WHITE EYE, A NEW SEX-LINKED MUTANT OF *AEDES AEGYPTI* [1]

SATISH C. BHALLA [2]
Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556

Since the discovery of the first sex-linked eye color mutant, white, in *Culex pipiens* (Gilchrist and Haldane, 1947), a few other mutants affecting eye color have been isolated in mosquitoes. Among them are included a sex-linked, red (Wild, 1963) and an autosomal, ruby (Iltis *et. al.*, 1965) of *Culex pipiens*. Another autosomal, white, is known in *Culex tarsalis* (Barr and Myers, 1966). K. Hartberg (personal communication) of this laboratory has isolated a sex-linked, currant, in *Aedes mascarensis* which can be incorporated in the genetic background of *Aedes aegypti*. McClelland (1962–1966) has reported an autosomal, olive, and two sex-linked, red and rust, of *A. aegypti*. Recently another sex-linked mutant, white, of *A. aegypti* has been isolated in our laboratory, a summary report of which was presented elsewhere (Bhalla and Craig, 1965). The present paper deals with the isolation, description, linkage and biochemical basis of this mutant. Refer to Figures 1 through 4 to illustrate the description which follows.

ISOLATION AND DESCRIPTION. The mutant white-eye, designated as *w*, appeared spontaneously in the F_2 progeny of a single pair in the cross involving certain chromosome III markers—*min blt* ♀ (miniature-appendages and black-tarsi) X *fz blp co* ♂ (fuzzy, black-palp and compressed antennae).

The gene *w* blocks all pigmentation in compound eyes and ocelli. There exists a slight similarity between this mutant and the colorless phenotype. The colorless eye occurs in individuals homozygous for red, rust and olive. Colorless eye shows a translucent pale pinkish color in late pupal and adult stages, whereas the white-eye phenotype is white in all stages of life history and can be distinguished even in the late embryo. However, adults older than one month may show a slight pink color.

LINKAGE. The white females were crossed to wild-type males and the offspring were inbred. F_2 progeny segregated as follows:

wild-type females	276
wild-type males	618
white females	200
white males	59
Total progeny	1153

These data are compatible with the hypothesis that white is a sex-linked recessive factor with complete penetrance and uniform expressivity. The ratio of wild-type: white (894:259) was close to an expected 3:1. The χ^2 test gave a value of 3.957 (χ^2 is reduced to 3.824 on using Yate's correction) which is fairly close to $\chi^2_{(1).05}=3.841$. There was a deficiency of about 10 percent in recovery of white in the F_2. Only 259 white individuals were recovered against an expected 288. Perhaps some white died during development; hence, the viability of white was somewhat reduced.

The F_2 data, upon analysis according to the method described by Bhalla and Craig (in press), gives a recombination value of $17.4 \pm .4$ between white and the sex locus. Note that sex in *A. aegypti* is determined by a single pair of alleles, *M* and *m*, or a small chromosome segment. According to this notation male is the heterogametic sex (*Mm*) and female homogametic (*mm*).

Linkage of white to sex is further confirmed by the appearance of a bilateral gy-

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[2] Present Address: E—1/70, Sector 14, Chandigarh, India.

nander (Fig. 1) in the progeny of a cross

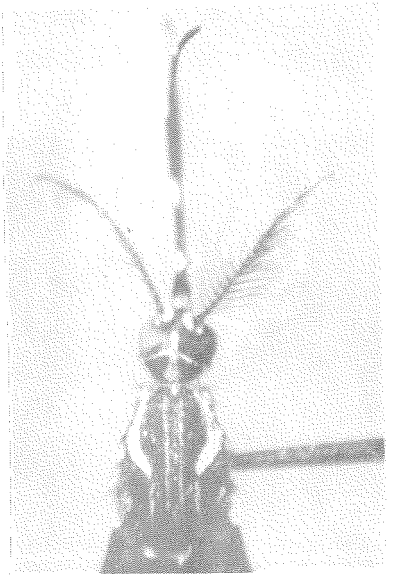

FIG. 1.—A bilateral gynander with white eye and female palp and antenna on left half and black eye and male palp and antenna on right half of the body.

