Teaching Notes

Deleterious mutations in natural populations of *Drosophila melanogaster.*

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It has been estimated that there are at least three new recessive deleterious mutations in each human (Crow, 2000; Eyre-Walker and Keightley, 1999; Kondrashov, 2001). Since these mutations reduce fitness even in the heterozygous state and can be carried for many generations before they are eliminated by selection, humans and other higher organisms carry an amazingly high load of deleterious alleles (Muller, 1950; Drake *et al.,* 1998; Lynch *et al.,* 1999). For example, Kondrashov (1995) suggests that each human carries about 100 deleterious mutations. Furthermore, in the model system *Drosophila melanogaster* it is estimated that up to one new deleterious mutation occurs per diploid genome (Mukai, 1972; Keightley, 1996; Kondrashov, 1997). Some of these mutations in *Drosophila* are caused by movement of transposable DNA elements, which give rise to new insertion or deletion events (Yodder *et al.,* 1997; Charlesworth and Langley, 1989) and chromosomal rearrangements (Mackay, 1985; Lyttle and Haymer, 1992; Caceres *et al.,* 1999).

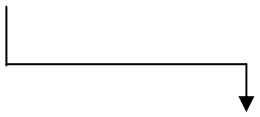
Hence, the mutational load in humans and *Drosophila* may seem surprisingly high based on what seems to be very fit organisms that we see everyday in shopping malls and garbage cans. Can we verify these reports of the high presence and occurrence of deleterious mutations in higher organisms by simple crosses using *D. melanogaster?* The answer is yes and the results show that flies, and probably other higher organisms including humans, are not as fit as they could be if they did not have new detrimental mutations occurring each generation in each of us.

In this report, we describe simple crosses that allow one to identify deleterious mutations that reduce viability, and in some cases kill their host, in a survey of approximately 80% of the genome in flies collected from a natural population of *D. melanogaster*. Deleterious mutations in laboratory lines of *D. melanogaster* have been studied previously using these crosses, where for example 194 (1.94%) new spontaneous recessive lethals were observed in a screen of more than 10,000 second and third chromosomes (Woodruff *et al.,* 1996).

To identify mutations on the second and third chromosomes of *D. melanogaster* from nature, males were captured by sweeping or aspiration of banana baits and were mated to five virgin females that contain translocated chromosomes in vials with standard cornmeal molasses medium according to the following mating scheme:

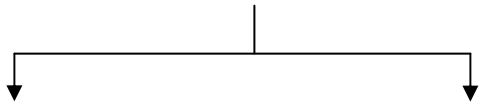
P

T(2;3)Al-W, *Cy L Ubx* / T(2;3)B18, *Pm Sb* × Single males
(Five virgin females) (from nature)



F1

T(2;3)Al-W, *Cy L Ubx* / T(2;3)B18, *Pm Sb* × Single +;+ / T(2;3)Al-W, *Cy L Ubx*
(five virgin females) males (vials are coded to identify parent)



F2

+;+ / T(2;3)Al-W, *Cy L Ubx* × +;+ / T(2;3)Al-W, *Cy L Ubx*
(eight virgin females) (eight males)

F3

Score F3 generation for wild type +/+;+/+ and +;+ / T(2;3)Al-W, *Cy L Ubx*
males and females

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 (Lindsley and Zimm, 1992; http://flybase.bio.indiana.edu). Similarly, T(2; 3)B18 segregates as a unit and carries the dominant markers Plum (*Pm*) eyes and Stubble (*Sb*) bristles. Mated flies were transferred to new food after two or three days and were subcultured a second time to increase the number of offspring from a given mating. In addition, inter-line F3 crosses were set up and F4 progeny were counted as controls, since deleterious mutations in each line should be in different genes. The results from these crosses are shown in Table 1 and Figure 1.

