A SEX-LIMITED ESTERASE IN THE ACCESSORY GLANDS OF MALES OF *ANOPHELES FUNESTUS*

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ABSTRACT. Electrophoresis of individuals of *Anopheles funestus* Giles, and a very closely related species from South Africa, and subsequent staining for non-specific esterases showed that the most intensely staining bands were limited to males. Dissection of individuals further showed these isozymes to be restricted to the accessory glands of the internal genitalia. The bands appear to represent the products of alleles segregating at a single, autosomal locus.

INTRODUCTION. Freyvogel et al. (1968) reported sexual dimorphism for esterase activity in *Anopheles* (*Cellia*) *stephensi* in which males showed a zone of enzyme action not present in females. They did not find a similar dimorphism in *A. (Anopheles) freeborni.*

During work on isozyme polymorphism in *A. funestus* we found a similar dimorphism for esterase activity; however it is not possible to directly compare our results with those of Freyvogel et al. (1968) because of technical reasons, and *A. stephensi* was not available to us. Due to the localization of an *A. funestus* esterase in accessory glands, and the interest in these organs in their role of inducing female monogamy (Craig 1967, Bryan 1972) and fertility (Adlakha 1975) I make the following report.

MATERIALS AND METHODS. Mosquitoes used in this work were the progeny of wild-caught females of (a) *A. funestus* caught biting man indoors at Kanyemba (15°40'S, 2030°E, n=16), and (b) an undescribed species caught biting man outdoors near Tzaneen, northern Transvaal (n = 22). This species was recently recognized from biological and genetic evidence, but seems to be a sibling species with *A. funestus*, i.e. morphologically indistinguishable from *A. funestus*. Two or more of each sex were run from each family.

Individual insects were homogenized in ca. 5 μl of distilled water and the homogenate absorbed onto chromotography paper cut into pieces 1.5mm x 6.0mm. These were inserted into 7.5% acrylamide gels. The gels are 1.5 mm thick, and 100.0 mm x 175.0 mm. The buffer system was modified from Barlow and Ridgeway (1971). The electrode buffer contained 12.4 m boric acid, 40.0 gm tris (hydroxymethyl aminomethane) and 4.0 g EDTA (disodium ethylene-diaminetetraacetic acid) pH 8.6. Five gels were polymerized from a mixture of the following solutions:

A. 15.00 g of acrylamide, and 0.45 g n,N'-methylene-bis-acrylamide dissolved in 100 ml of distilled water.

B. 5.00 g sucrose, and 0.15 ml of TEMED (N,N,N',N" - tetramethylethylenediamide) dissolved in 50 ml of electrode buffer.

C. 0.15 g ammonium persulphate dissolved in 50 ml of water.

Gels were run horizontally, and a constant current of 35 ma was maintained. Electrophoresis was carried out in a cold room at about 4°C. Human blood was used as a marker and electrophoresis continued until the haemaglobin had moved 2.5 cm from the origin. Esterase activity was induced by incubation of gels at 37°C in the following solution, 50 ml of 0.1 M tris maleate buffer, ph 6.4, containing 50 mg Fast Blue RR, and 2 ml of a 1% solution of alpha naphthyl acetate in acetone.

Some individuals were dissected in

distilled water, being divided into testes, accessory glands, and the rest of the body. Each individual thus occupied 3 contiguous slots in the gels.

RESULTS AND CONCLUSIONS. Plate 1 is a composite photograph of three gels, showing two males (slots 1 and 2) and two females (slots 3 and 4) from one South African family, one male dissected into accessory glands (slot 5), testes (slot 6), and the rest of his body (slot 7), and two males dissected into abdomen tip (containing the accessory glands; slots 8 and 10) and the rest of the bodies (slots 9 and 11). The unique, accessory glands esterases are the darkest bands in slots 1, 2, 5, 8 and 10. We always saw a trace of these esterases in testes preparations and do not know whether or not these represent contamination from the accessory glands during dissection. Slot 8 shows an individual with two bands. The family from which it came showed single and double banded individuals, as did some other families. These patterns are consistent with suggestion that the accessory glands esterases are products of alleles segregating at a single, autosomal locus. These products overlie another, polymorphic esterase system which is common to males and females but which can be seen in the former only if the accessory glands are removed.

