A MELANOTIC MUTANT OF *CULEX TARSALIS*

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ABSTRACT. The mutant *mel* (melanotic larva and pupa) is described from *Culex tarsalis*. It is inherited by a single, recessive, autosomal gene that is non-lethal. There is a wide range of expressivity that is primarily dependent on the time post ecdysis.

INTRODUCTION

Melanotic larvae have been observed to appear sporadically in at least 4 geographical strains of *Culex tarsalis* Coq. maintained at the Naval Biosciences Laboratory Insectary in Oakland, California. These densely melanotic larvae mimicked the melanotic larvae described by Laven and Chen (1956) and Craig and Gillham (1961) from *Cx. pipiens* and *Aedes aegypti,* respectively. Initial attempts to establish melanotic *Cx. tarsalis* failed because only small numbers of melanotic larvae were isolated and these usually died prior to pupation. On rare occasions adults of both sexes were obtained, but these could not be propagated. A melanotic strain was eventually established. This paper describes the melanotic mutant and the mode of inheritance.

MATERIALS AND METHODS

Mosquitoes were normally maintained at 26° C, 80% RH and under a controlled lighting cycle (LD 16:8). The larval diet was a 5% w/v suspension of TetraMin® tropical fish food until the third molt, then Purina® rat chow pellets were added. Adults were held in 3.8 liter paper cages with netted tops and maintained on raisins and moist cotton pads. The females were exposed overnight to 3 to 5-day-old White Leghorn chicks and egg rafts were collected 3 to 5 days later. A new cage was used for each pupal collection.

Populations taken from the *Cx. tarsalis* colony designated Sacramento Valley (SV) (synonymous with Chico), colonized in 1972 from field material collected in Butte County, California, were reared from the larval stage to pupation at 17° C, 26° C, and 32° C in an unrelated study. All adults emerged and reproduced at 26° C. The melanotic strain was established, without further selection, from 72 densely melanotic larvae that appeared in the F_8 generation of the SV strain being reared at 32° C. After isolation the melanotic strain was maintained continuously at 26° C. Seven viable egg rafts were obtained from the parent melanotic mosquitoes after 3 weekly exposures to chicks.

The heritability of melanism was investigated by genetic mating experiments between adults from the melanotic strain and a hybrid strain (WR FC − KL) of *Cx. tarsalis* (hybrid described by Hardy *et al.* 1978).

RESULTS

Teneral larvae of both wild and *mel* phenotypes were indistinguishable, but the *mel* individuals became distinct during the latter stage of each instar. Moderate to extensive cephalic melanization was common to both phenotypes in 1st and 2nd instar larvae. Cephalic melanization decreased greatly in wild 3rd and 4th instar larvae, while increasing in melanotic larvae, particularly in the postgenae. First instar *mel* larvae were moderately melanized on the siphon, saddle, abdomen, distal thoracic regions and tracheal trunks (Figure 1a). Pigmentation in these regions increased in the 2nd instar *mel* larvae (Figure 1b), and in later instars some larvae became completely melanized. Wild type larvae were mostly transparent with the continuously ob-
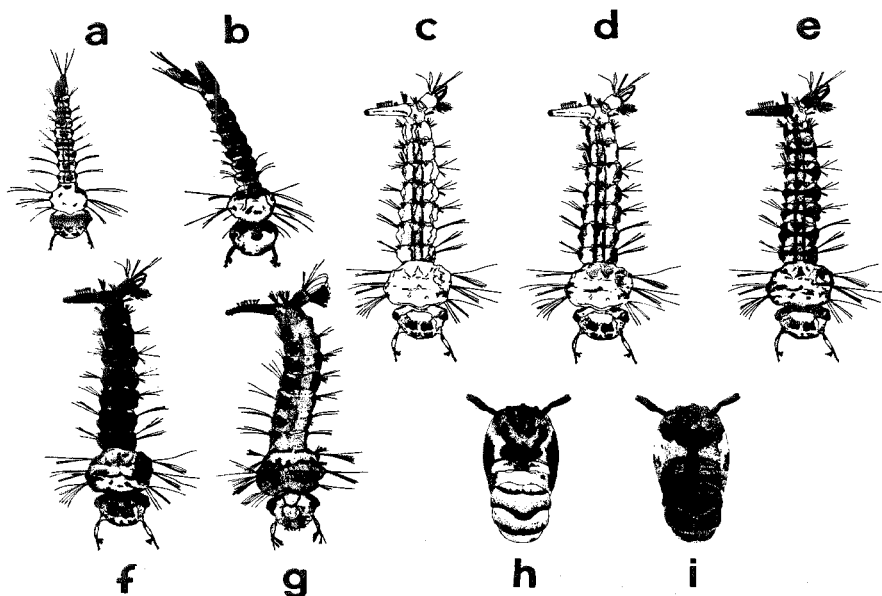
Figure 1. Melanotic (mel) and wild phenotypes (wild) of *Culex tarsalis*.
  a. First instar larva (mel).
  b. Second instar larva (mel).
  c. Fourth instar larva (wild).
  d. Fourth instar larva (mel: early stage, slight melanization).
  e. Fourth instar larva (mel: "banded effect").
  f. Fourth instar larva (mel: advanced melanization-dorsal view).
  g. Fourth instar larva (mel: advanced melanization-ventral view).
  h. Pupa (wild).
  i. Pupa (mel).

servable mesenteron being an obvious feature.

