LABORATORY COLONIZATION OF TWO BITING MIDGES, CULICOIDES ARAKAWAE (ARAKAWA) AND C. SCHULTZEI (ENDERLEIN) (DIPTERA: CERATOPOGONIAE)*

by

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Abstract: Two biting midges, *Culicoides arakawae* (Arakawa) and *C. schultzei* (Enderlein), have been colonized in the laboratory for six months and completed 6—7 generations and 4—6 generations, respectively. The adult females are placed in rearing cages made of bronze surrounded with nylon stocking material and set in a flat culture dish with several layers of moistened filter paper. The rearing cages are kept in dark cabinets at a constant temperature of 25°C and relative humidity of 80%. Blood-feeding is provided by exposure to young chicks. Oviposition results 4—5 days after feeding. Hatching occurs 4—5 days after oviposition. The medium for larvae is made of water (Cl-free and F-free tap water) and yeast blood agar base cake. No natural materials from the breeding habitat of the midges are added. Aeration is provided and larvae are shielded from light. The average life cycle of *C. arakawae* from egg to adult requires 26 days and of *C. schultzei* 30 days. Interspecific mating between these two midges with male *arakawae* and female *schultzei* has been sucessfully induced in the laboratory.

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INTRODUCTION

Rearing mosquitoes in the laboratory for disease vector studies has been widely developed since *Aedes aegypti* (Linnaeus) was first linked with yellow fever by Reed and other in 1900.[1] Beginning in 1959 *Culex tritaeniorhynchus* Giles, the most important vector of Japanese B encephalitis in Taiwan,[2] has been colonized for virological studies in the Entomology Laboratory at the United States Naval Medical Research Unit No. 2 (NAMRU-2), Taipei, Taiwan.[3] Several colonies of *Anopheles* species have also been established for the studies of malaria in the Department of Entomology, at the Taiwan Malaria Research Institute, Chao-chow, Pingtung, Taiwan.[4]

Wu and Wu claimed to have isolations of Japanese B encephalitis virus from a biting midge, *Lasiohelea taiwana* (Shiraki) at Fukien Province, China mainland.[5] Bergner and Jachowski succeeded in laboratory transmission of a filaria, *Macacanema formosana* Schad and Anderson by a midge, *Culicoides amamiensis* Tokunaga in the Parasitology Department, NAMRU-2, Taipei.[6] Lee *et al.* reported the biting mide, *Culicoides arakawae* (Arakawa) as the vector of a protozoan parasite, *Leucocytozoon caulleryi* among chickens in Taiwan.[7] Hurlbut and Sun have isolated several unknown viruses from *C. arakawae* and other ceratopogonids in Taiwan.[8] In order to study the host-parasite relationships of the diseases mentioned above clean laboratory-reared midges are needed.

Since 1915 several reports on the developmental stages of *Culicoides* have had only taxonomic objectives. Downes established the first laboratory colony of *C. nubeculosus* Meigen simulating the natural habitat of the larva with richly manured soil from their breeding place.[9] Megahed took over the care of Downes' colony of *C. nubeculosus* in 1951 and found that adding powdered charcoal and dried autolyzed yeast to the mud greatly increased the number of adults emerging.[10] The yeast presumably enriched the medium directly, by its content of growth-stimulating factors (vitamins of the B group), and indirectly, by encouraging the growth of bacteria and other micro-organisms. Jones has bee successful in the mass production of *C. variipennis sonorensis* (Cog.) by rearing the larvae on a mixture of cow manure, black soil, dried yeast and water.[11], [12] Hair *et al.* reported the laboratory colonization of *C. guttipennis* and reared the larvae in a medium consisting of decaying leaf-mold.[13]

Beginning in 1963 the author has reared a number of the immatures of *C. arakawae* (Arakawa), *C. schultzei* (Enderlein), and *C. homotomus* (Kieffer) to adults on several occasions in the Entomology Laboratory, at Tunghai University. Engorged females of these three spcies as well as of *Forcipomyia* (*Lasiohelea*) *taiwana* (Shiraki) laid eggs in the laboratory, which always died in the first larval stage. In June 1965, however, the author finally succeeded in rearing *F.* (*L.*) *taiwana* from eggs to adults on a medium of clay (laterite) and yeast.[14] *F.* (*L.*) *taiwana* is the most severe daytime biter afflicting man in Taiwan and has heen implicated in the transmission of Japaanese B encephalitis virus to man in Fukien Province, China mainland mentioned before.[5] *C. homotomus* also bites man and is known to transmit the filaria *Onchocerca cervicalis* to horses.[15] *C. schultzei* is a known vector for African horse-sickness virus in Africa and India and also for the filaria, *O. gibsoni* among cattle in Malaya.[15], [16], [17] *C. arakawae*, which is primarily ornithophilic,

is a vector for a protozoon causing leucocytozoonosis among chickens and is also the species from which the unidentified infant-mouse-killing viruses mentioned above have been isolated at NAMRU-2. [7][8]

