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NATURALLY OCCURRING PUPAL PHENOTYPES OF *ANOPHELES ALBIMANUS* AND THEIR SUSCEPTIBILITY TO *PLASMODIUM VIVAX* AND *P. FALCIPARUM*

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ABSTRACT. The pupal phenotypes found to occur naturally in 3 different strains of *Anopheles albimanus* from El Salvador were tested for their susceptibility to coindigenous strains of *Plasmodium vivax* and *P. falciparum.* The Melara strain contained both brown striped and brown nonstriped forms, and a significant difference was seen between these 2 phenotypes only with the mean percent infected in the *P. vivax* experiments. The CA109-A strain had brown and green forms which were homogeneous in their susceptibility to both *P. vivax* and *P. falciparum.* The Apastepeque strain contained brown striped as well as green and brown nonstriped forms. Significant differences in susceptibility to *P. vivax* were seen in comparisons between the base colony and each of the phenotypes as well as in comparisons between striped and nonstriped and the green phenotype. Variations in susceptibility between the base colony and the 3 phenotypes were less apparent with *P. falciparum* in that significant differences were seen only in comparisons between the base colony and green and between brown striped and green phenotypes. The importance of variable parasite susceptibility between and within strains of anophelines in the epidemiology of malaria is discussed.

Variations in the pupal morphology within populations of *Anopheles albimanus* have been reported and have been established in pure lines by selective breeding (Georghiou et al. 1967, Warren et al. 1975b). The relationship of these morphologic variations to such practical considerations as sensitivity to insecticides and susceptibility to human malaria parasites has been of interest. A previous report has shown that there are significant differences in susceptibility to *Plasmodium vivax* among selected morphologic lines from the Apastepeque strain of *An. albimanus* (Warren et al. 1977); the present report is concerned with the susceptibility to *P. vivax* and *P. falciparum* of naturally occurring morphologic phenotypes in insectary colonies of three strains of *An. albimanus.*

MATERIALS AND METHODS

The *An. albimanus* strains all originated in El Salvador and are maintained routinely in our insectaries. The Melara strain was isolated in 1971 from wild, gravid females collected in a cattle stable in Melara near La Libertad in a coastal area of El Salvador highly endemic for malaria. The CA109-A strain was established from a single female mosquito that was naturally infected with malaria. The capture was made inside a house located in a small beach village (Playa Cangrejera) in El Salvador that was undergoing a local but intense outbreak of *P. falciparum* malaria (Warren et al. 1975a). The Apastepeque strain was colonized from wild, gravid females collected from a cattle stable near Lake Apastepeque in the interior of El Salvador (Dame et al. 1974).

Each colony was examined for the presence of naturally occurring pupal phenotypes. The Melara strain had brown to brownish-gray specimens that were either striped or nonstriped. The CA109-A strain also had brown, brownish-gray forms and, in addition, a small percent of distinctly green pupae; there were no striped forms in this colony. The Apastepeque strain demonstrated greater variation, with brown striped and nonstriped as well as green forms.

The two malaria strains used in these studies were isolated from human infections in coastal El Salvador. The isolation and development in *Aotus* monkeys of both the El Salvador II strain of *P. vivax* (Collins et al. 1973 and 1974) and the Santa Lucia strain of *P. falciparum* (Collins et al. 1976) have been previously described.

The pupae collected each morning from the three mosquito strains were divided into 2 approximately equal portions. The specific pupal phenotypes to be studied were individually selected from 1 portion and placed into screened gallon cartons for emergence. The 2nd portion was reserved and fed as base colony controls. Females, when 3–5 days old, were transferred to pint cartons for feeding on infected monkeys.

Mosquito feedings for direct comparison were made at approximately the same time between 0830 and 1000 hours. One or more phenotypes and the base colony were fed in each experiment. Unfed mosquitoes were removed from feeding cartons and discarded. Mosquitoes were held at 25° C and fed on 5% sugar solution daily. After 7–9 days of extrinsic incubation, mosquitoes were dissected and the midguts examined for the presence of malaria oocysts.

Data collected included the percent infected in each lot of mosquitoes and the level of infection obtained. The latter observation was obtained by the dissection and enumeration of the number of oocysts on the guts of from 20 to 25 mosquitoes for each lot. The average number of oocysts per 100 guts for a particular lot is the gut infection index (Jeffery et al. 1954). Since there is a tendency for a skew in the mean gut infection index with an inordinate weight given to comparisons when mosquito infection is particularly heavy, the gut infection index was converted to the logarithmic value and a geometric mean determined. The antilog is the geometric mean gut infection index. Statistical analysis of the results used a paired t-test in which

$$t=\bar{x}\sqrt{\frac{n(n-1)}{Sx^2}}$$

where $\bar{x}$ is the difference between the two group means and SX^2 is the *pooled* sum of squares (Snedecor 1946).

