RUST-EYE, A NEW MUTANT IN THE *AEDES (STG.) SCUTELLARIS* COMPLEX[1]

MILAN TRPIS

The Johns Hopkins University, Department of Pathobiology, Laboratories of Medical Entomology, School of Hygiene and Public Health, Baltimore, MD 21205

ABSTRACT. Rust-eye, a new mutant in the *Aedes scutellaris* complex of mosquitoes, is described. The mutation effects color of eyes in 3 developmental stages: larvae, pupae and adults. This spontaneous mutant was isolated from a laboratory colony of *Aedes (Stg.) kesseli* and is easily transferable to other closely related species of the Polynesian group of the *Ae. scutellaris* complex. The rust-eye character is controlled by a single recessive, sex-linked gene. The linkage distance between sex locus (*M/m*) and rust (*ru*) locus was estimated from backcrosses and F_2 progeny were determined to be 30.5 ± 3.8 map units. The rust-eye mutant has good survival. Penetrance is complete, and expressivity is variable in intensity.

Several species of the *Aedes scutellaris* complex of mosquitoes possess a high vectorial capacity for transmission of *Wuchereria bancrofti,* the agent of a highly debilitating human disease in the South Pacific. One member of this complex, *Ae. polynesiensis* Marks is the principal vector of filariasis throughout Polynesia. *Aedes tabu* Ramalingam and Belkin and *Ae. kesseli* Huang and Hitchcock are particularly important vectors of filariasis in the Tonga group of islands. Susceptibility, refractoriness and genetic relatedness of the *Ae. scutellaris* complex is poorly understood. Usefulness of morphological mutants in various genetic studies in other groups of mosquitoes has been demonstrated by several mosquito geneticists (Craig and Hickey 1967, Kitzmiller and Mason 1967, Laven 1967, Bhalla 1968, Baker and Sakai 1974, Barr 1975, Coluzzi and Kitzmiller 1975, Rai and Hartberg 1975, Tadano 1976, Asman 1977 and others).

In the *Ae. scutellaris* complex 3 apparently sex-linked mutations concerning the eyes were described: a white eye mutant (*w*), in *Ae. cooki* Belkin by Wade (1977), a scrimpy eye mutant (*sy*) in *Ae. cooki* (Trpis 1979a) and a red eye mutant in *Ae. kesseli* (*Ae.* tafahi sp. at that time) (Trpis 1979b). The purpose of this presentation is to describe a new mutant, rust-eye (*ru*). Inheritance, linkage and the relative location of the (*ru*) mutant to other known mutants on the sex chromosome will be worked out. The rust-eye (*ru*) is a very useful mutant because of its high survival, good penetrance and it is easily recognized. The aim of this study is to determine the mode of inheritance of *ru* mutation, its linkage group and a chromosome location.

MATERIALS AND METHODS

The rust-eye (*ru*) mutant was isolated from a laboratory colony of *Ae. (Stg.) kesseli,* a member of the *Ae. scutellaris* complex. The *Ae. kesseli* colony was formed from biting females collected by Dr. J. Hitchcock on the island of Tafahi in the Tonga group in 1970. *Aedes kesseli* is an autogenous species and is fully compatible in the laboratory with several autogenous species such as *Ae. cooki, Ae. tabu,* and unidirectionally compatible with many colonies of *Ae. polynesiensis, Ae. pseudoscutellaris* (Theobald) and other members of the *Ae. scutellaris* complex. Rust-eye (*ru*) is a spontaneously occurring mutant. Its eye pigment is reddish-brown, fairly regularly scattered in ommatidia of the compound eyes (oculi), giving them a rusty color character. This mutation is easily recognizable in larvae, pupae and adults (Fig. 1).

In these experiments, mosquitoes were maintained in an insectary at temperature

[1] This study was supported in part by NIH research grant No. AI 13396.

