

The *Drosophila melanogaster cinnabar* Gene Is a Cell Autonomous Genetic Marker in *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT The *cinnabar* gene of *Drosophila melanogaster* (Meigen) encodes for *kynurenine hydroxylase*, an enzyme involved in ommochrome biosynthesis. This gene is commonly included as a visible genetic marker in gene vectors used to create transgenic *Aedes aegypti* (L.) that are homozygous for the *kh^w* allele, the mosquito homolog of *cinnabar*. Unexpectedly, the phenotype of cells expressing *kynurenine hydroxylase* in transgenic *Ae. aegypti* is cell autonomous as demonstrated by the recovery of insects heterozygous for the *kynurenine hydroxylase* transgene with mosaic eye color patterns. In addition, a transgenic gynandromorph was recovered in which one-half of the insect was expressing the *kynurenine hydroxylase* transgene, including one eye with red pigmentation, whereas the other half of the insect was homozygous *kh^w* and included a white eye. The cell autonomous behavior of *cinnabar* in transgenic *Ae. aegypti* is unexpected and increases the utility of this genetic marker.

KEY WORDS *Aedes aegypti*, *kynurenine hydroxylase*, *cinnabar*, *kh^w*, *piggyBac*

GENETIC TRANSFORMATION IS BECOMING an important tool for the investigation of medically important insects. Five gene vectors, *Hermes*, *Minos*, *mariner*, *piggyBac*, and *Tn5*, have been shown to function in at least one species of mosquito (Coates et al. 1998, Jasinskiene et al. 1998, Nolan et al. 2002, Rowan et al. 2004). Although a variety of gene vectors exist, there are few genetic markers available that have been used to detect the presence of an integrated transgene in nondrosophilids. The most widely used markers are genes encoding autofluorescent proteins, e.g., green fluorescent protein (GFP) and Ds Red, that are regulated by tissue-specific promoters such as 3XP3 promoter (Berghammer et al. 1999) or promoters with more global expression patterns (Grossman et al. 2001). The 3XP3 promoter is a synthetic regulatory region derived from the *eyeless* gene of *Drosophila melanogaster* (Meigen) and functions in a wide range of organisms. 3XP3::EGFP is an extremely useful promoter/marker combination in mosquitoes. The *kynurenine hydroxylase* gene (*cinnabar*) from *D. melanogaster*, which is involved in ommochrome biosynthesis, also can be used as a genetic marker in *Aedes aegypti* (L.). The *cinnabar* gene can complement the mutant allele *kh^w* in *Ae. aegypti* and is an effective visible genetic marker that can be used to recognize transgenic larvae, pupae, and adults (Bhalla 1968, Cornel et al. 1997, Coates et al. 1998). Typically, genes involved in pigment biosynthesis, such as *cinnabar*, do not act as cell autonomous markers (Sarkar and Collins 2000). That is, cells with a mutant genotype will not have a mutant phenotype if they are in an organism or population of cells

that has a wild-type genotype (Beadle and Ephrussi 1936). The lack of cell autonomy results from the transfer of gene products across cell membranes and tissue boundaries. Genes displaying cell autonomous phenotypes can be useful as genetic markers. The eye color gene, *white*, in *D. melanogaster* is an ATP-binding cassette transporter involved with pigment uptake in developing eyes and behaves cell autonomously. This characteristic of *white* has contributed to the utility and versatility of this gene as a genetic marker in a variety of applications, particularly those involving the creation of genetic mosaics. Cell autonomy is particularly useful when examining developmental interactions or signaling between cells, but it also is generally useful when genotypes of individual cells or small groups of cells need to be deduced from cell phenotypes. Here, we report that *cinnabar* from *D. melanogaster* acts cell autonomously in transgenic *Ae. aegypti* that are homozygous for the *kh^w* allele.

Materials and Methods

Two of the four transgenic lines described by O'Brochta et al. 2003 were used in this study (40D and 40L). The lines used here arose after the perfect cut-and-paste transposition of a *piggyBac* gene vector consisting of the intact *D. melanogaster cinnabar* gene regulated by its native promoter (Warren et al. 1996–1997) into the genome of *Ae. aegypti* homozygous for *kh^w*. Transgenic lines were maintained as homozygotes under standard rearing conditions for this species. Line 40D had a burgundy-red eye color, whereas

40L had a distinctly lighter eye color. Eye color phenotypes similar to these have been reported previously and reflect differing levels of *cinnabar* gene expression that results from poorly defined influences of the chromatin structure in the region of the transgene (Coates et al. 1998, Jasinskiene et al. 1998). Because the transgene is located in distinct positions in each line the level of expression is expected to be different, leading to different phenotypes. The lines have been maintained in the lab for ≈ 2 yr as stable homozygotes. Heterozygotes were created by outcrossing each transgenic line to a line homozygous for the sex-linked *kh^w* allele (this strain was originally referred to as *white-eye* and obtained from Dr. A. A. James, University of California, Irvine, CA). Adult heterozygotes were examined under a dissecting microscope, and eye pigmentation patterns were noted.

Results and Discussion

Most *Ae. aegypti* that were heterozygous for *cinnabar* in a *kh^w* genetic background usually had uniformly pigmented eyes, although the degree of pigmentation was line-dependent and varied as a function of the position of integration of the *cinnabar*-containing gene vector (Fig. 1B). Of the 1,232 heterozygous insects examined, 27 individuals had mosaic patterns of eye pigmentation. These mosaic animals were collected from each of the six families of insects from which heterozygotes were produced. The patterns of mosaicism varied, but notably eyes were observed to have pigmented and unpigmented regions of ommatidia. Some mosaic eyes had patches of dark red and light red ommatidia (Fig. 1E). On one occasion, a gynandromorph was recovered that had one-half of the head consisting of male tissue as indicated by the shape of the antenna and proboscis (Craig and Hickey 1967) (Fig. 1C). The eye on the male half of the head was red (*cn⁺*), whereas the eye on the female side was white (*kh^w/kh^w*) (Fig. 1D).

