

and Sioux Counties, lies near the northern border of the vast high plains region of short grass prairie. Extending through these counties in a generally east-west direction is the Pine Ridge, a prominent topographical escarpment whose mature stands of ponderosa pine distinguish the area from any other region in the treeless short grass prairie.

Studies of the mosquito fauna of the Pine Ridge and the surrounding area of northwestern Nebraska have shown the species composition of the two areas to

differ markedly. Results of the studies indicate that with respect to mosquitoes the Pine Ridge is an ecological island in the short grass prairie.

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CHANGES IN THE OVARIES OF CERTAIN BITING MIDGES (DIPTERA: CERATOPOGONIDAE) FOLLOWING COMPLETION OF THE GONOTROPHIC CYCLE

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INTRODUCTION. It has been known for a number of years that in certain mosquitoes, recognisable changes occur in the ovaries following the completion of each gonotrophic cycle. In particular, Russian workers have pioneered the study of changes in the ovarioles of *Anopheles* mosquitos, as a means for age determination. Russian literature on this subject has been reviewed by Gillies (1958), and a further account is given by Beklemishev *et al.* (1959) and also by Detinova (1962).

The Russian technique for age determination, by observing the condition of the ovaries, depends upon the fact that after oviposition, a small but distinct dilatation remains in the ovariole at the site recently occupied by the egg. Subsequent ovulations result in similar dilatations, so that after the completion of several gonotrophic cycles a string of

dilatations may be seen in the portion of the ovariole between the currently developing follicle and the point of attachment of the ovariole to the calyx. Each dilatation is separated from its neighbours by a distinct connective stalk, while the first formed dilatation is separated from the calyx by the pedicel. Details of these structures have been given by a number of authors, and a recent description, employing the currently accepted terminology, is provided by Bertram (1962), so that further elaboration is unnecessary here.

It is the purpose of this paper to report and describe the formation of the first dilatation in the ovarioles of three species of biting midge, namely *Leptocnops bequaerti* Kieffer, *Culicoides barbosai* Wirth and Blanton and *C. furens* Poey. These species were studied in Jamaica, where their man-biting activity constitutes a considerable problem. It should be emphasized that only the formation of the first dilatation (following the first ovi-

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position) was observed and studied in detail in the three species. The possibility of the formation of multiple dilatations (produced serially on completion of several gonotrophic cycles) will be discussed, but multiple dilatation formation was not investigated fully in the present work, which was secondary in importance to observations on the ovarian cycle in the three species.

The ovaries in midges are very similar in structure to those of mosquitoes, and after a blood meal has been taken they undergo similar development through a series of stages, (Amosova 1959; Glukhova 1958; Linley 1965, in press). Only Glukhova (1958) has published observations on the occurrence of changes in midge ovarioles after oviposition. According to this author, multiple dilatations are formed in the ovaries of *Culicoides griseescens* Edwards females, where it was possible to recognize the completion of four previous ovarian cycles. Lewis (1958) examined the ovaries of *C. barbosai* and *C. furens* females in Jamaica, but did not observe dilatations. Very probably, the females used were nulliparous.

MATERIALS AND METHODS. Hungry females of the three species were captured in the field using a small aspirator, and brought back to the laboratory in 3- x 1-inch tubes suitably insulated from a small quantity of ice in a large thermos flask. The insects were often dissected immediately, but when necessary they could be kept for several days, either in tubes containing a damp strip of filter paper and a ball of cotton wool dampened with dilute honey (*C. barbosai*, *C. furens*), or in tubes containing 1/2-inch of slightly damp sand together with a similar carbohydrate source separated from the sand by a small square of celluloid (*L. bequaerti*).

Dilatation formation in *L. bequaerti* females was studied by dissection at intervals after oviposition induced by decapitation. Females were fed in the laboratory and allowed to mature eggs at a suitable temperature. Gravid insects

were then decapitated after very light ethyl acetate anaesthetisation and kept on small damp filter paper squares enclosed in plastic pill boxes. Decapitation almost always induces immediate and usually complete oviposition. Furthermore, headless females often live for many hours. Groups of females, including only individuals visibly alive, were dissected at intervals after oviposition and the ovaries examined. Dissections were made at 1/2 hour after decapitation, that is, immediately after oviposition, and also at 8 and 16 hours after decapitation. At least 20 individuals were examined in each group.

With *C. barbosai*, it was unnecessary to decapitate gravid females in the laboratory because a locality was found where the biting females were almost 100 percent parous. Furthermore, when females were captured early in the morning at this locality, almost all individuals had very large sac-like or saccate dilatations (to be described shortly) in the ovaries, indicating very recent oviposition. These individuals provided perfect material for study and groups of 10 females, from a large number maintained in the laboratory, were dissected at 12-hourly intervals after capture.

