

# Stable transformation of the yellow fever mosquito, *Aedes aegypti*, with the *Hermes* element from the housefly

NIJOLE JASINSKIENE\*<sup>†</sup>, CRAIG J. COATES\*, MARK Q. BENEDICT<sup>‡</sup>, ANTHONY J. CORNEL<sup>‡§</sup>,  
CRISTINA SALAZAR RAFFERTY<sup>‡</sup>, ANTHONY A. JAMES\*, AND FRANK H. COLLINS<sup>‡¶</sup>

\*Department of Molecular Biology and Biochemistry, Bio Sci II, Room 3205, University of California, Irvine, CA 92697-3900; and <sup>‡</sup>Division of Parasitic Diseases, Centers for Disease Control and Prevention, 4770 Buford Highway, Chamblee, GA 30341-3724

Communicated by John H. Law, University of Arizona, Tucson, AZ, December 29, 1997 (received for review November 19, 1997)

**ABSTRACT** The mosquito *Aedes aegypti* is the world's most important vector of yellow fever and dengue viruses. Work is currently in progress to control the transmission of these viruses by genetically altering the capacity of wild *Ae. aegypti* populations to support virus replication. The germ-line transformation system reported here constitutes a major advance toward the implementation of this control strategy. A modified *Hermes* transposon carrying a 4.7-kb fragment of genomic DNA that includes a wild-type allele of the *Drosophila melanogaster cinnabar (cn)* gene was used to transform a white-eyed recipient strain of *Ae. aegypti*. Microinjection of preblastoderm mosquito embryos with this construct resulted in 50% of the emergent G<sub>0</sub> adults showing some color in their eyes. Three transformed families were recovered, each resulting from an independent insertion event of the *cn*<sup>+</sup>-carrying transposon. The *cn*<sup>+</sup> gene functioned as a semidominant transgene and segregated in Mendelian ratios. *Hermes* shows great promise as a vector for efficient, heritable, and stable transformation of this important mosquito vector species.

The recent application of molecular biological tools to the analyses of insect vectors of disease and vector–parasite interactions has required the development of efficient methods for germ-line transformation (1, 2). Although there have been preliminary reports of transformation (3–5), and a number of methods have been used for transient expression of gene products (6–8), a method for efficient, heritable, and stable integration of exogenous DNA into a mosquito genome has yet to be developed. Such a tool would enable study of the effects of various synthetic genes on parasite development in the insect and could contribute to the development of novel methods for the control of diseases caused by mosquito-borne pathogens (1).

Recently, it was shown that the class-II transposon *Hermes*, a member of the *hAT* family of elements isolated from the housefly, *Musca domestica*, is capable of plasmid-to-plasmid mobility in the embryonic soma of the yellow fever mosquito, *Aedes aegypti* (9). Here we report the results of experiments performed to determine if the observed mobility would translate into a method for the insertion of exogenous genes into the chromosomes of the whole organism. A critical feature of these experiments was the use of a marker gene that allowed the rapid and unequivocal detection of potentially transformed mosquitoes by screening for complementation of a mutant gene with an eye-color phenotype. Ommochrome pigments produce the deep purple color of the adult mosquito eye. Ommochromes are synthesized in a well-characterized series of enzymatically catalyzed steps from the precursor amino acid, tryptophan (10). Null mutations in two of the enzymes in

this pathway, tryptophan oxygenase and kynurenine hydroxylase, result in eye color mutant phenotypes in the adults of many insects (10, 11). An *Ae. aegypti* mutation, *white-eye* (designated *w*), was originally described by Bhalla (11) as a defect in the kynurenine hydroxylase that converts kynurenine to 3-hydroxykynurenine. The corresponding gene identified by this mutation was postulated to be homologous to the wild-type *cinnabar (cn)* gene described in *Drosophila melanogaster*. The product of the reaction, 3-hydroxykynurenine, is non-cell-autonomous and can diffuse from one cell to another. Therefore, we were able to show with a transient assay that the *D. melanogaster cn*<sup>+</sup> gene can complement the white-eye phenotype of the Bhalla *Ae. aegypti w* mutant strain (12). Microinjection of white-eye *Ae. aegypti* embryos with a 4.7-kb fragment of *D. melanogaster* genomic DNA that includes a wild-type copy of the *cn* gene (13) resulted in some adult mosquitoes with partial restoration of eye color. We have adopted the designation *kynurenine hydroxylase-white (kh<sup>w</sup>)* for the *Ae. aegypti* mutation described by Bhalla to distinguish this gene from others which when mutated may also cause a white-eye phenotype. Here we report the successful development of a germ-line transformation system for *Ae. aegypti* with the *Hermes* transposon in conjunction with the *cn*<sup>+</sup> gene.

