OBSERVATIONS ON *CULICOIDES OBSOLETUS* (MEIGEN) IN THE LABORATORY (DIPTERA:CERATOPOGONIDAE)[1]

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INTRODUCTION. "The black fly, when preparing to bleed you, can be seen; the mosquito warns you with his music; but punkies can be neither seen or heard, and if they could, what would it avail? You might, could you see them, kill an occasional one, but their funerals are marvelously well attended.

"They breed any time, and are ready for business in fifteen minutes after birth. They can drill a hole in a persons's skin as deep as their own bodies are long in just two-fifths of a second, eat a square meal off his blood and get away in another fifth, digest it and return hungry during the other two."

The foregoing quotation, from a book on the Adirondack Mountains of New York, was written in 1872 by H. Perry Smith and titled, "The Modern Babes in the Wood or Summerings in the Wilderness." It indicates that *Culicoides obsoletus* (Meigen), the punkie, is a serious pest in the Adirondacks and also that there is a considerable fund of "information" on its life history and habits.

A study of *Culicoides*, with particular reference to those attacking man, was undertaken by the New York State Museum and Science Service in 1959 and continued in 1960. The financial assistance of the Adirondack Entomology Research Fund and the field and laboratory assistance of Max McFadden in 1959, Thomas Watthews in early 1960, and Richard Riley in late 1960 in these studies is acknowledged. Collections of several thousand specimens attacking man from many locations in the Adirondacks during these two years were all identified by the author as *C. obsoletus* (Meigen).

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Indentification of representative specimens was confirmed by W. W. Wirth, U. S. National Museum.[2] In addition to *C. obsoletus*, two other species in the *C. obsoletus* group have been collected in the Adirondacks. Of these, *C. chiopterus* (Meigen) is not abundant and does not commonly bite man.[3] The other, an apparently undescribed species, is so uncommon that for purposes of this paper it is disregarded. The identification of species in the *C. obsoletus* group will be considered in a separate paper.

LONGEVITY OF WILD-CAUGHT FEMALES IN CAPTIVITY. It has become increasingly apparent in recent years that females of many species of biting Diptera do not invariably require a blood meal for the production of eggs and that many (if not most) feed on nectar as a source of energy (see review by Downes, 1958a).

One phase of our laboratory experiments with wild-caught *C. obsoletus* females was a study of their longevity, oöcyte development, and oviposition in the laboratory.

Punkies attacking man in search of a blood meal were captured and kept individually in cotton-stoppered four-dram

[2] The statement by Metcalf (1932) that *Culicoides biguttatus* (Coq.) is the important pest species in the Adirondacks was probably based on collections of adults not attacking man or on misidentified specimens. In our study, *C. biguttatus* was present in small numbers in light trap catches but was not collected attacking man.

[3] Only two references that mentioned biting by *C. chiopterus* were noted in the literature. Amosova (1958) cites Remm who recorded it as blood-sucking, particularly in the spring and autumn. Campbell and Pelham-Clinton (1960) state that they have only a few records of this species attacking animals but point out that specimens of *C. chiopterus* may well have been confused with *C. obsoletus* and overlooked.

vials. Some were allowed to engorge while others were collected before they could feed. A piece of moist cotton was placed in the bottom of each vial. The cotton in some vials was dipped in tap water and in others in a solution of refined cane sugar (sucrose). No attempt was made to maintain a standard strength solution because of subsequent changes caused by evaporation and the addition of more water to the cotton as it dried. The vials were kept inside the laboratory where the temperature averaged approximately 70 degrees during the test period.

Under these conditions, wild-caught females survived up to 51 days. Their survival times with or without blood meals and with or without sugar solution are shown in Figure 1. A convenient index to these differences is the time elapsed before 90 percent of the females died. These data are given in Table 1.

It can readily be seen from the figure and the table that a blood meal has little appreciable effect on longevity and that access to sugar solution greatly prolongs life.

