Identification of selfish genetic elements in natural populations of *Drosophila melanogaster*. I. P DNA elements.

Robinson, Bethany, Natalie Wiseman, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403.

All organisms have large pieces of DNA that have, or have had, the ability to move to new chromosomal locations. These transposable DNA elements make up a large proportion of the genomes of higher organisms, including 46% of the human genome and 5-15% of the *Drosophila melanogaster* genome (Kidwell, 2002; Burt and Trivers, 2006; Drosophila 12 Consortium, 2007). This high a proportion of genomes that could be transposable is surprising, since DNA element movements (insertion and excision events) often cause deleterious mutations and recombination between elements gives rise to chromosome rearrangements, including inversions, deficiencies, duplications, and translocations (Mackay, 1987; Babushok and Kazazian, 2007).

If all of the 4,000,000 transposable DNA elements in humans were active, even if they moved at a very low rate, these transpositions would cause extensive genetic damage that would reduce the health and fitness of humans. Hence, it is not surprising that the vast majority of transposable DNA elements in humans and other higher organisms are nonfunctional, mainly due to nucleotide mutations and deletions within the elements. For example, in humans only about 80 to 100 transposable DNA elements are active (Broushok and Kazazian, 2007). Still, as high as 1 in 10 mutations in humans have a novel insertion, and these DNA elements cause mutations that lead to cancer (Miki *et al*., 1992; Biemont and Vieira, 2006; Burt and Trivers, 2006). The impact of transposable DNA elements on genetic damage in the model organism *D. melanogaster* is even higher (Keightley, 1996).

In *D. melanogaster* there are 93 families of transposable DNA elements, and excisions and insertions of these elements are common inducers of mutation and sterility (Kaminker *et al*., 2002). Some inversions in natural populations of *D. melanogaster* and other *Drosophila* species are also caused by exchanges between DNA elements (Caceres *et al*., 1999). In fact, one of the first isolated visible mutations in *D. melanogaster*, the sex-linked, white-eyed recessive mutation identified by Thomas Hunt Morgan (Morgan, 1910), was caused by a *Doc* DNA element insertion in the *white* gene (Driver *et al*., 1989). One of the classical mutations studied by Mendel, wrinkled pea seeds, was also caused by a DNA insertion event (Bhattacharyya *et al*., 1990).

The first transposable DNA element identified at the genetic and molecular level in *D. melanogaster* was the P DNA element (Bingham *et al*., 1982). The P name for this element came from the observation that parental (P) males that contain the P DNA elements when crossed with maternal (M) females, which do not have active P DNA elements, give rise to a high frequency of P DNA movement in offspring, leading to germ cell death and sterility and to gene and chromosomal mutations in progeny (Henderson, Woodruff and Thompson, 1978; Kidwell and Novy, 1979). This syndrome of genetic damage events caused by P DNA element movement is called hybrid dysgenesis (Kidwell, Kidwell and Sved, 1977). The reciprocal cross of P females with M males, and crosses of M × M or P × P flies does not cause hybrid dysgenesis.

The molecular structure of the P DNA element is given in Figure 1. The functional P DNA element is 2,907 base-pairs long and codes for terminal repeats and a transposase that function in transpositions. There are also truncated P elements that can respond to transposase from other P

DNA elements. The P DNA element is active in germ cells, but not somatic cells, due to differences in splicing out of introns in the two cell types (Laski *et al*., 1986). The P DNA element was also used as the vector for the first gene therapy experiment in eukaryotes (Rubin and Spradling, 1982; Spradling and Rubin, 1982).

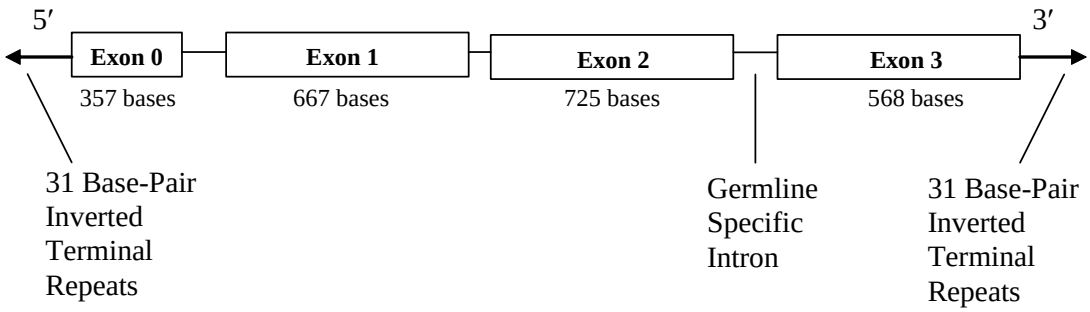

Figure 1. Molecular structure of the P DNA element of *Drosophila melanogaster*.

