Identification of selfish genetic elements in natural populations of *Drosophila melanogaster*. II. Segregation Distorter (*SD*).

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One of the Hardy/Weinberg equilibrium assumptions in population genetics is that meiosis is normal. By normal, it is assumed that if you have heterozygous (*Aa*) individuals in a population that is at Hardy/Weinberg equilibrium then the frequencies of gametes from these individuals will be one half *A* and one half *a*. Yet, there are examples of selfish genetic elements in natural populations in which one of the alleles (*A* or *a*) is driven to a high frequency in gametes each generation. This is sometimes referred to as meiotic drive or segregation distortion (Sandler and Novitski, 1957). In some cases one allele can end up in 95 to 99 percent of the gametes. See reviews of meiotic drive elements and other selfish genetic elements in Burt and Trivers (2006), Crow (1999), Kusano *et al*. (2003), Lyttle (1991), Presgraves (2007), Temin *et al*. (1991), and Wu and Hammer (1990).

One example of meiotic drive is the Segregation Distorter (*SD*) system of *Drosophila melanogaster* (Hartl and Hiraizumi, 1976; Lyttle, 1991). This is called a system, because it is controlled by a group of genes, including the *Sd* (Segregation distorter), *Rsp* (Responder of *Sd)* loci, and a number of modifiers of *Sd*, that are all located close to each other on the second chromosome (Burt and Trivers, 2006; Lyttle, 1991; Presgraves, 2007).

The Sd/Sd^+ males, which also have the Responder Sensitive, Rsp^s, allele on the Sd^+ second chromosome, transmit an excess of *SD* bearing chromosomes to their progeny; the proportion of *SD* bearing progeny can be as high as 99% (Hartl and Hiraizumi, 1996; Hiraizumi *et al*., 1960; Ganetzky *et al*., 1999; Sandler *et al*., 1959; Woodruff and Lyman, 1980). Conversely, females that are Sd/Sd^+ Rsp^s have normal second-chromosome segregation (Lyttle, 1991). The *Sd* locus is located near the centromere of the second chromosome and is found in about 1-3% of *D. melanogaster* males from nature (Hiraizumi and Thomas, 1984; Lyttle, 1991). In Sd/Sd^+ males, wild-type sperm fail to develop normally due to disruption of Sd^+ chromosomes (Burt and Trivers, 2006; Lyttle, 1991). The complete mechanism by which *Sd* causes the disruption of Sd^+ bearing chromosomes is not known, but it is known that *Sd* corresponds to a partial tandem duplication of the *RanGAP* gene, which codes for a RanGTPase Activator Protein (Kusano *et al*., 2003; Merrill *et al*., 1999). RanGTPase is involved in cell-cycle progression, spindle assembly, and chromosome packaging (Presgraves, 2007). In *SD* flies, the tandem duplication of genomic sequence codes for both wild-type *RanGA*P, an essential gene, and *Sd-RanGAP*, a dominant gain-of-function gene. *Sd-RanGAP* is an enzymatically active but truncated version of wild-type *RanGAP* whose mislocalization to the nucleus somehow leads to a developmental failure in sperm carrying a sensitive *Rsp* locus.

The Responder (Rsp^s) region, which responds to *Sd,* covers about 600,000 bases of DNA and is comprised of several hundred 240-basepair repeats (Houtchens and Lyttle, 2003). The RanGTP protein may bind to the Rsp^s region and causes meiotic drive (Burt and Trivers, 2006). What is not clear, however, is why *Sd* functions in males but not females.

It is the objective of this teaching exercise to attempt to identify Segregation Distorter (*Sd*) genes in natural populations of *D. melanogaster,* by use of F1 genetic screens with flies that have

easily identified phenotypes (red *vs* white eyes). We received a known *Sd* stock (*SD-72*/*CyO*) and a known *Rsp*^{s} stock (*Rsp*^{s} *cn bw/Rsp*^{s} *cn bw*) from Dr. Barry Ganetzky, University of Wisconsin. See a description of the mutant genes and balancer chromosomes used in this study in Lindsley and Zimm (1992). We tested these stocks for *SD* activity by crossing *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* virgin females, which are *Sd*$^+$/*Sd*$^+$, with *SD-72*/*Rsp*^{s} *cn bw* males, which are *Sd/Sd*$^+$, and screening progeny for *Sd/Rsp*^{s} *cn bw* (red eyed) and *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* (white eyed) flies. We recovered 1451 red-eyed progeny and 16 white-eyed progeny. This 99% (1451/1467) recovery of the *Sd* bearing second chromosomes from parental *Sd/Rsp*^{s} *cn bw* males confirmed that the *SD-72* chromosome had full *SD* activity and that the *Rsp*^{s} *cn bw* chromosome was sensitive to *Sd*. In addition, crosses of *SD-72/Rsp*^{s} *cn bw* females with *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* males gave normal second-chromosome segregation, with 157 *+/Rsp*^{s} *cn bw* (red eyed) progeny and 129 *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* (white eyed) progeny (55% red-eyed flies; see Table 1). Hence, we were then able to use the *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* stock in the following crosses to identify *SD* elements (*Sd* genes) from nature.

