MALE SPECIFIC ESTERASES IN CERTAIN ANOPHELINE MOSQUITOES

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ABSTRACT. Five anophelines viz., *Anopheles annularis, An. culicifacies, An. fluviatilis, An. stephensi* and *An. subpictus,* were studied with respect to non-specific esterases using polyacrylamide gel electrophoresis. Male specific esterases were found in *An. stephensi* and *An. subpictus* and not in the other three anophelines tested. In both the species, bands were localized in the accessory glands. In *An. stephensi,* the male specific esterase band appeared first in 18–20 hr old pupae while in *An. subpictus* they appeared in 0–1 hr old pupae. These bands persisted during the adult life of the male mosquitoes.

INTRODUCTION

Gene-enzyme studies of anophelines using the polyacrylamide gel electrophoresis were initiated in our laboratory about two years ago. One of the enzyme systems which has been extensively studied is non-specific esterases. These studies revealed considerable inter- and intrastrain variations. Following these studies, several strains homozygous for different allozymes have been isolated and genetically analyzed.

This paper reports the presence of male specific esterases and their localization in two species of the subgenus *Cellia, An. stephensi* Liston and *An. subpictus* Grassi. Ontogeny of this esterase in *An. stephensi* and *An. subpictus* is also reported.

MATERIALS AND METHODS

Mosquitoes were reared in an insectary maintained at 27–28°C and 70–80% RH following the standard procedures of colonization adopted in our laboratory. Adults were held in 30×30×30 cm cages and offered fresh water-soaked raisins and 1% glucose on cotton pads. They were also offered rabbits as a blood meal source. The following mosquito stocks were used in these experiments: 1) *An. stephensi:* Wild strains collected from Okhla (Delhi), Sonepat (Haryana) and Barhanpur (Madhya Pradesh); phenotypic markers, *red-eye, colorless-eye, green-larva* and *greenish brown-larva;* DDT and dieldrin resistant strains and dieldrin susceptible strain which have been isolated and maintained in our laboratory for about 4–6 years, 2) *An. culicifacies* Giles: Mosquitoes were collected and colonized from Mandora and Basantpur (Haryana), Okhla (Delhi), Aurangabad (Maharashtra), Gopalpur (Gujarat), and Sirolifarm in terai region (U.P.). Species A was isolated from Mandora and Basantpur villages (Haryana) and colonized for about one year. Other strains were of species B and colonized for about three years, 3) *An. fluviatilis* James: A wild strain collected from Sirolifarm in terai (U.P.) and colonized for about two years, 4) *An. annularis* Van der Wulp: A wild strain collected from Arthala near Delhi (U.P.) and colonized for about two years, and 5) *An. subpictus:* Progeny of wild collected adults from Basantpur (Haryana).

Polyacrylamide gel electrophoresis was carried out essentially following the techniques of Shaw and Prasad (1970) and Gabriel (1971). Adults, pupae and larvae were homogenized in 0.2 ml. 0.1M tris-borate EDTA buffer (pH 8.0) containing 10% sucrose and a drop of 0.1% bromophenol blue. The homogenate was centrifuged at 10,000 rpm at 0°C for about 10 min. The supernatant was placed on a polyacrylamide vertical column containing 7.5% running and 3.125% stacking gels. Electrophoresis was carried out at 4°C with a constant current of 3mA for about 3½ hrs. Esterases were detected by incubating gels in 100 ml of 0.1M phosphate buffer (6.5 pH) containing fast blue RR (100 mg), 1-naphthyl acetate (30 mg) and 2-naphthyl acetate (35 mg) for about 30 min. at room temperature. Further characterization of isozymes were carried out separately with 1-naphthyl acetate, 2-naphthyl acetate and 1-naphthyl propionate. After staining, gels were fixed in alcohol gel fixative.

Inhibitor studies were carried out for the characterization of male specific esterases following the methods of Holmstedt (1971) and Metcalf et al. (1972). The inhibitors were: eserine, diisopropyl fluorophosphate (DFP), 1,5-bis- (4-Allyldimethylammonium phenyl) - pentan-3-one diiodide, tetraisopropylpyrophosphoramide, Paraoxon (0,0 - diethyl 0- (4 nitrophenyl phosphate), parahydroxy mercuribenzoate (pHMB). Each inhibitor was used in two different concentrations of 10^{-4}M and 10^{-5}M. In these experiments, gels were first treated with a buffered inhibitor solution for 30 min. and after washing, the gels were placed in a mixture of diazonium salt, substrate and inhibitor.

RESULTS AND DISCUSSION

Sexual dimorphism for non-specific esterase bands was observed in *An. stephensi* and *An. subpictus*. Figures 1A and 1B represent the zymograms of *An. stephensi* and *An. subpictus* respectively. Figure 1C represents the relative electrophoretic mobility of male bands in these two species.

