Teaching Notes

The identification of nearly neutral mutations in *Drosophila melanogaster*: The bw^{75} and bw^{1} alleles of Buri.

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The identification of nearly neutral mutations with only small differences in fitness is difficult in higher organisms. In a classical experiment Buri (1956) was able to follow changes in the bw^{75} and bw^{1} alleles at the second-chromosome brown locus of *Drosophila melanogaster* over 19 generations due to drift, because he estimated that the two alleles were neutral, *i.e.,* the bw^{75}/bw^{75}, bw^{75}/bw^{1}, and bw^{1}/bw^{1} flies (that were also homozygous for the third-chromosome mutant scarlet, *st/st*) had equal fitness. "No evidence consistent with an hypothesis of selection appeared in either series when the sample frequencies were grouped according to donor frequency classes. From this standpoint gene frequency changes could safely be attributed wholly to accidents of sampling." (Buri, 1956).

We synthesized the Buri $bw^{75}/bw^{1};st/st$ stock from stocks of bw^{75}/bw^{75}, bw^{1}/bw^{1}, and *st/st*, and tested the fitness (viability) of the genotypes $bw^{75}/bw^{75};st/st$ (orange eyes), $bw^{75}/bw^{1};st/st$ (yellow eyes), and $bw^{1}/bw^{1};st/st$ (white eyes). The *st/st* genotype will be assumed in subsequent crosses. We mated single bw^{75}/bw^{1} virgin females with single bw^{75}/bw^{1} males in vials and counted the three possible genotypes in F1 progeny. The results of two experiments are shown in Table 1.

The P values indicate that the results are not significantly different from the Hardy/Weinberg expectations (317, 634, and 317 in experiment A; and 305, 610, and 305 in experiment B) if bw^{75} and bw^{1} were neutral alleles. Hence, as in the study of Buri (1956) it seems that the bw^{75} and bw^{1} alleles are neutral, or nearly neutral, mutations.

Table 1. Frequencies of brown locus genotypes.

Experiment (vials)	bw^{75}/bw^{75}	bw^{75}/bw^{1}	bw^{1}/bw^{1}	P value
A(25)	330	620	318	0.81
B(28)	310	606	304	0.97

Buri (1956) also tested the fitness of the three genotypes of the brown locus using population cage experiments and observed no difference in fitness. "Considering the group of cage experiments as a whole, there is no indication of a difference in relative selective value between the two alleles …". Hence, we ran two population cage experiments beginning each cage with a frequency of bw^{75} and bw^{1} of 0.5; the cages were each started with 200 bw^{75}/bw^{1} heterozygous flies (100 virgin females and 100 males). The cages contained 15 population cups, and every seven days the five oldest cups were replaced with new cups and the first 300 flies that eclosed from the five older cups were identified as bw^{75}/bw^{75}, bw^{75}/bw^{1}, or bw^{1}/bw^{1} every fifth generation up to generation 25. We then determined the frequencies of the bw^{75} and bw^{1} alleles. The results are shown in Table 2. The changes in the frequency of the bw^{1} allele over time are shown in Figure 1.

Table 2. Frequencies of brown locus alleles in two population cages over time.

	Generation	bw^{75}/bw^{75}	bw^{75}/bw^{1}	bw^{1}/bw^{1}
Cage 1:	5	87	144	69
	10	85	161	54
	15	88	141	71
	20	83	158	59
	25	93	152	55
Cage 2:	5	95	147	58
	10	93	152	55
	15	119	135	46
	20	109	153	38
	25	103	143	54

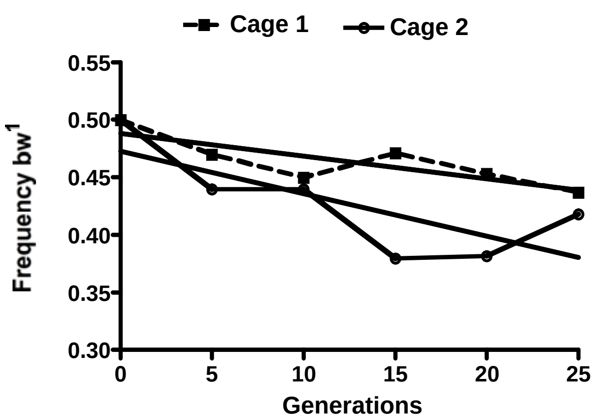

Figure 1. Frequency of the bw^{1} allele over time in two population cages.

