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Lethal mutations and their elimination by selection in natural populations of *Drosophila melanogaster*.

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Lethal mutations are surprisingly frequent in all organisms. For example, up to 70% of *Drosophila melanogaster* in nature carry at least one recessive lethal mutation (Crow, 1993a,b; Lynch *et al*., 1999; Azad *et al*., 2003), and new lethal mutations arise in about six percent of these flies (Simmons and Crow, 1977; Woodruff *et al*., 1983, 1984, 1996; Fu and Huai, 2003; Gao *et al*., 2011). In humans, recessive mutations that can cause death in homozygotes or hemizygotes (X-linked in males) before reproductive maturity are numerous (Morton, 1981; Strachan and Read, 2004; also see Online Mendelian Inheritance in Man (OMIM) at the National Center for Biotechnology Information at http://www.ncbi.nim.hih.gov.omim). Examples of such mutations in humans include Duchenne muscular dystrophy, Lesch-Nyhan syndrome, congenital erythropoietic porphyria, and cystic fibrosis (Morton, 1981; Cummings, 2009).

In addition, most lethal mutations are not completely recessive (Muller, 1950; Simmons and Crow, 1977; Crow and Simmons, 1983; Crow, 1993a,b; Garcia-Dorado and Caballero, 2000). Organisms that are heterozygous for a recessive lethal mutation (*Ll,* with *l* being the lethal mutant allele) have a fitness that is lower than those with homozygous dominant alleles (*LL*). This can be modeled as follows where *L* is the wild- type allele, *l* is the recessive deleterious mutant allele, *s* is the selection coefficient (with s = 1 for lethals), and *h* is the dominance coefficient.

	For autosomal mutations			For X-linked mutations		
	LL	*Ll*	*ll*	*LL female*	*Ll female*	*lY male*
Fitness =	1	1-*hs*	1- *s*	1	1-*hs*	1-*s*
Fitness =	1	1-*h*	0	1	1-*h*	0

A completely recessive mutant allele would have $h = 0$ in heterozygotes, making the fitness of the heterozygotes the same as the *LL* homozygotes. Most lethal mutations, however, have *h* values greater than zero, *i.e*., they are not completely recessive and the heterozygotes (*Ll*) have a fitness that

is less than the *LL* homozygotes. In addition, lethals that occur on X chromosomes are eliminated almost entirely in hemizygous males (one X and one Y chromosome), whereas, almost all autosomal lethal mutations, which are rare, are eliminated in heterozygotes.

What do we know of the values of *h* for lethal mutations in higher animals? For lethal mutations in *D. melanogaster*, the *hs* value on average is about 0.025; hence, *h* is also about 0.025. This makes the fitness of *Ll* flies about 98% of *LL* flies (Crow, 1993a,b). In addition, the genomic (X, second, and third chromosomes) lethal mutation rate per gamete is about 0.016 (Woodruff *et al*., 1983, 1984, 1996; Fu and Huai, 2003; Hedrick, 2011; Gao *et al*., 2011). This rate comes from the lethal rate for X chromosomes of about 0.001 and the autosomal mutation rate of about 0.015 for the second and third chromosomes. Hence, about one fly in 15 will have a new genomic lethal mutation (a mutation can occur either in the female or male parent) and a typical recessive autosomal lethal will persist for about 40 generations ($1/hs = 1/0.025 = 40$) (Crow, 1993a,b). In addition, since it has been estimated that there are about 1,200 genes that can mutate to lethality in *D. melanogaster* (Abrahamson *et al*., 1980; Gao *et al*., 2011), the per gene mutation rate for lethals (*u*) in *D. melanogaster* is about 1.3×10^{-5} ($0.016/1200 = 0.000013$).

Estimations of dominance for autosomal lethals are especially important because these mutations are usually low in frequency and are, therefore, almost always in the heterozygous state. Hence, their mutation/selection equilibrium frequencies are dependent almost entirely on their dominance (Muller, 1950; Crow 1993a,b). For autosomal recessive lethals ($h = 0$), the expected mutation/selection equilibrium value for the frequency of the recessive deleterious allele *l* is $(u/s)^{1/2}$, or $(u)^{1/2}$ for lethals, whereas for mutations with dominance ($h > 0$) the equilibrium value for *l* is $u/(hs)$, or u/h for lethals (Hedrick, 2011). For example, using the *s* and *h* values given above for *D. melanogaster* lethals (1 and 0.025), and *u* of 0.000013 per gene, the mutation/selection equilibrium frequency for lethals with dominance ($u/hs = 0.000013/0.025 = 0.00005$) would be about eight times lower than that for completely recessive ($h = 0$) deleterious mutations [$(us)^{1/2} = (0.000013)^{1/2} = 0.004$]. The expected frequency of X-linked lethals in nature would be much lower.

