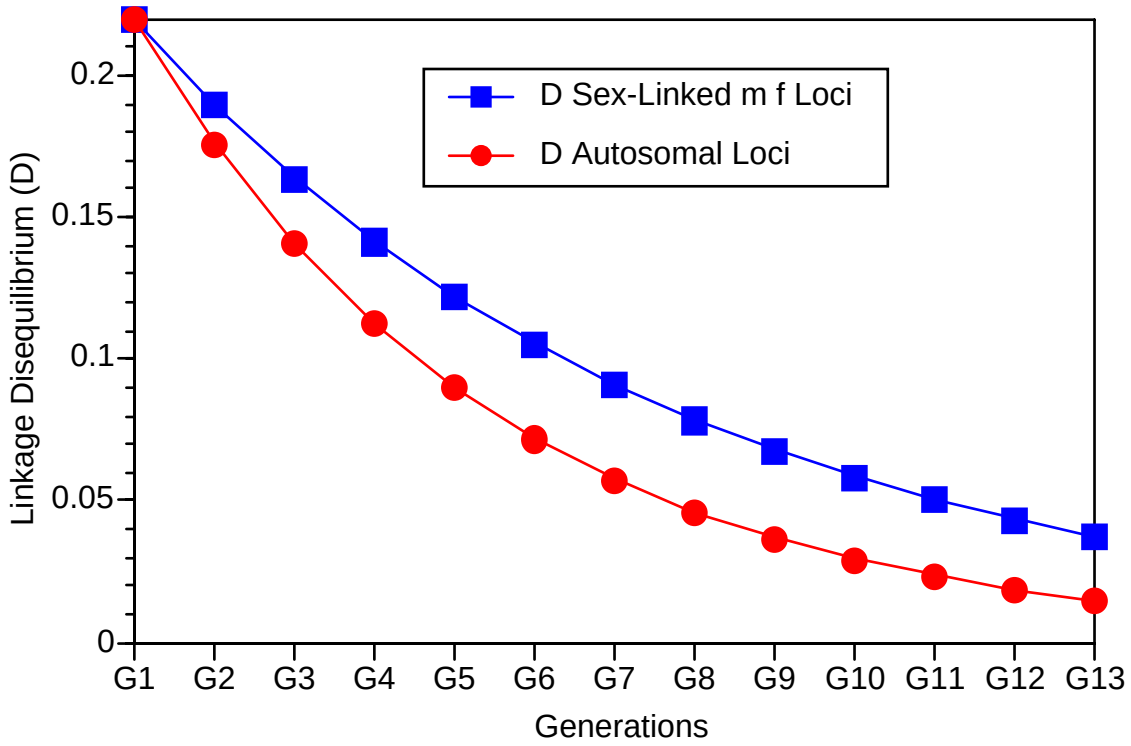

Figure 2. Expected changes in linkage disequilibrium (D) over time for two sex-linked loci (*w* and sn^3), where recombination only occurs in females, using equation (9), and for two autosomal loci, where recombination occurs in both sexes, using equation (6). In both cases, r = 0.2 and D_o is 0.22. D values are from Table 1.

After seven days at 25°C, the adults were removed from each bottle and the D haplotype values determined by counting male progeny up to day 14 from time of initiation of the cross. There are four possible phenotypes of males that are equivalent to the four possible haplotypes: $\underline{+\ +}$ / Y = red eyes and long bristles; $\underline{w\ sn^3}$ / Y = white eyes and short bristles; $\underline{w\ +}$ / Y = white eyes and long bristles; and $\underline{+\ sn^3}$ / Y = red eyes and short bristles.

Flies were raised on a standard cornmeal-molasses medium supplemented with yeast. After the initial generation, each subsequent generation bottle was set up with all female and male progeny collected up to day 14 from the time of initiation of each cross, including the males used to measure haplotype frequencies. The progeny were collected and maintained in a bottle until all flies are transferred to a new bottle on day 14. The collection bottle was then discarded.

At the end of the experiment the observed D results over time from the *w sn*^{3}crosses will be compared with the expected theoretical changes of D over time from equation (9). It is our hypothesis that the two curves will not be significantly different. Each bottle will also be tested for significant amounts of linkage disequilibrium by use of the chi-square test as discussed by Hedrick (2005) using the Prism statistical program.

