A classroom test of predictions about Segregation Distorter (SD) activity in *Drosophila*.

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Mendelian genetics is based on the assumption that chromosome combinations will occur in predictable ratios. For example, monohybrid crosses will result in a 1:2:1 genotypic ratio. There are a few select genes that manage to distort the expected ratio in their favor. These have been called "cheaters" (Crow, 2005) and include the Segregation Distorter (SD) gene of *Drosophila melanogaster*. The SD gene cannot display this distortion alone; it requires a cofactor, the Responder (RSP) gene. When both factors are present, past studies showed that the SD gene can be passed to as many as 95% of the progeny (Crow, 1962). On the other hand, studies of natural populations of *Drosophila melanogaster* show the frequency of chromosomes carrying the SD gene is lower than expected at only 1-5% (Hiraisumi and Thomas, 1984; Lyttle, 1991; Hao *et al*., 2000). Other factors must be preventing the SD gene from spreading through a natural population with the same rapid rate as in monitored crosses. The experiment we conducted screened both natural and caged populations of *Drosophila melanogaster* for the SD gene. Our objective was to calculate the frequency of the SD gene in the local population (near Norman, Oklahoma) and in segregating caged populations that had had time to produce multiple generations. SD screening was done by PCR as described by Robinson *et al*. (2008). This technique has not previously been used to screen for the SD gene in natural populations of *Drosophila* and should produce more accurate results.

The frequency of SD chromosomes was monitored in replicated population cages, constructed of Styrofoam tubs with holes for food tubes and gas exchange. Each cage had a clear viewing panel that served to seal the top. Cage populations were initiated with 100 pairs of adults and an SD chromosome frequency of 10%. The "Control" cages began with 20 pairs of SD/CyO and 80 pairs of Canton-S. The "Experimental" cages included segregating Responder (RSP) chromosomes marked with *cn bw* (20 pairs of SD/CyO, 40 pairs of Canton-S, and 40 pairs of *cn bw* SD sensitive). The prediction was that SD frequency would increase much more rapidly in the Experimental cages due to the presence of the segregating Responder chromosome.

The presence of SD was determined by PCR, as described by Robinson *et al*. (2008). Random samples were taken from each cage (5 PCR reactions per student, working in groups of three for each cage; total 60 PCR reactions per time period) at week 5 and at week 10 after setup. DNA was extracted from individual flies using the technique of Gloor and Engels, 1992; mashing individual flies in microfuge tubes in 10 mM Tris HCl (pH 8.2), 1 mM EDTA, 25 mM NaCl and 200 µg/ml Proteinase K; incubating the tube at about 37°C for at least 20 min; and transfer to 95°C water bath for 1-2 minutes to inactivate the proteinase K). The primers were: frwd, GAACGACTGGAAGTTATCGAC; rev, CCGTGAGAAATACCGCACTTGTCTTGG (Merrill *et al*., 1999; Cynthia Staber, unpubl., as referenced in Robinson *et al*., 2008). The PCR cycle was 95°C for 15 minutes; 40 cycles of 95°C for 45 sec, 60°C for 45 sec, 72°C for 45 sec; 72°C for 10 minutes

final amplification extension;   programmed storage at 4°C until transferred to the freezer. Electrophoresis was done on 1.5% agarose gels or 5% polyacrylamide gels at 110 volts.

To our surprise the SD DNA was detected in almost all flies from the four cages (see, for example, Figure 1).   The explanation came from noting SD amplified DNA in negative controls (Canton-S does not carry this sequence; right end of second row of samples in Figure 1). With a large number of people sharing material, group class pipetting may have contributed to contamination of some common reagent, an important lesson in laboratory class preparation. In a final sample in week 12 with new reagents, however, only one sample, from the Control cage, tested positive for SD, suggesting an overall decline in SD frequency in the cages. We plan to repeat this experiment in the future.
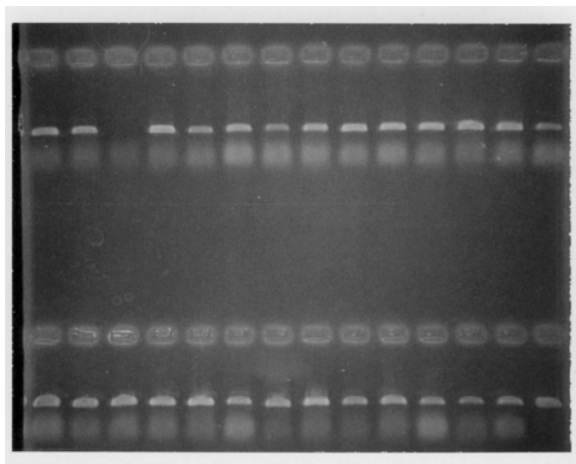