## MATERIALS AND METHODS

**Plasmid Construction.** The transformation vector pH[*cn*], which includes the *cn*<sup>+</sup> gene fragment from *D. melanogaster* (13) flanked by the terminal inverted repeats of the *Hermes* transposon, was developed as follows. All *Hermes* fragments have been described by Warren *et al.* (14). A 1.6-kb *EcoRI/BamHI* fragment containing ≈0.8 kb of the left arm of *Hermes* and 0.8 kb of flanking *Musca domestica* genomic DNA (derived from the E1 fragment) was ligated into *EcoRI/BamHI*-digested pUC19. The unique *EcoRI* site was eliminated by *EcoRI* digestion and filled by using the Klenow DNA polymerase. This plasmid was amplified by using a long reverse oligonucleotide primer (5'-CACAGGAAACAGCTATGAC-CATG-3') and a second primer complementary to the region at the 5' end of the *LacZ* gene of pUC19 (5'-GCGGTACCT-GGCCAACGCGCGGGGAGAGGC-3'), which added a unique *KpnI* site. This product and a 0.8-kb fragment containing 0.5 kb of the right half of *Hermes* and ≈0.3 kb of flanking genomic DNA (derived from the B6 fragment) were

Abbreviations: *cn*, *cinnabar*; *kh<sup>w</sup>*, *kynurenine hydroxylase-white*; LH, left hand probe.

A commentary on this article begins on page 3349.

<sup>†</sup>Permanent address: Institute of Biochemistry of the Academy of Sciences of Lithuania, Mokslininkų 12, 2600 Vilnius, Lithuania.

<sup>§</sup>Present address: University of California, Mosquito Control Research Laboratories, Kearney Agricultural Experimental Field Station, 9240 South Riverbend Avenue, Parlier, CA 93648.

<sup>¶</sup>To whom reprint requests should be sent at the present address: Department of Biological Sciences, Galvin Life Sciences, University of Notre Dame, Notre Dame, IN 46556. e-mail: Frank.H.Collins.75@nd.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

© 1998 by The National Academy of Sciences 0027-8424/98/953743-5\$2.00/0  
PNAS is available online at <http://www.pnas.org>.

digested with *SalI/KpnI* and ligated. The resulting plasmid contained unique *BamHI* and *SalI* sites between the two arms into which the *LacZ* alpha peptide of pBluescript SK (Stratagene) was inserted as a *BglII/XhoI*-digested fragment produced by PCR by using the primers, 5'-GCAGATCTAT-CAGGGCGATGGCC-3' and 5'-GCCTCGAGCTGGCAC-GACAGGTTTCCCG-3'. This plasmid, pH[LacZ], was modified further by cutting with *XbaI* and *SacII*, and inserting a 4.7-kb *XbaI/SacII* fragment of *D. melanogaster* genomic DNA containing a wild-type copy of the *cn* gene (13). The resulting plasmid pH[*cn*] and the transposase encoding helper plasmid pSHHH1.9 (15) were purified through a cesium chloride gradient by ultracentrifugation, washed five times with isopropanol saturated with NaCl, and precipitated in three volumes of 70% ethanol. The dried pellet was resuspended in sterile, distilled H<sub>2</sub>O.