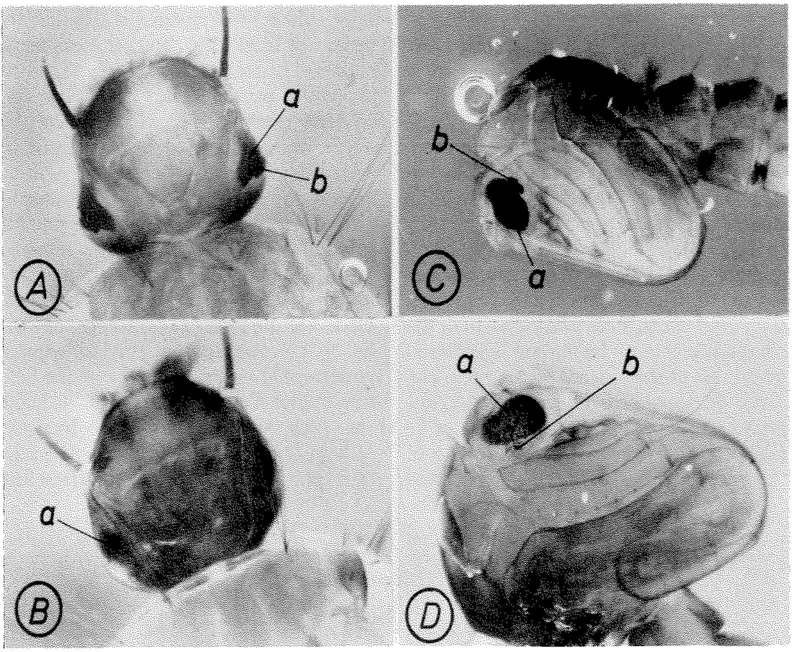

Fig. 1. Eye-characters of wild type and mutant larvae and pupae of *Aedes kesseli*.
A: Wild type larva,
B. Mutant larva,
C: Wild type pupa,
D: Mutant pupa.
a—Compound eye (Oculus); b—Simple eye (Ocellus)

26 ± 1°C and RH of 80 ± 10%. Photoperiod was maintained at 18 hr light and 6 hr dark using a sunrise/sunset simulator from TATRA Electronics Systems. Larvae were fed on a suspension of liver powder and adults were fed on honey mixed with cellulose fibers. Since *Ae. kesseli* is autogenous, females were fed on guinea pigs only for second and subsequent egg batches as required. Individuals for crosses were sexed and separated while in the pupal stage. Analysis and counting of the mutants and wild phenotype was also done in the pupal stage. Crosses were done usually within 48 hr after emerging. Any delay in crosses would cause oviposition of unfertilized eggs. Approximately 30 adult pairs were placed into carton cylindrical cages 180 mm in diam and 180 mm high with a sleeve opening on the side. After mass mating, females were separated into smaller cylindrical cages 90 mm in diam and 90 mm high, covered with netting, and provided with a shell-vial lined with paper towel and filled with tap water for oviposition. Eggs from individual females or pools of 5 females were reared separately in round white enamel pans, 30 cm in diameter and 10 cm deep.

RESULTS AND DISCUSSION

DESCRIPTION OF THE MUTANT STRAIN. The rust-eye (*ru*) mutation is manifested in 3 developmental stages: larva, pupa and adult. In normal larvae, the compound eyes (oculi) are located posterior to the antennae. The compound eyes consist of a large group of ommatidia (Fig. 1Aa). Below the compound eyes another small group of ommatidia (ocelli) are located (Fig. 1Ab). The compound eyes of the mutant larvae can be easily recognized. Ocelli are hardly recognizable in the mutant larvae (Fig. 1B). Boundaries of the compound eyes in normal pupae are very sharply delineated. A small patch of ommatidia (Fig. 1Cb) forming the ocelli is located dorsally to the compound eyes (Fig. 1Ca). Reddish-brown pigment of the compound eyes in the mutant is scattered over most of the surface of the eyes (Fig. 1Da). In the ocelli of pupae only few grains of reddish-brown pigment can be found (Fig. 1Db), thus the boundaries of the ocelli are not formed. In the adult stage, the eyes of normal males and females are black. The mutant lacks black pigment. Instead of black, brown-reddish pigment is present. Thus the mutant can be easily recognized in all developmental stages which might be advantageous in those circumstances when segregation of normal and mutant strains is desirable in early developmental stages. The mutant has uniform expression in both sexes.

Both wild type and the *ru* mutant are autogenous; the females do not take blood for the first batch of eggs, but will do so for the second and subsequent gonotrophic cycles. Females of the wild mosquitoes will lay an average 50.3±6.0 eggs per clutch (test of 157 females). Mutant females laid an average 47.6±9.0 eggs per clutch, (test of 113 females),

Table 1. Segregation of rust-eye (*ru*) mutants and wild type (±) mosquitoes.