$\frac{mw}{mw} \times \frac{mw}{M+}$. The left half of the gynander had white eyes and female palp, whereas the right half had a black eye and male palp. It could have been produced as a result of double fertilization by two sperms—one fertilizing the egg and the other a polar body (Fig. 5.) A similar hypothesis for gynander production has been proposed by Rai and Craig (1963).

GENETIC BLOCK IN TRYPTOPHAN METABOLISM. Two different types of pigmentary systems can be distingusihed in the insect eye—the ommochrome and the pterin. These systems differ in behavior towards solvents and in the time of formation during development (Daneel, 1941; Hadorn and Ziegler, 1958).

The ommochromes are found in the eyes of all arthropods (Butenandt *et al.*, 1958; Butenandt, 1959). There are two different types of ommochromes—ommatins and ommins. They differ in molecular weights. Their chemical structures and metabolic pathway leading to their formation have been worked out in a number of insects. Various steps in the pathway are shown in Fig. 6.

The proof that these compounds are on the pathway to ommochrome synthesis comes from the fact that they are not formed in various eye color mutants because of the lack of specific enzymes, synthesis of which is controlled by the normal alleles of mutated genes, e.g. v^+ (vermilion), cn^+ (cinnabar), st^+ (scarlet) of *Drosophila* and $w_1{}^+$ and $w_2{}^+$ (white) of *Bombyx*. Ommochrome formation may be induced either by injecting or feeding the mutants the necessary constituent involved in determination of eye color. Various steps in the pathway can also be demonstrated by various biochemical assays.

In order to see if the mutant *w* of *A. aegypti* results from a block in tryptophan metabolism, two kinds of experiments were performed:

(a) A brew of the wild-type (black-eyed) ground up larvae was fed to the white-eyed larvae. Sporadic dark brown spots appeared in the ommatidia of a few individuals (Fig. 2). This shows

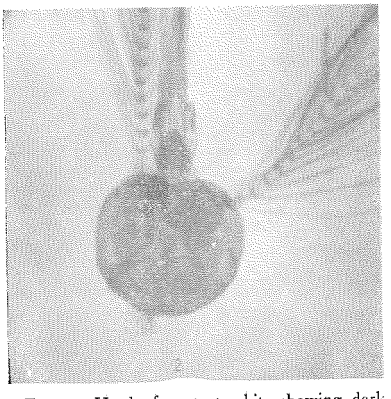

FIG. 2.—Head of mutant white showing dark spots in the eyes. The larvae were fed on ground up homogenate of wild-type larvae (X 30).

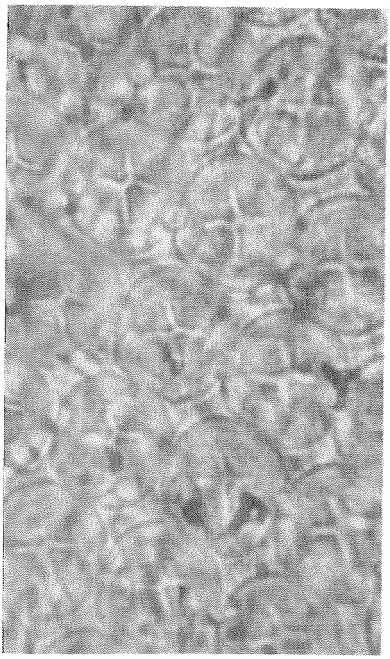

FIG. 3.—Facets of the normal (untreated) white eye (X 125).

that some dietary constituent involved in eye color determination was lacking in white and was provided by wild-type.

(b) In another series of experiments, various compounds involved in the pathway (Fig. 6), i.e., tryptophan, formylkynurenine, kynurenine and 3-hydroxyknurenine, were injected in the early white pupae. Results are shown in Table 1. A uniform darkening of the ommatidial walls was achieved with 3-hydroxykynurenine (Fig. 4). Presumably *w* blocks the