**Embryo Microinjection.** Embryos homozygous for the *kh<sup>w</sup>* mutation were collected and prepared for microinjection in the manner described by Morris (16). Embryos were injected with a solution of the plasmids, pH[*cn*] and pSHHH1.9, with a final concentration of 0.5 mg/ml and 0.3 mg/ml, respectively, in 5 mM KCl and 0.1 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 6.8). Microinjected G<sub>0</sub> embryos were exposed to a heat shock of 39–41°C for 1 h to induce the expression of the *Hermes* transposase, which is under the control of the *D. melanogaster hsp70* gene promoter. Embryos were then placed at 27°C, 80% relative humidity, and estivated for 5 days before hatching.

**Mosquito Rearing.** General aspects of mosquito rearing are described by Munstermann (17). The *kh<sup>w</sup>* strain was obtained from the University of Notre Dame *Ae. aegypti* stock center in 1992 by the senior author. In the course of validating that the *kh<sup>w</sup>* strain contained the same mutation described by Bhalla (11), several thousand individuals were examined without detecting any segregation of colored eyes. Individual G<sub>0</sub> males with colored eyes were mated with 15 *kh<sup>w</sup>* strain virgin females. Single G<sub>0</sub> females with colored eyes were mated with 3–5 *kh<sup>w</sup>* strain males. Males and females with white eyes were mated together in several pools. Females were allowed to feed on blood and the G<sub>1</sub> eggs collected. The adult mosquito eye starts differentiation during the later larval instars and pigment is readily visible by the late fourth instar. Phenotypic differences in eye color among the *kh<sup>w</sup>* strain, wild-type mosquitoes, and putative transformants may be scored at the late fourth instar; however, routine screening of eye color was done on adults.

**Southern Blot Analysis.** Southern blot analyses were done as described in Sambrook et al. (18). The various probes that were used are illustrated in Fig. 2A. The *Hermes* left-hand probe (LH) was made by gene amplification of a 0.76-kb DNA fragment with the following primers: 5'-GATACTTAT-GCGCCACTTGC-3' and 5'-CCGACAATCTCGTAC-CACCC-3', with pH[*cn*] as the template. The *cn* probe (*cn<sup>+</sup>*) was the complete 4.7-kb *XbaI/SacII* *D. melanogaster* fragment purified from pH[*cn*]. Probes to the *M. domestica* genomic DNA flanking the *Hermes* element in pH[*cn*] (MD) were made by gene amplification of the left-hand sequence (E1) with the following primers, E1REV, 5'-ACAGACTGCATTGCAGT-TGG-3', and E1FOR, 5'-AAAGCTGGTACCAGATCTGC-3', and the right-hand sequence (B6) with the primers B6REV, 5'-ATCTCCGAACCTCGATCTAAG-3', and B6FOR, 5'-GGTCAAACCGTGCCTAGTCC-3'. By using these primers and pH[*cn*] as template DNA, probes of 0.9 and 0.21 kb in length were produced for the left- and right-hand sequences, respectively.

## RESULTS

**Germ-Line Transformation and Genetic Analysis.** A total of 925 preblastoderm embryos were microinjected with pH[*cn*] and the helper plasmid, pSHHH1.9, and subsequently heat-shocked for 1 h. *Hermes* transposase produced from

pSHHH1.9 can act in trans to mobilize the *Hermes* construct in pH[*cn*]. A total of 120 adults (G<sub>0</sub> generation) resulted from the microinjected embryos, and of these, 60 (31 males, 29 females) showed color in their eyes. These mosquitoes were used as single founders of families by mating them with homozygous *kh<sup>w</sup>* individuals. The 60 white-eyed G<sub>0</sub> adults were mated together in pools. Only 24 (40%) of the single founder families produced G<sub>1</sub> progeny. Poor fertility and fecundity were also observed in the pooled white-eyed G<sub>0</sub> families. Either late fourth instar larvae or adult G<sub>1</sub> progeny were screened visually for changes in eye color that might result from integration of the *cn<sup>+</sup>* transgene into the germ line of a G<sub>0</sub> mosquito. Four of the founder families, 32, 35, 36, and 64, and one of the pools produced G<sub>1</sub> progeny with colored eyes (Table 1). The eye colors were all different, varying from light to dark red. Two families, 32 and 36, showed mosaicism in their eye color. Families 35 and 64 produced multiple G<sub>1</sub> progeny with colored eyes, a "cluster" phenomenon observed in other transposon-mediated transformation experiments with *D. melanogaster* and the Medfly, *Ceratitis capitata* (19, 20). A total of 63 G<sub>1</sub> progeny with colored eyes were found in ≈6,800 mosquitoes screened.