In August, 1965 during his summer visit at NAMRU-2, the author has established a laboratory for rearing *Culicoides* in the Department of Medical Ecology. Two species of *Culicoides: C. arakawae* and *C. schultzei* were successfully colonized in the laboratory and maintained for 6 months. On December 1965, the first laboratory reared F_1 adults of both species emerged and by April 1966, *C. arakawae* completed 6—7 generations and *C. schultzei* 4—6 generations. Unfortunately, the colonies were destroyed in a explosion of May 1966 at NAMRU 2. A new laboratory at Tunghai University to develop the colonization of midges of medical and veterinary importance was established by the author in 1968.

MATERIALS AND METHODS

Adult Midge Collection and Maintenance

Adult midges were collected from the field by using light traps of the New Jersey type, which were located near pig or chicken shelters providing blood source to attract these blood-sucking insects. Living specimens were brought back to the laboratory for identification. Female midges were fed on laboratory-hatched young chickens and they were kept in bronze or galvanized iron rearing cages, 11 x 15 cm, surrounded with nylon stocking material and covered by a moistened cloth to maintain a relative humidity of about 80% inside the cage. The rearing cage was set in a flat culture dish with several layers of filter paper. As the gravid females will never oviposit on a dry substrate, the filter paper was kept constantly moistened. A glass bulb containing glucose solution (5%) was inserted in the top of the cage for feeding. Glucose was renewed three times per week. The cages were kept in dark cabinets at constant temperature of 25°C, and relative humidity of 80%.

Mating and Oviposition

Mating of the midges takes place readily in the rearing cages;conjugating pairs are often seen in the cages flying or moving about on the walls of the cage; duration of mating is about 3—5 minutes. A blood meal is necessary for the development of the ovaries and the maturation of the eggs. Blood feeding was initated two days after the emergence of adult females; one end of the rearing cage covered by a piece of nylon was held in contact with the feather-free skin on the back of an anesthetized laboratory-hatched young chicken. The feeding condition was maintained in the dark for an hour and repeated twice a week.

Larval medium

The medium for larvae was prepared from chlorine-free and fluorine-free tap water mixed with yeast-blood agar base cake. The cake was made from 50 gm of blood agar base (Difco) and 0.5 gm of dried yeast (Taiwan Sugar Co.) plus 30 cc of distilled water and boiled for an hour. A finger bowl (Turtox) 11 x 4.5 cm containing 120 cc of Cl-free and F-free tap water was added by one gram of the medium cake. For the newly hatched larvae adding medium cake once every week is enough and for the grown instars three adding times per week are needed. Aeration was

supplied by glass tubes connected by rubber tubing from an air-pump. All larval instars were shielded from light.

RESULTS AND DISCUSSION

Life cycle

The average life cycle of *C. arakawae* under these laboratory conditions requires 26 days (24—28) as follows: egg to larva, 4—5 days; larva to pupa, 18—20 days; and pupa to adult, 2—3 days. The development of *C. schultzei* is similar to that of *C. arakawae* except for lengthening of larval time to 22—24 days under these conditions resulting in an average life cycle of 30 (28—32) days.

Oviposition usually results within four or five days after feeding. The number of eggs per one oviposition varies greatly from a few to over two hundred eggs. The eggs are laid singly and dispersed on the filter paper in a linear sequence. (Fig. 1) The filter paper with eggs is placed in larval medium.

Hatching occurs in 4—5 days after oviposition beginning at the upper third of the anterior end where the first instar breaks its way out. (Fig. 2)

The larvae are transparent with head capsule yellow. They swim actively in the water by eel-like motion and can move slowly through the gelatinous mass of the medium. Their forward protruding mouth and sharp-pointed mandibles are used for piercing prey, as the *Culicoides* larvae are primarily carnivorous and sometimes cannibal. (Fig. 3)

The pupae are greenish in color immediately after pupation and gradually change into brown and continue to darken in color before emerging. They usually float on the surface of water so that they are easily seen and removed from the larval bowl by a medicine dropper and placed on a moist pad in a rearing cage. Pupae usually emerge to adult midges in two or three days. (Fig. 4)

Artificial insemination

Artificial insemination of the midges has been successfully tested and may be used to insure the fertilization of eggs and thus increase the hatching rate. The method uses a decapitated male adult put into bodily contact with a female adult.