RESULTS

The results of these experiments are presented in Tables 1–3. In the Melara strain (Table 1) there were no significant differences in susceptibility of the striped and nonstriped phenotypes and the base colony. The nonstriped phenotype did show a significantly greater percentage of mosquitoes infected with *P. vivax* than did the striped form, but there were no significant differences in the levels of infection (gut infection index).

With the CA109-A strain, there were no significant differences in susceptibility to *P. vivax* or *P. falciparum* between the green and brown phenotypes or between these phenotypes and the base colony (Table 2).

Table 1. Comparisons between different base colony pupal phenotypes of the Melara strain of *Anopheles albimanus* with regard to susceptibility to *Plasmodium vivax* and *P. falciparum.*

Plasmodium	Pupal Phenotypes	Number of Comparisons	Mosquitoes Examined	Mean Percent Infection	Geo. Mean Gut Infection Index
vivax	BASE : STRIPED	7	200 : 186	31.3 : 27.0	48 : 44
	BASE : NONSTRIPED	7	200 : 193	31.3 : 35.9	48 : 66
	STRIPED : NONSTRIPED	9	228 : 248	25.8 : 38.5*	43 : 79
falciparum	BASE : STRIPED	5	142 : 130	22.6 : 21.6	25 : 26
	BASE : NONSTRIPED	4	113 : 100	27.4 : 16.1	42 : 38
	STRIPED : NONSTRIPED	6	156 : 148	21.6 : 12.8	40 : 30

* Statistically significant difference at the 5 percent level.

Table 2. Comparisons between different base colony pupal phenotypes of the CA109-A strain of *Anopheles albimanus* with regard to susceptibility to *Plasmodium vivax* and *P. falciparum.*

Plasmodium	Pupal Phenotypes	Number of Comparisons	Mosquitoes Examined	Mean Percent Infection	Geo. Mean Gut Infection Index
vivax	BASE : BROWN	9	234 : 213	49.6 : 45.6	129 : 112
	BASE : GREEN	8	234 : 132	47.5 : 52.1	110 : 123
	BROWN : GREEN	9	195 : 153	39.0 : 41.1	78 : 71
falciparum	BASE : BROWN	11	298 : 276	27.4 : 23.1	60 : 33
	BASE : GREEN	11	298 : 226	27.4 : 28.9	60 : 59
	BROWN : GREEN	11	276 : 226	23.1 : 28.9	33 : 59

Table 3. Comparisons between different base colony pupal phenotypes of the Apastepeque strain of *Anopheles albimanus* with regard to susceptibility to *Plasmodium vivax* and *P. falciparum*.

Plasmodium	Pupal Phenotypes	Number of Comparisons	Mosquitoes Examined	Mean Percent Infection	Geo. Mean Gut Infection Index
vivax	BASE : STRIPED	15	311 : 320	39.4 : 59.5**	112 : 269*
	BASE : NONSTRIPED	16	339 : 405	39.8 : 59.7**	123 : 269**
	BASE : GREEN	16	340 : 340	39.7 : 76.9**	107 : 479**
	STRIPED : NONSTRIPED	19	393 : 437	56.2 : 54.1	219 : 200
	STRIPED : GREEN	22	454 : 467	52.2 : 63.4**	186 : 316**
	NONSTRIPED : GREEN	20	461 : 427	53.7 : 66.1*	195 : 339**
falciparum	BASE : STRIPED	7	82 : 86	28.8 : 26.6	60 : 52
	BASE : NONSTRIPED	6	68 : 123	26.4 : 32.6	55 : 87
	BASE : GREEN	8	106 : 167	26.2 : 41.6**	54 : 170**
	STRIPED : NONSTRIPED	8	122 : 180	24.8 : 26.1	49 : 47
	STRIPED : GREEN	10	150 : 208	25.1 : 39.1*	51 : 83
	NONSTRIPED : GREEN	9	212 : 206	26.7 : 33.5	52 : 79

* Statistically significant difference at the 5 percent level.
** Statistically significant difference at the 1 percent level.

In the Apastepeque strain significant differences in susceptibility were more apparent with *P. vivax* than with *P. falciparum* experiments. All 3 phenotypes tested were more receptive to *P. vivax* than the base colony from which they were derived, with the green phenotype being the most susceptible. The responses were quite different with *P. falciparum.* Once again, the green phenotype was most receptive, but the other 2 forms (striped and nonstriped) were not different from either the base colony or from each other.