Test crosses were set up with colored-eyed G<sub>1</sub> mosquitoes by crossing them to homozygous *kh<sup>w</sup>* individuals. In the case of families 35 and 64, where multiple colored eye G<sub>1</sub> animals were available, intercrosses also were made between colored-eyed siblings. In the test crosses, families 32 and 36 failed to produce G<sub>2</sub> progeny with colored eyes. However, families 35 and 64, and the three colored-eye G<sub>1</sub> mosquitoes from the pooled G<sub>0</sub> animals produced G<sub>2</sub> progeny distributed in phenotypic classes with numbers consistent with the conclusion that each contains a single insertion capable of segregation (Table 2).

Family 64 produced colored-eye progeny that could be interpreted to result from complementation by a gene with a semidominant effect. Three distinct eye-color phenotypes, dark red, medium red, and white, could be seen in the progeny of the intercross, and these were interpreted to result from the homozygous dominant (two copies of the transgene), heterozygous (one copy of the transgene), and homozygous recessive (no copies of the transgene), progeny respectively (Fig. 1, Table 2).

The analysis of the G<sub>2</sub> progeny of family 35 was made more complex by the presence of at least four distinct eye color phenotypes. The lighter colored eyes were remarkable in that they showed mosaic patches of darker facets among a lighter pink background eye color. However, dividing the animals into two phenotypic classes, colored eyes and white eyes, resulted in numbers consistent with a 3:1 ratio also indicative of a single integration event (Table 2).

**Southern Blot Analysis.** Southern blot analyses were performed on genomic DNA isolated from families 35, 64, and the pool to determine if the presence of DNA fragments corresponding to the transgene was correlated with complementation of the phenotype and if these fragments had the structure characteristic of a transposition event. Three types of DNA fragments were used as probes in the Southern blot analysis

Table 1. G<sub>0</sub> families showing colored eyes in the G<sub>1</sub> progeny

G <sub>0</sub> family	No. of G <sub>1</sub> with colored eyes	No. of G <sub>1</sub> with mosaic eyes	Total G <sub>1</sub> screened
32	0 (0%)	1 (0.2%)	357
35	27 (56%)	0 (0%)	48
36	0 (0%)	1 (0.8%)	117
64	31 (19%)	0 (0%)	163
Pool*	3 (11%)	0 (0%)	26

\*The three G<sub>1</sub> progeny with colored eyes were mated separately to *kh<sup>w</sup>* strain individuals and lines were established. Subsequent Southern analysis showed that all three G<sub>1</sub> progeny resulted from a single event, and G<sub>2</sub> offspring from these lines were combined.

Table 2. Results from test crosses and intercrosses of G<sub>1</sub> colored-eye progeny of families 35 and 64, and the pool

Experiment	Family	Phenotypic class	No. of animals	Total	$\chi^2$
Test cross*	35	Colored-eye	142	253	3.78†
		White-eye	111		
	64	Colored-eye	39	85	0.57†
		White-eye	46		
	Pool	Colored-eye	121	282	5.67†
White-eye		161			
Intercross‡	35	Colored-eye	133	169	1.23§
		White-eye	36		
	64	Dark red-eye	28	139	5.24¶
		Red-eye	65		
		White-eye	48		

\*G<sub>1</sub> colored-eye mosquito crossed with homozygous recessive *kh<sup>w</sup>*.

†Phenotypic distributions are consistent with 1:1 segregation of a single integrated marker gene as evaluated by  $\chi^2$ , df = 1.

‡G<sub>1</sub> colored eye mosquitoes crossed with one another.

