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The identification of hidden genetic variation (recessive visible mutations) in a natural population of *Drosophila melanogaster*.

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"It is clear that descriptions of the genetic variation in populations are the fundamental observations on which evolutionary genetics depends." (Lewontin, 1974).

Dobzhansky in 1955 compared two hypotheses for the genetic structure of natural populations: the classical school, which predicted that most genes are homozygous for wild-type alleles, because most mutations are deleterious and are selected against, and the balanced school, which predicted that there are numerous heterozygotes for many genes, mainly due to overdominance (heterozygotes are more fit than homozygotes) (see discussion in Ford, 1964; Lewontin, 1974). It is now known from protein electrophoresis and DNA analyses that there is an immense amount of genetic variation in most species and populations, but most of this variation is probably not maintained by overdominance (see Hedrick, 2005, for a discussion of this topic).

Before the advent of protein electrophoresis and DNA analyses, which allowed for the differentiation of heterozygotes and homozygotes, how did Dobzhansky and others know that there was hidden genetic variation in natural populations, hidden because this variation was due to recessive mutations that were not expressed in heterozygotes? Dobzhansky (1955) pointed out that when one looks at *Drosophila* from nature, few, if any, visible mutations are observed: "…natural populations of *Drosophila* show scant variability in externally visible traits" (Dobzhansky, 1955). He then explains how this hidden genetic variation was first observed.

> "The pioneer work of Chetverikov, Timofeeff-Ressovsky, and Dubinin and his collaborators during the late twenties and the early thirties demonstrated that the paucity of overt phenotypic variability does not mean genotypic uniformity. When the *Drosophila* flies collected in nature are inbred in laboratory cultures, a fair proportion of them prove to be heterozygous for recessive mutants affecting the visible external morphology of the fly. Many of the classical mutants which grace the pages of genetics manuals were thus shown to exist concealed in natural populations." (Dobzhansky, 1955; see a detailed discussion of these studies in Dobzhansky, 1937; Spencer, 1947; Lewontin, 1974).

Others, including scientists from the USA, also identified recessive visible mutations in wild *Drosophila* (see Mickey, 1954; Spencer, 1947, 1957).

The objective of this study is to attempt to identify hidden, recessive visible mutations in a natural population of *D. melanogaster*. Are these mutations in nature now as they were in the earlier experiments of the Russian and American scientists? The answer is yes.

We collected *D. melanogaster* by sweeping bananas in Perrysburg (Wood County), Ohio on October 10, 2008. Eighty-two presumably mated females were placed the next day singly in vials of

Drosophila food, and 65 (79%) of the females gave progeny. Three of these females were *D. simulans*, a sibling species of *D. melanogaster*, that can be identified by differences in the structure of the male genitalia (Ashburner, 1989).

No visible phenotypic changes were observed in 1,436 F1 male progeny from these wild *D. melanogaster* females. This was expected, since any recessive X-linked visible mutations that are deleterious would be quickly selected against in nature in hemizygous males. Hence, none of the 62 fertile, wild females were heterozygous for recessive sex-linked visible mutations.

In an attempt to make homozygous any autosomal recessive visible mutations that were heterozygous in the original wild females, or their mates, four vials of single F1 females were mated with single F1 sibling males from 40 of the 62 wild lines, for a total of 160 F1 sibling crosses. One hundred and fifty nine of the 160 crosses produced F2 progeny. In some of these F2 progeny, recessive visible mutations, if present, should become homozygous and be expressed because of inbreeding. We screened at least 20 F2 progeny from each F1 cross for altered adult morphologies. Seventeen of the 159 F1 crosses had one or more F2 flies with altered phenotypes, suggesting uncovered recessive visible mutations. We next determined if these 17 presumptive visible mutations bred true by mating F2 sibling males and females and scoring F3 progeny for visible mutant phenotypes.

Of the 17 possible visible mutations, 10 bred true in subsequent F3 progeny. These 10 mutations were present in nine of the parental, wild females from nature; *i.e.*, 11% (9/80) of the fertile parental females from nature, or their mates, carried one or two hidden recessive visible mutations as autosomal heterozygotes. This is probably an underestimation of the true frequency of recessive visible mutations in the Perrysburg, Ohio, population, because not all mutations in the wild flies will be made homozygous by the mating scheme of this study. Using four F1 sibling matings for each parental line in this study allows for only about 68% of visible mutations in nature as heterozygotes to become homozygous and expressed in F2 flies (Spencer, 1947).

