

## COLONIZATION STUDIES OF *LEPTOCONOPS KERTESZI*,<sup>1</sup> BITING GNATS

R. D. SJOGREN<sup>2</sup> AND J. D. FOULK

Department of Entomology, University of California, Riverside, California

### INTRODUCTION

*Leptoconops kerteszi* Kieffer is a minute, diurnal biting gnat which breeds in damp, sandy habitats in western North America. It attacks both wild and domestic mammals, and is especially annoying to man. The species has not been incriminated as a vector of disease, but its bites at the hairline and on the ears and neck of man frequently produce painful, persistent swellings. The present paper is a progress report on colonization studies of this gnat whose importance as a public health pest in Southern California has paralleled the increased use of desert recreation areas by the expanding urban population.

No species of *Leptoconops* has been colonized. Smith and Lowe (1948) described the life stages and discussed the hatching of *L. kerteszi* eggs, molting of third to fourth larval instars, and pupation and emergence. They did not observe oviposition nor mating. Whitsel and Schoepfner (1965, 1966), studying the biology and control of *L. torrens* Townsend in the Central Valley of California, observed natural oviposition and induced oviposition by decapitating gravid females, but they were unable to effect subsequent development of ova to adult gnats. Davies and Linley (1965), working in Jamaica, were unable to rear sizable numbers of *L. bequaerti* Kieffer from eggs to adults. At least three species of the closely related genus *Culicoides* have been colonized. These are *C. varii-*

*pennis* Coquillett, a vector of blue-tongue disease of sheep (Jones, 1957, 1960), *C. nubeculosis* (Meigen) by Downes (1950), and most recently *C. guttipennis* by Hair and Turner (1966).

### METHODS

*Collection of Specimens for Study.* Both adult and immature stages were easily collected in quantity from a breeding ground within the Santa Ana River basin near Riverside. Surface scrapings from moist sandy depressions were washed through .35 mm and .71 mm soil sieves to remove the gravel and silt. The clean sand retained on the smaller sieve was flooded with 40 percent sucrose (sp. gr. 1.13) and stirred, and the larvae and pupae which floated to the surface were removed with a small pipette. Few eggs were recovered by this technique.

Adults were captured with a portable suction device developed by Foulk and Sjogren (1967). Hundreds of female specimens were collected unharmed as they swarmed to attack near the head of the collector; males were aspirated directly from mating swarms. Adults were transferred to feeding chambers or were retained in fine mesh insect nets for removal to the laboratory. During the summer it was necessary to carry them in cool containers to prevent mortality.

*Feeding of Adults.* Initial attempts to feed adults on restrained rabbits, mice, rats, baby chicks or a human hand placed in cloth-sleeved, clear plastic cages were unsuccessful. The polyethylene feeding chambers shown in Figure 1 were next tried, and these proved most satisfactory. The chambers were Titesal® glass vial

<sup>1</sup> Diptera: Ceratopogonidae.

<sup>2</sup> Present address: Northwest Mosquito Abatement District, P.O. Box 3211, Riverside, California.

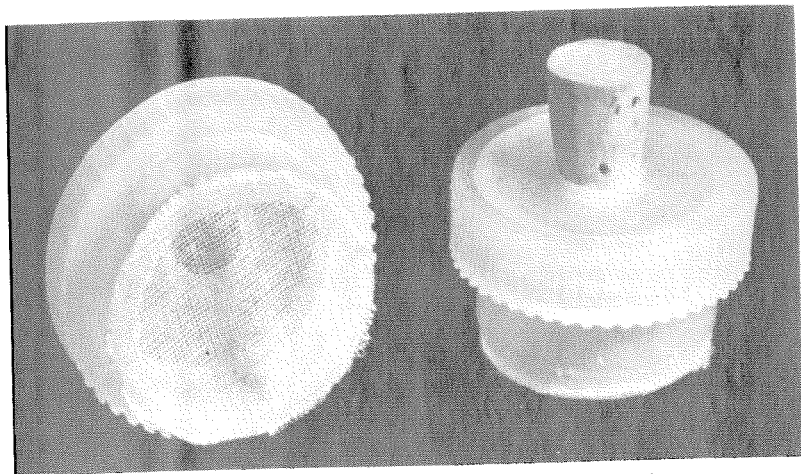


FIG. 1.—Gnat feeding chamber constructed of a modified glass vial top.