If the sexual dimorphism in *A. stephensi* reported by Freyvogel et al (1968) is similar to the phenomenon reported here, then it may be of phylogenetic interest that *A. stephensi* and the *A. funestus* species belong to the subgenus *Cellia* whilst *A. freeborni* belongs to *Anopheles*. However no such sex limited esterase is present in at least four of the species of the *A. gambiae* complex (Mahon et al. 1975), and these belong to *Cellia*.

In thinking of the function of this accessory glands protein it should be remembered that the hydrolysis of the ester, alphanaptyl acetate to its alcohol, used in this work to visualize esterase activity, is an artificial reaction in that it does not occur in vivo. (Shaw 1965).

ACKNOWLEDGMENTS. I would like

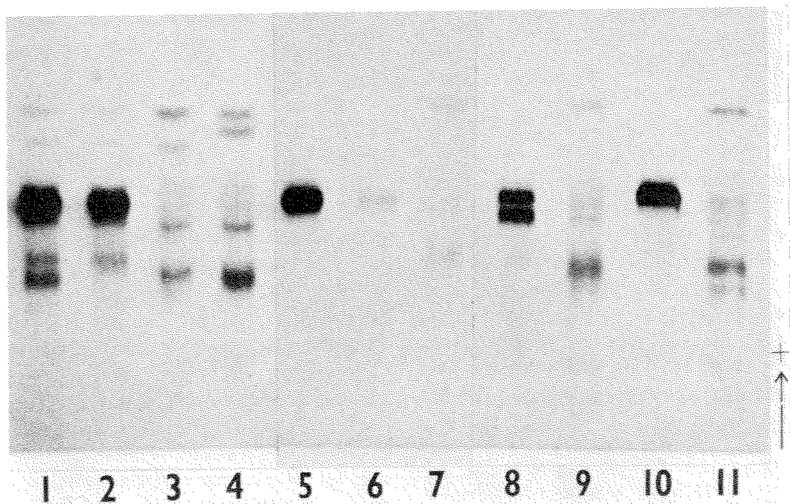

Plate 1. Nonspecific esterases of *Anopheles funestus* and a closely related South African species. See text for explanation.

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THE EHRENBERG PIGEON TRAP AS A SAMPLER OF *CULEX* MOSQUITOES FOR ST. LOUIS ENCEPHALITIS SURVEILLANCE[1]

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ABSTRACT. The Ehrenberg pigeon trap, a portable unit which utilizes a pigeon as bait for collecting mosquitoes, was utilized as a vector surveillance tool during the 1975 outbreak of St. Louis encephalitis in New Jersey. The trap was highly selective for *Culex* species and the majority were *Culex pipiens*. Ovarian dissections using the Detinova technique revealed that the trap collected parous and nulliparous *C. pipiens* in nearly equal numbers. The utility of the trap as well as its selectivity for *C. pipiens* of broad physiological age make this trap an efficient tool for St. Louis encephalitis vector and virus surveillance.

INTRODUCTION. Baited traps gained recognition when Bellamy and Reeves (1952) used a modified lard can baited with dry ice to trap mosquitoes. A number of authors have since utilized the lard can trap with a variety of animal baits (Dow et al., 1964; Easton et al., 1968; and Taylor et al., 1966). Many trap types have also been designed for specific purposes using a variety of animals as bait (Ehrenberg 1966; Pillai and MacNamara 1968; and Service 1969).

The Ehrenberg pigeon trap (Ehrenberg 1966) which uses a pigeon as bait (Fig. 1), was designed primarily to monitor *Culex* populations and has been used regularly by the Bergen County Mosquito Extermination Commission in New Jersey as part of its surveillance program. During the 1975 outbreak of St. Louis encephalitis (SLE) in New Jersey a sampling technique was needed to monitor *Culex* populations and collect large numbers for virus assay. The suspect vector of SLE, *Culex pipiens* Linn., is not readily collected for

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