TABLE 1.—Number of days from capture to ninety percent mortality of wild-caught *C. obsoletus* females with and without blood and sugar

Number of specimens	Blood meal	Sugar available	Days to 90% mortality
200	+	+	29.6
100	—	+	28.5
100	+	—	4.8
100	—	—	5.7

These data agree well with those of Parker (1950) who found that *C. obsoletus* gp. adults in Scotland survived an average of three weeks in moist environments when given access to nutrients in the form of a raisin or piece of prune. He records a maximum longevity of three and one-half months for a *C. obsoletus* group specimen and also notes that the other species of *Culicoides* tested survived

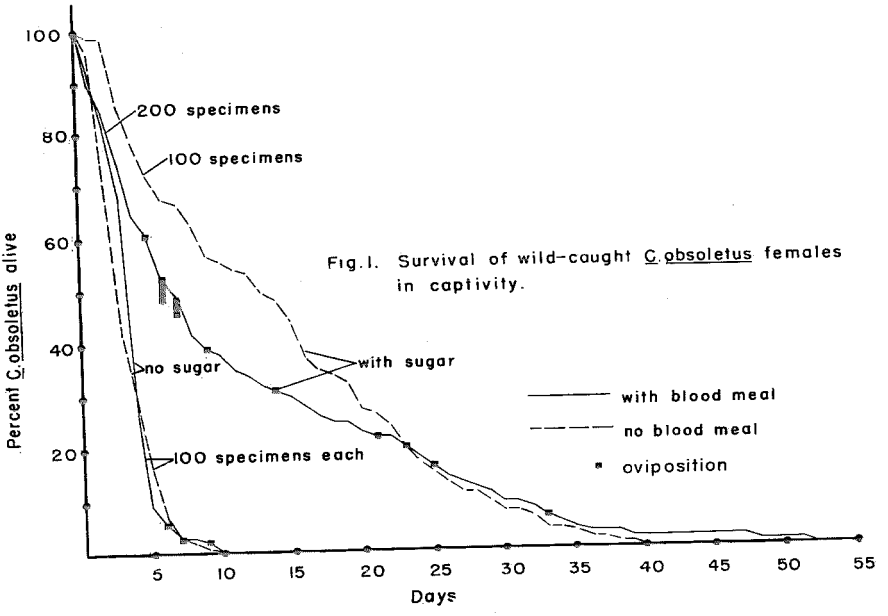

Fig. 1. Survival of wild-caught C. obsoletus females in captivity.

for much shorter periods (nine to 11 days) under the same conditions.

The abdomens of wild-caught specimens attacking man are usually much flattened. When placed on cotton moistened with sugar solution they almost immediately inserted their mouthparts into the cotton to feed. Their abdomens rapidly filled and became swollen with sugar solution. In contrast, specimens placed on cotton moistened only with water did not attempt to feed and when observed thereafter still had flattened abdomens.

DEVELOPMENT OF OÖCYTES IN RELATION TO THE BLOOD MEAL. Although the abdomens of punkies attacking man are usually flattened, when specimens are preserved in 90 percent alcohol they become swollen by osmosis. Since the walls are partially transparant some of the internal structure can be discerned. The mid- and hind-gut are inconspicuously appressed to the inner dorsal wall of the abdomen and the ovaries are small so that the abdomen is almost empty at this time. After a blood meal the abdomen is filled by the blood-distended stomach. Then as the blood is digested and the by-products eliminated the maturing oöcytes tend to keep the abdomen full.

The rate of blood digestion and oöcyte development was investigated by examining females killed at regular intervals after a blood meal. At least five females were killed on each of the 10 days following a blood meal. Others were killed at less regular intervals thereafter. Examination of living and preserved specimens indicated that there was little change in the volume of the blood meal during the first two days although it solidified and became opaque. By the third day small black-pigmented granules and rods (digested blood by-products, see Megahed, 1956) could be seen in the stomach along with apparently undigested blood. By the fourth day the blood was completely digested and the by-products largely or entirely eliminated. Megahed (1956) reported a similar pattern of digestion for *C. nubeculosus* Meigen except that digestion began within 24 hours and was completed within 72 hours.