A strain of *An. stephensi*, homozygous for an esterase allozyme Est-4C, was used to demonstrate the sexual dimorphism. In Fig. 1A, the gels represent esterases of adult female (a), adult male (b), male without the reproductive organs (c), total reproductive organs (d), and accessory glands (e) respectively. Esterase band 'M' shown in Fig. 1A (e) was observed in males of all the strains studied of *An. stephensi* but not in the females. When male reproductive organs and males without the reproductive organs were subjected to electrophoresis, an esterase band with an electrophoretic mobility identical to the male specific esterase band was observed in the reproductive organs (d). This band was absent in males without the reproductive organs (c). The male specific esterase band was further investigated for its tissue specificity within the reproductive system. The testes lobes with vasa deferentia, accessory glands and ejaculatory bulb were run separately in the polyacrylamide gel electrophoresis system. The 'M' band was observed only in the gel with accessory glands (e). This indicated that the male specific esterase was active in the accessory glands of the reproductive system. Freyvogel et al. (1968), while investigating the non-specific esterases in *An. stephensi*, observed an extra esterase band in males but made no further observations. In *An. funestus* Giles, another member of the subgenus *Cellia*, a male specific band was localized in the accessory glands (Green 1977).

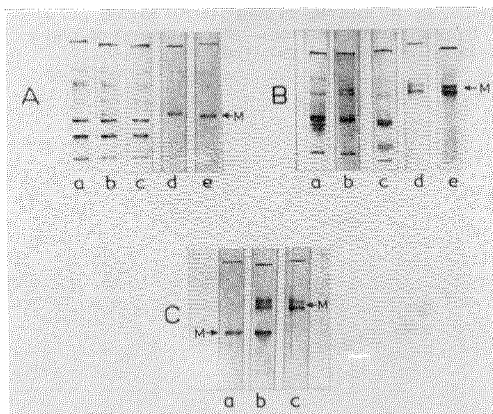

Fig. 1A: a-e Zymograms of *An. stephensi* esterases. a) Adult female, b) Adult male, c) Male without the reproductive organs, d) Total reproductive organs, e) Accessory glands; Fig. 1B: a-e Zymograms of *An. subpictus* esterases. a) Adult female, b) Adult male, c) Male without the reproductive organs, d) Total reproductive organs, e) Accessory glands; Fig. 1C: a-c Composite zymograms of the accessory glands of *An. stephensi* and *An. subpictus*. a) Male band of *An. stephensi*, b) Male bands of both *An. stephensi* and *An. subpictus*, c) Male bands of *An. subpictus*.

Figure 1B represents esterases of adult female (a), adult male (b), male without the reproductive organs (c), total reproductive organs (d), and accessory glands (e) respectively of *An. subpictus*. Results of this study were similar to those observed in *An. stephensi* except that two bands were seen instead of one. To confirm whether the two bands observed are the allelic products at a single locus, as in *An. funestus* (Green 1977), or not, sixty individual males were tested. In all males both the bands appeared, thereby suggesting that they are not the products of alleles segregating at a single autosomal locus.

The relative electrophoretic mobility of male bands of *An. stephensi* and *An. subpictus* was studied by running the accessory glands of both the species in the same gel. The three gels in Fig. 1C i.e., a, b, and c represent male bands of *An. stephensi*, *An. stephensi* + *An. subpictus* and *An. subpictus* respectively. The electrophoretic mobility of the male specific esterase band of *An. stephensi* was more anodal than that of *An. subpictus* and their Rf values were 0.63 for *An. stephensi* and 0.39, 0.34 for the 2 bands of *An. subpictus*.

Ontogenic study of the male specific esterase bands in *An. stephensi* and *An. subpictus* revealed that they are absent in III and IV instars (larvae can be sexed at this stage). In *An. stephensi* the enzyme is expressed for the first time in pupae which are 18–20 hrs old while in *An. subpictus*, they are expressed even in 0–1 hr old pupae. Once the enzymes were expressed, they persisted throughout the pupal and adult life of the males. This suggested that the gene responsible for this esterase was activated at the mid-pupal stage in *An. stephensi* while in *An. subpictus* it was activated at an early pupal stage. Substrate specificity tests revealed that the male specific esterase band of *An. stephensi* and *An. subpictus* were capable of hydrolyzing 1-naphthyl acetate, 2-naphthyl acetate and 1-naphthyl propionate. However, with 1-naphthyl propionate the esterase bands were more prominent suggesting that this substrate

is hydrolyzed at a faster rate. Enzyme inhibitors viz., eserine, diisopropyl fluorophosphate, tetraisopropyl pyrophosphoramide and 1.5 bis pentane 3-one-diiodide had no effect while paraoxon completely inhibited and pHMB enhanced the activity of this band. This suggests that this enzyme has unique properties and does not resemble any of the esterases described so far.

Sexual dimorphism for non-specific esterases was not observed in three of the anopheline species of the subgenus *Cellia* viz., *An. annularis, An. fluviatilis,* and *An. culicifacies.* Further, in *An. annularis* and *An. fluviatilis* esterase bands were not observed in the male reproductive organs. However, in *An. culicifacies* there were esterase bands in the male reproductive organs but these were not specific to the reproductive organs. Esterase profile in males without the reproductive organs was same as in whole males.

All three species, *An. stephensi, An. subpictus* and *An. funestus*, which exhibited sexual dimorphism for nonspecific esterases belong to the subgenus *Cellia* but not to the same series. Even though *An. annularis* and *An. stephensi* belong to the Neocellia series, sexual dimorphism was observed only in *An. stephensi*. Likewise, *An. funestus* belonging to the Myzomyia series, exhibited sexual dimorphism while *An. culicifacies* and *An. fluviatilis* belonging to the same series did not. From the limited information available it may not be possible to attach any phylogenetic importance to the phenomenon of sexual dimorphism in these species.

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