DISCUSSION

Studies on *An. albimanus* from El Salvador and other parts of Middle America have shown that variations in pupal coloration are identifiable markers indicating genetic differences within a particular local population of the species. Usually, pupae of *An. albimanus* are brown to brownish-gray. In El Salvador, at least, some specimens may show a distinct unpigmented central stripe on the dorsal side and some strains show a variable percentage of green forms. In this study, we worked with 3 color phenotypes occurring naturally in the mosquito strains under investigation: brown and green nonstriped and brown striped. Green striped forms were not found to occur naturally in any of the 3 strains. Studies reported earlier (Collins et al. 1976) had indicated that the Apastepeque strain was the most susceptible to the El Salvador II strain of *P. vivax,* followed by the CA109-A, and then the Melara strain of *An. albimanus.*

The two coastal strains, Melara and CA109-A, were found to be genetically homogeneous in terms of malaria susceptibility. The Melara nonstriped phenotype had a significantly higher percentage of mosquitoes infected with *P. vivax* than the striped form, but these phenotypes showed no difference in the susceptibility to *P. falciparum.* The CA109-A strain proved to be the least variable in its morphology and in its receptivity to malaria. This is perhaps to be expected since the colony was originally isolated from a single wild caught female.

Results with the Apastepeque strain were much more variable in respect to both morphology and receptivity to malaria. Since all the phenotypes examined were more susceptible than the base colony forms, the specimens not readily falling into any of the 3 phenotypic categories were less susceptible to *P. vivax*. Morphologically, this less susceptible segment of the Apastepeque population consisted of mostly brown to brownish-gray nonstriped or partially or indistinctly striped forms. In contrast to the difference seen in the Melara strain, the brown striped and nonstriped forms from the Apastepeque strain were similar in their susceptibility to *P. vivax*. Also, the green phenotype from this strain was almost invariably more susceptible to *P. vivax* or *P. falciparum*, in contrast to the absence of differences seen in the CA109-A strain.

A previous report on the Apastepeque strain (Warren et al. 1977) examined the susceptibility to *P. vivax* and *P. falciparum* of purified lines selectively bred for these color characters through 6 or 7 generations. Surprisingly, the results reported here for phenotypes extracted directly from the Apastepeque strain base colony are somewhat inconsistent with those seen previously with the lines selected from the same colony. In the previous study, the selected green line was the least susceptible of the phenotypes in all parameters studied; in the present study, it appeared to be the most susceptible. In the selected lines, the striped phenotype was the most receptive to the malaria parasite, while in the present study receptivity was approximately the same in striped and nonstriped isolates. It seems apparent that the selection for the specific phenotype over a number of generations resulted in the elimination of some and the purification of other factors, and that one or more of these inapparent factors were responsible for parasite susceptibility and others for the particular color character being selected. It should be pointed out that during the selection procedure, the characters selected become more pronounced with each selection; i.e., the "green" becomes greener and the stripe character becomes more distinct. Thus, it cannot be concluded that susceptibility is necessarily related to "greenness," even though the results showed this phenotype to be the most susceptible. If related to any of the phenotypes studied, susceptibility would more likely accompany the stripe character, since in the selected strain again 2 characteristics seemed to coexist.

The idea that all mosquitoes in a particular vector population are not equally susceptible to a given species or strain of *Plasmodium* is not new. We were fortunate in having identifiable markers in *An. albimanus* which could be evaluated in their association with malaria parasite receptivity. These results suggest the presence of genetic lines within local mosquito populations that vary consistently in their response to *P. vivax* and to a lesser extent *P. falciparum*. If the proportion of such genetic segments is influenced by seasonal changes, insecticide pressures, etc., then the transmission capacity of a proven malaria vector could vary from season to season or from place to place on a limited geographic scale. It is clear that it is not valid to regard even local vector populations as homogeneous in their capacity to transmit malaria.

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SURVIVAL OF *AEDES TRISERIATUS* PUPAE IN THE ABSENCE OF FREE WATER[1]

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ABSTRACT. Although pupae are able to survive in the absence of free, unabsorbed water, the timing of disappearance of free water relative to age post-ecdysis is an important factor. Pupae are more vulnerable to the disappearance of free water within 1 hr following ecdysis than subsequently. Most pupae exposed to conditions devoid of free water die during ecdysis.

It is well-established that immature stages of mosquitoes can survive to adult emergence under conditions devoid of free, unabsorbed water, e.g. in muck, moist soil, etc. (Al-Azawi & Chew 1959; Bick 1948; Bick & Penn 1947; Gunstream & Chew 1957; Headlee 1945; Husbands 1953; Markos & O'Berg 1958; Nielsen & Nielsen 1953; Schoof, Schell & Ashton 1945; Thurman & Mortenson 1950; Woodard & Fukuda 1971).

The purpose of this study was to determine survival of *Aedes triseriatus* (Say) pupae in the absence of free water as a function of age post-larval-pupal ecdysis. We particularly wanted to establish whether or not it would be feasible to hold pupae on moist filter paper for experimental purposes.

Ae. triseriatus is found throughout the eastern U.S. and most of the Great Plains, is the most abundant tree hold breeder in North America and will oviposit in almost any artificial aquatic habitat (Jenkins & Carpenter 1946). Tree holes form in various ways including rot cavities where branches have been removed, rotted-out tree stumps and the trunks of adjacent trees growing together. Since tree holes and similar habitats are likely to be temporary water containers, *Ae. triseriatus* is

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