§All colored-eye phenotypes were treated as equal for family 35, and the phenotypic distributions are consistent with 3:1 segregation of a single integrated marker gene as evaluated by  $\chi^2$ , df = 1.

¶Phenotypic distributions are consistent with the 1:2:1 ratio of genotypes as evaluated by  $\chi^2$ , df = 2.

(Fig. 2A). A 0.76-kb fragment, LH, complementary to the left-hand end of *Hermes* was used to verify the presence of the inverted repeat region of the transformation construct. The 4.7-kb fragment, *cn<sup>+</sup>*, of *D. melanogaster* DNA containing the wild-type copy of the *cn* gene was used to verify the presence of the marker gene. Two probes, MD, were made by gene amplification of the *M. domestica* DNA flanking the *Hermes* element in pH[*cn*]. These fragments were used to verify that the integrations into the genome did not include the flanking plasmid DNA, as would be expected of precise excision and transposition.

Genomic DNA was digested with *Xba*I, which cuts once in the putative transposed fragment, or with *Xba*I and *Sac*II, which results in the precise excision of the 4.7-kb fragment containing the *D. melanogaster cn<sup>+</sup>* gene (Fig. 2A). Families 35, 64, and the pool all have unique hybridizing fragments of  $\approx$ 12, 11 and 10 kb, respectively, in the *Xba*I-digested DNA hybridized with the LH or *cn<sup>+</sup>* fragments (Fig. 2B and C). Additional strong hybridizing fragments were seen in both experimental and control DNAs when using the LH fragment (Fig. 2B). Family 64 and the pool have the expected *Xba*I/

*Sac*II signal at 4.7 kb representing the internal *cn<sup>+</sup>* fragment. However, family 35 shows an anomalously smaller signal suggesting loss of DNA within this internal fragment (Fig. 2C). Diagnostic digests with *Sty*I and hybridization with the 4.7-kb fragment show loss of DNA in the portion of the *D. melanogaster* genomic fragment that is outside the coding region and to the 5' end of the *cn* gene (data not shown). Hybridization to *Xba*I-digested genomic DNAs with the MD probes failed to produce a signal (Fig. 2D).

## DISCUSSION

These data indicate that transformation constructs based on the *Hermes* transposable element can integrate into the genome of the mosquito *Ae. aegypti* in an efficient and heritable manner. Three stable independent insertion events were recovered, two of which were from single founder families, resulting in a transformation efficiency of  $\approx$ 8% (2/24) of single fertile G<sub>0</sub> founders or 0.3% (3/925) when expressed in terms of events per injected embryo. The transgenic lines have been stable through more than 10 generations at the time of submission of this report (data not shown).

In addition, we have confirmed that a wild-type allele of the *cn* gene from *D. melanogaster* functions as a semidominant transgene when introduced into the *kh<sup>w</sup>* strain of *Ae. aegypti*. The transgenic phenotype is readily observed, and it is possible in family 64 to discriminate between homozygous and heterozygous animals. It is quite probable that the *D. melanogaster cn<sup>+</sup>* gene also can be used as a transformation marker in other Diptera, and possibly other orders that contain species with kynurenine hydroxylase mutants. Of particular note, both colored-eyed G<sub>0</sub> founders (represented by the single families) and white-eyed G<sub>0</sub> founders (represented by the pool) produced transformed families. Therefore the presence or absence of color in a G<sub>0</sub> animal is not an accurate indication that it has been genetically transformed.

Genetic analysis of G<sub>1</sub> families showing complementation demonstrated that the changes in eye color were heritable and could segregate as Mendelian factors, suggesting that the transformation events were caused by insertions in chromosomes. The results of test crosses revealed that the three families produced G<sub>2</sub> progeny in the 1:1 phenotypic ratio expected for a single chromosomal insertion of the transgene. However the insertion observed in the pool family appears to have occurred in a chromosomal region that results in a reduction in the number of colored-eyed individuals observed.

