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A NEW METHOD FOR RECOGNIZING NULLIPAROUS FEMALES OF
ANOPHELES GAMBIAE

by

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In the common oviduct of recently fertilized gambiae it is possible to detect a small plug of albumen-like material, that is apparently secreted by the male at the time of fertilization. This "mating plug" is about 1/2 mm long and persists for about 24 hours. It is present in all females shortly after mating and can be observed in about 80 per cent. of them on the following morning (see photo I). By the second morning it has been absorbed in practically all females.

This plug can be seen in wild-caught and laboratory-bred mosquitos. In the latter case, presumably owing to the confined space in a cage, double plugs are sometimes formed, indicating fertilization by more than one male (see photo II). This does not seem to occur in nature.

The presence of this plug in wild-caught females indicates that mating took place the previous night, and it provides an objective character for recognizing one section of the nulliparous population. If ~~recently~~ fed females are dissected they can readily be divided up into three categories:

- (a) unfertilized,
- (b) fertilized, plug positive, and
- (c) fertilized, plug negative.

Categories (a) and (b) are obviously entirely nulliparous; (c) is largely composed of multipars, but it has been found that it contains a proportion of nulliparous females that appears to vary according to the behaviour pattern of gambiae in that

particular district. On the East African coast, for instance, two blood meals are normally taken during the first cycle and the majority of females mate after the first feed. Nulliparous females will be recognized therefore after their first feed, by being unfertilized and, after the second, by the presence in 80 per cent. of them of mating plugs. In another district (South Pare), on the other hand, while some require only one blood meal, those taking two blood meals are very often fertilized before the first feed. So that by the time they have fed a second time, the plug will have been absorbed and they will appear in category (c).

It is not possible by this method to recognize all nullipars in a population except by further examination of category (c) (those without plugs). This may be done by study of other qualitative characters in the oviducts or perhaps by measurement of the ampullae. Once the proportion of plug negative to plug positive nullipars has been established in a particular district, it will only be necessary after that to record the incidence of unfertilized and of plug positive females. The total number of nullipars can be derived from this by simple calculation.

It is suggested that this method may be of value in estimating age changes occurring in gambiae populations under the influence of insecticides. For comparative purposes, before and after spraying say, it would be enough simply to record the proportions of unfertilized and of plug positive females before and after treatment. But it would obviously be better to be able to determine the absolute level of nullipars by one of the two more difficult methods mentioned above. Once this has been done, the work can be handed over to less experienced workers, whose job would be simply to record the proportion of females in categories (a) and (b) (unfertilized and plug positive) present.

Technique

Fed females are dissected in normal saline. The ovaries should be extracted without tearing the oviduct and examined under a cover-slip. The degree of ovarian development should be recorded first as exactly as possible, using the finer categories

defined by Macan.* The spermatheca is looked at next, and the common oviduct examined under the low power objective. The plug is usually readily visible, but it is sometimes necessary to exert gentle pressure on the cover-slip with a dissecting needle while the preparation is being examined. This will make the plug more visible and demonstrate its solid nature. After a few plugs have been seen, no difficulty should be experienced in recognizing them.

A useful way of tabulating results is set out below. All the older age groups are thus contained within the lower right hand spaces.

	Sperm negative	Sperm positive	
		Plug positive	Plug negative
Stage I or early II			
Mid II			
Late II			
Stage III			

* Early stage II. Yolk present, only visible under high power objective.

Mid II. Yolk readily visible under low power objective.

Late II. Yolk visible under binocular dissecting microscope (low power).

Stage III. Yolk occupying more than half of the follicle.