In summary, nine of the twelve (66.7%) tested natural population lines of *Drosophila melanogaster* contained one or more recessive deleterious or lethal mutations, with five lines (#2, 3, 7, 9 and 11) segregating lethal homozygotes (5/12 = 0.42%) and four lines (#1, 5, 6 and 10) showing

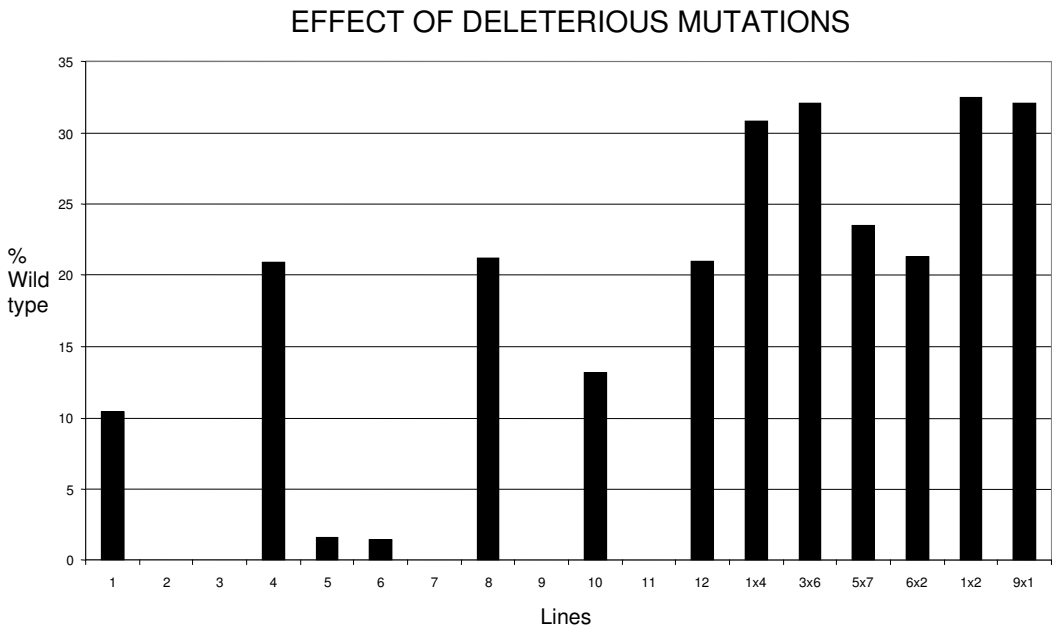


Figure 1. Graph of lines versus percentage wild type.

a considerable reduction in viability. The average percentage wild type of the intercrosses was 28.72%, whereas the average percent wild type of the natural population lines was 7.47%. These results clearly show that natural population lines of *D. melanogaster* carry a significant genetic load from new and pre-existing mutations. In some of the intercross results (5×7 and 6×2), the percentage wild type was below the expected 33.3%, suggesting that some deleterious mutations from nature reduce viability as heterozygotes. The surviving homozygotes could further be examined for reduced fertility and developmental rates and for abnormalities in external morphology caused by deleterious mutations.

Table 1. Results from crosses.

Natural population lines	Total number of flies	% Wild type
1	299	10.37
2	330	0
3	246	0
4	195	20.88
5	191	1.58
6	138	1.45
7	131	0
8	240	21.25
9	214	0
10	129	13.18
11	199	0
12	212	20.95
Intercross lines		
1x4	230	30.87
3x6	249	32.10
5x7	185	23.52
6x2	147	21.28
1x2	310	32.5
9x1	290	32.06

These results, plus those from the previous identification of the rate of second and third chromosome lethal mutations (over 1%; Woodruff *et al.,*1996), clearly show that the model organism *D. melanogaster* has a high frequency of pre-existing and new deleterious mutations. Since humans contain at least twice the number of genes as *D. melanogaster* (Ewing and Green, 2000), the previous estimation of high mutation loads in humans seem reasonable.

A class discussion of the results of these crosses could include the following topics. In modern human societies selection against deleterious mutation has been reduced due to advanced

medical facilities and care, which in turn could possibly increase our mutation load (Crow, 1997). In addition, somatic mutations, which are not scored in the assay of this study, could increase cancer rates and reduce lifespan (Yang *et al.,* 2003; Morley, 1995). At the same time, thousands of new industrial chemicals are introduced into our environment every day, most of them being potent mutagens (for example chemicals from tobacco use and some pesticides), thereby increasing the mutation rate in germinal tissues. These increases in mutations might, in turn, increase the genetic load in future generations.