The process of formation of single dilatations in *C. furens* was not followed in the same detail as with the other two species, but as exactly the same structures were observed in ovaries of *furens* females taken in the field, it seems reasonable to suggest that the stages in dilatation formation are the same.

Dissections of the ovaries were performed in a drop of saline under a binocular microscope, using a magnification of $\times 100$. The two ovaries were removed intact by light posterior traction of the terminal two abdominal segments, then separated cleanly for more detailed examination. In order to view the basal portion of each ovariole, that is, the portion between the currently developing follicle and the calyx, it is necessary to stretch the ovariole very lightly, or more correctly to extend it so that its whole

length may be seen. This operation can be performed with very fine needles, and is facilitated if the ovary is first divided into clumps, each consisting of a few ovarioles (4 or 5 is an ideal number). It is important that during examination, the ovarioles are only very lightly extended. Extreme stretching causes the dilatations to become less obvious, and very frequently leads to tearing of the ovariole away from the calyx. Dilatations can be recognised without staining, but application of a stain, as used by Giglioli (1963) would undoubtedly render the structures more easily visible.

FORMATION OF THE FIRST DILATATION. Since the process of formation of the first dilatation is the same in *L. bequaerti* and

C. barbosa females, and is almost certainly similar in *C. furens*, a single description will be given, with the aid of Fig. 1.

The appearance of an ovariole containing a mature egg (just before oviposition) is shown in Fig. 1A. Immediately after oviposition (Fig. 1B), the position recently occupied by the egg is marked by a very large sac-like or saccate dilatation, which contains a quantity of granular material derived from the follicular epithelium. This stage was observed in *L. bequaerti* females immediately after oviposition induced by decapitation, in *C. barbosa* females caught in the early morning and dissected immediately on return to the laboratory, and in several

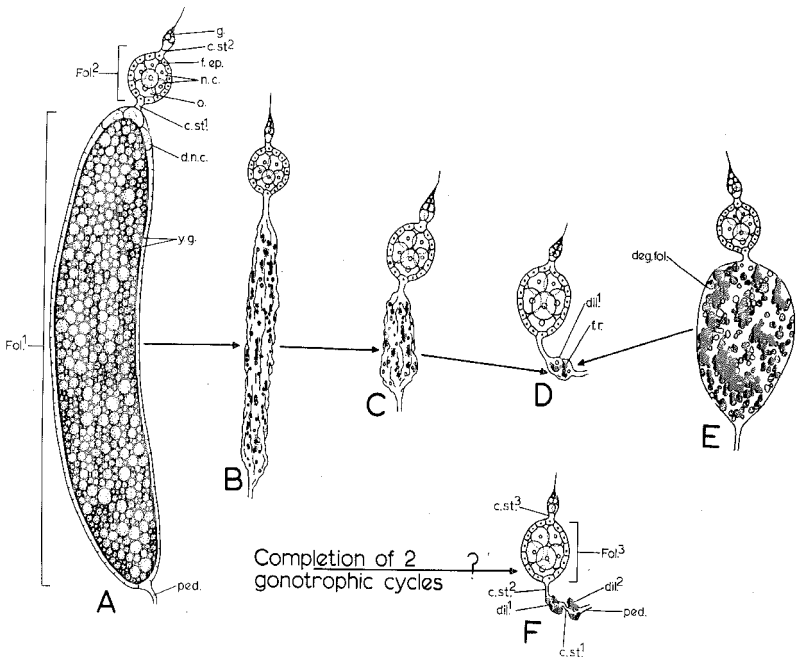


FIG. 1.—Stages in the formation of the first dilatation in the ovaries of *L. bequaerti*, *C. barbosa* and *C. furens*. A—ovariole containing a mature egg; B—ovariole with a saccate dilatation, (just after oviposition); C—ovariole with half contracted dilatation; D—ovariole with fully contracted dilatation; E—ovariole containing a degenerating follicle; F—ovariole possibly showing 2 distinct dilatations. c.st.¹, ², ³—first, second and third connecting stalks respectively; dil.¹, ²—first and second dilatations respectively; deg.fol.—degenerating follicle; d.n.c.—degenerating nurse cell; f.ep.—follicular epithelium; Fol.¹, ², ³—primary (first), second and third follicles respectively; f.r.—follicular relics; g.—germarium; n.c.—nurse cells; o.—oocyte; ped.—pedicel; y.g.—yolk granules.

furens females dissected soon after capture. In fact, the presence of large saccate dilatations can be observed without particularly close scrutiny of each ovariole. An ovary having dilatations in this condition has a distinctly milky or clouded appearance while still intact, and the dilatations are immediately obvious when groups of ovarioles are separated.