caps, 24 mm diam. and 13 mm in height. The plastic bottoms of the caps were cut out, and pieces of 70 mesh bolting silk were fused to the rim with a wood-burning pencil. No more than 25 unengorged females were placed in a chamber via a small hole cut in the top. This hole was plugged with a cork, and the feeding chambers were inserted silk-side down into the pinna of a rabbit's ear. Rubber bands held the chamber against the ear.

Approximately 100 females could be fed at one time by attaching 2 chambers to each ear. The rabbit was apparently undisturbed by the gnat bites, since it did not shake nor scratch its ears. The same rabbit was used in every feeding period, and it may have been a particularly insensitive animal since both brush and jack rabbits subjected to attack by *L. kerteszi* in the field shake their heads and scratch their ears almost continually. Gnats also received human and avian blood meals by taping the feeding chambers to a bared forearm, and the shaven breast of baby chicks, respectively. After the gnats had fed to repletion, the chambers were removed and placed into a 1 quart clear plastic oviposition-rearing container 1/3 filled with larval media. The friction-tight vial cap tops were removed to release the

engorged gnats, and a lid was placed on the container.

*Larval Medium.* A suitable culture medium was formulated for *L. kerteszi* in 1964. Equal numbers of field-collected larvae were placed in water-saturated sand alone, and in mixtures of sand plus vermiculite, sand plus alfalfa leaf meal, and sand, vermiculite, and alfalfa leaf meal. The greatest survival, pupation and emergence of adults occurred in the last mixture. The formula used in our rearing studies was 200 grams of clean white sand (20-40 mesh), plus 2 grams each of vermiculite and alfalfa leaf meal. No salts were added. After thorough mixing, sufficient distilled water was added to completely saturate the medium. A 3 mm surface layer of sand was finally added to reduce the entrapment of newly-engorged females in the wet sand and to render the minute, banana-shaped ova more visible.

*Rearing Conditions and Mating Chambers.* The dual purpose oviposition-rearing chambers were held in an environmental chamber at  $90 \pm 3^\circ \text{F}$ ., 80-90 percent relative humidity, and a 12-hour fluorescent light cycle. Adults became stuck in droplets of condensation in closed chambers, but this problem was solved by venting the sides of the containers with cloth-

covered holes. Clear plastic food containers were used in mating attempts. They were 2 cu. ft. in volume and were sleeved and vented.

#### RESULTS AND DISCUSSION

Soon after the start of feeding, the females' abdomens became distended and red with the ingested blood meal. More than 85 percent of the gnats frequently fed to repletion and withdrew their mouthparts from a host in less than 10 minutes. A comparison of body weights of engorged and unengorged *L. kerteszi* gnats has been reported by Foulk (1967). The duration of three separate instances of undisturbed feeding in the field on a human forearm was 7 mins. 20 secs., 8 mins. 5 secs., and 8 mins. 20 secs. When gnats contained in a feeding chamber lacking a cloth membrane were placed on the ventral surface of a human forearm, they crawled about and were unable to gain sufficient purchase with their legs to feed satisfactorily. They readily fed here, however, when confined in a chamber with a floor of bolting silk. This may explain the preference of this species to attack man on the hirsute portions of his body.

Replete gnats excreted minute waste droplets soon after withdrawing their mouthparts, and for several hours thereafter. As the blood meal was digested the abdomen lost its swollen shape, and changed in color from a brilliant red to a dark blue or black. Digestion was completed within 72 hours, and oviposition began approximately 88 hours post-feeding. Females fed on rabbits usually laid 70-75 eggs, but carbon dioxide anesthesia—even for brief periods—adversely affected egg production.