References: Caceres, M., J.M. Ranz, A. Barbadilla, M. Long, and A. Ruiz 1999, Science 285: 415-418; Charlesworth, B., and C.H. Langley 1989, Annu. Rev. Genet. 23: 251; Crow, J.F., 1997, Proc. Natl. Acad. Sci. 94: 8380-8386; Crow, J.F., 2000, Nat. Rev. Genet 1: 40-47; Drake, J.W., B. Charlesworth, D. Charlesworth, and J.F. Crow 1998 Genetics: 645-663; Eyre-Walker, A., and P.D. Keightley 1999, Nature 397: 344-347; Ewing, B., and P. Green 2000, Nature Genet. 25: 232-234; Keightley, P.D., 1996, Genetics 144: 1993-1999; Kondrashov, A.S., 1995, J. Theor. Biol. 175: 583-594; Kondrashov, A.S., 1997, Annu. Rev. Ecol. Syst. 28: 391-435; Kondrashov, A.S., 2001, Trends Genet. 17: 75-77; Lindsley, D.L, and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster, New York: Academic Press, Inc; Lynch, M., J. Blanchard, D. Houle, T. Kibota, S. Schultz, L. Vassilieva, and J. Willis 1999, Evolution 53: 645-663; Lyttle, T.W., and D.S Haymer 1992, Genetica 86: 113-126; Mackay T.F., 1994, Genetics 111: 351-374; Morley, A.A., 1995, Mutat. Res. 338:19-23; Mukai, T., 1972 Genetics 72: 335-355; Muller, H. J., 1950, Am. J. Hum. Genet. 2: 111-176; Yang Z., R. Simon, and B. Rannala 2003, Genetics 165: 695-705; Yodder, J.A., C.P. Walsh, and T.A. Bestor 1997, Trends Genet. 13: 335-340; Woodruff, R.C., H. Huai, and J.N. Thompson, jr., 1996, Genetica 98: 149-160.

Recombination mapping of P-element transposon inserts: A new set of laboratory exercises for an undergraduate genetics course.

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Despite the large number of P-element transposon insertion lines available to *Drosophila* researchers and the good location data associated with these insertions on the cytogenetic and genome maps (Spradling *et al*., 1999), very few of these inserts have been formally placed on the recombination map (but see Marcus, 2003 for exceptions). FlyBase (2003) does provide a table for the conversion of cytological map position to recombination map position (http://flybase.bio.indiana.edu/maps/lk/cytotable.txt), but the resolution of this conversion table (generally 1 map unit) is rather coarse. Because of the high potential utility for conducting transposon mutagenesis of genes with unknown cytogenetic locations, it would be desirable to accumulate a set of transposon insertions with known locations on both the cytogenetic and recombination maps

This is not a difficult task because many transposon inserts are marked with easily traceable markers (such as w^+). In fact, there are hundreds of transposons that can be traced using the same eye color marker, but which exist in many different locations. The P-element insertion stock collection therefore represents a very useful opportunity to give undergraduate genetics students unique, but equivalent genetic unknowns that can be used for recombination mapping exercises. In some sense, this represents an advance over other types of eye color unknowns (*e.g*., MacIntyre, 1974; Pye, 1980), because it eliminates a frustration that many genetics students express when doing *Drosophila*

genetics laboratory exercises: that they are re-mapping genes that have been mapped before hundreds of times, and that the labor-intensive exercises are a waste of time.

A series of *P[lacW]* transposon insertion lines (in a w^- background) were selected for this exercise that spanned the right arm of the third chromosome, and were mapped against a common mapping strain w^{1118}; h^1 ri^1 e^s. Insertion lines were selected such that the interrupted genes associated with the insertion had been identified, but not genetically mapped. Students created F1 hybrids by crossing males carrying *P[lacW]* with non-virgin females of the mapping strain. Then virgin females F1 hybrids with pigmented eyes were backcrossed to males from the mapping strain and the F2 progeny were scored for eye color, body color, and wing vein phenotypes. The h^1 phenotype was not scored, because it was not easily visible through our student-grade dissecting microscopes, and was also more than 50 map units from most of the transposon inserts making it uninformative for recombination mapping. At Western Kentucky University, faculty proctor most teaching labs themselves, so the instructor was present to assist students in identifying phenotypes, and was able to enforce quality control over student data.