There was a wide range of melanism in all larval instars; this was most apparent in the larger, longer-lived 4th instar (Figure 1d − g). The range was from individuals having only slight lateral melanization (Figure 1d) which closely mimicked the wild phenotype (Figure 1c) through melanotic larvae having a banded appearance (Figure 1e) to complete or nearly complete melanization (Figure 1f). The pleural regions were not melanized (Figure 1g) and the last distinctive region to melanize was oftentimes the frontocypeus. Not all individuals reached the advanced melanotic stage prior to pupation.

Melanization was reduced during the pupal molt, but teneral *mel* pupae were distinct from teneral wild pupae. Mature wild pupae exhibited a wide range of expressivity, from melanization exclusively in the pronotal trumpets to the stage illustrated in Figure 1H. The *mel* pupae (Figure 1i) were densely pigmented except for the pleural regions and the lateral cephalothorax was transparent to translucent orange.

Melanotic adults had the full complement of silver scales characteristic of the species, but were darker than the wild

Table 1. Phenotypic ratios of hybrid fourth instar larvae.

Parent Cross	F_1 Cross	Wild:mel	X^{2a}	P
$\frac{mel}{mel}$ ♀	mel/+ ♀ x mel/mel ♂	657:596 (8)[b]	2.970	.10–.05
x	mel/mel ♀ x mel/+ ♂	845:829 (8)	0.153	.70–.50
$\frac{+}{+}$	mel/+ ♀ x mel/+ ♂	825:281 (6)	0.202	.70–.50
$\frac{+}{+}$ ♀	+/mel ♀ x mel/mel ♂	617:567 (8)	2.111	.20–.10
x	mel/mel ♀ x +/mel ♂	853:774 (6)	3.658	.10–.05
$\frac{mel}{mel}$ ♂	+/mel ♀ x +/mel ♂	1118:373 (6)	2.684	.20–.10

[a] Tests hypothesis that melanism is determined by a single regressive gene.
[b] Number of sibling groups phenotypically classified.

phenotype adults. This was most evident in teneral adults, however, highly subjective in aged adults.

Autogeny was observed in the melanotic strain. A viable autogenous population was maintained for 2 generations before being discontinued.

The heritability of melanism was indicated when F_2 and F_1 backcross mel larvae segregated out in expected Mendelian ratios. That the condition was recessive was indicated by the absence of melanotic larvae in the F_1 generation. The phenotypic ratios of F_1 larvae derived from reciprocal backcrosses were not significantly different from the expected 1:1 ratio for single gene inheritance with full penetrance ($X^2 = 2.970$, 1 df, .10>P>.05). The phenotypic ratios of F_2 larvae derived from F_1 sibling crosses fit the expected 3:1 ratio expected from the

above hypothesis ($X^2 = 2.684$, 1df, .20>P>.10). The autosomal nature of mel was indicated by the sex ratios of inbred and hybrid melanotic adults (Table 2); these ratios fit the 1:1 ratio expected of an autosomal gene ($X^2 = 1.255$, 1df, .30>P>.25).

DISCUSSION

Melanism in Cx. tarsalis was heritable and determined by a single, recessive, autosomal gene that was not lethal in the homozygous state. Melanotic larvae have been described from cultures of Cx. pipiens (Laven and Chen 1956), Aedes aegypti (Craig and Gillham 1961), Anopheles stephensi (Mason and Davidson 1966), An. quadrimaculatus (Seawright and Anthony 1972), and An. albimanus (Rabbani et al. 1976). The anopheline mutants were de-

Table 2. Sex ratio determination of hybrid melanotic adults.

Derivation	Phenotype	Adults ♀ ♂		X^{2a}	P
Mel colony	mel	292:305 (10)[b]		0.283	.70–.50
Mel/+ X mel/+	wild	318:331 (6)		0.260	.70–.50
	mel	110:94 (6)		1.255	.30–.25
mel/+ X ml ♂	wild	102:92 (3)		1.030	.70–.50
	mel	81:80 (3)		0.012	.95–.90
+/mel X mel ♂	wild	107:120 (3)		0.149	.70–.50
	mel	83:93 (3)		0.281	.70–.50

[a] Determines fit to 1:1 ratio.
[b] Number of sibling groups sexed.

scribed as "black larvae." All of these melanotic mutants share the single-recessive-autosomal gene aspect of heritability, except that melanism is polygenically determined and semidominant in *An. stephensi*. There is no specific evidence that any of these 6 mutations were not spontaneous.