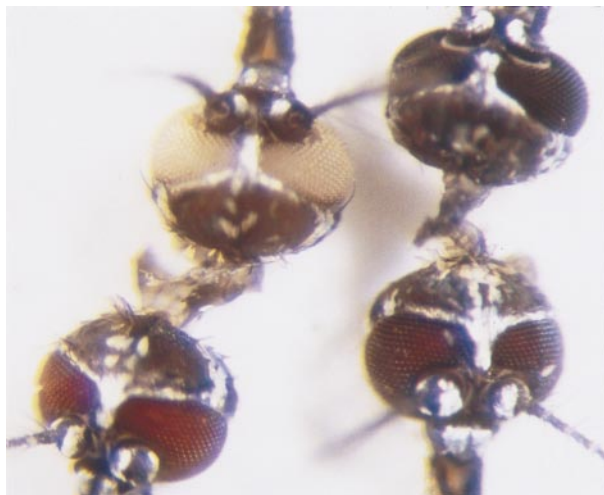


FIG. 1. Eye color phenotypes resulting from the G<sub>1</sub> intercross of family 64. Animals were photographed with incident light under a dissecting microscope. (Upper Left) *kh<sup>w</sup>* recipient strain white eye phenotype. (Upper Right) Wild-type eye color. Transformed members of family 64 carrying two copies (Lower Right) and one copy (Lower Left) of an insertion.

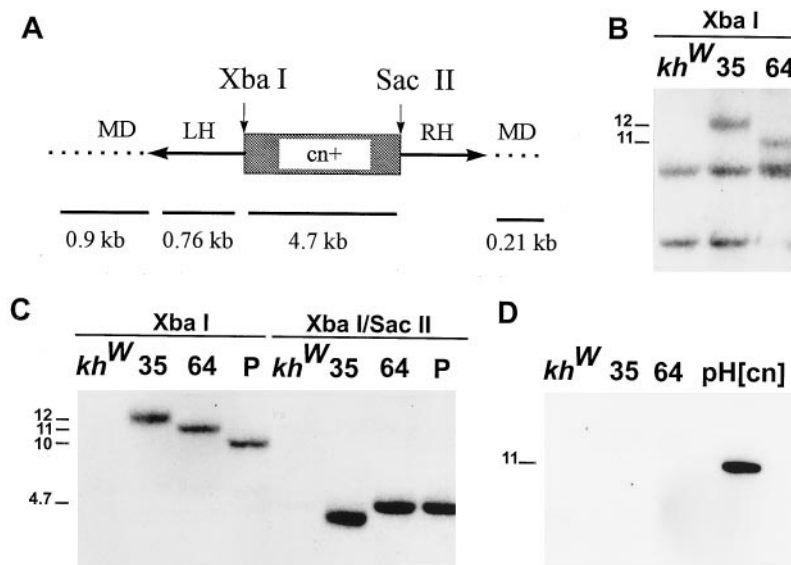


FIG. 2. (A) A representational map of the partial structure of the *Hermes* construct, pH[*cn*]. The *Hermes* left (LH) and right (RH) inverted terminal repeats (arrows) flank the 4.7-kb genomic fragment of *D. melanogaster* DNA carrying a wild-type copy of the *cinnabar* gene (*cn*<sup>+</sup>) (boxed region). This potentially mobile portion of the pH[*cn*] construct is flanked by *M. domestica* genomic DNA (MD, dotted lines). The positions of two restriction endonuclease-cleavage sites (*Sac*II and *Xba*I) are indicated. The relative extents and sizes of the DNA fragments used as probes in the Southern analyses are shown below the map (numbers and solid lines). (B) Southern analysis of genomic DNA isolated from G<sub>1</sub> progeny of transformed families 35 and 64, and the control recipient strain, *kh*<sup>W</sup>, digested with *Xba*I, which cuts once in the integrated fragment, hybridized with the 0.76-kb LH-labeled fragment. Unique hybridization signals associated with insertions in families 35 and 64 are seen at 12 and 11 kb, respectively. Lower molecular weight signals corresponding to cross-hybridizing fragments are seen in the transformed families and recipient strain. (C) Southern blot analysis of genomic DNA isolated from G<sub>2</sub> mosquitoes of families 35, 64, and the pool (P), and the recipient strain digested with either *Xba*I or *Xba*I/*Sac*II, and hybridized with the 4.7-kb *cn*<sup>+</sup> labeled fragment. High molecular weight signals corresponding to fragment lengths of 12, 11, and 10 kb are seen in the *Xba*I digests of DNA from families 35, 64, and the pool, respectively. The expected 4.7-kb fragment size is evident in the *Xba*I/*Sac*II digest of DNA from family 64 and the pool. However, a truncated signal at 4.5 kb is seen in family 35 DNA, indicating loss of some DNA. (D) Southern blot analysis of genomic DNA isolated from G<sub>2</sub> mosquitoes of families 35, 64, and the recipient strain, *kh*<sup>W</sup> digested with *Xba*I, and hybridized with the MD-labeled fragments. The positive control is *Xba*I-digested pH[*cn*].