Figure 1.   PCR amplified SD sequences detected in all but one of 28 lanes in 1.5% agarose gel.   Right end of second row includes Canton-S (negative control) with SD band present, confirming contamination.

Very interesting data came from separate assays of *Drosophila* collected from the natural population near Norman, Oklahoma, during August and September 2008. *D. melanogaster* was among several species collected by sweep-netting over fermenting banana and other fruit in a rural area.   To avoid including D. simulans in the sample, only males were used. Flies were individually frozen in microfuge tubes until tested.

A total of 8 samples tested positive from 43 wild-caught males screened (18.6%; Figure 2). This is a higher frequency of SD than the 0 to 5% detected using assays for distorted segregation patterns (*c.f.*, Hiraisumi and Thomas, 1984; Lyttle, 1991; Hao *et al.*, 2000). In addition, some extracts yielded a faint band, which was detectable in re-tests of the samples. These faint bands were distinctly lighter than the normal amplification products from SD, although they appear to be the same size (Figure 3), making it unlikely that they are due to contamination. One possible explanation is that the population is segregating a polymorphism in one of the primer hybridization sites so that hybridization, thus PCR amplification, efficiency is reduced. An interested follow-up question for class discussion is how to test that hypothesis, since DNA sequencing reactions begin from the primers and does not independently sequence the primer binding sites themselves. One solution is to identify new primers from within the SD amplified region and sequence outwards through the original primer binding sites. The laboratory class exercise thus provides opportunities for both protocol trouble-shooting and design of future experiments.
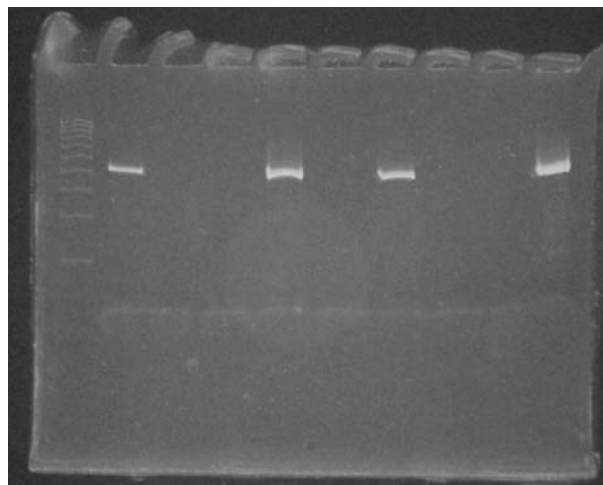

Figure 2. Representative 5% polyacrylamide gel with samples from wild-caught *Drosophila*. Lane 1, ladder; Lanes 2 through 8, *D. melanogaster* from natural population; Lane 9, Canton-S (negative control); Lane 10, SD (positive control).
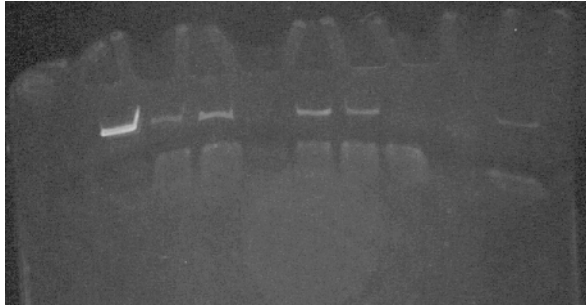
Figure 3. Re-test of natural population samples showing five lanes with light bands; typically bright SD band in Lane 2; Lane 1 blank.

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