Melanism in *Ae. aegypti* (Craig and Gillham 1961) was determined by one of a multiple series of alleles determining body color, and expressivity of heterozygotes could be environmentally influenced. Expressivity in *Cx. tarsalis mel* can be influenced by starvation (Apperson 1977). Laven and Chen (1956) described the melanotic mutant of *Cx. pipiens* as having slight expressivity, thus less than *Cx. tarsalis mel*. In melanotic *An. albimanus* (Rabbani et al. 1976) larval expressivity did not vary between instars; the pupae were also melanized. In melanotic *An. quadrimaculatus* (Seawright and Anthony 1972) both pupa and adult were melanized. Information on the range of larval expressivity of melanotic *An. stephensi* and *An. quadrimaculatus,* and the pigmentation of the pupal and adult species not previously mentioned, was not found in the literature.

Melanotic *Cx. tarsalis* differed from all previously described melanotic mutants in viability. Melanism in *Cx. pipiens* (Laven and Chen 1956), *An. stephensi* (Mason and Davidson 1966), *An. quadrimaculatus* (Seawright and Anthony 1972), *An. albimanus* (Rabbani et al. 1976) was highly lethal in the homozygous state with predominant mortality occurring in the 4th instar; indeed, no mutant adults were ever obtained from *An. albimanus*. The same mortality pattern has been reported from uncultured populations of *Cx. nigripalpus* and *Ae. taeniorhynchus* (Rabbani et al. 1976). Laven and Chen (1956) were not able to obtain sufficient numbers of melanotic *Cx. pipiens* adults to perform the traditional genetic experiments, but they deduced the mode of inheritance using homozygous wild and heterozygote crosses. The traditional genetic crosses were performed with melanotic *Cx. pi-*piens by Tadano and Barr (1975). Melanotic *Ae. aegypti* larvae (Craig and Gillham 1961) were severely retarded, sluggish, and developed into small adults with a reduced reproductive potential, a condition described as homozygously semilethal. This trait was initially considered lethal, but continuous rearing eventually yielded melanotic *Ae. aegypti* adults (Craig and Gillham 1961). Relative to the other 5 melanotic species *Cx. tarsalis mel* is not lethal; however, it is not as viable as its ancestral strain (Apperson 1977). It has been possible to rear large numbers of *Cx. tarsalis mel* females (i.e., 1500 plus) from 40 or fewer egg rafts.

Barr and Myers (1966) described a yellow larval color mutant from *Cx. tarsalis.* Other mutants from *Cx. tarsalis* were summarized by Asman (1976). The melanotic mutant is the 2nd larval color mutant described from *Cx. tarsalis* and the most visible yet described from the species and should be of value in genetic experimentation.

ACKNOWLEDGMENT

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COLONIZATION OF NORTH AMERICAN *AEDES TOGOI*

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ABSTRACT. Asian *Aedes togoi* (Theobald) has been colonized. Procedures for the successful colonization and laboratory mainte-

nance of *Ae. togoi* from British Columbia, Canada, are described.

INTRODUCTION

Aedes (Finlaya) togoi (Theobald) occurs in Siberia, China, Formosa, Korea, Japan, Marcus Island, Ryukyu-Retto, Ogasawara Gunto, Malaysia, U.S.S.R., Bonin Island and Thailand in Asia (Knight and Stone 1977). Larvae develop in saline, rock-pools along rocky sea shores. In Korea, larvae have also been recorded from freshwater, man-made containers in port cities and in settlements hundreds of kilometers inland (Petrishcheva 1948). *Ae. togoi* has recently been collected along the south-west coast of Canada. Meredith and Phillips (1973) collected larvae from rock-pools at Victoria, Vancouver Island, and we have collected larvae from rock-pools in West Vancouver, at Horseshoe Bay, and on South Pender Island. *Ae. togoi* was probably collected earlier from rockpools in the Vancouver area by Hearle (1926) as *Aedes (Ochlerotatus) dorsalis* (Meigen).

Ae. togoi is a natural vector of bancroftian and brugian filariasis in China and Japan and of Japanese B encephalitis in the Far-eastern U.S.S.R. (Smith 1973). Gerberg (1970) described procedures for

laboratory maintenance of *Ae. togoi* from Asia. This paper describes the colonization and laboratory maintenance of North American *Ae. togoi*.

COLONIZATION

Our colony was established using large numbers (>300) of 4th-instar larvae and pupae collected from rock-pools at Lighthouse Park, West Vancouver (49°20′N., 123°15′W.), during August and September 1977. Adults were held in a 23×23×23 cm cage and provided with water, dry sucrose and a guinea pig for blood. Twilight was not simulated. No mating was observed, although viable eggs were laid.

LABORATORY MAINTENANCE

Seventy-five adults of each sex are held in a 23×23×23 cm plexiglass cage. Dry sucrose is provided as carbohydrate. Water-soaked paper toweling held in a 100-ml beaker (water-wick) provides water and maintains the RH in the cage at 55–60%. Temperature is 25°C and the photoperiod (17.5L:06.5D) approximates