The ovarian follicles of specimens attacking man were small, the largest measuring about 32 x 34 microns. After a blood meal the ovaries are pressed against the dorsolateral wall of the abdomen and are difficult to locate. In one such specimen the largest follicles measured about 42 x 32 microns the day after a blood meal. After three days the oöcytes nearly fill the follicles which have increased in size so that they can readily be seen in dissections and often through the wall of the abdomen. By the third day the largest oöcytes average about 112 x 46 microns. By the fourth or fifth day they reach approximately their pre-oviposition length of 262 to 340 microns and width of 46 microns and have assumed their characteristic cigar-like shape.

DEVELOPMENT OF OÖCYTES WITHOUT A BLOOD MEAL. When reared, unfertilized females that had not been given a blood meal were dissected it was surprising to find that the ovarian follicles were larger than those of wild-caught females. The largest follicles measured about 50 x 34 microns compared to 32 x 34 microns for those of wild-caught females. The enlarged follicles and the considerable amount of stored food material present in the abdomen of newly-emerged females suggested that the first ovarian cycle might be autogenous. Five reared unmated females that were given sugar solution survived for 6, 8, 17, 21 and 37 days respectively. The specimens that died on the eighth and twenty-first days after emergence contained large, apparently mature oöcytes measuring about 285 microns for the former and about 340 microns for the latter. The oöcytes of the other three specimens had not increased in size. These observations show that *C. obsoletus* females do not require blood for development of the first batch of eggs. Although it is highly probable that the first ovarian cycle is autogenous this cannot be assumed until it can be demonstrated that these oöcytes develop into viable eggs. Downes

(1958b) notes that the first ovarian cycle of *Culicoides gigas* Root and Hoffman is autogenous and suggests that, since it bites man, the second ovarian cycle requires a blood meal. Amosova (1958) notes that *C. dendrophilus* Amosova follows a similar pattern and cites records of autogeny for *C. circumscriptus* Kieffer and *C. riethi* Kieffer.

OVIPOSITION IN CAPTIVITY. When annoying wild-caught *C. obsoletus* females were allowed to take a blood meal and then held in captivity their oöcytes almost always developed to pre-oviposition size if the females survived for five or six days. However, most of the females died without laying eggs. Viable eggs were laid by seven percent of 200 wild-caught females that were given a blood meal and had access to sugar solution and by three percent of 100 similar females that had a blood meal but no sugar solution.

In contrast, no eggs were laid by 200 wild-caught females that were not given a blood meal. Of these, one half had access to sugar solution and the other half did not. Unlike those of the blood-fed females, the oöcytes of these females did not increase in size when held in captivity.

Eggs were laid between the fifth and thirty-third day following a blood meal. Nine of the 17 ovipositions occurred on the sixth or seventh day (Figure 1). These observations agree with those of Parker (1950), who noted that *C. obsoletus* group females generally laid eggs one to two weeks after a blood meal. Hill (1947) states that females lay eggs about two and one-half weeks after a blood meal.

The 17 females mentioned above laid an average of only 14.7 eggs (range 2–42) while 10 wild-caught gravid females captured in light traps contained an average of 36.1 oöcytes (range 18–58). In examining gravid females it was interesting to note that while both ovaries usually were filled with large oöcytes, sometimes one ovary had large oöcytes and the other undeveloped ones.

Hill (1947) states that *C. obsoletus* lays about 75 eggs (range 35–120) while Parker (1950) records 30–55 eggs per batch for *C. obsoletus* group specimens.

The reluctance of the females to lay eggs in captivity in our studies suggests that some modification of the conditions in the vials is desirable. Moist filter paper was used as a substrate in holding chambers by Hill (1947) and Parker (1950), apparently with considerable success in obtaining oviposition from *Culicoides* females including *C. obsoletus* gp. species.

In our studies the eggs were laid singly on moist cotton, usually all within a 24-hour period. The females usually became entangled in the cotton and died within 24 hours of oviposition, and in some cases before laying their full complement of eggs. The longest that a female survived after oviposition in our studies was six days. In contrast Parker (1950), using a moist filter paper substrate, was able to keep *C. obsoletus* group females (but not other *Culicoides*) alive for a reported average of 5.5 weeks following oviposition. This statement by Parker may be incorrect since he states in the same paragraph that *C. obsoletus* group females live an average of three weeks in captivity.