The results of intercrosses performed for families 35 and 64 also were consistent with a single chromosomal insertion of the transgene. Family 35 has an interesting insertion that results in variable expression of the phenotype, reminiscent of either position-effect variegation or somatic excision of the transposon (21). Due to this effect it was not possible to determine which animals were homozygous or heterozygous for the transgene; therefore the results were evaluated based on a 3:1 ratio of colored eye/white eye phenotypic segregation. For family 64 the distribution of phenotypes produced the expected dark red eye/medium red eye/white eye ratio (1:2:1) for a semidominant transgene.

Southern blot analyses of the transformed lines confirmed the presence of the transgene and provided further evidence for integration into mosquito chromosomes. The results observed with the LH and *cn*<sup>+</sup> probes indicate that hybridizing fragments containing both the *Hermes* element and the *cn*<sup>+</sup> marker gene were integrated. The size and number of the observed fragments were consistent with a single insertion of the transformation construct into the genome. The different sizes of the *Xba*I fragments from each of the lines demonstrates that they are independent insertions. The single unique hybridizing fragment in each case demonstrates that only a single copy of the transgene was integrated. The lack of hybridization to MD probes is a result consistent with transposase-mediated insertion as only sequences delimited by the *Hermes* inverted terminal repeats would be expected to have transposed into the genome.

The nature of the DNA producing the common hybridizing signal with the LH probe has not been determined, but it may represent cross-hybridization of the probe with an endogenous *hAT* element. If such an endogenous *hAT* element does exist, activity of its transposase may have been responsible for a number of the results observed, including the small deletion in

family 35, the absence of transformants in the G<sub>2</sub> generation of families 32 and 36, as well as the observed mosaicism. Although the *cn*<sup>+</sup> gene product and its enzymatic substrate and product are thought to be cell nonautonomous, it appears that they can display "local autonomy," presumably as a result of the product of the enzyme reaction, 3'-hydroxy kynurenine, being immediately available for the next step in the pigment biosynthetic pathway and thus does not diffuse out of the cell.

We have presented both genetic and molecular evidence that we interpret to demonstrate that the observed phenotypic complementation is associated with single insertions of the transgene into mosquito chromosomes of three independent lines. Given the previous demonstration of the ability of the *Hermes* element to accurately transpose within the embryonic soma of *Ae. aegypti* (9), it is probable that the integration events observed in these experiments were also caused by a *Hermes*-mediated transposition event. However, *Hermes* integration events that included flanking plasmid sequences have been observed (A.A.J., unpublished data). Various protocols including inverse and universal PCR were performed to determine the nature of the junction between the integrated transgene and the mosquito genomic DNA. These attempts were unsuccessful and the characterization of these junction fragments is the basis of ongoing research.

The *Hermes* transposon coupled with the *cn*<sup>+</sup> marker gene and *kh*<sup>W</sup> recipient strain, along with the similar development of *mariner*-based transformation in *Ae. aegypti* (22), provide powerful tools for the molecular analysis of this major vector mosquito species. They also make it possible to develop stable lines of virus-resistant mosquitoes (8) that can be used in genetic control strategies to interfere with the transmission of pathogenic agents.

