decticus and *Culiseta morsitans* had not been reported from the area according to Procter 1946. *Aedes atropalpus* previously reported from Bar Harbor was found to occur quite commonly wherever there are rocky ledges extending above the high tide level.

The Tabanidae are represented by 22 species and five genera. *Chrysops montana, Hybomitra frontalis, Merycomyia whitneyi* and *Tabanus nivosus* are new records both for Mt. Desert Island and for Maine. *Hybomitra aurilimba* is a new record for the Island.

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References

JOHNSON, C. W. 1925. List of the Diptera or two-winged flies. Fauna of New England 15. Occ. Pprs. Boston Soc. Natl. Hist. VII:3–326.

PHILIPS, C. B. 1947. A catalog of the blood-sucking fly family Tabanidae (horse flies and deer flies) of the Nearctic Region north of Mexico. Am. Mid. Natl. 37:257–324.

PROCTER, W. 1946. Biological survey of the Mt. Desert region. VII. Wistar Inst. Anat. & Biol. 506 pp., illus.

NOTES ON THE TECHNIQUES OF HANDLING MOSQUITOES IN THE LABORATORY [1]

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In the laboratory, techniques are often a matter of personal preference. However, a communication of this nature may nevertheless be very useful. For extensive information on mosquito culture techniques and experimental procedures, Trembley's paper (1955) should be consulted. Reported here are some experiments and techniques which either are not covered by Trembley or are considered improvements by the author.

CAGE. For the conventional screen-type of mosquito cage, nylon is the most desirable, because it is lighter than wire, more durable than cheesecloth, and above all, offers better visibility than either. Since adequate humidity is essential for the survival of the adult mosquitoes, ordinarily it is supplied to this type of cage by providing wet toweling or by putting the cage in a specially humidified room. Frequently the cage becomes moldy and difficult to clean.

In our laboratory, cages with favorable humidity but with no mold contamination have been maintained in their original condition for the past five years.

The cage is very simply constructed. It is composed of a plywood top and bottom, four corner bars, and an extra horizontal bar to square off a lower opening for the nylon sleeve. The rest of the framed area is walled with "Clear Acetate" sheet, a plastic that is transparent, durable, non-inflammable and easily cleaned by a damp sponge.

Moisture inside the cage is supplied from a piece of cheesecloth drawn out from a water bottle. The cloth is spread and hung over a glass rod resting on two hooks screwed on the corner bars of the cage. In such a closed cage, the relative humidity

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can be kept above 60 percent with one piece of cheesecloth, and above 80 percent with two pieces, one on each side of the cage. For efficient transpiration, distilled water should be used in the bottle and the cloth should be bleached or replaced when it becomes stiff.

The wood frame is painted with enamel paint, and hence the cage can be cleaned easily by wiping with a damp cloth or washing, and kept in top condition at all times.

ADULT TRAPPING TUBES. For general routine in our laboratory, a suction tube attached to the compressed air line was used instead of a mouth suction tube. In picking up adults for experimental purpose, a simple glass trapping tube was employed.

This tube had a bore of 1 cm. and a length of about 45 cm., depending upon the size of the cage in which it was to be used. It was slightly flared at one end over a flame. About 6 cm. from this end it was bent broadly to an angle of about 112°.

For trapping mosquitoes, the handling end of the tube was plugged with cotton and the flared end was brought directly over the mosquito. This was quickly lowered onto the mosquito, which in trying to take flight, was trapped at the bent section of the tube. A gentle tapping sent this mosquito to the other end, and the tube was ready to catch the next one.

After a little practice three or four mosquitoes could be caught in a minute. It was found convenient to have many such tubes made, and thus mosquitoes trapped in small lots of 5 or 6 could be handled more easily for experimentation. This method was especially good for picking up engorged females which might be injured by suction.

ANAESTHESIA. Carbon dioxide gas was preferred to ether or chloroform because it could be easily passed through the trapping tube, and if it was applied to a closed chamber such as lamp chimney, it did not have an area of high concentration that could kill the mosquitoes falling close by.

