GENETIC STUDIES ON MECHANISMS INFLUENCING THE SUSCEPTIBILITY OF ANOPHELINE MOSQUITOES TO PLASMODIAL INFECTION

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ABSTRACT. More than forty years ago it was suggested by Huff that resistance of mosquitoes to plasmodial infections could be a genetic character behaving as a Mendelian dominant. Even now we do not possess a clear explanation of why different species or strains of mosquitoes differ in susceptibility to infection by different species or strains of plasmodia. We have performed and analyzed genetic crosses and tried to establish biochemical profiles in two strains of *Anopheles stephensi*, one selected for susceptibility and another for resistance to *Plasmodium gallinaceum* with the aim of improving our knowledge on the assumed but not sufficiently proven genetic control of vector capacity. Our genetic data suggest that susceptibility and resistance of *Anopheles stephensi* to *Plasmodium gallinaceum* are phenotypic expressions at a single genetic locus, confirming Huff's conclusions that resistance behaves like a Mendelian dominant phenotype.

It has long been well established that different species or strains of mosquitoes may greatly differ in their susceptibility to infection by given species or strains of plasmodia (Ward 1963, Kilama and Craig 1969). However in spite of the fact that a certain amount of attention has been paid to the mechanisms that make one mosquito species a dangerous vector while other species are not, we do not currently have a clear explanation of this phenomenon.

Among the investigations performed during the past years dealing with comparisons of differential susceptibilities of several mosquito species to plasmodial infection, special attention should be given to those carried out by Huff (1927–1935). This author stated that two strains of *Culex pipiens*, respectively susceptible and resistant to plasmodial infection, could be established by artificial selection. It was furthermore suggested by Huff that immunity of mosquitoes to plasmodia was a hereditary phenotype behaving as a Mendelian dominant.

Starting from this suggestion and attempting to improve our knowledge on the mechanisms which control the susceptibility of mosquitoes to plasmodia we have analyzed genetic crosses and established biochemical profiles in 2 strains of *Anopheles stephensi* selected for susceptibility and resistance to *Plasmodium gallinaceum*.

MATERIAL AND METHODS

Preliminary selection work was carried out in the laboratory of Parasitology of the "Istituto Superiore di Sanità," Rome, by Professor A. Corradetti, who some years ago was able to isolate highly susceptible strains and to whom we are greatly indebted for having very kindly supplied the material and generously given important technical advice. Selection was further carried out in our laboratory. It was possible to obtain a strain, arbitrarily named "A," the females of which contained in their midgut an average of 30 plasmodia oocysts 7 days after ingestion of chick-infected blood. Another strain "B," highly refractory to *Plasmodium gallinaceum* infection and constantly showing very low values of susceptibility was also isolated. In this strain only 3% of the females fed on infected blood contained oocysts. Susceptible and resistant colonies were then maintained in subsequent generations but without applying strong selection pressure. The *Plasmodium gallinaceum* strain was maintained in chicks by transfusion of infected blood or, alternatively, by using infected susceptible mosquitoes as vectors. To diagnose

the presence of *Plasmodium* in artificially infected chicks, blood smears were stained with Giemsa.

To detect the presence of oocysts, midguts freshly dissected from females fed on infected chicks 5 to 9 days before dissection were directly observed with a phase-contrast light microscope.

Electrophoretic characterization of enzymes including esterases, malate dehydrogenases, glucose-6-phosphate dehydrogenases, xanthine dehydrogenases and peptidases have been carried out alternatively on starch and polyacrilamide slabs or on cellulose acetate strips. Methods for electrophoretic incubation and staining to detect catalytically active zones were those already described in the literature: (Beckman and Johnson, 1964; Hubby and Lewontin, 1966; Lewis and Harris, 1967). Midgut protease activity was determined in homogenates each consisting of 100 dissected midguts by applying the method of Kunitz (1947).

BIOCHEMICAL APPROACH. Esterases (EST_1), Malate Dehydrogenases (MDH), Glucose-6-Phosphate Dehydrogenases G6-PD), Xanthine Dehydrogenases (XDH), Dipeptides and Tripeptides (Val-Leu, Leu-Ala, Leu-Gly-Gly), Hydrolyzing enzymes, have been screened electrophoretically in both strains.

No consistent differences between the two strains have been detected by studying the MDH, G6PD, XDH or Pep zymograms. In 5 experiments on protease activity however, using homogenate obtained from 100 midguts dissected from freshly emerged unfed females, a slightly higher trypsin-like activity was found in the midguts of specimens belonging to the "B" resistant strain, (Bianchi and Rinaldi, unpublished data).

