REARING AND DISTRIBUTION OF MARYLAND *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) [1]

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INTRODUCTION. The importance of *Culicoides* (Diptera, Ceratopogonidae) as vectors of several diseases of veterinary importance makes their laboratory colonization and mass rearing a necessary step before undertaking a study in depth.

DuToit (1944), Price and Hardy (1954), Foster *et al.* (1962) and Jones (1965) demonstrated *Culicoides* to be a vector of blue tongue virus in sheep.

Messersmith (1961) demonstrated their relationship to avian infectious synovitis in poultry in Virginia.

Culicoides are also intermediate hosts of *Haemoproteus* in ducks (Fallis *et al.*, 1957, 1960, 1961). Filaria such as *Acanthocheilonema perstans* (Hopkins and Nicholas, 1952), *Microfilaria perstans* and *Filaria perstans* (Sharp, 1927, 1928) are also known to be transmitted by *Culicoides*.

Scanlon (1960) studied their relationship to the virus of eastern equine encephalitis.

Rearing them in large numbers or even past the first generation is made difficult by their small size and complex ecological requirements. In an attempt to develop new techniques, a small colony of *C. guttipennis* (Coq.) was maintained in the laboratory for five months using techniques adapted from Jones (1957, 1960, 1964) and Hair and Turner (1966).

MATERIALS AND METHODS. Larvae of *Culicoides* were collected in the wild throughout the State of Maryland and brought back to the laboratory in waxed pint or gallon containers. Contrary to the method outlined in Hair *et al.* (1966) the contents of the tree and stump holes were not entirely removed. In wet tree-holes only the water in which the larvae could be seen swimming was taken and in the few instances where debris was removed, additional dead leaves were added to the hole. These holes were inspected several weeks later and third as well as fourth instar larvae were again plentiful. In moist tree-holes only a portion of the debris was sampled for the same reason.

Mud from the edges of ponds, streams and salt marshes was scooped up to a depth of 1 to 2 inches. Leaves from stream runoffs, moist wood shavings and mosses were also sampled.

All tree-holes were individually tagged in the field for quick reference as to their exact location. Upon arrival in the laboratory an emergence cage was placed on top of the containers. These were numbered and the date and location of the sampling was entered in a logbook.

The emergence cages consisted of a lid from a pint or gallon ice cream container on which was taped a pint container, its top being replaced with bolting cloth, and a stoppered hole permitting easy removal of the emerged adults.

A vial filled with tap water, stoppered with a dental wick and a cork to which raisins are pinned, was inserted in the side of the container. These cages were then placed on the collecting containers, a hole providing easy access to the emergence cages. Water vials were refilled every 2 or 3 days, while the raisins were changed every week or whenever they became mouldy or dry.

Culicoides guttipennis (Coquillett) were

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kept alive for colonization while all others were removed and preserved in 70 percent alcohol for further study.

Emergence data were collected and recorded from each cage even though all adults taken on the same day were placed in a common container. These holding cages are similar to the emergence cages but they lack a hole through the bottom.

After a minimum wait of 4 days adults of both sexes were placed in a feeding vial. This is a 1¼" × 2¼" plastic vial with two holes punched through the sides. One is big enough to admit the tip of an aspirator by means of which the *Culicoides* are transferred from the holding cage. The other, directly opposite, is fitted with a cotton plug. As the punkies are gently blown into the feeding vial they hit the cotton. This greatly reduces the incidence of injuries and shock to the flies. The top of the vial is covered with a piece of ordinary nylon stocking held in place by a rubber band.

The adults were left undisturbed for a period of 15 minutes prior to feeding since the females would not take a blood meal immediately after being transferred. The vial was then taped to the inner surface of a rabbit's ear. Feeding was observed immediately after and since the majority of the females had fed within an hour with very little feeding observed later, the vial was always removed after this lapse of time.

Contrary to the observations made by Jones (1957), no mating was ever seen in the vial even though both sexes were present and a crowded condition prevailed.

After the vial was carefully removed from the ear it was left standing for another 15 minutes or so. The adults were then transferred to an oviposition cage. To do this the adults were first attracted to one end of the vial by means of a strong light and the nylon removed. The vial was then quickly turned over the hole in the bolting cloth of the oviposition cage and transferring was effected by gently tapping down the adults. This eliminated the shock of being aspirated immediately after feeding.

The oviposition cages consist of two parts: the upper, similar to the emergence cages; the lower, a pint container with a lid taped to the bottom. This lid fits snugly over a plastic petri dish top. Dead leaves were taped to the inside of the bottom portion to provide hiding and resting places for the adults.

These cages were kept in a dark closet where temperature and humidity fluctuated.

A tissue moistened with stump water was placed in the petri dish to provide a suitable place for oviposition. This water was prepared by letting dead leaves settle for a minimum of 2 weeks in distilled water. The broth was then carefully strained to exclude all foreign larvae and eggs. Microscopic examination of the water revealed numerous rotifers, protozoa and nematodes. Since nematodes are known to parasitize *Culicoides* and their presence could destroy the colony they were taken to the U.S.D.A. Ag. Res. Ser. Division of Nematology at Beltsville and identified by Dr. A. M. Golden as *Rhabditis* sp. which feed on decaying matter.

The tissue was inspected daily for eggs. If present the eggs were counted and a new dish with paper and stump water was inserted into the bottom of the cage. Dead adults were removed whenever possible and preserved in 70 percent alcohol. The dish containing the eggs was then flooded with stump water and placed in another dish with a tight-fitting lid. This dish contained tap water to minimize evaporation.