Table 1. Segregation Distorter (SD) results from *Drosophila melanogaster*.

Males or Females Tested	*+/Rsp*^{s} *cn bw* (red eyed)	*Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* (white eyed)	% red-eyed	P values[a]
SD-72 Males	1,451	16	99%	
SD-72 Females	157	129	55%	
Totals for 91 Natural Population Males	7,659	4,839	61%	
JO-30 Males				
G2	78	5	94%	<0.0001
G3	122	15	89%	<0.0001
G4	99	28	78%	=0.003
G5	103	5	95%	<0.0001
G6	146	10	94%	<0.0001
Total	548	63	90%	<0.0001
JO-30 Females	95	72	57%	

[a]P values were from a comparison with the average observed frequencies of red- and white-eyed flies from the 91 natural population males using the Fisher exact probability test.

Three *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* females were mated with 55 single wild-type males (P-1 to P-55) captured in Perrysburg, Ohio (Wood County) in August 2007 and from 36 single wild-type males (JO-1 to JO-36) captured in Jeffers Orchard, Grand Rapids, Ohio (Wood County) in September 2007. Single G1 *+/Rsp*^{s} *cn bw* males were then mated with three *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* virgin females and G2 males and females were scored for *+/Rsp*^{s} *cn bw* and *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* genotypes, as shown below.

P 3 *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* females × Single +/+ males from nature

↘

G1 3 *Rsp*^{s} *cn bw/Rsp*^{s} *cn bw* females × Single *+/Rsp*^{s} *cn bw* males

↓

G2 female and male progeny scored for red eyes (*+/Rsp*^{s} *cn bw)* and white eyes (*Rsp*^{s} *cn bw/Rsp*^{s} *cn bw)*

In addition, single G2 and single G3 *+/Rsp^s cn bw* males from each parental cross were mated with three *Rsp^s cn bw/Rsp^s cn bw* females and G3 and G4 progeny were scored for red and white eye colors. The G2 and G3 crosses were performed in an attempt to remove any suppressor of *Sd* activity that previously has been found to be associated with *Sd* in some populations (Burt and Trivers, 2006; Lyttle, 1991; Presgraves, 2007).

The proportion of *+/Rsp^s cn bw* progeny out of the total for the G1, G2 and G3 crosses of the 91 lines was 7,659/12,498 (61.3%)(Table 1). Among the crosses from the 91 males from nature one male (JO-30) gave results showing *SD* activity. The results from these crosses are shown in Table 1. Additional G4, G5 and G6 crosses were performed with the JO-30 line to confirm that it continued to show *SD* activity. These results are also shown on Table 1. The JO-30 line gave consistent segregation distortion results each generation, with a range of 89% to 95% red-eyed progeny and an average of 90% (548/611)(Table 1).

We also tested for *SD* activity in females by mating JO-30/*Rsp^s cn bw* females with *Rsp^s cn bw/Rsp^s cn bw* males and screening for red and white-eyed progeny. If JO-30 is a SD line with a *Sd* gene on the second chromosome, based on previous results and the results with *SD-72/Rsp^s cn bw* females in this study, *SD* activity should be absent in the JO-30/*Rsp^s cn bw* females. Similar to the SD-72 results, 57% of progeny were red eyed. This supports our hypothesis that JO-30 contains *Sd* that only functions in males (see Table 1).

To confirm that JO-30 carries the SD element, we performed a polymerase chain reaction (PCR) analysis using primers SdRG F1 (GAACGACTGGAAGTTATCGAC) and SdRG R2 (CCGTGAGAAATACCGCACTTGTCTTGG)(Merrill *et al*., 1999; Cynthia Staber, unpublished). We used the SD-72 line as a positive control and the long-term wild-type stock Canton-S as a negative control. Based on preliminary experiments by Cynthia Staber, we predicted that a DNA band of 459 base pairs would be amplified from chromosomes containing the SD element, whereas no DNA product, and no band, would be recovered from chromosomes that did not contain the SD element.