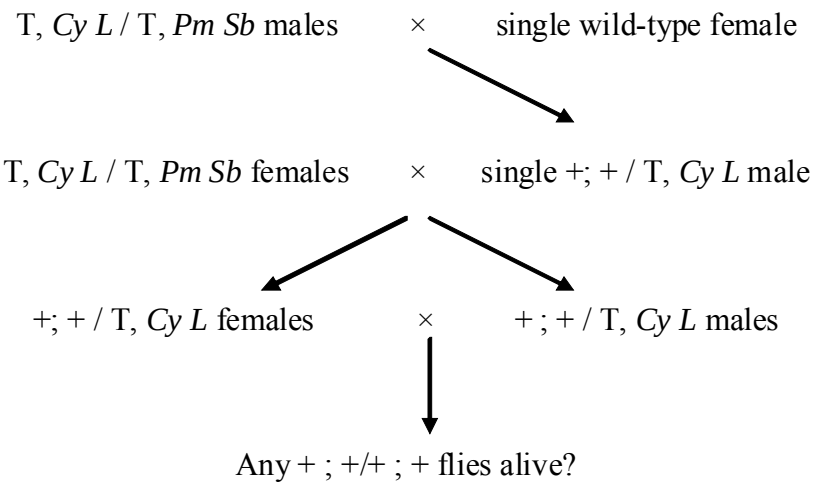
For X-linked lethals, where selection will occur in hemizygous males, the equilibrium frequency for *l* is $3u/s$ (Crow and Kimura, 1970) and will be about 0.00004 (3*0.00013/1). Hence, the expected equilibrium frequency of recessive X-linked lethals in nature will be about 13 times lower than autosomal lethals with dominance (0.0005/0.00004) and 100 times lower than completely recessive autosomal lethals (0.004/0.00004). Because of hemizygous selection for X-linked lethals in males *vs*. selection in heterozygotes for autosomal lethals, one would expect, therefore, a much lower frequency of X-linked lethals in nature compared to autosomal lethals. Is this true?

In this study we attempted to determine if the frequency of X-linked lethal mutations is significantly lower than the frequency of autosomal lethal mutations in natural populations of *D. melanogaster*, as predicted by population genetic theory. Female *D. melanogaster* were captured by sweeping bananas in Perrysburg, Ohio on November 5, 2009 and July 30, 2010. From these flies, isofemale lines were set up from single flies, and, after one generation, single virgin females from each line were tested for preexisting second and third recessive lethal mutations and single virgin sibling females were tested for preexisting X-linked lethal mutations by the following two sets of crosses.

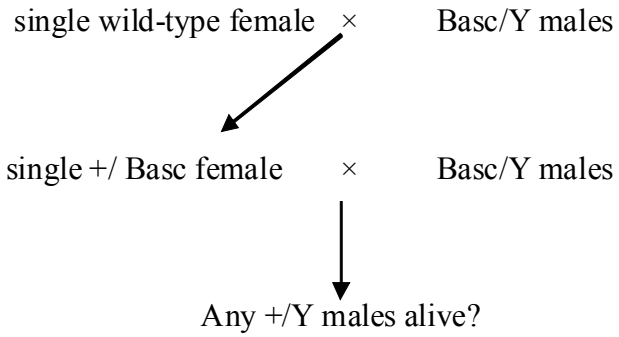
In these crosses, + = a wild-type X, second, or third chromosome from nature; T(2;3)A1-W, *Cy L* is a translocation for the second and third chromosomes that contains the dominant visible markers *Cy* (curly wings) and *L* (lobed eyes) (*Cy* and *L* are also recessive lethals), plus multiple inversions that make it a balancer for the second and third chromosomes; T(2;3)B18, *Pm Sb* is a translocation for the second and third chromosome that contains the dominant visible markers *Pm* (plum eyes) and *Sb* (stubble bristles) (*Pm* and *Sb* are also recessive lethals); Basc is a balancer for the X chromosome that contains the dominant marker *B* (bar eyes), the recessive white-apricot mutation

(with the symbol *a*, for w^a, which causes white eyes), and multiple inversions associated with the *sc* (scute) mutation. See Lindsley and Zimm (1992) for descriptions of these mutants, chromosomal rearrangements, and balancer chromosomes. Since T(2;3)A1-W, *Cy L* and Basc are balancer chromosomes, recombination events in heterozygotes will be eliminated (see a discussion of this topic in Klug *et al*. 2010). This keeps new lethal mutations from being moved from the wild-type chromosomes by recombination. In these crosses, the T(2;3) chromosomes will be given the symbol T and all females are virgins.

For the identification of preexisting second or third chromosome lethal mutations:



For the identification of preexisting X-linked lethal mutations:



The absence of +;+/+;+ (wild-type, non-*Cy* and non-*Sb*) flies or the absence of +/Y (wild-type, red eyed) males in the final generations indicate the presence of a preexisting recessive lethal mutation on the autosomes or X-chromosome, respectively. Additional crosses to confirm lethals were made by mating +; +/T, *Cy L* females and males or by mating +/ Basc females with Basc/Y males. A presumptive lethal was declared a lethal if no wild-type (+;+/+;+) flies or no wild-type (+/Y) males were observed among at least 100 progeny.