It is the objective of this teaching exercise to attempt to identify active P DNA elements from natural populations of *D. melanogaster* by screening for P DNA element induced gonadal dysgenesis (atrophied ovaries) in F1 females from crosses of males from nature to laboratory females that do not have active P DNA elements (M line). Woodruff and Thompson (2001) have discussed how to identify active *mariner* DNA elements in natural populations of *D. simulans* by screening for mosaic eye spots caused by the ability of active *mariner* elements from nature to excise a *mariner* insert in the *white* locus.

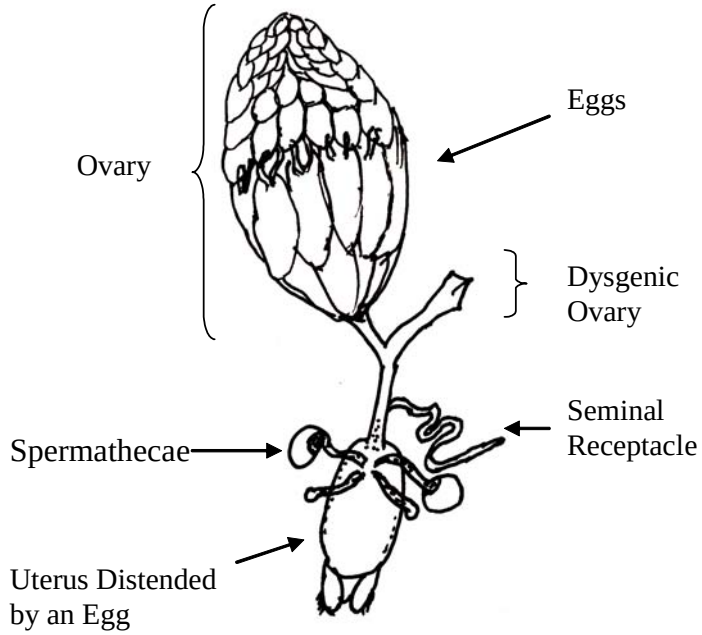

Figure 2. Female reproductive system of *Drosophila melanogaster* with one normal ovary and one dysgenic ovary (atrophied gonad) caused by P DNA element induced germ-cell death. Spermathecae and seminal receptacle are storage organs for sperm. Modified from Miller (1965).

To identify gonadal dysgenesis due to P DNA element induced germ-cell death and atrophy of female gonads, we double mated single *D. melanogaster* males collected in two Northwest Ohio sites in 2007 (Perrysburg, Lucas County, and Jeffers Orchard in Grand Rapid, Wood County) to P DNA element control females (Harwich-w, P line with the white-eyed mutation and active P DNA elements and *y* w^{67c23}, M line with yellow-body and white-eyed mutations and without active P DNA

elements). The P DNA element control lines were obtained from Dr. Michael Simmons at the University of Minnesota. These crosses were performed at 29°C, which gives the highest rate of P DNA element movement (Kidwell *et al*., 1977).

2-3 Harwich-w (P) females × Single males from nature × 2-3 *y* w^{67c23} (M) females

↓ ↓

The F1 females were collected, aged for four days at 29°C, and dissected in 0.7% NaCl. The paired gonads of each F1 female were scored for normal or atrophied gonads. Each female gave two data points that were normal/normal, normal/atrophied, or atrophied/atrophied. Figure 2 is a drawing of a paired female gonad, with one being normal in structure with developing eggs and the other atrophied with no eggs. A similar mating scheme has been used to screen for P DNA induced gonadal dysgenesis in crosses with *D. melanogaster* males from Australia (Boussy *et al*., 1998; Itoh *et al*., 1999).

The following crosses were also performed at 29°C to verify that the two control lines were true P and M lines.