DNA was isolated from single flies by mashing a fly in a 0.5 ml microfuge tube with a pipette tip containing 50 µl of squishing buffer (10mM Tris Cl pH 8.2, 1mM EDTA, 25mM NaCl), without expelling any liquid for 5-10 seconds. Then the squishing buffer was expelled into the tube (Gloor and Engels, 1992). One µl of proteinase K was added and the tube was incubated at 25^o or 37^{o}C for 20 to 30 minutes. Placing the tube at 95^{o}C for one to two minutes inactivated the proteinase K.

For PCR amplifications, the reaction mixture (50 µl total) contained 5 ul fly DNA, 25 µl Taq polymerase mixture, 1 µl primer SdRG F1, 1 µl primer SdRG R2, and 18 µl dH_2O. The PCR Cycle was: 94^{o}C for 45 seconds, 60^{o}C for 45 seconds, 72^{o}C for 45 seconds, for a total of 40 cycles. Electrophoresis was performed on 1.5% agarose gels (0.75g agarose and 50 ml 1× TBE), plus ethidium bromide, for 75 minutes at 110 volts.

The results of the PCR runs are shown in Figure 1. The SD-72 and JO-30 lines clearly contain SD elements, whereas Canton-S does not. This confirms our identification of JO-30 as a true SD line by genetic crosses and by molecular analysis. We plan next to determine if the PCR analysis can be used as a quick means of determining the frequency of SD in other natural populations of *D. melanogaster*.

A class discussion of the results of this teaching exercise could include the following topics: 1) With the SD chromosome occurring in over 90% of the progeny of SD/SD$^+$ males, why does the SD element not go to 100% in natural populations? Many SD chromosomes carry an inversion that contains a recessive lethal mutation. 2) There is an ongoing debate on the level of action of natural selection, with some saying it is the individual and some saying it is the gene that is selected. How

would the results from this study fit into this debate? 3) Have students read about the *t* haplotype system in mice, which also shows meiotic drive (Burt and Trivers, 2006).

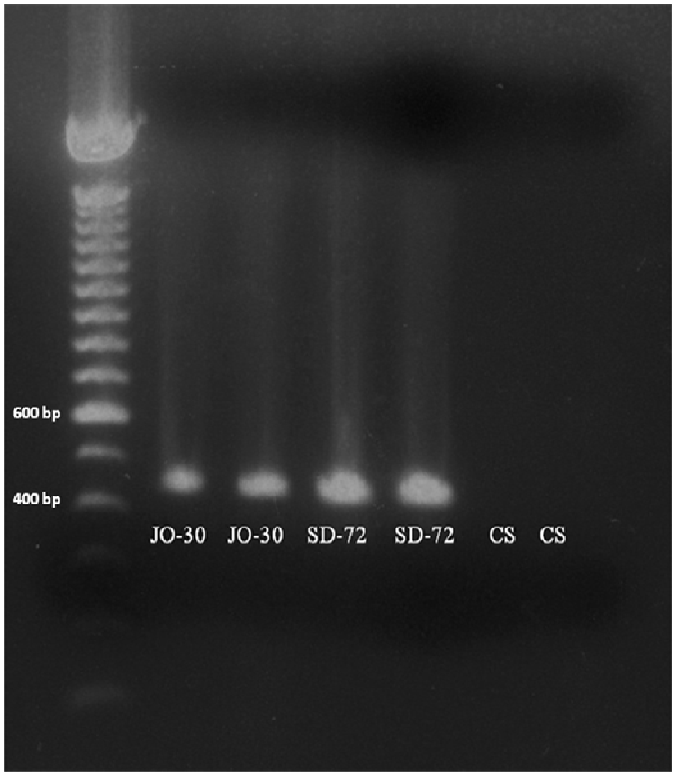

Figure 1. Polymerase chain reaction results with Sd primers. JO-30 and SD-72 contain Sd, whereas CS (Canton-S) does not.

References: Burt, A., and R. Trivers 2006, *Genes in Conflict*. The Belknap Press of Harvard University Press, Cambridge, MA; Crow, J.F., 1999, Science 283: 1651-1652; Gloor, G.B., and W.R. Engels 1992, Dros. Inf. Serv. 71: 148-149; Houtchens, K., and T.W. Lyttle 2003, Genetica 117: 291-302; Kusano, A., C. Staber, H.Y.E. Chan, and B. Ganetzky 2003, BioEssays 25: 108-115; Lindsley, D.L., and G.C. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press, New York; Lyttle, T.W., 1991, Annu. Rev. Genet. 25: 511-557; Merrill, C., L. Bayraktaroglu, A. Kusano, and B. Ganetzky 1999, Science 283: 1742-1745; Presgraves, D.C., 2007, BioEssays 29: 386-391; Woodruff, R.C., and R.F. Lyman 1980, Am. Nat. 116: 297-304.

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