



Rare male mating advantage in *Drosophila melanogaster*.

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Introduction

An understanding of how genetic variation is maintained in populations, including humans, is important because organisms cannot evolve to meet changes in the environment without genetic variation or new beneficial mutations. For example, organisms with low amounts of genetic variation will be less likely to evolve to maintain defenses against new parasites and microbial infections. This is especially important in endangered animals and plants, where the population size is low (Frankham *et al.*, 2002), and in humans where infections with new antibiotic resistant bacterial strains have become more common (Maree *et al.*, 2007).

One mechanism to maintain genetic variation in populations is frequency dependent selection (Hedrick, 2005; Hamilton, 2009). As the frequencies of alleles at a gene change in nature, there is selection for low frequency alleles. For example, if a gene has alleles A^1 and A^2 , the A^1 allele or the A^2 allele (or both alleles) would be selected for when it is in a low frequency. This keeps both alleles in the population and helps to maintain standing genetic variation. For example, rare flower color alleles in orchids are selected by insect pollinators (Gigord *et al.*, 2001).

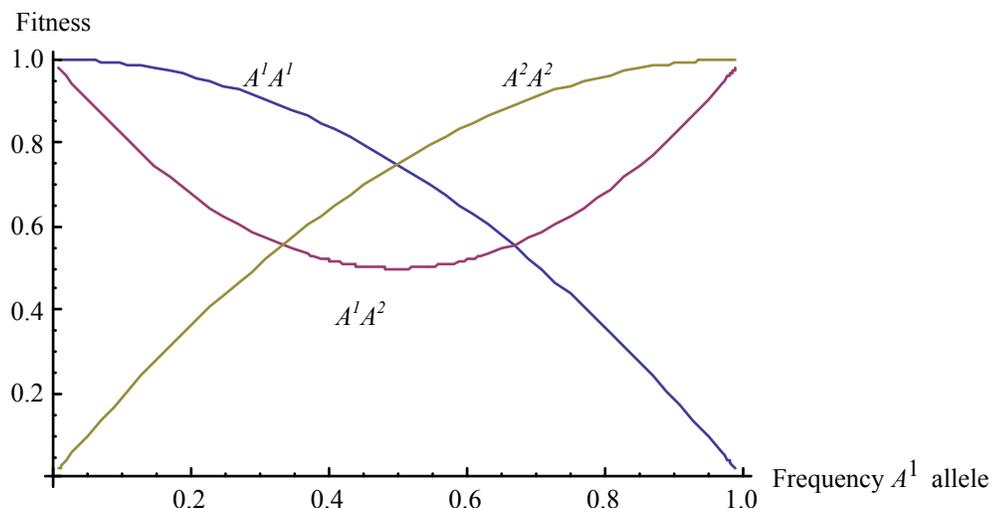
Frequency dependent selection can be modeled for a gene with two alleles as follows (see Hamilton, 2009). Let p be the frequency of the A^1 allele and q be the frequency of the A^2 allele. At Hardy/Weinberg equilibrium the frequency of $A^1A^1 = p^2$, the frequency of $A^1A^2 = 2pq$, and the frequency of $A^2A^2 = q^2$. Also let selection against $A^1A^1 = s_{11}$, selection against $A^1A^2 = s_{12}$, and the selection against $A^2A^2 = s_{22}$.

$$\begin{array}{l} \text{Fitness of } A^1A^1 \\ 1 - s_{11}p^2 \end{array}$$

$$\begin{array}{l} \text{Fitness of } A^1A^2 \\ 1 - s_{12}2pq \end{array}$$

$$\begin{array}{l} \text{Fitness of } A^2A^2 \\ 1 - s_{22}q^2 \end{array}$$

For example, as the frequency of A^1A^1 goes down, their fitness goes up, as shown below.



A mechanism for frequency dependent selection is the rare male mating advantage (Powell, 1999; Hedrick, 2005). If rare males also carry rare alleles, their increased mating ability, above the expected based on their frequency, will help to maintain a balance where more than one allele will be present in populations.

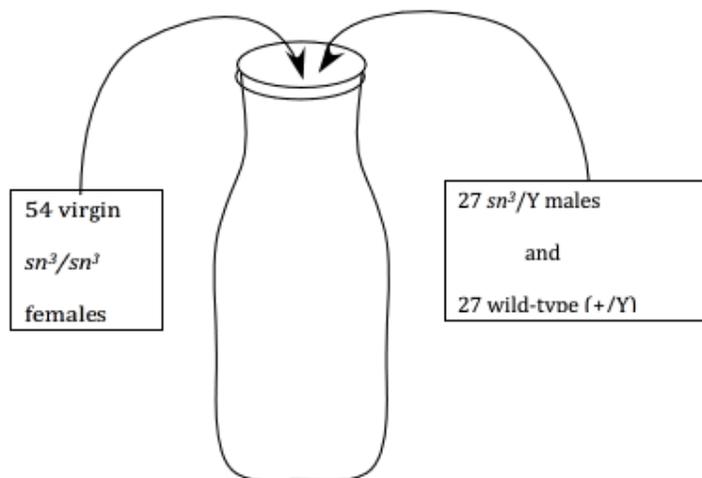
Rare male mating advantage has been reported in over 10 species of *Drosophila* and in a few vertebrates (reviewed in Spiess and Kruckeberg, 1980; Powell, 1999; Som and Singh, 2005). Yet, others have not observed such an effect (Markow *et al.*, 1980), and some have concluded that rare male mating advantage has not been shown to be an important mechanism for the maintenance of genetic variation (Bryant *et al.*, 1980).