While the crosses were in progress, students used the FlyBase and NCBI databases to learn about their particular interrupted genes in a bioinformatics exercise and during the course of the experiments, students were asked to do three writing assignments related to these exercises: first to summarize what they were able to learn about their insertion from the bioinformatics exercise, second to summarize their own recombination data and calculate the map position of a single *P[lacW]* insertion, and finally to create and describe a map that summarizes all of the recombination data collected by the entire class. This approach to teaching genetics allows students to develop a strong identification with "their gene", introduces them to some of the modern tools of genetics research, and allows students to make small but real contributions to our knowledge of the *Drosophila melanogaster* genome. By and large, the students seemed to be very enthusiastic about these exercises, including several students who were not generally enthusiastic about more typical laboratory and lecture experiences in my genetics course.

There were 26 students in my Fall 2003 undergraduate genetics course, and a total of 27 transposon insertion lines were mapped (one for each student, plus a spare, in case one of the crosses did not work and the instructor needed to provide a student with a replacement). This, coincidentally, represents all of the transposon insertion stocks currently available from chromosome arm 3R that fit the four criteria used to select lines: the stock carries a *P[lacW]* insertion, the insertion is associated with a particular open reading frame of known function, the stock is available from the Bloomington Stock Center, and the insertion has not been mapped by recombination before. All recombination map positions for P-element insertions that were obtained by students were verified in a parallel set of genetic crosses conducted by the instructor. Student data that was clearly faulty was discarded, but the majority of student data closely matched the data gathered by the instructor and the data sets were combined to generate the map positions presented here.

The recombination map positions that were obtained in these experiments are shown in Table 1. Maximum likelihood standard errors for each meiotic recombination map distance were calculated according to Weir (1996). Due to a minor inconsistency in how FlyBase (2003) reported the recombination map position for one insertion line *P[lacW]Pp1-87*^{Bj6E7}, this line was inadvertently included among the student unknowns even though its recombination map position (3-51.1 ± 0.5, Reuter *et al*, 1986) was already established. As is typical of such occurrences, a student realized this before the instructor, but she completed the mapping experiment herself to see how her results compared to those published previously. Reuter *et al.* (1986) mapped their mutation in *Pp1-8* using a locus (*kar*) that was closer than the mapping loci used in our experiments, so their result is probably a better estimate of the actual genetic map position.

Table 1. Map positions of *P[lacW]* transposon insertions on chromosome arm 3R. Underlined entries indicate markers that had already been assigned meiotic map positions at the beginning of this study. The map position in brackets is a predicted map position, based on cytogenetic position. These data, and cytogenetic data for the markers used in this study, were obtained from Flybase (2003). SE is the standard error of each of the recombination frequency calculations, and N is the number of flies scored to calculate the map positions. The student who mapped each mutation is listed in the final column.