Descriptions of the visible mutants recovered in this study are given in Table 1, and photographs of three of these mutants (dark eyes, outstretched wings, and halteres that developed wing tissues) are shown in Figures 1, 2 and 3.

Table 1. Descriptions of recovered visible mutations from a natural population of *D. melanogaster*.

F1 Cross	Visible Mutant Recovered in F2 and Confirmed in F3 flies
A4*	Dark trident-like structure on thorax
C2	Dark trident-like structure on thorax
C3	Dark trident-like structure on thorax
I1	Black thorax
Q2	Wings out (see Figure 1)
5D	Wings flattened at ends
8D	Wings out
13D	Black eyes (see Figure 2)
16B	Bright red eyes
21B	Halters as small wings (see Figure 3)

*A4 = the F1 vial four from parental, wild-type female A.

The frequency of visible mutations per tested wild female and their mates is too high (10 mutations in 40 lines) to be due to new mutations. Visible mutations occur at a rate of about 1 to 3 per 100,000 gametes in *D. melanogaster* (Woodruff, Slatko, and Thompson, 1983). The identified visible mutants are more likely due to preexisting recessive mutations carried as heterozygotes. Evidence in support of this hypothesis comes from a separate study that is currently in progress in our laboratory to measure the influence of new deleterious mutations on viability. In this study, two females and two of their brothers were mated each generation for 16 generations from an original yellow-body stock that had been maintained by single brother-sister crosses for 159 generations. Hence, this inbred stock was homozygous for all, or almost all, genes. Any recessive visible mutants would, therefore, have to arise by new genetic changes, because the original inbred stock was homozygous and did not carry any visible mutations as heterozygotes. We observed no new visible mutations among 14,257

progeny from these inbred yellow flies. This highlights the rare rate of new visible mutations in *D. melanogaster* and gives support to the hypothesis that the 10 visible mutants observed in this study were due to preexisting genetic variation in the 40 Perrysburg, Ohio, natural population lines. Up to 10%, or more, of previous collections of *D. melanogaster* in Russia and the USA carried recessive visible mutations as heterozygotes (Dobzhansky, 1937; Spencer, 1947; Lewontin, 1974). After reviewing a number of published studies, Lewontin (1974) concluded that every female *D. melanogaster* in nature has on average one recessive visible mutation carried as a heterozygote.

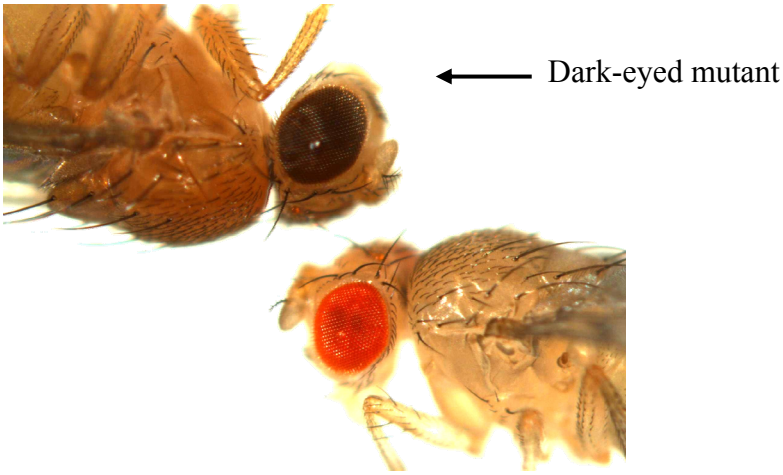

Figure 1. Recessive autosomal dark-eyed mutant from nature compared to a fly with wild-type eyes.

Figure 2. A recessive autosomal outstretched-wing mutant from nature.

Figure 3. Two flies with a recessive autosomal visible mutation causing halteres to develop into wing-like tissue (a homeotic mutant).

The seven possible visible mutants that did not breed true (folded wings, dark thorax, wings curved up, three with wings held out, and altered eye color) were probably caused by interactions of multiple genes that were uncoupled in F2 flies, environmental influences (not likely, since the environment was similar in all crosses), or developmental differences that were not inherited.