The two slopes are not significantly different (P = 0.10). In both population cages the frequency of bw^{1} went down with time. The slope of cage 1 was significantly different from zero (P = 0.04), whereas the slope of cage 2 was not significantly different from zero (P = 0.07).

If we assume the following fitness model for the three brown genotypes, with s being the selection coefficient,

	bw^{75}/bw^{75}	bw^{75}/bw^{1}	bw^{1}/bw^{1}
Fitness	1	1	1 –s

then one can estimate the s value by using the following equation (Hedrick, 2011, equation 3.6c),

$$s = \frac{1}{n}\left[\left(\frac{q_0 - q_n}{q_0 q_n}\right) + \ln\left(\frac{q_0(1-q_n)}{q_n(1-q_0)}\right)\right]$$

where q_0 is the frequency of *bw*[1] at the beginning of the population cage experiment (0.5), q_n is the frequency of *bw*[1] at the end (generations 25) of the population cage experiment (0.43 from an average of Cages 1 and 2), and n is the number of generations (25). Hence,

$$\mathbf{s = \frac{1}{25}\left[\left(\frac{0.5 - 0.43}{(0.5)(0.43)}\right) + \ln\left(\frac{0.5(1 - 0.43)}{0.43(1 - 0.5)}\right)\right]}$$

$$\mathbf{s = 0.024}$$

The *bw*[1]/*bw*[1] flies have 98% of the fitness of the *bw*[75]/*bw*[75] and *bw*[75]/*bw*[1] flies. This supports *bw*[1] as a nearly neutral allele.

Since *bw*[1] and *bw*[75] are almost neutral alleles, we can also get an estimation of the effective population size (N_e)(the ideal population size in which all parents have an equal expectation of being the parents of any progeny; Hedrick, 2011) of the flies in the population cages by measuring the drop in the frequency of heterozygotes over time (Hedrick, 2011, page 197), with H_0 = 0.5, t = 25, and the Cage 1 and Cage 2 combined change in the frequency of heterozygotes from 0.5 to 0.49 (295/600) in 25 generations was 0.49 (H_t = 0.49):

$$\frac{H_t}{H_0} = e^{-t/2N_e}$$

$$0.98 = e^{-25/2N_e}$$

$$0.98 = e^{-12.5/N_e}$$

$$N_e = \frac{-12.5}{\ln 0.98}$$

$$N_e = 618$$

Since we estimated that there were at least 2,000 flies in each cage each generation after the beginning generation, the effective population size (N_e) was about 31 percent of the census population size (N). Malpica and Briscoe (1981) have estimated that the effective population size in cages with about 5,000 flies is as low as 190. Frankham (1995) has estimated that N_e/N is on average about 1/10 in natural populations of higher organisms.

A class discussion of the results of this teaching exercise could include 1) a discussion of the genetics of neutral alleles, including how the data of the population cage experiments would have differed over time if the three genotypes (*bw*[75]/*bw*[75], *bw*[75]/*bw1*, and *bw*[1]/*bw*[1]) had the same fitness, 2) how does the observation that N_e is much smaller than N influence the conservation of endangered species (see discussions in Frankham *et al.*, 2010), and 3) since Buri's experiment is considered a classical experiment showing genetic drift, how do the results of this study alter his study. Keep in mind that the *bw*[75]/*bw*[1]*;st/st* stock used in this study would have a different genetic background from the stock used by Buri.

References: Buri, P., 1956, Evolution 10: 367-402; Frankham, R., 1995, Genet. Res. 66: 95-107; Frankham, R., J.D. Ballou, and D.A. Briscoe 2010, *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge; Hedrick, P.W., 2011, *Genetics of Populations*. Jones and Bartlett Publishers, Sudbury, MA; Malpica, J.M., and D.A. Briscoe 1981, Experientia 37: 947-948.