5. Results:

The changes in linkage disequilibrium (D) over time for *w* and *sn*^{3} are shown in Tables 2 and 3 and in Figure 3. In preliminary crosses we observed 19.22% recombination (551/2,867) between *w* and *sn*^{3} in our stocks at 25^{o}C and, therefore, used r = 0.1922. Linkage disequilibrium (D) values decreased significantly (P < 0.02) over time in each of the four bottles, but the decrease was significantly (P < 0.05) faster than the theoretical expectation. Part of the reason why the drop in D was faster than theoretical may be because not all haplotypes (*+ +*, *+ sn*^{3}, *w +*, and *w sn*^{3}) had the same viabilities. In crosses of *w + / + sn*^{3} females with *w +* / Y males, instead of equal proportions of the nonrecombinants haplotype progeny males we observed: 421/1349 (0.31) *w +* / Y males and 688/1349 (0.51) *+ sn*^{3} / Y males (P < 0.0001). In addition, we did not observe equal frequencies of recombinant male progeny*:* 157/1349 (0.12) *+ +* / Y males and *w sn*^{3} = 83/1349 (0.06) *w sn*^{3} / Y males (P < 0.001).

Table 2. Observed changes in genotypes over time.

Bottle	Generation	Genotypes				D
		+ +	*w sn*^{3}	*w +*	*+ sn*^{3}	
A	1	84	84	6	6	0.22
	2	178	96	30	28	0.147
	3	110	45	37	68	0.036
	4	184	41	31	65	0.054
	5	188	42	51	55	0.045
	6	187	4	39	61	-0.018
	7	224	16	31	28	0.012
B	1	84	84	6	6	0.22
	2	205	122	38	41	0.135
	3	142	61	33	35	0.104
	4	141	25	17	25	0.072
	5	207	35	59	49	0.035
	6	222	14	39	32	0.023
	7	266	20	33	51	0.027
C	1	84	84	6	6	0.22
	2	157	122	43	45	0.128
	3	190	53	25	43	0.093
	4	192	44	24	42	0.082
	5	238	30	40	61	0.093
	6	188	19	33	27	0.038
	7	219	13	46	30	0.013
D	1	84	84	6	6	0.22
	2	189	127	44	42	0.137
	3	85	64	15	37	0.120
	4	142	38	11	29	0.104
	5	175	55	39	29	0.096
	6	195	23	37	31	0.041
	7	190	20	38	29	0.034

Table 3. Observed changes in linkage disequilibrium (D) over time for the sex-linked genes *w* and *sn*^{3}. The theoretical model is based on a recombination (r) value of 0.1922 between the two loci and D_o is 0.22, using equation (9).

Generations	Theoretical	Bottle A	Bottle B	Bottle C	Bottle D
1	0.22	0.22	0.22	0.22	0.22
2	0.191	0.147	0.135	0.128	0.137
3	0.166	0.036	0.104	0.093	0.12
4	0.144	0.054	0.072	0.082	0.104
5	0.125	0.045	0.035	0.093	0.096
6	0.109	-0.018	0.023	0.038	0.041
7	0.095	0.012	0.027	0.013	0.034