This finding is undoubtedly attractive and interesting, but at present it is considered by us with caution and as a simple hypothesis for further work.

THE "STRIPED" LARVAE. The problem of "striped" and "non-striped" larvae and its hypothetical correlation to susceptibility represented an interesting subject. We found that almost all resistant larvae at the fourth stage were "striped"; that is, they showed a thin continuous dark line on the abdomen. On the other hand this line was missing in almost all larvae belonging to the susceptible strain.

In a laboratory strain derived from a wild Indian population of *A. stephensi,* it was possible to isolate 47 "striped" females in the larval stage. All these specimens were reared in the laboratory and as adults stage were fed *P. gallinaceum* infected blood and analyzed for oocyst presence. Only 1 out of 47 females was found to be infected. On the other hand 8 out of 50 females having a normal gray "non-striped" abdomen, which had been randomly sampled from the Indian strain, were also found to be infected. There is no obvious correlation between the character and susceptibility.

FORMAL GENETICS. Since both the strains maintained their phenotypic susceptibility and resistance without selection pressure, we thought it reasonably correct to assume the presence of Mendelian factors controlling these characters. To test this assumption experimentally we performed several crosses by mating individuals derived from a single inseminated female randomly collected from each strain. The results are given in Table 1. Each founder female was tested for susceptibility or resistance immediately after deposition by inspection of the midgut (Fig. 1). Among the offspring obtained by crossing A females to B males and the reciprocal cross, 14 susceptible females were observed (Table 1). If resistance behaves as a Mendelian dominant phenotype, no susceptible females should have appeared in the offspring of these crosses. Likewise the classes of suscetpible and resistant segregants obtained by backcrossing AB females to A males do not fit well the expected 1:1 ratio ($X^2 = 4.05$, $P < 0.045$), (Table 1). In this regard, however, we want to stress that different variables (male genotype, modifiers) may interfere with the expected Mendelian distribution of this particular phenotype.

On the other hand, the picture obtained from the other crosses seems to indicate

Table 1. Crosses made to study the inheritance of susceptibility of *A. stephensi* adult females to *P. gallinaceum*.

Parental phenotype	Phenotype of offspring susceptible (A)	resistant (B)	X^2 1 df
A ♀♀ x B ♂♂ and reciprocal	14	169	1.07 P<0.2
AB x AB	195	620	0.49 P<0.5
AB ♀♀ x A ♂♂ and reciprocal	59	83	4.05 P<0.045
AB ♀♀ x B ♂♂ and reciprocal	0	79	

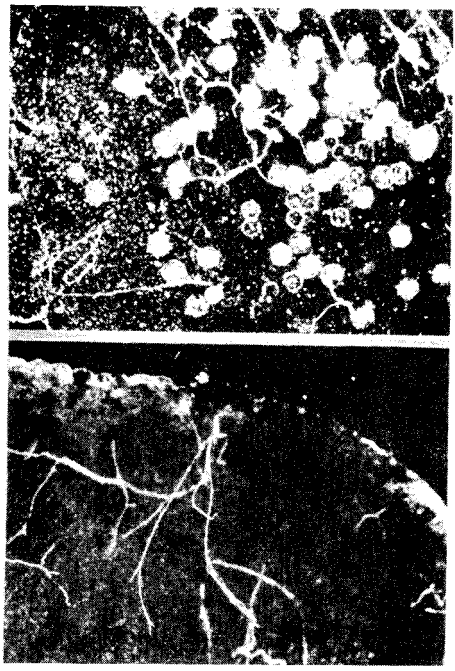

Fig. 1. Midguts of two *Anopheles stephensi* females fed, 7 days before dissection, on the same infected chick. *Above:* midgut of the female belonging to the susceptible strain showing many oocysts. *Below:* midgut of the female belonging to the resistant strain showing complete absence of oocysts.

that resistance is controlled by a single dominant genetic locus. This conclusion is particularly suggested by the values of segregation fitting well the 3:1 expected ratio ($X^2 = 0.49$, $P < 0.5$) obtained by crossing AB to AB individuals, and by the lack of susceptible females among the offspring obtained by backcrossing AB females to B males and the reciprocal (Table 1).

To conclude, we think that in general our data may support the hypothesis stating that resistance of *A. stephensi* to *P. gallinaceum* is a dominant phenotypic expression at a single locus or possibly the result of coordinated action of a group of closely linked loci.

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