Cross	Parental genotype: Female		Male	No. of families	Offspring–Phenotype: Females ru	Females +	Males ru	Males +	Total progeny	% recombination
A	ru m / ru m	×	+ m / + M	30	0	711	0	751	1462	—
B	+ m / + m	×	ru m / ru M	22	0	475	0	511	986	—
C	ru m / + m	×	ru m / + M	23	147	453	178	426	1204	29.6 ± 3.0
D	+ m / ru m	×	+ m / ru M	18	144	340	124	342	950	30.3 ± 3.4
E	ru m / ru m	×	ru m / + M	18	252	120	114	265	751	31.1 ± 3.6
F	ru m / ru m	×	+ m / ru M	21	231	468	506	211	1416	31.2 ± 5.4
G	+ m / + m	×	+ m / ru M	17	0	365	0	386	751	—
H	+ m / + m	×	ru m / + M	12	0	289	0	260	549	—
I	+ m / ru m	×	+ m / + M	18	0	396	0	429	825	—
J	+ m / ru m	×	ru m / ru M	13	156	142	145	159	602	—

which is not significantly different from wild type females. Some females may go through as many as 4 gonotrophic cycles, which brings up their fecundity to nearly 150 eggs. This is relatively low in comparison with anautogenous species of the *Ae. scutellaris* complex, where fecundity can be as high as 700–800 eggs per female.

MODE OF INHERITANCE. The data presented in Table 1, specifically cross A and B, indicate that rust-eye phenotype is recessive. Crosses C, D, E, and F suggest that rust is controlled by single pair of alleles. F_2 progeny resulting from selfing of F_1 gave 2 kinds of phenotypes, wild type and the mutant, with the ratio of approximately 3:1 (Table 1, cross C and D, Table 2, cross C and D). The backcrosses of the 2 types of F_1 male progeny to the homozygous rust-eye females gave both the wild type and mutant offspring in numbers that were not significantly different from a 1:1 ratio (Table 1, and Table 2, cross E and F). These backcross data as well as data from F_2 progeny (Table 1, crosses E, F and C, D respectively) indicate that the gene controlling the rust-eye character is about 30 map units from the sex locus (M/m). The χ^2 analysis of data concerning sex ratio, the 3:1 ratio of F_2 and the 1:1 ratio of backcrosses are given in Table 2.

Aedes kesseli interbreeds in the laboratory freely with some other autogenous species of the *Ae. scutellaris* complex, such as *Ae. cooki* and *Ae. tabu*. Thus the rust-eye mutant can be a very useful genetic marker also in species other than *Ae. kesseli*. Since anautogenous species of the *Ae. scutellaris* complex also interbreed, at least unidirectionally (Hitchcock and Rozeboom 1973, Hoyer and Rozeboom 1976, Trpis 1978) with *Ae. kesseli*, the rust-eye character can be transferred also to *Ae. polynesiensis*, *Ae. pseudoscutellaris*, and other members of the *Ae. scutellaris* group, and may become a useful tool in genetic studies in those species.

Table 2. Chi-square analysis (1 df) of crosses involving sex ratio, 3:1 of F_2, and 1:1 of backcrosses.

	Single factor					
	1:1 M,m		3:1 +/ru		1:1 +/ru	
Cross	χ^2	P	χ^2	P	χ^2	P
A	1.094	0.70	—	—	—	—
B	1.314	0.75	—	—	—	—
C	0.013	0.09	2.606	0.89	—	—
D	0.341	0.44	5.222	0.98	—	—
E	0.652	0.58	—	—	0.481	0.51
F	0.114	0.26	—	—	2.376	0.88
G	0.587	0.56	—	—	—	—
H	1.532	0.78	—	—	—	—
I	1.320	0.75	—	—	—	—
J	0.598	0.56	—	—	—	—

ACKNOWLEDGMENT

I express my gratitude to Miss Karen L. Parker for assistance in crossing experiments.

References Cited

Asman, M. 1977. Two sex-linked mutations in *Culex tarsalis*. J. Hered. 68:195–197.

Baker, R. H. and R. K. Sakai. 1974. Genetic studies on *Culex tritaeniorhynchus*. pp. 133–182 *in* R. Pal and M. J. Whitten, eds. The use of genetics in insect control. Elsevier Publishing Co., New York.

Barr, R. 1975. Mosquitoes and flies of genetic interest—*Culex*. pp. 347–375 *in* R. C. King, ed. Handbook of genetics. Vol. 3. Plenum Press, New York.

Bhalla, S. C. 1968. White eye, a new sex-linked mutant of *Aedes aegypti*. Mosq. News 28:380–385.

