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A one-generation, *white-peach* assay for *mariner* DNA element activity in *Drosophila simulans*.

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Transposable DNA elements are common in all organisms from prokaryotes to eukaryotes, including humans. When these elements move they cause genetic damage such as insertions (which can disrupt genes and their control regions) and excisions (which can cause loss of nucleotides and chromosome breakage) (Lambert *et al.*, 1988; Berg and Howe, 1989; McDonald, 1993). Recombination between DNA elements can also lead to chromosome rearrangements, such as inversions (Caceres *et al.*, 1999). In humans, about 40% of the DNA is comprised of these elements and about 40-60 active elements are present in each genome (Prak and Kazazian, 2000). The movement of DNA elements has been identified to cause mutations in a number of human genes, including Duchenne muscular dystrophy, type 2 retinitis pigmentosa, and hemophilia A. The movement of DNA elements in somatic cells can also cause cancer (Miki *et al.*, 1992). Hence, these DNA elements are a major source of spontaneous genetic damage and disease in humans.

However, it is difficult to identify the presence or activity of DNA elements in higher organisms in a teaching environment, unless one has the equipment and supplies to perform molecular techniques, such as Southern blot analysis or polymerase chain reactions. To overcome this limitation, we describe here a one-generation assay that can be used in a classroom laboratory to identify somatic movement of the *mariner* transposable DNA element in *D. simulans* obtained from natural populations. The movement is observed as mosaic eye-color spots in adults.

The *mariner* element is present in most organisms that have been tested and is found in the genome of humans (Oosumi *et al.*, 1995; Robertson, 1995). This DNA element is especially common in *Drosophila* species, including the cosmopolitan species *D. simulans* (Hartl, 1989). The *mariner* element has also been used as a vector to transfer DNA from one species to another (Warren and Crampton, 1994; Gujeiros-Filho and Beverley, 1997; Plasterk *et al.*, 1999).

To identify *mariner* elements that produce an active transposase protein, single *D. simulans* males captured on banana baits in nature are mated individually to females that are homozygous for the *white-peach* (w^{pch}/w^{pch}) mutation. The only *Drosophila* species that resembles *D. simulans* is *D. melanogaster*, but these species can be differentiated by the amber-colored clam-shaped claspers of the male genitalia in *D. simulans* that are not seen in *D. melanogaster* when the male abdomens are viewed from the side. If *D. melanogaster* males are placed together by mistake with *white-peach D. simulans* females, this will not be a problem, because the two sibling species almost never mate. If they were to mate, however, only sterile female progeny survive (Ashburner, 1989). Furthermore, *mariner* is not found in *D. melanogaster* (Brunet *et al.*, 1994).

The *white-peach* mutation is caused by a 1,300 base pair insertion of an inactive *mariner* element into the *white* gene on the X chromosome. This mutation was originally isolated in *D. mauritiana* and was then placed into *D. simulans* by repeated backcrosses (Haymer and Marsh, 1986; Capy *et al.*, 1990). This insertion can be activated to excise out of the *white* gene in germ and somatic cells by *mariner* transposase in *D. simulans* males from natural populations (Giraud and Capy, 1996). This leads to red mosaic spots on a *white-peach* color background (see Haymer and Marsh, 1986, their Figure 1, p. 287; Hartl, 1989, his Figure 2, p. 534).

The *mariner* element has previously been identified in *D. simulans* lines world-wide (Capy *et al.*, 1990; Russell and Woodruff, 1999). In this teaching exercise, we captured *D. simulans* males from four locations in the USA: St. George Island and East Point, Florida (April 13, 2001); the University of Oklahoma Biological Station on Lake Texhoma, Willis, Oklahoma (May 21, 2001); Perrysburg, Ohio (September 10, 2001). The males were captured by sweeping or aspiration of banana baits. Males were mated individually with two or three, virgin w^{pch}/w^{pch} females in vials on standard cornmeal, molasses medium. The F1 progeny were screened to be sure that *white-peach* males and wild-type, red-eyed females were present. Non-virgin females would give male and female progeny with *white-peach* eyes. Each eye of the *white-peach* males was then scored for the presence or absence of red spots on the *white-peach* color eye background. A male was recorded as having spots if at least one ommatidium was red. Most of the positive males had one or two small spots per eye, but some had up to 8 spots on one eye and many had spots that covered at least one-third of an eye. The results from the crosses are shown in Table 1.

In summary, all natural populations of *D. simulans* tested contained somatically active *mariner* elements, showing that the w^{pch} assay is a one-generation assay that can be easily used to identify active DNA elements in nature.

Table 1. Results from the crosses.

	% of Natural Population *D. simulans* Males with Active Mariner Elements	Number of F1 Males with spots/Total Males (%)
St. George Island, Fl.	100 (8/8)	95/ 354 (26.8)
East Point, Fl.	100 (8/8)	81/ 274 (29.6)
Univ. Okla. Biol. Station	91 (10/11)	128/ 259 (49.4)
Perrysburg, Ohio	100 (11/11)	99/ 390 (25.4)
Totals:	97 (37/38)	403/1,277 (31.6)

As a follow-up discussion, the class might be asked if they believe the somatically active *mariner* elements could cause genetic damage that would reduce the fitness of the F1 males. Hint: they do seem to reduce lifespan (Nikitin and Woodruff, 1995), and similar somatic movement of P DNA elements in *D. melanogaster* has been reported to reduce lifespan, mating activity, locomotion and fitness (Woodruff, 1992; Woodruff and Nikitin, 1995; Woodruff *et al.*, 1999). This could naturally lead to discussions of broader topics like the effects of mutagenic agents on somatic events like cancer and the possible role of increased mutation rates on life history in general.

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