EFFECTS OF LARVAL AND ADULT DIET PLUS MATING/INSEMINATION UPON OVARIAN DEVELOPMENT OF LABORATORY-REARED *ANOPHELES PHAROENSIS* IN EGYPT

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ABSTRACT. A study was conducted in Egypt on the role of sugar and blood in oogenesis of *Anopheles pharoensis*. Also studied was the significance of mating vs. nonmating on oocyte maturity. Ovarian development in this species was influenced by larval diet. Those with inadequate larval nutrition emerged at Christophers' stage I and required either a sugar or blood meal to reach the resting stage. A subsequent complete blood meal was needed to take these females to stage V of Christophers (gravid). Females which emerged from well-nourished larvae were at the resting stage and became gravid via a single complete blood meal. Mating did not effect ovarian development since both inseminated and virgin females developed in like manner. At emergence, sugar feeding followed by a complete blood meal seems the best way for *An. pharoensis* to mature a first batch of eggs.

INTRODUCTION

In anautogenous species of mosquitoes, blood is an important and essential food. The role of the blood meal is to activate the ovaries and provide nutrients for the developing oocytes. Gonotrophic discordance, the requirement for multiple blood meals in the course of a single gonotrophic cycle, was observed by the authors among laboratory-reared *Anopheles pharoensis* Theobald females. The main objective of this investigation was to ascertain why some *An. pharoensis* females require multiple blood meals to produce their first batch of fertile eggs while others require only a single complete blood meal.

Nielsen and Haeger (1954) and Nayar (1969) produced evidence that in certain mosquitoes undersized adults result from inadequate larval nutrition. This in turn affects the stage of ovarian development in the emerging female.

The growth of the egg follicle in anautogenous mosquitoes consists of 2 periods: 1) Development to the resting stage, and 2) further development to form a mature egg which begins shortly after the female has taken a blood meal (Mer 1936, Rosay 1969, El-Said 1975[3], El-Akad 1985[4]).

Ovarian development beyond the resting stage also consists of a two-step process of initiation and promotion; development is initiated by taking a blood meal, but promotion of the oocytes to full term depends on the release of a hormone, presumably from the corpora allata (Detinova 1945, Mednikova 1952) or from the neurosecretory cells of the brain (Gillett 1956b, Hagedorn 1974). It seems likely that release of this hormone into the hemolymph results in mobilization of nutrient materials present in the blood meal for further development of the oocytes.

This study investigated: 1) the role of carbohydrates and blood in oogensis, 2) whether the presence of sperm in the spermatheca is a prerequisite for ovarian development in *An. pharoensis* and 3) whether a partial blood meal would induce sugar-fed females to mature any eggs.

MATERIALS AND METHODS

Mosquitoes used in these experiments were obtained from a long-established laboratory colony of *An. pharoensis* collected as larvae from Fayioum Governorate, southeast of Cairo, Egypt. This species was suitable for the study since it develops eggs only after ingesting blood. It is visually easy to determine the degree of blood engorgement from the amount of distention of the female abdomen (partial engorgement was considered <50% engorgement).

All stages of the mosquito were maintained at an average of 27–30°C and 70–75% RH. Procedures for rearing larvae were those of Gaaboub et al. (1970), El-Said (1975[3]) and El-Akad (1981[5]). Human blood via the senior author's fingers provided blood meals for the females; 10% sugar solution was provided for both sexes via a cotton wick.

Prior to the experimentation, 2 types of females were reared. Type A emerged from well-nourished larvae and type B from poorly nour-

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ished larvae. Larval diet was 2 parts dried milk: 2 parts bread crumbs: 1 part dried yeast. Two hundred larvae/pan were reared in Nile River water. Type A larvae were fed 3 times/day, type B larvae only once/day. Adult mosquitoes were housed in 50-cm cubical cages covered with fine nylon netting.

Ovarian maturation relative to mating and insemination: Two cages with 100 newly emerged type A females in each were established. One hundred males were added to one cage and kept there for 2 days to insure mating. The other 100 females remained virgin throughout the experiment.

On day 2 following emergence, both the mated and the virgin females were each subdivided into 4 groups of 25 and were offered various diets: water only, sugar solution only, a single blood meal, or sugar solution on day 2 followed by a blood meal on day 3. On day 6 the females were dissected and ovarian stages determined using Christophers' method (Detinova 1962). Spermathecal dissections were performed to assure matings.