2-3 *y* w^{67c23} M females × Harwich-w P males

↓

Expected to show high frequency of gonadal dysgenesis in F1 females

2-3 Harwich-w P females × *y* w^{67c23} M males

↓

Expected to show no, or low, frequency of gonadal dysgenesis in F1 females

Results

1. Control Crosses:

As predicted, the crosses of *y* w^{67c23} (M) females with Harwich-w (P) males gave 40 atrophied gonads and no normal gonads among 20 F1 female progeny, and crosses of Harwich-w (P) females with *y* w^{67c23} (M) males gave 40 normal ovaries among 20 F1 female progeny. These results confirm that Harwich-w is a P line with functional P DNA elements and that *y* w^{67c23} is an M line with no functional P DNA elements. Hence, these two control lines can be used to identify gonadal dysgenesis in crosses with natural population males, as shown below.

2. Crosses with Natural Population Males:

The F1 gonadal dysgenesis results from double crosses of Perrysburg and Jeffers Orchard males with *y* w^{67c23} M females and with Harwich-w P females are shown in Table 1 and Figure 3. A total of 20-25 males from each of the populations were each double mated with the two control females, but only eight of the males from Perrysburg and seven from Jeffers Orchard produced

progeny from both crosses. A significant increase in gonadal dysgenesis caused by P DNA element induced germ-cell death and subsequent gonad atrophy was observed in both natural populations.

In the Perrysburg sample, total atrophy in crosses with M females (15.8%) was significantly higher ($P < 0.0001$) than in crosses with P females (0.9%). There were two males (Perrysburg 6 and 7) that had significant increases in gonadal dysgenesis in crosses with M females compared to crosses with P females (33% to 0%, and 39% to 0%, $P < 0.01$). In addition, one male (Perrysburg 8) had a high frequency of gonadal dysgenesis in the cross with M females as compared to P females (39% to 0%), but the increase was not significant because of the low number of tested flies in the latter cross (we were only able to score two F1 progeny due to low fecundity).

Table 1. Gonadal dysgenesis in F1 females.

Natural Population Males	Gonads from Crosses with *y* w^{67c23} (M) Females			Gonads from Crosses with Harwich-w (P) Females		
Perrysburg, Ohio	Atrophied	Normal	%	Atrophied	Normal	%
1	0	40	0	2	38	5
2	1	25	4	0	18	0
3	2	18	10	0	36	0
4	0	40	0	0	40	0
5	2	36	5	0	26	0
6	16	32	33	0	16	0
7	7	11	39	0	40	0
8	14	22	39	0	4	0
Total	42	224	15.8[a]	2	218	0.9[a]
Jeffery Orchard	Atrophied	Normal	%	Atrophied	Normal	%
1	14	10	58	0	36	0
2	13	26	33	0	40	0
3	3	37	8	0	40	0
4	7	17	29	0	40	0
5	0	14	0	0	40	0
6	2	38	5	2	38	5
7	3	37	8	1	39	3
Total	42	179	19.0[b]	3	273	1.1[b]

[a]$P < 0.0001$, [b]$P < 0.0001$

In the Jeffers Orchard, Grand Rapid sample, total atrophy in crosses with M females (19.0%) was significantly higher ($P < 0.0001$) than in crosses with P females (1.1%). There were three males (Jeffer Orchard 1, 2 and 4) that had significant increases in gonadal dysgenesis in crosses with M females compared to crosses with P females (58% to 0%, 33% to 0%, and 29% to 0%, $P < 0.01$).

These gonadal dysgenesis results indicate that the two Northwest Ohio populations of *D. melanogaster* had active P DNA elements. The frequencies of males with active P DNA elements in the two populations (25% and 43%) and the frequencies of gonadal dysgenesis in the P DNA active lines (15.8% and 19%) are similar to other natural populations (Kidwell, 1994).

A class discussion of the results of these crosses could include the following topics: 1) How could P DNA elements that cause deleterious mutations and chromosomal rearrangements be maintained in natural populations? Would you expect organisms that contain these elements to be selected against? 2) Is there evidence that DNA elements are ever beneficial? You might read about the enzymes that are involved in the movement of segments of DNA to make mature antibody genes in humans (Burt and Trivers, 2006). 3) How could P DNA elements cause germ-cell death? You

might see the photographs of P DNA induced chromosome damage in germ cells of *D. melanogaster* in Henderson, Woodruff and Thompson (1978). 4) Discuss the possible evolutionary implications of DNA elements, such as P DNA elements, that move in germ cells but not in somatic cells. 5) Ask students to go to http://www.med.upenn.edu/genetics/kazazianlab_home.shtml for a list of DNA insertion mutations in humans.