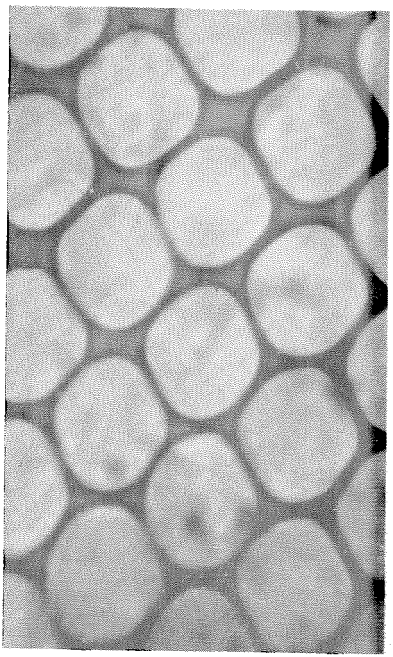

FIG. 4.—Facets of treated white eye. Pupae were injected with 3-hydroxykynurenine. Note the deposition of dark pigment on the ommatidial walls (X 125).

formation of 3-hydroxykynurenine from kynurenine, thus preventing the formation of ommochromes.

DISCUSSION. The mutant white seems to be widely distributed among insects. In addition to a series of white alleles in *Drosophila,* it has been reported in dipterans such as *Lucilia cuprina* (MacKerras,

TABLE 1.—Results of injecting early white-eyed pupae of *A. aegypti* with compounds from the Tryptophan cycle.

Compound injected	Concentration (% in water)	Number of individuals		
		Injected	Survived	Showing darkening of facets
Tryptophan	1.0	38	11	0
Formyl-L-Kynurenine Trihydrate	1.0	25	22	0
L-Kynurenine Sulfate H_2O	1.0	46	21	0
3-Hydroxy-Kynurenine	0.2	34	19	6

1933); *Phormia regina* (Dickler, 1943); *Culex molestus* (Gilchrist and Haldane, 1947); *Calliphora erythrocephala* (Tate, 1947); and *Musca domestica* (Zingron, 1955-quoted from Hiroyoshi, 1960). Ziegler (1961) lists a number of other non-dipteran insects which show this mutation. Among them are included *Ephestia kühniella, Bombyx mori, Periplanata americana* and so forth.

The phenotype white may be produced in a different variety of ways in different insects. For example, it may be the result of absence of "core granules," i.e., the ribonucleoprotein granules where the final steps of synthesis of both pteridines and ommochromes occur, as in the case of *Ephestia kühniella* (Hadorn and Kühn, 1953). On the other hand, "core granules" may be present as is the case in *D. melanogaster* (Ziegler and Janaicke, 1959) and *A. aegypti*, but the phenotype may still be white. In the latter cases the white phenotype may be the result of genetic blocks in the pathways involving the synthesis of pteridines and ommochromes.

A good example where pteridine synthesis is blocked is white *D. melanogaster*. The pteridines appear in small amounts

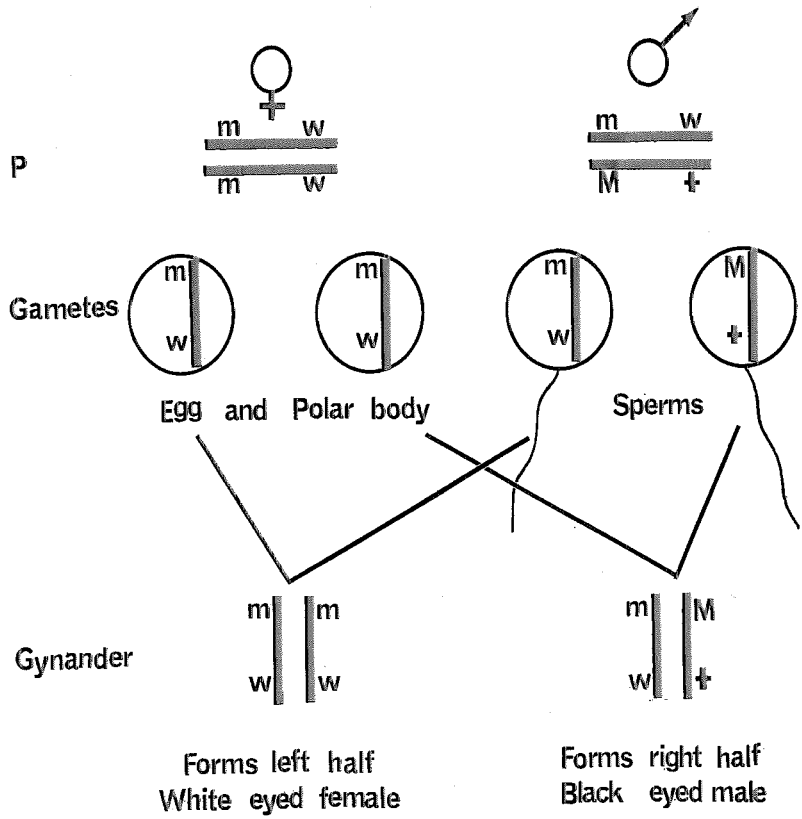

FIG. 5.—A scheme showing the probable mechanism by which a bilateral gynander, shown in FIG. 1, was produced. Note that the two egg products have similar genotypes whereas the two sperms carry different genotypes.