Contraction of dilatations from the saccate stage proceeds quite rapidly, at least in *L. bequaerti* and *C. barbosai*. The half contracted state (Fig. 1C) was observed in most of the decapitated *L. bequaerti* after 8 hours, and in the wild-caught *barbosai* after 12 hours. Contraction continues until the dilatation reaches the fully shrunken state (Fig. 1D), when no further shrinkage takes place. The dilatation at this point is more or less oval in shape and contains a few refractile granules, or follicular relics. Though quite small, dilatations are easily recognisable if dissections are performed carefully. Even if the particular ovariole under examination tears away from the calyx, and owing to the delicate nature of the material this frequently occurs, the dilatation often remains visible at the end of the short connective stalk attached to the separated follicle. Fully contracted dilatations were observed after 16 hours in headless *L. bequaerti* and after 24 hours in the *barbosai*. Similar structures were seen in a number of wild-caught *furens* females.

Work on the ovarian cycle in the three species (Linley 1965, in press and unpublished observations on the ovarian cycle in *C. barbosai* and *C. furens*) has shown that single dilatations are also formed when follicles start to develop, but later degenerate before reaching maturity, as indicated in Fig. 1E. Degenerating follicles are easily distinguishable because of irregular shape and the appearance of the yolk granules, which are aggregated into loose masses disposed throughout the entire follicle.

FORMATION OF MULTIPLE DILATATIONS. According to Glukhova (1958), multiple

dilatations, formed serially on completion of successive gonotrophic cycles, can be recognised in the ovarioles of *C. grise-cens* females. However, a similar occurrence should not be assumed for other midges without careful investigation.

In the present work, no *barbosai* or *furens* female was encountered with more than one dilatation in each ovariole. With *L. bequaerti*, out of a sample of 119 wild-caught females, dissected in the hope of seeing multiple dilatations, 93 were judged to be nulliparous (no dilatations), 23 had one dilatation, and tentatively, 3 individuals had two dilatations. The appearance of an ovariole from one of the individuals with two dilatations is shown in Fig. 1F. It would seem from this that multiple dilatations may be formed in *L. bequaerti*, but before this is accepted as a basis for further work, definite confirmation is needed through the examination of females known to have laid two batches of eggs. Multiple dilatations were not observed in *barbosai* and *furens* in the present work, but it is possible that they are formed, and again, this possibility should be carefully investigated.

SUMMARY. In females of *Leptoconops bequaerti*, *Culicoides barbosai*, and *C. furens*, a distinct dilatation remains in the limiting membrane (the tunica) of each ovariole after the completion of the first gonotrophic cycle, to mark the position recently occupied by the mature egg. The formation of this first dilatation is described. Single dilatations are also formed following the complete degeneration of follicles that fail to reach maturity.

Dissections of wild-caught *L. bequaerti* females suggest that multiple dilatations, produced serially on completion of successive gonotrophic cycles, may be formed in this species, though this needs confirmation. Multiple dilatations were not observed in *C. barbosai* or *C. furens* females, but further investigation is needed.

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CHIRONOMID CONTROL BY CARP AND GOLDFISH

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INTRODUCTION. This study on the use of carp, *Cyprinus carpio* Linnaeus, for the control of midges was undertaken as the result of observations on midge-carp relations at Lake Elsinore, California (Miller, 1951). There, in the year 1950, following a die-off of carp, enormous outbreaks of chironomid midges soon harassed local residents.

Although carp are omnivorous bottom feeders, their preference for chironomid larvae has often been observed. Thiennemann (1954) reported that chironomids are purposely cultured in Asian rice fields for the production of carp. Sigler (1958) stated that chironomid larvae apparently constitute the chief food item of carp. Johnson and Munger (1930) found that carp were the heaviest consumers of *Chironomus plumosus* (Linnaeus) larvae in Lake Pepin, Minnesota. Assman (1960) found carp, roach, bream, and goldfish to have a decreasing order of ef-

fectiveness as predators on chironomid larvae.

The present study was conducted to determine the near and long-term value of carp and goldfish in chironomid control. The study was made at the Rio Hondo water spreading grounds of the Los Angeles County Flood Control District near Whittier, California, during the spring and summer of 1960. Water at these grounds is intermittently impounded for ground water replenishment. Due to the instability of the aquatic environment thus formed, enormous populations of chironomids are at times able to develop and create a nuisance before natural controls can become established. Anderson and Ingram (1960) were the first to study this problem and found carp to be more effective than brown bullheads, *Ictalurus natalis* (LeSueur), in reducing numbers of midge larvae.

MATERIALS AND METHODS. Tests 1 and 2 were made in 1/50-acre earthen basins excavated 4 feet deep in alluvial sand. Except for the checks, treatments were unreplicated because of insufficient basins. Tests 3 and 4 were made in earthen basins 1/80-acre by 2 feet deep, and all treatments were replicated 3 times.

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