Craig, G. B. and W. A. Hickey. 1967. Genetics of *Aedes aegypti*. pp. 67–131 *in* J. W. Wright and R. Pal, eds. Genetics of insect vectors of disease. Elsevier Publishing Co., Amsterdam.

Coluzzi, M. and J. B. Kitzmiller. 1975. Mosquitoes and flies of genetic interest—Anopheline mosquitoes. pp. 285–309 *in* R. C. King, ed. Handbook of genetics. Vol. 3. Plenum Press, New York.

Hitchcock, J. C. and L. E. Rozeboom. 1973. Cross-breeding of *Aedes (S.) polynesiensis* Marks with an autogenous species of the *Ae. scutellaris* group. Bull. W.H.O. 49:367–370.

Hoyer, L. C. and L. E. Rozeboom. 1976. Inheritance of autogeny in the *Aedes scutellaris*

subgroup of mosquitoes. J. Med. Entomol. 13:193–197.
Kitzmiller, J. B. and G. F. Mason. 1967. Formal genetics of anophelines. pp. 3–15 *in* J. W. Wright and R. Pal, eds. Genetics of insect vectors of disease. Elsevier Publishing Co., Amsterdam.
Laven, H. 1967. Formal genetics of *Culex pipiens*. pp. 17–65 *in* J. W. Wright and R. Pal, eds. Genetics of insect vectors of disease. Elsevier Publishing Co., Amsterdam.
Rai, K. S. and W. K. Hartberg. 1975. Mosquitoes and flies of genetic interest—*Aedes*. pp. 311–345 *in* R. C. King, ed. Handbook of genetics. Vol. 3. Plenum Press, New York.
Tadano, T. 1976. The inheritance of a mutant yellow larva in the mosquito *Aedes togoi*. Ann. Trop. Med. Parasitol. 71:361–365.
Trpis, M. 1978. Genetics of haematophagy and autogeny in the *Aedes scutellaris* complex of mosquitoes. J. Med. Entomol. 15:73–80.
Trpis, M. 1979a. Scrimpy-eye, a sex-linked mutant in the *Aedes scutellaris* complex of mosquitoes. J. Hered. 70:51–56.
Trpis, M. 1979b. The inheritance of red-eye, a sex-linked mutant of the *Aedes scutellaris* complex (Diptera: Culicidae). Canad. J. Genet. Cytol. 22:223–229.
Wade, J. O. 1977. The genetics of the white-eye, a sex-linked mutant of *Aedes (St.) cooki* Belkin. Ann. Trop. Med. Parasitol. 71:483–485.

NONCORRELATION OF INSECTICIDE AND REPELLENT TOLERANCES IN REPRESENTATIVE SPECIES AND STRAINS OF MOSQUITOES[1]

L. C. RUTLEDGE, M. A. LAWSON, L. L. YOUNG AND M. A. MOUSSA

Division of Cutaneous Hazards, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129

ABSTRACT. Median effective dosages (ED50) of the repellent diethyl toluamide and median lethal concentrations (LC50) of the insecticides DDT and malathion were determined for 13 strains of *Anopheles albimanus, An. stephensi, An. quadrimaculatus, Aedes aegypti, Ae. taeniorhynchus, Culex tarsalis* and *Cx. pipiens.* Correlations of the ED50's of diethyl toluamide with the LC50's of DDT and malathion were not statistically significant. The results indicate that diethyl toluamide would be equally effective against insecticide-resistant and insecticide-susceptible mosquitoes in the field.

A number of thiocyanate, pyrethroid, organophosphate and chlorinated hydrocarbon insecticides have been shown to be repellent to different species of *Anopheles, Aedes, Armigeres, Culex* and *Mansonia* (Rudolfs 1930, Roy et al. 1942, Metcalf et al. 1945, Ribbands 1946, Johnson 1947, Kennedy 1947, Wharton and Reid 1950, Vigueras and Corzo 1960, Busvine 1964, Hudson and Esozed 1971).

[1] Opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Conversely, Sarkaria and Brown (1951) demonstrated significant knockdown of *Aedes aegypti* (Linnaeus) by 32 repellents, including dimethyl phthalate, dimethyl carbate, Indalone® (butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate) and citronella oil. Elliott (1964) demonstrated that dimethyl phthalate and ethyl hexanediol were weakly insecticidal to *Anopheles gambiae* Giles, and Kuraishy et al. (1962) reported that a repellent cream containing diethyl toluamide and cetyl trimethyl ammonium bromide produced 7 to 9% mortality in unspecified field-collected anopheline and culicine mosquitoes.