Mutation	Cytogenetic Positon	Meiotic Map	SE	N	Student
ri^{1}	077E03	46.8			
P[lacW]Karybeta^{3j3A4}	082D01-02	47.1	0.34	293	Thomas Thacker
P[lacW]ksr^{j5E2}	083A05-06	52.1	1.18	358	Nicole Weathers
P[lacW]noi^{j3E7}	083B01-02	47.0	0.17	575	Erica White
P[lacW]Atus^{1938}	083B04-07	47.6	0.55	254	Amanda Maupin
P[lacW]sec23^{j13C8}	083B06-07	47.9	0.56	352	Shawn Peavie
P[lacW]cas^{j1C2}	083C01-02	58.5	1.36	556	
P[lacW]Dhod^{s3512}	085A05-07	48.0			
P[lacW]neur^{j6B12}	085C09-10	48.5			
P[lacW]pum^{bem}	085C04-D01	48.5			
P[lacW]TfIIFbeta^{j3C1}	086C03-04	59.1	2.10	244	Jaivonna Crook
P[lacW]Vha^{55j2E9}	087C02-03	51.7			
P[lacW]Pp1-87^{Bj6E7}	087C11-13	57.2	2.27	182	Susannah Craig
P[lacW]l(3)87Egs^{2149}	087E10-11	[53]			
P[lacW]sqd^{j6E3}	087F02-03	55.6	1.83	383	Lindsay Gardner
P[lacW]flfl^{L4179}	087F07-08	57.0	1.86	342	Jaime Crocker
P[lacW]B52^{s2249}	087F07-08	54.8	2.15	289	David Arboe
P[lacW]trx^{j14A6}	088B01	54.2			
P[lacW]MRG15^{j6A3}	088E11-12	62.1	2.00	197	James Heltsley
P[lacW]CSN5^{L4032}	089D01-02	61.0	1.33	495	Kelly Bowersox
P[lacW]Dad^{j1E4}	089E10-11	59.0	1.50	460	Chris Carter
P[lacW]Trap80^{s2956}	090F01-02	62.5	1.39	389	Matt King
P[lacW]nos^{j3B6}	091F07	66.2			
P[lacW]bon^{S048706}	092E	70.7	0	140	Tommy Crockett
P[lacW]Rab11^{j2D}	093C01-02	61.3	1.70	297	Megan Jackson
e^{s}	093D01	70.7			
P[lacW]mod(mdg4)^{L3101}	093D09-10	70.7			
P[lacW]how^{E7-3-4}	093F13	77.7	1.64	242	Joey Oliver
P[lacW]Dph5^{L4910}	094B04-05	80.5	1.39	457	Margaret Au
P[lacW]CycB3^{L6540}	096B03-05	84.3	1.78	369	Christina Archey
P[lacW]OstStt^{3j2D9}	096B19-20	81.7	1.87	281	Alecea Davis
P[lacW]scrib^{j7B3}	097B08-09	81.1	1.38	490	Jennifer Dennison
P[lacW]Takr99D^{s2222}	099D01-02	97.8	3.07	210	Cheri Watson
P[lacW]hdc^{Fus-6}	099F	98.6	1.98	513	Janie Baxter
P[lacW]dco^{j3B9}	100B02-04	88.2	1.82	435	Kim Phillips
P[lacW]S057302	100C	100.5	2.59	312	Priscilla Hamilton
P[lacW]awd^{j2A4}	100E01-02	98.0	2.18	417	Kate Hertweck

In most cases, the 95% confidence intervals for the meiotic map positions of each locus calculated from the class data (calculated after Snedecor and Cochrane, 1989; data not shown) overlapped with the map location predicted by FlyBase (2003). However, in other cases, our calculated map position differs significantly from what was expected. The observed disparities may be due to viability differences between F2 genotypes, the rather large distances between some of the transposon inserts and our mapping markers (which will tend to systematically underestimate recombination map distance (Haldane, 1919)), or to sampling errors caused by the small samples sizes of F2 progeny for some of the transposon insertion lines.

Overall, the data presented here are consistent with the meiotic recombination map positions predicted by FlyBase (2003) and confirm that students remain capable of making contributions to the

field of *Drosophila* genetics as a part of their undergraduate coursework (Wright, 1932). Future student laboratory exercises will extend these investigations to other chromosome arms.

References: FlyBase, 2003, *The* Drosophila *genetic database* (http://flybase.bio.indiana.edu); Haldane, J.B.S., 1919, J. Genet. 8: 299-309; MacIntyre, R., 1974, Dros. Inf. Serv. 51: 158; Marcus, J.M., 2003, Genetics 163: 591-597; Pye, Q., 1980, Dros. Inf. Serv. 55: 171; Reuter, G., R. Dorn, G. Wustmann, B. Friede, and G. Rauh 1986, Mol. Gen. Genet. 202: 481-487; Snedecor, G.W., and W.G. Cochran 1989, *Statistical Methods*, Iowa State University Press, Ames, Iowa, Eight Edition; Spradling, A.C., D. Stern, A. Beaton, E.J. Rhem, T. Laverty, N. Mozden, S. Misra, and G.M. Rubin 1999, Genetics 153: 135-177; Weir, B.S., 1996, *Genetic Data Analysis II: Methods for Discrete Population Genetic Data*, Sinauer, Sunderland, Mass., Second Edition; Wright, S., 1932, Am. Nat. 66: 282-283.

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