Alternative odors: propyl butyrate (CAS: 105-66-8), pineapple odor (ethyl butyrate, CAS: 105-54-4), nail polish remover (ethyl acetate, CAS: 141-78-6).

Preparation of Material

Larvae: Flies are allowed to lay eggs in small food vials for 24 hours. After oviposition, flies are removed and egg-containing vials incubated at 22°C on a 12h-12h light cycle. (These growing conditions are recommended but not essential for the success of the experiment). In 5-6 days larvae reach the 3rd instar stage of development. They are easier to handle than the smaller larvae and also show a robust chemotaxis response. If wandering larvae start to appear on the walls of the food vial remove them with a paintbrush before the experiment, since at this stage they no longer forage and may behave differently towards the odor.

Odors: In this assay we pipetted 10 ul of a 1/40 dilution of isoamyl acetate in paraffin oil. (You can also use a dropper; in this case do the preparation tests with the dropper also). As the response may change depending on species (or even between different strains of the same species) it is advisable to carry out a test beforehand with a battery of dilutions, select the dilution that gives the clearest response. If the odor is too weak, larvae will take longer to respond; if it is too strong, the gradient may not be steep enough for the larvae to detect its direction – which can also result in a low attraction index. Testing of different concentrations can also be carried out by the students as a preparatory experiment.

More details about alternative set ups and tips on this assay can be found in Louis *et al.* (2012).

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Genetic drift leading to fixation of the *bw*^{1} neutral allele of *Drosophila melanogaster*.

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We have previously observed a reduction in heterozygosity over time due to genetic drift for two neutral alleles (*bw*^{1} and *bw*^{75}) of the brown locus of *Drosophila melanogaster* in the presence of the scarlet (*st*) mutation (Clendenin *et al.*, 2014; Woodruff and Boulton, 2011). We also screened for losses and fixations of the two neutral alleles over time and observed one fixation for the *bw*^{75} allele (Clendenin, 2014). Yet, it was

difficult to differentiate the *bw*^{1}/*bw*^{75}*; st/st* yellow-eyed phenotype from the *bw*^{75}/*bw*^{75}*; st/st* orange-eyed phenotype among older flies. The *bw*^{1}/*bw*^{1}*; st/st* flies, however, have a distinct white-eye color and should not be confused with other phenotypes. See Lindsley and Zimm (1992) for a description of the mutant genes and alleles used in this study.

Hence, in this study we screened for fixation of the *bw*^{1} allele only, by setting up 99 vials with a starting frequency of 0.5 for the *bw*^{1} and *bw*^{75} alleles, using all *bw*^{1}/*bw*^{75}; *st*/*st* flies and selecting eight flies at random for the next generation by lining up the progeny and picking the first four females and first four males (Buri, 1956). If all subsequent progeny from any one vial are white eyed (*bw*^{1}/*bw*^{1}*; st/st*), there was a fixation for the *bw*^{1} allele. The results of five generations of drift are shown here.

Generation	Number of lines with *bw*^{1} and *bw*^{75} alleles	Number of lines with only *bw*^{1} alleles
1	99	0
2	98	1
3	97	2
4	95	4
5	93	6

Hence, we saw six fixations for the *bw*^{1} allele in five generations, with an average of 46 white-eyed flies per fixation vial. These six fixations compare to one fixation that was observed in the first four generations by Buri (1956), who selected eight females and eight males per vial each generation (in a total of 107 vials).

The results of this study, therefore, support the use of the *bw*^{1} allele for observations of fixations of neutral alleles in small populations of four females and four males as a teaching exercise in a one-semester laboratory course.

A classroom discussion of the results of this study could include:

1) What would be the expected number of fixations of the *bw*^{75} (instead of the *bw*^{1} allele) allele in this study? The number of fixations should be the same as for the *bw*^{1} allele, since they are neutral mutations and the frequencies of each allele was 0.5 at the start of the experiment.

2) What is the predicted probability of fixation of the *bw*^{1} allele over time in this study if all the progeny were added to each vial every generation? Since half of the alleles in the original cross were *bw*^{1}, the probability of fixation would be one half.

3) Woodruff and Boulton (2011) observed that *bw*^{75}/*bw*^{75}*; st/st* and *bw*^{1}/*bw*^{75}*; st/st* flies were about two percent more fit than the *bw*^{1}/*bw*^{1}*; st/st* flies. With that in mind, what would be the expected probability of fixation of the *bw*^{75} allele if all the progeny were added to each vial every generation? Haldane (1927) showed that the probability of fixation of a beneficial mutation (such as *bw*^{75}) is 2*s*, with *s* being the selection coefficient in favor of the *bw*^{75}/*bw*^{75}*; st/st* flies. Hence, the probability of fixation of the *bw*^{75} allele would be about four percent ($2s = 2 * 0.2 = 0.4$) (see Hedrick, 2011, for a discussion of this topic).

4) Students could be asked if the population size that is picked each generation to set up the next generations would influence the probability of fixation of the *bw*^{1} allele. With an increase in population size, there would be a reduction in the frequency of lines that go to fixation for the *bw*^{1} allele (Hedrick, 2011). With this in mind, a class could be divided up into three groups and have one group select at random four females and four males for the next generation, one group select five females and five males, and one group select six females and six males. It is expected that with time the group with the smaller number of females and males picked each generation would have the largest frequency of *bw*^{1} fixations.

5) Finally, students could be asked to go to the literature and see if they can find examples of genetic drift in nature. An example where an organism has lost alleles over time in small populations is the greater prairie chicken in the USA. Compared to older museum specimens, small natural populations of greater prairie chickens have lost a number of microsatellite alleles, which are tandem repeats of two, three, or four nucleotides and are neutral alleles (Bouzat *et al*., 1998).

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