OPERATIONAL AND SCIENTIFIC NOTES

TESTING FOR ASSORTATIVE MATING BETWEEN TWO *CULEX TARSALIS* STRAINS REARED IN DIFFERENT ENVIRONMENTS[1]

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Field and laboratory tests indicate that laboratory colonization of *Culex tarsalis* Coquillett leads to assortative mating, females mating more frequently with males of their own genotype (McDonald et al. 1979; Reisen et al. 1981, 1982). Although selection of laboratory-adapted strains is clearly a factor, some data suggested non-genetic factors could be involved since changes occurred in the direct progeny of females mated in the field and reared in the laboratory (Reisen et al. 1982). Non-genetic factors in the rearing environment that might contribute to assortative mating include nutrients—missing or oversupplied—in one diet versus another, or differences in biochemical development dependent upon rearing temperature, humidity, and/or fluctuations of both. Unpublished observations have indicated that males reared in the environment where mating tests took place had a competitive edge over males coming from another environment.

A study was done to determine if 2 laboratory strains of mosquitoes known to mate randomly when reared in the same environment would mate assortatively when each strain was reared under a different condition.

The strains used were carmine/black eye (*car/ble*) and wildtype (Br 80). The eye-colored mutants are both recessives, and can be detected easily when both are homozygous in the same individual (Asman 1975). Both strains are long-established, laboratory-adapted colonies.

Both colonies were maintained in 2 different rearing rooms for at least 3 generations prior to tests. One room had variable temperature (18-35°C) cycling from an afternoon high to a predawn low, and humidity cycling between 40% RH in daytime and 90% RH at nighttime. Larvae were fed a mixed diet consisting of ground rat-chow, rabbit chow, liver powder, TetraMin® and yeast. Large cages (0.65 m^3) were used for colonies in this environment. The second insectary had constant temperature (24°C), constant humidity (75% RH) and a simple larval diet of ground rat chow. The colonies in this environment were maintained in smaller screened cages (0.03 m^3). Both rearing rooms had a photoperiod of 14 h light, 1 h each "dusk" and "dawn" (15 watt bulb only), and 8 h dark.

The Br 80 and *car/ble* larvae used in each test were reared synchronously in the 2 insectaries. Pupae were checked for eye color and held so that adults emerged in the same conditions under which they were reared as larvae. Adults were separated by sex within one day of emergence to insure virginity, and then were held for 3 days to insure mating readiness. Crosses were made in each environment to give *car/ble* females from one rearing environment a choice of males from each of the 2 rearing environments, using the eye color of progeny as a marker to determine which males succeeded in mating with the females. Fifty to 70 females were used in each competitive test. The number of males in each test was identical to the number of females but represented equal numbers of the 2 strains from different environments. The adults in each cross were combined in 35 x 17 cm cartons and placed in the environment in which the females had been reared. Mating, oviposition, and the subsequent rearing of the F_1 generation took place under those conditions. Parental females were provided 2 blood meal opportunities. Egg rafts were collected in oviposition cups, and each egg raft was transferred to an individual rearing container, a 700 ccm plastic box.

Eighteen tests were conducted, 9 under each environment. In addition, 4 control tests, in which all males and females were reared under the test environment, were done in each environment. Eye color of the F_1 was scored during the late larval stage when the mutant eye color

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Table 1. Competitiveness of *car/ble* males in assortative mating tests.

Competition between	Replicates	Rearing environment			Test environment	Competitiveness
		car/ble ♂♂	+ ♂♂	*car/ble* ♀♀		
Constant *car/ble* and variable +	2	C (constant)	V (variable)	C	C	1.06
	2	C	V	C	V	0.84
	3	C	V	V	V	1.26
	3	C	V	V	C	0.54*
Variable *car/ble* and constant +	2	V	C	C	C	0.66
	2	V	C	C	V	0.91
	2	V	C	V	V	1.29
	2	V	C	V	C	1.60
Both constant	4	C	C	C	C	1.06
Both variable	4	V	V	V	V	1.05

* Competitiveness of *car/ble* males significantly less than 1.0 ($P = 0.035$).

is easily distinguished from the darker wild type. All the offspring of a given raft were expected to have eyes of the same color, carmine/black resulting when a *car/ble* male mated with the female, or wild type resulting when a Br 80 male mated with the female. Under the null hypothesis of no mating assortativeness, equal numbers of carmine and wild type rafts are expected from each cross; that is, *car/ble* and Br 80 males should mate in equal numbers with the *car/ble* females. Differences from the expected 1:1 ratio were tested by Chi-square.

No statistically significant differences were found between replicates of any type of comparison, so results from each type were combined for analysis (Table 1). All tests in the variable environment showed equal competitiveness of *car/ble* males against Br 80 males, regardless of the rearing environment of females or either type of male. In the constant environment tests, *car/ble* males reared in the constant environment failed to compete equally against Br 80 males reared in the variable environment for females from the variable environment, but all other comparisons showed no statistically significant differences from the expected equal competitiveness.

Although these results cannot reflect the possibility of interaction of strains in a field environment, the findings show that assortative mating is not a factor between the 2 strains used in this experiment under the 2 laboratory environments described here.

One possible reason why assortative mating was not demonstrated in the laboratory despite evidence from field trials is that the field larval diet could not be duplicated in these experiments. More recent studies suggest that the larval diet might play a prominent role in the adult mosquito's ability to fly which in turn relates to swarming, a premating requisite. As we continue to improve the rearing diet there is every possibility that assortative mating could be demonstrated between adults reared in the old standard laboratory environment and those reared in an environment more closely simulating that encountered in nature.

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