It is the objective of this teaching exercise to measure rare male mating advantage in the model organism *Drosophila melanogaster* using easily recognizable visible genetic markers.

Control Crosses

As a control, we will first determine if two phenotypically distinct types of males (sn^3 males with short bristles and wild-type, Canton-S, males with long bristles) have similar mating abilities when they are at the same frequency. If their mating abilities are not equal, this will be taken into consideration when analyzing subsequent matings where each male is placed in a rare frequency.

In the following control crosses in half-pint bottles, note that the Y chromosome does not contain a gene pairing partner for the X-linked *singed* gene and that the sn^3 allele is a recessive mutation. In addition, sn^3/sn^3 females and sn^3/Y males have very short bristles, whereas $sn^3/+$ females and $+/Y$ (wild-type) males have long bristles.



After two or three days, each female was placed individually, without males, into separate vials. The parental females were then cleared from each vial after seven days, and the F1 progeny of each female were scored as follows to determine if they:

1. Mated with sn^3/Y males:
Would have sn^3/sn^3 (short bristles) female offspring and sn^3/Y (short bristle) male offspring.

2. Mated with wild-type (+/Y) males:
Would have wild-type ($sn^3/+$; long bristles) female offspring and sn^3/Y (short bristles) male offspring.
3. Mated with both sn^3/Y and wild-type (+/Y) males:
Would have a mixture of sn^3 and wild type females and sn^3/Y male progeny.

The ratio of sn^3 matings to wild-type matings in these control crosses gives an estimation of the relative mating ability of the sn^3 and wild-type parental males. If they have equal mating abilities, then $\frac{1}{2}$ of the 54 females in a bottle will mate with sn^3 males and $\frac{1}{2}$ with wild-type males. Double matings will be recorded, but not included in the final data analysis.

Experimental Crosses

A. 45 sn^3 / 9 + Crosses:

In each bottle we mated:

54 sn^3/sn^3 virgin females × 45 sn^3/Y males and 9 +/Y (wild-type) males

As in the control crosses, after 2-3 days single females were placed, without males, into separate vials.



If the previously observed relative mating ability of sn^3 males and wild-type males in the control crosses is the same, it is expected that 5 in 6 crosses will be with sn^3 males and 1 in 6 will be with wild-type males. A rare-male mating effect will give a significantly higher mating frequency for wild-type males than 17% (1 in 6) of the total matings.

B. 9 sn^3 / 45 + Crosses:

In each bottle we mated:

54 sn^3/sn^3 virgin females × 9 sn^3/Y males and 45 +/Y (wild-type) males

As in the control crosses, after 2-3 days single females were placed, without males, into separate vials.



If the previously observed relative mating ability of sn^3 males and wild-type males in the control crosses is the same, it is expected that 1 in 6 crosses will be with sn^3 males and 5 in 6 will be with wild-type males. A rare-male mating effect will give a significantly higher mating frequency for sn^3 males than 17% of the total matings.

Data Analysis

The frequencies of matings were analyzed using the chi-square test by comparing the expected frequencies of mating based on the control crosses with the observed frequencies in the two rare male experimental crosses using the Prism program.

Table 1. Test of rare male mating advantage.

	Control		45 sn^3 / 9 +		9 sn^3 / 45 +	
	Crosses		Crosses		Crosses	
	# Mating Males		Crosses		# Mating Males	
	sn^3	+	sn^3	+	sn^3	+
Observed	107	103	120	57	27	136
Expected	105	105	147	30	28	135
	p = 0.84		p = 0.001		p = 0.88	

Results

A total of 16 bottles were set up in the control crosses; 13.1 females per bottle survived and produced progeny. A total of 10 bottles were set up for the 45 sn^3 / 9 + experiment; 17.7 females per bottle survived and produced progeny. A total of 10 bottles were set up in the 9 sn^3 / 45 + experiment; 16.3 females per bottle survived and produced progeny.

The results of the control crosses (54 sn^3/sn^3 females \times 27 sn^3/Y males and 27 +/Y males), the 45/9 crosses (54 sn^3/sn^3 females \times 45 sn^3/Y males and 9 +/Y males), and the 9/45 crosses (54 sn^3/sn^3 females \times 9 sn^3/Y males and 45 +/Y males) are shown in Table 1 and in Figures 1, 2 and 3. Since the observed frequencies of sn^3/Y and +/Y male matings were not significantly different from the expected frequency (1:1) in the control crosses ($P = 0.84$), the mating ability of each male type was the same when the two genotypes are of equal proportions. Hence, if there is no male mating advantage for either genotype (sn^3 or + males) when their frequencies are equal, the expected frequency of matings in the 45 sn^3 / 9 + crosses is expected to be 5:1 (five times as many expected matings of sn^3/Y males as compared to matings of +/Y males). In addition, in the 9 sn^3 / 45 + crosses the expected frequency of matings is expected to be 1:5 (five times as many expected matings of +/Y males as compared to matings of sn^3/Y males).