Ovarian maturation relative to type of adult food available: Five batches each of 25 newly emerged type A and B females were offered 5 different meals: water only, sugar solution only, one complete blood meal, two successive blood meals (one on the day of emergence, the second at 3 days of age) and sugar solution at emergence followed by a complete blood meal on day 2. On day 6 ovarian stages were determined via Christophers' method.

Ovarian maturation relative to the amount of blood taken: Four batches each of 25 newly emerged type A females were offered 4 different meals: sugar solution followed by a partial blood meal (<50% engorgement), sugar solution followed by a complete blood meal, water followed by a partial blood meal and water followed by a complete blood meal. On day 6 ovarian stages were determined.

RESULTS AND DISCUSSION

Ovarian maturation in type A females was not dependent upon or affected by mating/insemination. When fed on water only or sugar solution only, both unmated and mated/inseminated females did not develop past the resting stage, which was the ovarian condition at emergence. Both mated and unmated specimens then developed mature eggs when given either a single blood meal or a sugar meal followed by a blood meal; this latter pattern is the normal sequence of feeding for *An. pharoensis* following emergence (El-Akad et al. 1988).

Follicles from females which emerged from well-nourished larvae were at the resting stage shortly after emergence. When these females were fed only on water or on sugar no further ovarian development was noted. A single complete blood meal, however, promoted development to mature eggs. A second complete blood meal had no additional discernible effect. Type B females which had fed previously on sugar matured their follicles shortly after feeding on blood.

Follicles from type B females were at the preresting stage of development (stage I of Christophers) at emergence. When the females fed only on water, the follicles remained at the preresting stage; when they fed on sugar, however, the follicles grew to the resting stage. After a complete blood meal the follicles developed to the resting stage. After two successive blood meals, the eggs matured. Type B females which had fed previously on sugar matured their eggs shortly after feeding on blood.

The quantity of blood imbibed by type A females was important to ovarian development. A complete blood meal was necessary for egg maturation to occur. Females normally fed for about 10 min. They deposited a blood droplet from the anus when engorgement was completed. If their feeding was interrupted at 5 min, their ovaries never developed beyond the resting stage. In type A females a single complete blood meal was required to produce the first batch of eggs.

It is clear from the results recorded in this study and the studies of Christophers (1911), Mer (1936), Detinova (1945, 1962), Mednikova (1952), Gillett (1956a, 1956b), Volozina (1967), and El-Akad (1985[4]) that for many anautogenous mosquitoes the growth of the follicle falls into 2 successive periods:

1) Development to the resting stage utilizes the nutritional reserves accumulated during the larval stage. Sugar feeding by the adult also allows the follicle to develop to the resting stage. In the absence of larval reserves, the effect of a single blood meal seems to be the same as that of feeding on sugar.

2) Ovarian development beyond the resting stage consists of a two-step process of initiation and promotion. Development is initiated and promoted by taking a complete blood meal which leads to distention of the abdomen, probably of the midgut itself. This physical pressure stimulates afferent nervous impulses leading directly or indirectly to the release of gonadotrophic hormone in the hemolymph which results in mobilization of nutrient materials present in the blood meal for further development of the oocytes (Larsen and Bodenstein 1959).

Previous studies by Roy (1936), Detinova (1942), Hosoi (1954) and Volozina (1967) claimed that when engorgement with blood is

incomplete, only a fraction of the potentially active follicles mature and the number of eggs laid is proportional to the size of blood meal. *Anopheles pharoensis* females do not respond in this manner. In this study a partial (incomplete) blood meal did not stimulate the development of any resting stage follicles. Evidently, full engorgement is required for hormonal release in this species.

Earlier studies claimed that the presence of sperm in the spermathecae was a prerequisite for ovarian development (Roy 1940). The ovaries of virgin *An. pharoensis* females, however, developed normally after feeding. Rosay (1969) reported similar results for *Aedes nigromaculis* (Ludlow) and *Culex quinquefasciatus* Say.

Larval diet definitely had an effect on the stage of ovarian development at emergence. Adult diet affected the terminal stage to which the ovaries could develop. Unless *An. pharoensis* females emerged from well-nourished larvae and had reached the resting stage via reserves accumulated during larval life, a single complete blood meal was not sufficient to complete oogenesis and produce mature eggs.

Nayar and Sauerman (1971) reported that utilizing blood both as an energy source and for ovarian development decreases the longevity of mosquitoes, thus reducing their reproductive potential. Unpublished studies by the authors found similar results for *An. pharoensis* fed only on blood. Therefore, it was reproductively advantageous for both type A and type B females to feed on both sugar and blood. Sugar feeding followed by a complete blood meal is likely the best way for *An. pharoensis* females to mature their first batch of eggs.

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