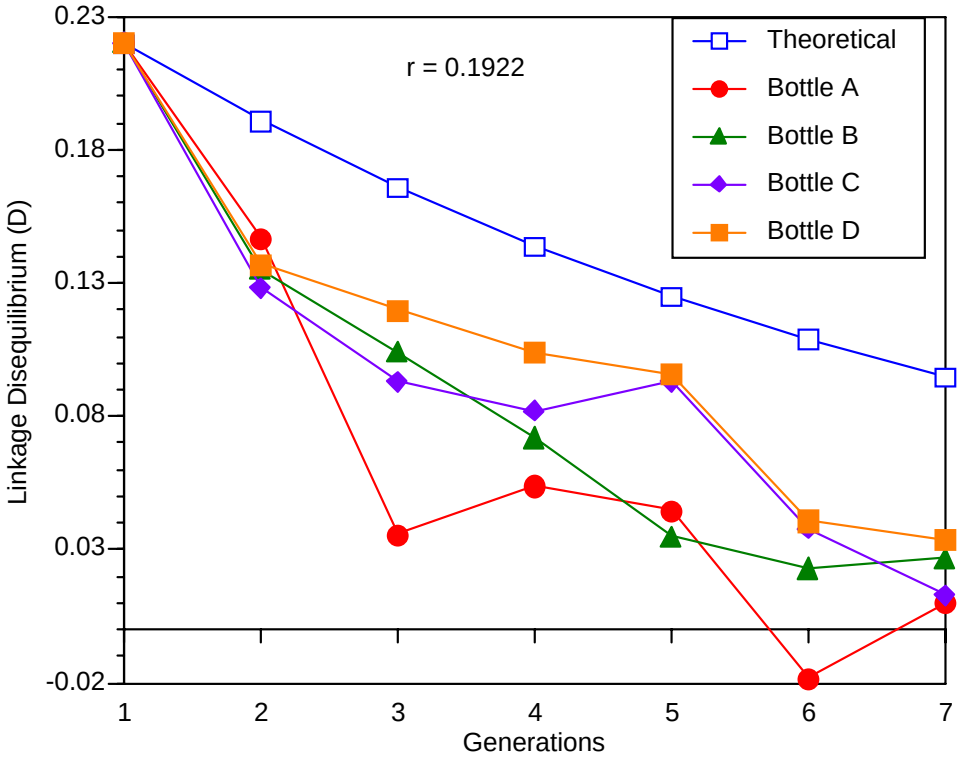

Figure 3. Observed changes in linkage disequilibrium (D) over time for two sex-linked loci (*w* and *sn*^{3}), where r = 0.0.1922 and D_o is 0.22, using equation (9). D values are from Table 3.

Although linkage disequilibrium (D) dropped from 0.22 to low levels by the seventh generation (0.0102, 0.027, 0.013 and 0.034), there were still significant amounts of linkage disequilibrium in each bottle population ($P < 0.001$).

A class discussion of the results of these crosses could include the following topics: 1) Discuss how linkage disequilibrium is used in evolutionary/population genetics and in human genetics. Linkage disequilibrium is used to locate closely-linked mutant genes in humans that cause genetic disorders (Kruglyak, 1999), to determine effective population sizes (Hedrick, 2005), to estimate the age of mutations in humans and other organisms (Rannala and Bertorelle, 2001), and to

locate genes that have evolved by adaptive selection (Wang *et al*., 2006). These topics related to linkage disequilibrium are discussed in Pritchard and Przeworski (2001), Reich *et al*. (2001), Ardlie *et al*. (2002), Nordborg and Tavar (2002), Weiss and Clark (2002), Schlotterer (2003), and Hinds *et al*. (2006).

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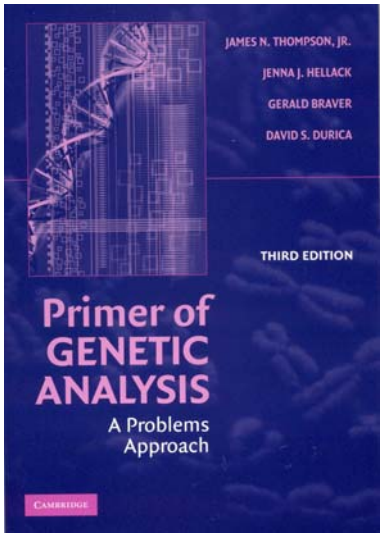

Primer of Genetic Analysis: A Problems Approach, 3rd edition.

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This manual provides students with guided instruction in the analysis and interpretation of genetic principles and practice in problem solving to test their understanding. Each question is accompanied by a detailed explanation. Study Hints and a list of Key Terms introduce topic areas from mitosis and meiosis, to DNA and RNA structure, genetic transmission, probability, pedigree analysis, linkage and mapping in viruses, bacteria, and diploids, mutation, changes in chromosome number and structure, protein synthesis, gene regulation, quantitative genetics, and population genetics. Additional practice problems and a Glossary help students prepare for genetics course examinations or review for tests like the Medical Candidacy Aptitude Test (MCAT), Graduate Record Exam (GRE) subject tests, and other assessments.