The presence of relict eggs, indicating a prior ovarian cycle was observed in three specimens out of several hundred dissected for various purposes. Parker (1950) reported that two *C. obsoletus* group females took a second blood meal in captivity following oviposition and laid eggs a second time. It seems likely that *C. obsoletus* has more than two ovarian cycles but this has not been demonstrated to date.

EGGS AND NEWLY HATCHED LARVAE. The hatching time for 20 eggs laid by *C. obsoletus* females in captivity ranged from four to seven days at room temperatures averaging about 70° F. Hill (1947) reported a range of 30 hours to 11 days for hatching; and Parker (1950), two to three days at temperatures averaging about 63° F. In our studies ten of the twenty eggs observed individually hatched on the fifth day. They hatched equally well under water or on moist cotton exposed to the air. Three of the eggs were placed on

damp cotton which was allowed to dry out. The cotton and eggs were apparently dry after 24 hours. One of the eggs placed in water at this time hatched in a total of five days. The other two were allowed to dry for two more days. At this time the eggs had started to collapse inwardly because of the drying. The eggs were immersed in water and two apparently normal larvae emerged, one six days after oviposition and the other a day later. These observations tend to support the more detailed studies of Parker (1950) with *C. pulicaris* group eggs. He found that the eggs may survive considerable desiccation and that drying prolongs the egg stage.

The individual eggs are medium brown in color and cigar-like in shape. Egg shells measured after larval emergence averaged 366 microns in length and 49 microns in maximum width (range in length 330–390 microns). Hill (1947) records a length of 380 microns (range 310–410 microns) and width of 76 microns for *C. obsoletus* eggs. The apparent difference in egg width between Hill's measurements and ours may be due to an increase in width due to flattening by the pressure of a cover slip in the case of Hill's measurements. When eggs of Adirondack specimens were slightly flattened by the pressure of a cover slip on a slide they measured 75 microns in width instead of the unflattened 49 microns.

Under high magnification about 10 longitudinal irregular rows of short thick stalks can be seen on the surface of the egg shell. These are enlarged apically and look like small mushrooms when examined under the microscope at about 970 X. These stalks are somewhat longer toward the anterior and posterior ends of the egg and range from less than one to about three microns in length. They are not so long as those illustrated and described by Hill (1947). In this connection Parker (1950) noted that the "suckerlike" structures on *C. obsoletus* gp. egg shells were not so long as indicated by Hill (1947) and suggests that this may be due to species differences within the *C. obsoletus* group.

The recently hatched larvae are pale white. The head capsule is barely distinguishable from the rest of the body under low magnification. Only the eye spots are dark and distinct. Ten of these larvae averaged 546 microns in length. The head capsules averaged 73 microns in length and 51 microns in width. Hill (1947) states that the larval head length of British specimens is about 0.5 mm and the head 77 microns long and 70 microns wide. This difference in width between Hill's measurements and ours may again be due to flattening by the cover slip. The larvae have a prothoracic proleg the details of which are difficult to make out. It has 2 or 3 pairs of long slender spines (about 9 microns) and about five transverse rows of fine combs. The presence of a proleg in first instar larvae of *C. obsoletus* was first reported by Kettle and Lawson (1952) who cite an unpublished communication from Parker.

SUMMARY. Wild-caught *C. obsoletus* females survived up to 51 days in captivity. Those that did not have a blood meal survived about as long as those that did. Those that were given sugar solution lived about five times longer than those that were not. Specimens attacking man usually have small oöcytes. After a blood meal the oöcytes grow rapidly so that in four to five days they fill the abdomen. The oöcytes of newly-emerged, reared, unmated females were larger than those of wild-caught females collected attacking man. In two instances they grew to preoviposition size when the females were kept alive and given sugar solution but not a blood meal. It seems probable that the first ovarian cycle of *C. obsoletus* is not dependent on a blood meal.

Among the wild-caught females captured while attacking man and kept in captivity, seven percent given blood meal and sugar solution laid eggs and three percent given a blood meal but no sugar solution laid eggs. None of those that were not given a blood meal laid eggs.

C. obsoletus females laid eggs between five and 33 days after a blood meal. The eggs hatched in four to seven days. The newly hatched larvae have a distinct proleg equipped with spines.

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