A class discussion of the results of this study could include the following topics:

1) Do humans also carry recessive visible mutations that are hidden as heterozygotes? The answer is yes, because recessive visible mutations are expressed in homozygotes in a higher frequency in human pedigrees that include matings between closely related individuals (Hedrick, 2005).

2) Do humans also carry other recessive deleterious mutations as heterozygotes? It has been estimated that each human carries a number of such deleterious mutations. For example, the proportion of infant deaths in the progeny of first-cousin matings is higher than in progeny of unrelated humans, due to inbreeding and homozygosis of recessive mutations (Hedrick, 2005).

3) How are recessive deleterious mutations, such as those identified in this study, maintained in natural populations? If they are deleterious, one might expect them to be eliminated over time. One possible answer is that the deleterious effects of these mutations are hidden when they are in heterozygotes, *i.e.*, the mutations are completely recessive. If *A* is the wild-type allele and *a* is the mutant allele, a population genetics model of a recessive deleterious mutation with no dominance is (*s* is the selection coefficient against the *aa* homozygotes, and the *AA* and *Aa* individuals have on average the largest number of offspring):

	AA	*Aa*	*aa*
Fitness =	1	1	1-*s*

Even if the deleterious mutations do reduce the fitness of heterozygotes a small amount, *i.e.*, the deleterious mutations have some dominance, there will be a balance between mutations that produce new deleterious alleles and selection that removes deleterious alleles. This can be shown in the following model (*h* is the dominance coefficient; if *h* is greater than zero but less than one, the heterozygotes will be less fit than the *AA* homozygotes, but more fit than the *aa* homozygotes):

	AA	*Aa*	*aa*
Fitness =	1	1-*hs*	1-*s*

At equilibrium the recessive mutation allele (*a*) will have a frequency ($\hat{q}$) equal to (Hedrick, 2005):

$$\hat{q} = \frac{u}{hs}$$

It has been estimated that in *D. melanogaster*, *u* per gene is about 1×10^{-5}, and for slightly deleterious mutations *h* is about 0.33 and *s* is about 0.03 (Simmons and Crow, 1977), giving:

$$\hat{q} = \frac{0.00001}{(0.33)(0.03)}$$
$$\hat{q} = 0.001$$

Hence, if the sampled Perrysburg, Ohio population were at Hardy/Weinberg equilibrium, the expected frequencies of flies with the *A* and *a* alleles would be:

$$AA = p^2 = (0.999)^2 = 0.99800$$
$$Aa = 2pq = 2(0.999)(0.001) = 0.00199$$
$$aa = q^2 = (0.001)^2 = 0.000001$$

Only about one fly in 1,000,000 would, therefore, be expected to express the recessive visible mutation (*aa*). On the other hand, about one fly in 500 would be expected to be heterozygous (*Aa*). In this study we observed that about one to three flies in 80 carried a specific recessive visible mutation as a heterozygote. The reason(s) for this difference in frequencies of heterozygotes is unknown, although changes in *u* or *hs* could bring the two values closer.

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The *BLOG*: A new electronic resource for teaching in the XXIST century.

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Abstract

This paper describes the potential of a *blog* as a tool for curriculum innovation in the context of university attendance. It describes an experimental implementation of this service on the subject of Genetics in the career of Biology at the Faculty of Sciences, UNAM. The results indicate the contribution of this tool to improve the quality of teaching-learning process.

Introduction

Students all over the world today have access to the world wide web and thus many things are competing for their attention. As our students' world changes, so too must the methods by which we teach and engage our students (Lara, 2005; García-Manzano, 2006). In this article, we propose one way that teaching can be significantly enhanced by using one of these web services, the blogs.

Blogs, a contraction of the words *web* and *log*, is a type of website usually maintained by an individual with regular entries of commentaries or news of a particular subject based on the idea of a newspaper that collects texts and files in chronological order. They are regularly updated by its author or authors. Blogs provide the functionality that enables people to publish their thoughts and ideas easily online. One of the primary reasons for the rapid adoption of blogs is that they are quick to setup and easy to use, requiring absolutely no programming knowledge or HTML skills (Gallego-Torres, 2006).