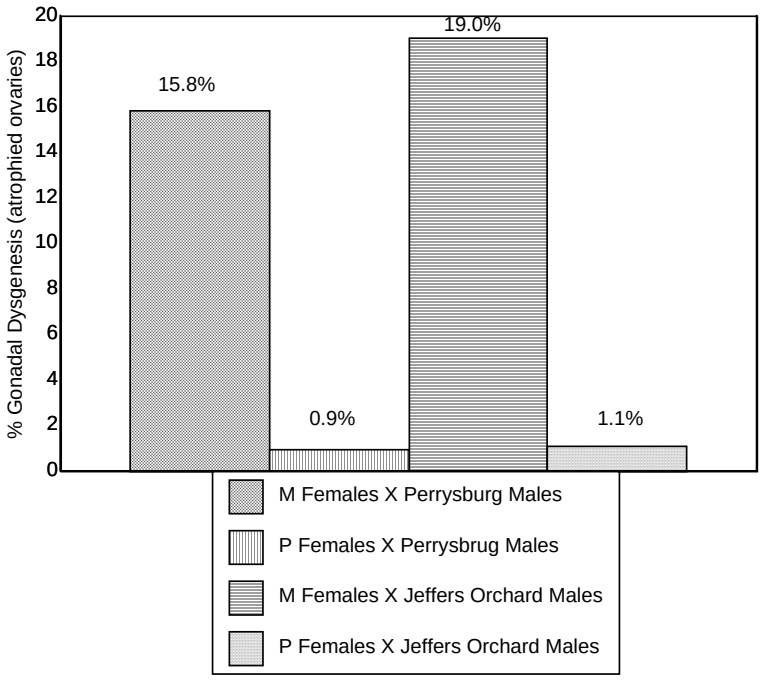

Figure 3. Gonadal dysgenesis levels in crosses of *Drosophila melanogaster* natural population males with females that do or do not contain functional P DNA elements.

References: Babushok, D.V., and H.H. Kazazian, Jr. 2007, Human Mutation 28: 527-539; Bhattacharyya M.K., A.M. Smith, T.H.N. Ellis, and C. Martin 1990, Cell 60: 115-122; Biemont, C., and C. Vieira 2006, Nature 443: 521-524; Bingham, P.M., M.G. Kidwell, and G.M. Rubin 1982, Cell 29: 995-1004; Boussy, I.A., M. Itoh, D. Rand, and R.C. Woodruff 1998, Genetica 104: 45-57; Brouha, B. *et al*., 2003, Proc. Nat. Acad. Sci. USA 100: 5280-5285; Driver, A. *et al*., 1989, Molecular General Genetics 220: 49-52; Burt, A., and R. Trivers 2006, *Genes in Conflict*, Cambridge, Massachusetts: The Belknap Press of Harvard University Press; Caceres *et al*. 1999, Science 285: 415-418; Drosophila 12 Genome Consortium 2007, Nature 450: 203-218; Henderson, S.A., R.C. Woodruff, and J.N. Thompson, Jr. 1978, Genetics 88: 93-107; Itoh, M., R.C. Woodruff, M.A. Leone, and I.A. Boussy 1999, Genetica 106: 231-245; Keightley, P.D., 1996, Genetics 144: 1993-1999; Kidwell, M.G., 1994, J. Heredity 85: 339-346; Kidwell, M.G., 2002, Genetica 115: 49-63; Kidwell, M.G., J.F. Kidwell, and J.A. Sved 1977, Genetics 86: 813-833; Laski, F.A., D.C. Rio, and G.M. Rubin 1986, Cell 44: 7-19; Mackay, T.F.C., 1987, Genetical Research 49: 225-234; Miller, A., 1965, The internal anatomy and histology of the imago of *Drosophila melanogaster*, In: *Biology of* Drosophila (Demerec, M., Ed.) Hafner Publishing Company, New York; Morgan, T.H., 1910, Science 32: 120-122; Rubin, G.M., and A.C. Spradling 1982, Science 218: 348-353; Spradling, A.C., and G.M. Rubin 1982, Science 218: 341-347; Woodruff, R.C., and J.N. Thompson, Jr. 2001, Dros. Inf. Serv. 84: 213-215.