DE-WINGED ADULTS. In our laboratory, it was found that *Culex tarsalis* and *C. pipiens* lived a normal length of life after their wings were pulled out at the base. With two pairs of forceps, such an operation could easily be performed while the insects were still under the influence of CO_2. Judged from the mortality immediately following the operation and thereafter, the mosquitoes apparently were not harmed. If they were supplied with food, they could be raised as walking insects in Petri dishes.

Kept as de-winged adults, *C. tarsalis* females laid viable eggs as usual after a blood meal on a malarial canary. Likewise, the sporogonous development of *Plasmodium relictum* in the mosquito was completed in a normal manner. We have found that such a technique certainly streamlined our dissection work, and we hope it will be found useful by fellow "mosquiteers."

EGG COUNTS. It is impractical, if not impossible to count the eggs while they are floating on the surface of water. In egg-hatching studies on *Culex* mosquitoes in our laboratory, the egg cases were submerged in a thin layer of 70 percent alcohol and the eggs were counted under a dissection microscope, after the hatching was finished.

If great accuracy is not demanded, the percentage of hatching can be determined by counting only the eggs in the peripheral layer of the raft. By this method, counts can be done under the dissection microscope while the rafts are still floating on the surface of water without being teased apart. The hatched and unhatched eggs are very apparent in the unbroken raft.

In insects which lay circular or near circular egg masses, this counting method is very accurate. One may choose to count the eggs along the diameter or around the circumference in an egg mass to get values both for the total number of eggs and the hatching ratio.

MOSQUITOES IN THE DARK ROOM AT 18° C. *C. tarsalis* and *C. pipiens* went through many generations in a dark room at a constant temperature of 18° C. although the populations and life processes

(biting, egg laying and hatching, length of each life stage) were subnormal. Mortality in the first larval instar was high, but emergence was normal and the pupae and adults were larger than those at room temperature (above 26° C.).

Our *C. pipiens* is an autogenous strain, and the autogenous character is demonstrable under the above mentioned conditions. Our *C. tarsalis* is capable of laying viable eggs without a blood meal at room temperature (Chao, 1958) but a blood meal is necessary for viable eggs in the dark at 18° C.

In answer to a mosquito control problem, Shute (1959) pointed out that *Culex molestus* bred autogenically in air raid shelters in London during the last world war. Earlier, Jobling (1937) reported that *C. pipiens, C. fatigans,* and *Aedes argenteus* could develop in complete darkness, and a second generation was produced by the autogenous *C. pipiens*. It is evident that some autogenous strains of mosquito can reproduce in the absence of light, a fact that should be taken into consideration in mosquito eradication programs.

EXPERIMENTS IN HYBRIDIZATION OF *C. tarsalis* AND *C. stigmatosoma*. Since our laboratory had both colonies of *C. tarsalis* and *C. stigmatosoma* in 1955 (Ball and Chao, 1956) several attempts were made at that time to cross them. The results of the trials were negative regardless of in which direction the cross was made. No sperm was found in the spermacecae of the females of either species. Egg cases were laid after a blood meal but the eggs were not viable.

SUMMARY. Reported in this paper are an improved mosquito cage, techniques of catching and de-winging of the adults, and the method of egg counting. The raising of mosquitoes in a dark room at reduced temperature is reported. Attempts to hybridize *Culex tarsalis* and *C. stigmatosoma* were unsuccessful.

Literature Cited

BALL, G. H., and CHAO, J. 1956. Laboratory colonization of *Culex stigmatosoma*. Mosq. News 16(4):306.

CHAO, J. 1958. An autogenous strain of *Culex tarsalis* Coq. Mosq. News 18(2):134–136.

JOBLING, B. 1937. The development of mosquitoes in complete darkness. Trans. R. Soc. trop. Med. & Hyg. 30(4):467–474.

SHUTE, P. G. 1959. (A letter to the Editor.) Mosq. News 19(1):28.

TREMBLEY, H. L. 1955. Mosquito Culture Techniques and Experimental Procedures. Amer. Mosq. Control Assoc., Bull. No. 3. 73 pp.