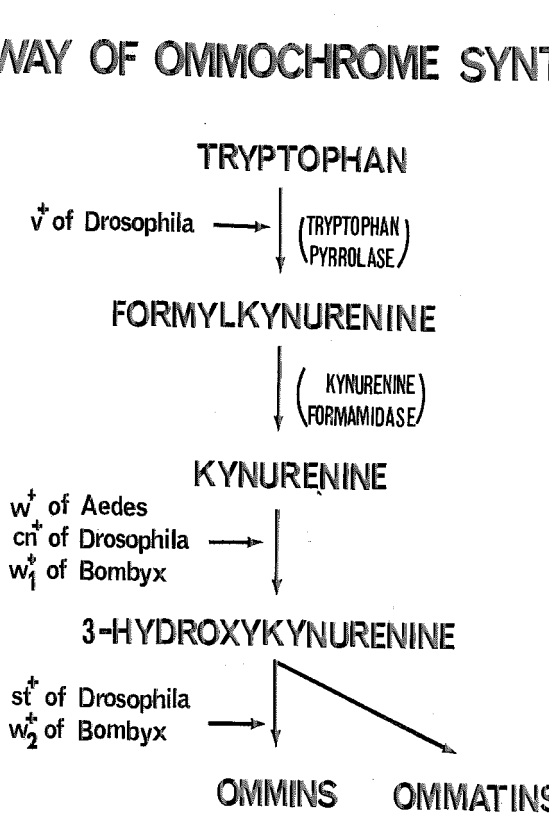

FIG. 6.—Pathway of tryptophan metabolism, leading to synthesis of ommochromes (adapted from Gilmour, 1965. Points of gene action already known in *Drosophila* and *Bombyx* are shown. Note that w^+ of *Aedes* regulates the conversion of kynurenine to 3-hydroxykynurenine.

in the 20-hour old pupae but disappear after about 50 hours (Hadorn and Ziegler, 1958). Apparently the "core granules" can neither convert the precursors of eye pterins—delivered by haemolymph—into eye pterins, nor convert the precursors into ommochromes. A number of examples where ommochrome synthesis is blocked are shown in Fig. 6, i.e., vermilion, cinnabar and scarlet of *Drosophila* and white of *Bombyx mori*. White of *Periplanata americana* and snow of *Apis mellifica* are also the results of similar genetic blocks.

Frequently the gene *w* may show pleiotropy and affect both the pathways, e.g., *Calliphora erythrocephala*. First, it blocks the conversion of kynurenine to 3-hydroxykynurenine thus preventing the synthesis of ommochromes. Second, it causes a shift in the equilibrium of yellow pterin ⇄tetra-hydrobiopterin, i.e., the relation of these two pteridines is reversed; wild-type shows much more tetrahydrobiopterin while white shows a threefold increase of yellow pterin (Autrum and Langer, 1958). The gene *w* of *A. aegypti* is similar to *C. erythrocephala* in that it prevents the conversion of kynurenine to 3-hydroxykynurenine. It also shows a pleiotropic effect in blocking synthesis of all pteridines except 2-amino-4-hydroxypteridine which appears in abnormally large quantities (Bhalla, 1966).

SUMMARY AND CONCLUSIONS

1. A new spontaneously-occurring mutant, white (*w*), was isolated in *Aedes aegypti*.

2. The gene *w* is a sex-linked recessive and shows complete penetrance and uniform expressivity. It belongs to linkage group I and is located at about 17 units from sex locus.

3. The mutant white blocks the ommochrome synthesis in compound eyes and ocelli. A genetic block, preventing the formation of 3-hydroxykynurenine from kynurenine, in the metabolic pathway leading from tryptophan to ommochrome formation, has been demonstrated.

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