The results of the screens for autosomal and X-linked lethals are shown in Table 1.

As predicted by population genetic theory, in nature there was a significantly higher frequency (30%) of autosomal lethals as compared to X-linked lethals (0%). Part of the reason for the high frequency of autosomal lethals in nature may be due to some lethals within a collection being the same mutation (are allelic). For example, the mutations could be in siblings or the original

mutation could have arisen as a premeiotic cluster (see Woodruff *et al*., 1996, for a discussion of this topic). Hence, by interline crosses we also tested the allelism of the five lethals from the 11/05/2009 collection and the five lethals from the 7/20/2010 collection. All of the lethals in each collection were unique (non-allelic). Hence, all of the autosomal lethals were probably of independent mutational origin. Again, because of selection against X-linked lethals in hemizygous males and autosomal lethals in heterozygotes, the results of this study clearly show that X-linked lethals are in a lower frequency than autosomal lethals in nature.

Table 1. Frequencies of autosomal (2nd and 3rd) and X-linked recessive lethal mutations in a natural population (Perrysburg, Ohio) of *Drosophila melanogaster*.

Collections	Autosomal Lethals/ Total	X-Linked Lethals/Total	P
11/05/2009	5/17	0/29	0.011
7/20/2010	5/13	0/21	0.015
Total	10/30	0/50	0.0001

A classroom discussion of the results of this teaching exercise could include the following questions. 1) If the expected equilibrium frequencies of autosomal and X-linked lethal mutations were similar in *D. melanogaster* and humans, what would be the expected frequency of humans that are homozygous for an autosomal lethal mutation that t kills in young children? For autosomal lethals, each parent would have to carry the recessive mutation and then there would be a one-fourth chance that their offspring would be homozygous for the recessive lethal mutations. Hence, for a completely recessive autosomal lethal: $0.004 \times 0.004 \times 0.25 = 0.000004$ or one in 250,000 humans would be homozygous. For an autosomal lethal mutation with a dominance of 0.025 ($h = 0.025$), $0.00005 \times 0.00005 \times 0.25 = 0.0000000006$ or one in 1,600,000,000 humans would be homozygous. 2) What is the expected frequency of human males that are hemizygous for a recessive lethal mutation that t kills in young children? For a preexisting sex-linked lethal to appear in a male, there would need to be a female parent that is heterozygous for the lethal, and then she would have a one-half chance of having a son with the recessive lethal. Hence, $0.00004 \times ½ = 0.00002$ or one in 50,000 human males. 3) This teaching exercise has only considered lethal mutations. How many deleterious mutations, that are not lethal, occur in each human? The answer is that there may be as many as ten new deleterious in each human (Reed and Aquadro, 2006). 4) James Crow (Crow, 1999) has stated that we now live in an environment with improved living conditions that reduces selection against deleterious mutations. What impact would this have on future humans? Would the frequency of humans with homozygous lethal and deleterious mutations increase?

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The role of sexual reproduction and recombination in adaptive evolution.

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The evolution and maintenance of sexual reproduction that leads to recombination of chromosomes at meiosis is a evolutionary puzzle. Why should a fit organism give up half of its genes and experience possible harm (for example, sexually transmitted diseases and increased risk of predation) when it could reproduce asexually? With asexual reproduction all of the genes of an individual are passed to its offspring, and there is no harm that occurs in the search for a mate (Muller, 1964; Maynard Smith, 1978; Ridley, 1993; Michod, 1995; Barton and Charlesworth, 1998).

There are two main hypotheses for the evolution and maintenance of sexual reproduction: 1) Sexual reproduction brings together favourable alleles of different genes by recombination, increasesing the fitness of offspring and the rate of adaptation to new environments; 2) Sexual reproduction can bring together deleterious alleles of different genes by recombination. These deleterious alleles can then be eliminated from the population in bunches by negative selection more quickly than can a combination of deleterious alleles that are removed one at a time in the absence of recombination (for reviews of this topic see Crow and Kimura, 1965; Barton and Charlesworth, 1986; Kondrashov, 1988; Otto and Lenormand, 2002; Rice, 2002; Gillespie, 2004).

The objective of this proposed study is to test the first hypothesis listed above (combining favourable genes by recombination) by measuring the rates of selection response in the presence and absence of recombination in the model system *Drosophila melanogaster*. Rice and Chippindale (2001) have shown that beneficial alleles that increase offspring numbers in *D. melanogaster* accumulate faster in populations with recombination than in populations without recombination.

We took advantage of the natural lack of recombination in *D. melanogaster* males and the aviability of balancer chromosomes with multiple inversions that eliminate recombinant gametes in *D. melanogaster* females. With the appropriate crosses, as shown below, we tested a model for adaptive evolution, selection response for bristle numbers, in the presence and in the absence of recombination. As part of this model, it was assumed that flies with decreased or increased bristle numbers are more fit. This proposed study is partially based on the materials, methods, and results in