These data show that in a genetic background homozygous for the *kh^w* allele, the *cinnabar* gene behaves as a cell autonomous marker. Despite the presence of a large number of cells (one-half of the organism in the case of the gynandromorph) expressing wild-type *kynurenine hydroxylase*, ommatidia with a homozygous mutant genotype had a mutant phenotype. Although the cell autonomy of *kynurenine hydroxylase* was unexpected based on the tissue transplantation studies of Beadle and Ephrussi (1936), it was not unprecedented. Paton and Sullivan (1978), while screening *D. melanogaster* for ethyl methane-sulfonate (EMS)-induced *kynurenine hydroxylase* mutants in a genetic background homozygous for the mutation *red*, found that >80% of the 122 phenotypically mutant F₁ individuals contained mosaic eyes, indicating that *cinnabar* can be cell autonomous under certain conditions (Paton and Sullivan 1978). EMS induces mutations postmeiotically, and mosaics are thought to arise after mutagenesis of one strand of a DNA duplex. After fertilization and DNA replication, a population of cells with mutated chromosomes will

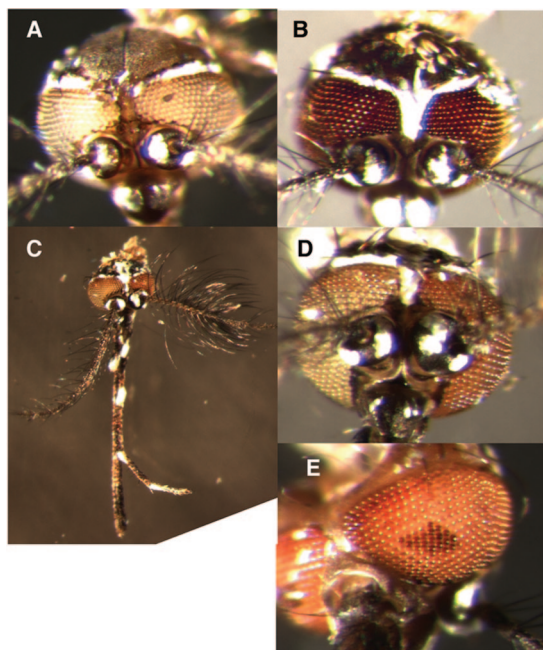


Fig. 1. Photograph of an individual homozygous for *kh^w* (A); an individual from line 40D that is homozygous for *kh^w* and a *piggyBac* gene vector expressing the *D. melanogaster cn* gene (B); the head of a gynandromorph arising from a transgenic *kh^w* individual (from line 40L) heterozygous for a *piggyBac* gene vector expressing the *D. melanogaster cn* gene (C); a close-up of the head shown in C showing the male side of the head with pigmented ommatidia and the female side with white ommatidia (D); and the head of a transgenic *kh^w* individual heterozygous for a *piggyBac* gene vector expressing the *D. melanogaster cn* gene in a clone of cells that developed into ommatidia with a pigmented phenotype (E).

coexist with a population of nonmutated chromosomes (Jenkins 1967). Paton and Sullivan (1978) proposed that the autonomy of *cinnabar* in mosaic flies could be explained by irreversible uptake and conversion of 3-hydroxykynurenine in the Malpighian tubules of larvae, resulting in the depletion of the hemolymph pool of 3-hydroxykynurenine. During pupation, when pigment uptake takes place, they proposed that there is not enough 3-hydroxykynurenine for pigment synthesis by the mutant eye tissue (Paton and Sullivan 1978). This is a possible explanation for the observed autonomy of *cinnabar* in transgenic mosquitoes. Alternatively, 3-hydroxykynurenine may not be secreted in transgenic mosquitoes and therefore may not be available in the hemolymph.

The genetic mechanisms by which mosaic *Ae. aegypti* arose in this study are not clear. Somatic recombination or somatic transposition of the integrated *piggyBac* vector containing the *cinnabar* transgene is expected to have resulted in twin spots with one clone lacking the *cinnabar* transgene (and having a white phenotype) and the other possessing two copies (and having a dark red phenotype). Somatic transposition of the *cinnabar*-containing *piggyBac* vector seems un-

likely because there was no known source of *piggyBac* transposase in the *cinnabar* heterozygotes, and independent experiments specifically designed to induce somatic and germline movement with functional *piggyBac* transposase have failed to detect a single transposition event (unpublished data). In addition, twin spots were never observed, which is inconsistent with mitotic recombination being responsible for the observed mosaicism. Mosquito gynandromorphs are thought to result from double fertilization and the fusion of two sperm nuclei with two products of female meiosis in a single egg (Craig and Hickey 1967).

Regardless of the mechanism by which mutant clones arose, it is clear that the *cinnabar* gene behaves autonomously under these conditions. This type of behavior potentially increases the versatility of *cinnabar* and permits its use in ways analogous to those described for the *white* gene of *D. melanogaster*. For example, the cell autonomous behavior of *kynurenine hydroxylase* could facilitate the analysis of transposable element mobility in *Ae. aegypti* by providing a means by which somatic transposition events could be detected in eye tissue. This would, under some conditions, eliminate the need to rely on methods requiring the detection of germline transposition events and could facilitate efforts to modify and improve existing insect gene vectors.

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