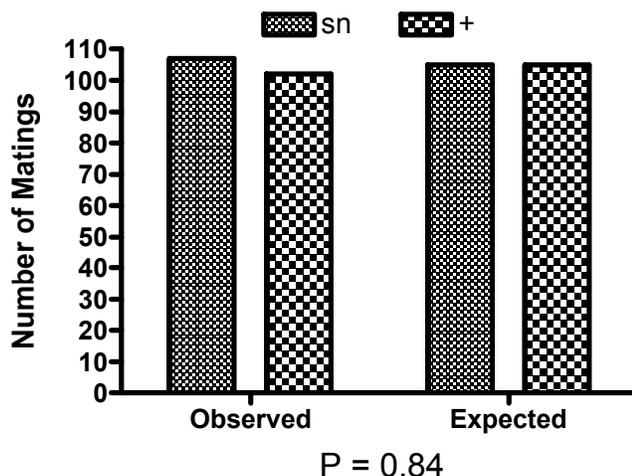


Figure 1. Control Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 27 sn^3/Y males and 27 wild-type (Canton-S) males per bottle.

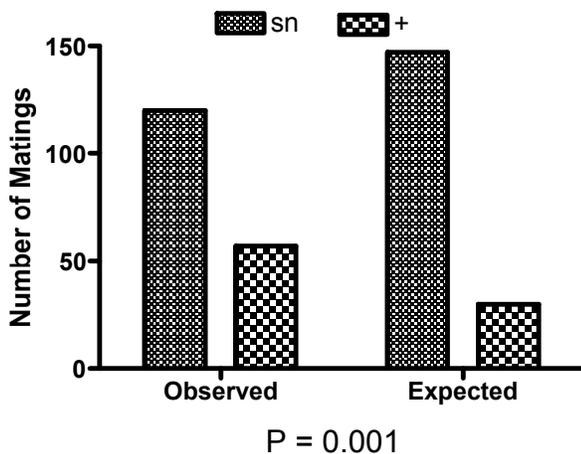


Figure 2. 45 sn^3 / 9 + Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 45 sn^3/Y males and 9 wild-type (Canton-S) males per bottle.

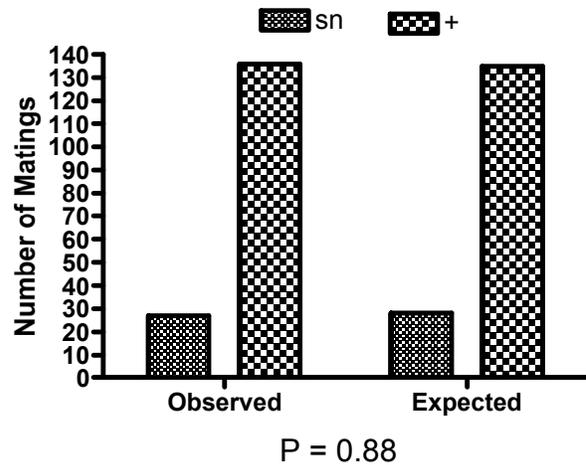


Figure 3. 9 sn^3 / 45 + Crosses: Frequencies of male matings in crosses of 54 sn^3/sn^3 females with 9 sn^3/Y males and 45 wild-type (Canton-S) males per bottle.

As shown in Table 1, the frequency of wild-type (Canton-S) male matings was significantly higher than expected ($P = 0.001$) in the 45 sn^3 / 9 + crosses. Hence, there is a rare male mating advantage for the wild-type males. On the other hand, in the 9 sn^3 / 45 + crosses there was not a significant mating advantage for the sn^3/Y males ($P = 0.88$). Hence, there was no rare male mating advantage for the sn^3/Y males; also see Figures 1, 2 and 3.

In summary, in crosses with sn^3/Y and $+/Y$ (Canton-S) males there was a significant rare male mating advantage for the wild-type (Canton-S) males, but not for the sn^3/Y males. This rare male mating advantage for Canton-S males does not seem to be due just to higher mating activity of these males, because Canton-S males did not mate more than sn^3/Y males in the control crosses, where there were equal frequencies of the two males ($P = 0.84$). Others have also observed one-sided rare male mating advantage in *D. ananassae* for visible markers and for chromosomal inversions (Som and Singh, 2004, 2005).

As a follow-up of this study, we plan to determine if the Canton-S males also show a rare male mating advantage with sn^3 males in experiments where mating pairs of flies are identified during copulation instead of scoring for progeny phenotypes (Ehrman, 1970). It would also be interesting to compare the rare male mating behavior of Canton-S males with males from other stocks that contain sex-linked visible markers, such as *forked* (small bristles), *yellow* (yellow body color), and *white* (white eyes).

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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