Interspecific mating

Out of approximately 1,000 copulating pairs of *Culicoides* collected in light traps four cases of interspecific mating were noted. In each case, the male was *C. arakawae*. In two cases, the females were *C. schultzei*; in one case, the female was *C. duodenaris* Kieffer; and in the most interesting case, the male *arakawae* was copulating with a female gall-gnat (Diptera: Cecidomyiidae). (Figs. 5, 6, 7)

Experiments of interspecific mating in the laboratory were carried out. Two or three male *arakawae* and 10 female *schultzei* were brought together and confined to a ten-centimeter length of a glass tube (5 mm in diameter) by filling both ends of the tube with small cotton balls in order to minimize the central space inside the tubing and ensure maximal contact between the sexes. Several cases of interspecific mating have been induced in this manner. In only one case, however, was oviposition observed after the aberrant copulation and, in that case, no eggs hatched.

SUMMARY

Two biting midges, *Culicoides arakawae* (Arakawa) and *C. schultzei* (Enderlein), have been colonized in the laboratory for six months and have completed 6—7 generations and 4—6 generations respectively. A larval medium made of water mixed with yeast-blood agar base cake has been developed. Interspecific mating with male *arakawae* and female *schultzei* has been successfully induced in the laboratory.

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荒川氏庫蠓與舒爾茲氏庫蠓之實驗室培育

荒川氏庫蠓與舒爾茲氏庫蠓在臺灣甚屬普遍，每於日沒前後成群飛出，刺螫人體及家禽家畜。荒川氏庫蠓傳遞鷄白冠病原蟲爲養鷄業之大害，舒爾茲氏庫蠓能傳遞非洲馬瘟病毒及數種絲虫。著者於五十一年曾與病毒學家 Dr. Hurllbut 合作，自荒川氏庫蠓分離出病毒四株（尙未定名），更進一步顯示出此類昆蟲在醫學、衞生上之重要性。

著者利用捕蟲燈將成體捕獲而飼育於實驗室中。經發現其完全之生活史分別爲 26 日（荒川氏庫蠓）及 30日（舒爾茲氏庫蠓），前者共飼育6——7代，後者4——6代，在飼育過程中，一種合成容易，完全不用蠓虫孳生地天然物質的幼蟲媒地、已經實驗成功。

在自然環境下，雄性荒川氏庫蠓之性活動，非常積極，除與本種雌體交配外，並和數種異種者（甚或異科者）交配。著者在實驗室中曾誘導雄性荒川氏庫蠓與雌性舒爾茲氏庫蠓交配，獲得成功，其中並有一組產卵，惟卵未孵化。