It was placed in an incubator at 25± 1° C. and inspected daily until the eggs hatched. The contents of the petri dish were then placed in an aquarium half filled with the same stump water. In addition, dead leaves were held to the bottom by a screen. An aerator running at night kept the water circulating and provided aeration. The aquaria were painted flat black and covered with plywood tops on which rested an emergence cage similar to those already described. Each succeeding generation was kept in a separate aquarium.

Emerged adults were removed and placed in a holding cage. Feeding and oviposition techniques were the same for each generation.

RESULTS. In the 5 months during which the experiment lasted more than 2,600 adults of *C. guttipennis* emerged, 13,000 eggs were laid and 7,500 hatched.

Two thousand wild adults (W) emerged from larvae brought into the laboratory. These adults laid 8,700 eggs during a 3-month period; 6,000 of these eggs hatched (F_1, 2nd generation larvae) representing approximately 70 percent of the eggs laid. Subsequently 446 F_1 adults emerged representing 7.5 percent of the larvae or 5 percent of the number of eggs laid by the wild adults. The 446 F_1 adults laid 3,000 eggs; 1,400 of these eggs hatched (F_2, 3rd generation larvae) representing approximately 50 percent of the number of eggs laid by the F_1 adults. One hundred and sixty F_2 adults emerged representing 10 percent of the larvae or 5 percent of the number of eggs laid by the F_1 adults. The 160 F_2 adults laid 1,500 eggs; 40 of these hatched (F_3, 4th generation larvae) or 2 percent of the number of eggs laid by the F_2 adults. Two adults emerged (F_3) representing 5 percent of the number of larvae or 0.15 percent of the number of eggs laid by the F_2 adults.

Ten other species were reared in the laboratory from samples taken while collecting *Culicoides guttipennis.* These were:

C. arboricola Root and Hoffman, *C. baueri* Hoffman, *C. biguttatus* (Coquillett), *C. furens* (Poey), *C. haematopotus* Malloch, *C. hollensis* (Melander and Brues), *C. piliferus* gp. Root and Hoffman, *C. spinosus* (Root & Hoffman), *C. stellifer* (Coq.), *C. villosipennis* Root and Hoffman. See Table 1.

In addition three other species were collected while biting, one each of *C. obsoletus* (Meigen), *C. sanguisuga* (Coquillett) and *C. paraensis* (Goeldi).

It is also interesting to note that one male specimen of *Culicoides villosipennis,* ordinarily a tree-hole breeder, emerged in the laboratory from a soil sample from Glenwood, Howard Co. This sample contained decaying vegetable matter and mud from a swampy area. No other specimens were recovered. It is possible that a lone female unable to find a suitable oviposition site laid her eggs in the dead leaves and that this was the only larva surviving in an unnatural habitat.

DISCUSSION. Two factors seem to have caused the loss of the colony.

There was a marked decrease in the number of males emerging as the colony progressed. Of the second generation adults 17 percent were males as compared with only 5 percent in the third generation. It is well known that the males are less hardy than the females; for this reason they may have been drowned during emergence. A careful examination of the surface of the water in the aquaria did not reveal any number of dead males. It is, however, possible that they sank to the bottom upon dying and were not notice-

TABLE 1.—Species of *Culicoides* reared in the laboratory.

	Males	Females	Total	Substrates
arboricola	2	1	3	Tree-holes
baueri	..	1	3	Swamp. Dead leaves and mud
biguttatus	27	46	73	Swamp. Dead leaves and mud
furens	5	5	10	Stream edge in salt marsh
guttipennis	?	?	2000+	Tree-holes
haematopotus	6	5	11	Soil
hollensis	1	13	14	Pond edge near salt marsh
piliferus gp.	23	27	50	Swamp. Dead leaves and mud
spinosus	5	..	5	Swamp. Dead leaves and mud
stellifer	4	5	9	Swamp. Dead leaves and mud
villosipennis	350	500	850+	Tree-holes

able. It does seem unlikely that such is the case since these flies will generally float on the surface of the water even after having been dead for days but it is very possible that the aerator caused enough disturbance to prevent their emergence during the night.

One note of interest is the fact that the females which emerged from these aquaria were much bigger than the emerged or reared females which had been brought into the laboratory as larvae. An investigation of the sex ratio of *Culicoides* in relation to larval nutrition may be a worthwhile undertaking.

The second factor affecting the colony was the untimely appearance of Pharaoh ants *Monomorium pharaoins* (Linné). These notorious pests have been known to destroy insect collections and colonies. The emergence cages on the aquaria contained many wings, legs and antennae, the only remains left after a single night's visit by the foraging ants. The oviposition cages were also raided and adults were lost in the same way, the ants penetrating through holes too small for the *Culicoides*. Placing the cages on an island in water pans also proved unsuccessful since the surface tension was great enough to permit the ants to walk across the surface of the water.

Tanglefoot or other sticky substances to provide a protective barrier should have been applied but even this has been known not to deter the ants. In any case it was already too late since most of the colony had already been destroyed by morning. Inauguration of a new project precluded continuation of the rearing studies.

CONCLUSION. Even though the colony was destroyed the techniques and materials used proved very successful.

A minimum amount of time was required each day to remove and blood feed the adults and inspect the oviposition cages. The materials were cheap and easily obtainable and cages were easy to construct and maintain.

Feeding time was reduced to a minimum, yet still allowed the majority of the females to take a blood meal. It is felt that placing the oviposition cages in a controlled environment room will increase the oviposition rate and the viability of the eggs.

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