We acknowledge the pioneering efforts of Drs. Alison C. Morris and Kurt Yardley in the development of mosquito transgenesis, Ms. L.

Miyashiro and Ms. P. Nguyen for mosquito care, Dr. A. J. Howell for the gift of the *D. melanogaster* *cn<sup>+</sup>* gene, Drs. D. O'Brochta and P. Atkinson for the *Hermes* plasmids, Dr. D. Prager for continuous support of the project, and L. M. Olson for help in preparing the manuscript. This research was supported by grants from the National Institutes of Health (AI29746 and AI32730 to A.A.J.), the United Nations Development Programme/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases (to F.H.C. and M.Q.B.), and the John D. and Catherine T. MacArthur Foundation (to F.H.C. and A.A.J.).

1. Collins, F. H. & James, A. A. (1996) *Sci. Med.* **3**, 52–61.
2. Meredith, S. E. O. & James, A. A. (1990) *Ann. Parasitol. Humaine Comp.* **65**, S1:113–118.
3. Miller, L. H., Sakai, R. K., Romans, P., Gwadz, R. W., Kantoff, P. & Coon H. G. (1987) *Science* **237**, 779–781.
4. McGrane, V., Carlson, J. O., Miller, B. R. & Beaty, B. J. (1988) *Am. J. Trop. Med. Hyg.* **39**, 502–510.
5. Morris A. C., Eggleston P. & Crampton J. M. (1989) *Med. Vet. Entomol.* **3**, 1–7.
6. Morris, A. C., Pott, G. B., Chen, J. & James, A. A. (1995) *Am. J. Trop. Med. Hyg.* **52**, 456–460.
7. Shotkoski, F., Morris, A. C., James, A. A. & French-Constant, R. H. (1996) *Gene* **168**, 127–133.
8. Olson, K. E., Higgs, S., Gaines, P. J., Powers, A. M., Davis, B. S., Kamrud, K. I., Carlson, J. O., Blair, C. D. & Beaty, B. J. (1996) *Science* **272**, 884–886.
9. Sarkar, A., Yardley, K., Atkinson, P. W., James, A. A. & O'Brochta D. A. (1997) *Insect. Biochem. Mol. Biol.* **27**, 359–363.
10. Beard, C. B., Benedict, M. Q., Primus, A. P., Finnerty, V. & Collins, F. H. (1995) *J. Heredity* **86**, 375–380.
11. Bhalla, S. C. (1968) *Mosq. News* **28**, 381–385.
12. Cornel, A. J., Benedict, M. Q., Salazar Rafferty, C., Howells A. J. & Collins, F. H. (1997) *Insect Biochem. Mol. Biol.* **27**, 993–997.
13. Warren, W. D., Palmer, S. & Howells, A. J. (1996–1997) *Genetica* **98**, 249–262.
14. Warren, W. D., Atkinson, P. W. & O'Brochta, D. A. (1994) *Genet. Res.* **64**, 87–97.
15. O'Brochta, D. A., Warren, W. D., Saville, K. J. & Atkinson, P. W. (1996) *Genetics* **142**, 907–914.
16. Morris, A. (1997) in *The Molecular Biology of Insect Vectors of Disease*, eds. Crampton, J. M., Beard, C. B. & Louis, C. (Chapman & Hall, London), p. 423–429.
17. Munstermann, L. E. (1997) in *The Molecular Biology of Insect Vectors of Disease*, eds. Crampton, J. M., Beard, C. B. & Louis, C. (Chapman & Hall, London), pp. 13–20.
18. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
19. Lidholm, D. A., Lohe, A. R. & Hartl, D. L. (1993) *Genetics* **134**, 859–868.
20. Loukeris, T. G., Livadaras, I., Arca, B., Zabalou, S. & Savakis, C. (1995) *Science* **270**, 2002–2005.
21. Henikoff, S. (1996) *BioEssays* **18**, 401–409.
22. Coates, C. J., Jasinskiene, N., Miyashiro, L. & James, A. A. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 3748–3751.