Fig. 1 *Culicoides arakawae* eggs deposited in laboratory

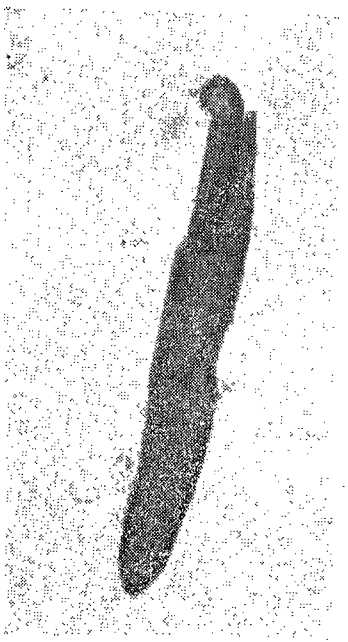

Fig. 2

C. arakawae egg hatched

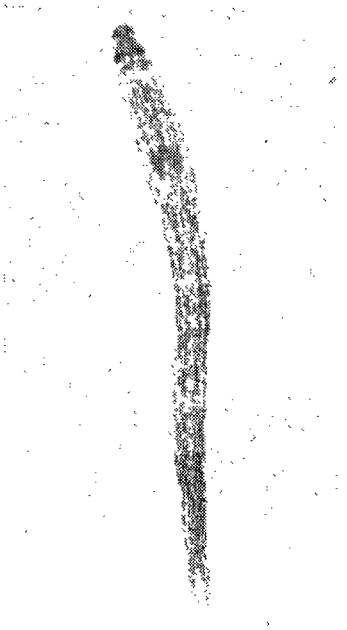

Fig. 3

C. arakawae larva

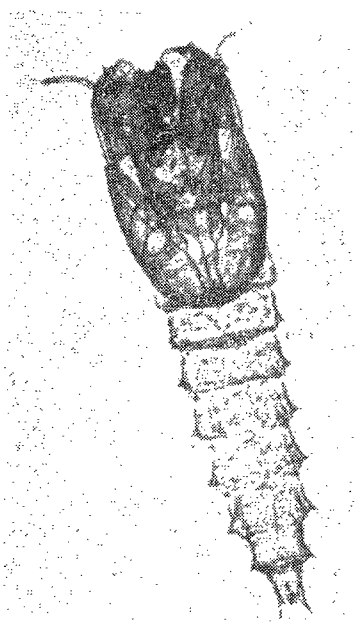

Fig. 4

C. arakawae pupa

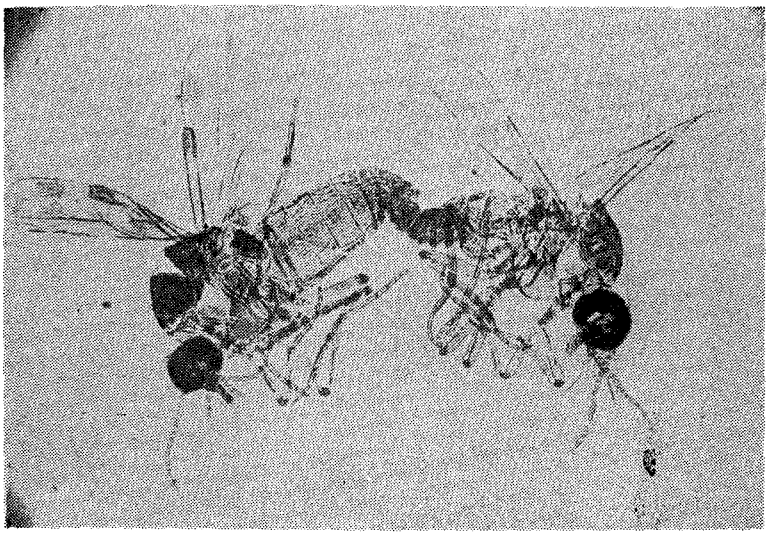

Fig. 5
◁Male *C. arakawae* mating female *C. schultzei*

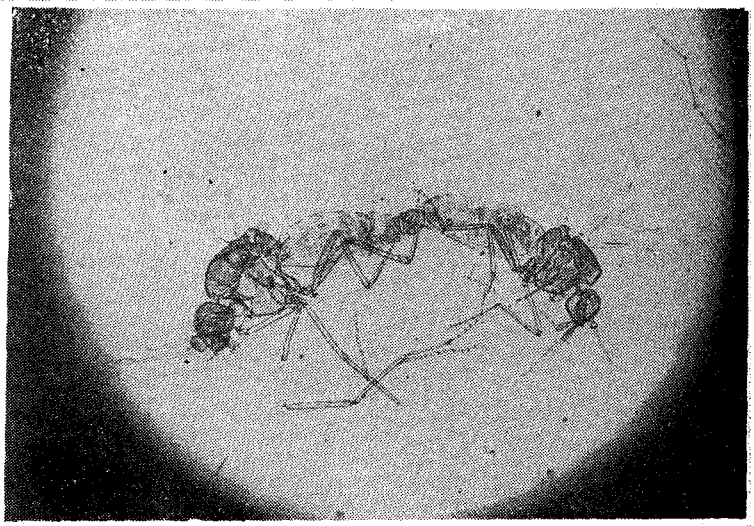

Fig. 6
Male *C. arakawae* mating▷ female *C. duodenaris*

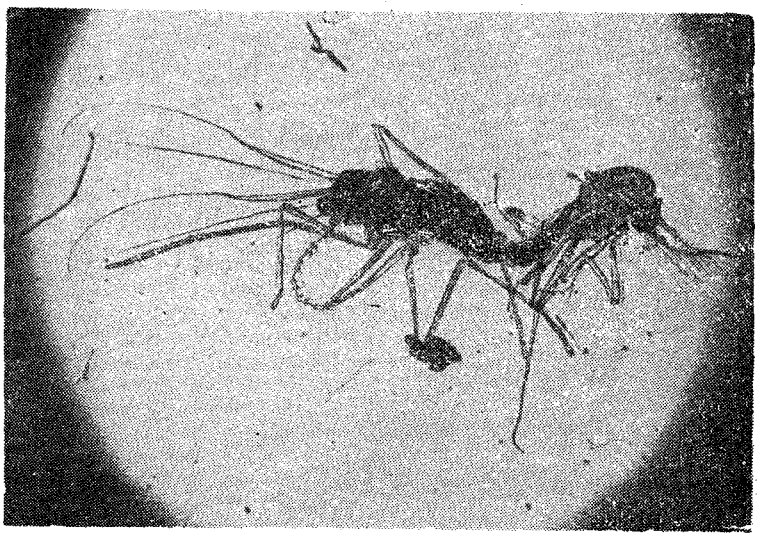

Fig. 7
◁Male *C. arakawae* mating female gall-gnat (Cecidomyiidae)