Viable eggs were also produced following feedings on mice, man, and baby chicks. *L. kerteszi* is not believed to be naturally ornithophilic. Nucleated erythrocytes have never been observed in squash preparations of engorged females collected in the field, and in a serological analysis of more than 20 field-engorged specimens

conducted by the University of California School of Public Health (Tempelis, 1966) no bird feedings were demonstrated.

Eggs were oviposited singly or in groups of four or five in the interstices of surface grains of sand. The females remained stationary and thrust the tip of the abdomen down between loose grains of sand to oviposit. The mean size of 10 eggs was .06 mm by .03 mm. Females died shortly after ovipositing, so no second feedings were possible. Eggs hatched in  $2\frac{1}{2}$  days at  $90 \pm 3^\circ$  F. The egg case split longitudinally, and the colorless 1st instar larva remained partially enclosed by the case for several hours before crawling out into the medium. The percentage of egg hatch was high, but was not determined quantitatively. Pupae became visible at the surface of the media 20 days after the start of eclosion, and subequal numbers of males and females began emerging about 2 days later. Therefore, starting with the time of feeding, an  $F_1$  generation of adults was produced in about 24 days. The length of this gnat's life cycle under laboratory conditions differs markedly from the estimate of 8-10 months made by Smith and Lowe (1948) in their study of the species at Bodega Bay on the California Coast. The desert strain of *L. kerteszi* differs morphologically from the larger coastal strain found in Orange County, on the Monterey peninsula, or at Bodega Bay (Wirth, 1965), and may also differ physiologically as well. Study of the population dynamics and natural life cycle of *L. kerteszi* is presently under way along the north shore of the Salton Sea, and incomplete results suggest that a complete generation is produced here in less than 8 weeks during the months of March-May.

Twenty-five squash examinations of the spermathecae of field-collected unengorged females were examined for spermatozoa. The ovoid, sclerotized spermathecae were dissected from live gnats, crushed in a small drop of distilled water, and examined microscopically. Eighteen (72 percent) of the gnats contained spermatozoa,

suggesting that mating occurs in the field prior to the females taking a blood meal.

Newly emerged, virgin females from the rearing containers were enclosed with field-collected males in a mating chamber floored with dry sand. The gnats were left undisturbed in bright light for 12 hours, then the females were removed and fed on a rabbit. Cotton wicks saturated with sugar water were provided, but neither sex was observed feeding on them. Mortality of the gnats was high in this trial, and eggs laid by the few females which lived long enough to oviposit were unviable. The spermathecae of 5 females which had been contained with the males were negative for sperm. No matings were observed. In additional tests in subdued light, adult activity was greatly reduced.

The limited production and short life span of the virgin females, as well as the failure of successful matings to occur were the major factors preventing colonization. Further attempts in chambers sufficiently large to contain male swarms of this eurygamous gnat will be made during the present year.

#### SUMMARY

Certain aspects of the biology of *L. kerteszi* biting gnats were studied in the laboratory. Adult gnats contained in cloth-floor feeding chambers readily fed on rabbits, mice, man and baby chicks. Field-collected females had mated before feeding. Egg production from a rabbit blood

meal averages 70-75 eggs per female, and an egg-to-adult generation was obtained in a damp sand-vermiculite-alfalfa meal rearing mixture in approximately three weeks at  $90 \pm 3^\circ$  F. and r.h. 80 percent. No viable eggs were produced by blooded virgin females. Matings between newly-emerged adults contained in 2 cu. ft. volume plastic containers were not observed. Males swarm afield and larger mating chambers may be the answer to successful laboratory colonization.

#### References Cited

- DAVIES, J. B. 1965. Personal communication.
- DOWNES, J. A. 1950. Habits and life-cycle of *Culicoides nubeculosus* Mg. Nature. 166:510-511.
- FOULK, J. D., and SJOGREN, R. D. 1967. A collection device for *Leptoconops kerteszi* (Diptera: Ceratopogonidae) biting gnats. J. Med. Entomol. In Press.
- . 1967. Blood meal size of *Leptoconops kerteszi* (Diptera: Ceratopogonidae). Mosq. News. 27:(3)424.
- HAIR, J. A., and TURNER, E. C., Jr. 1966. Laboratory colonization and mass-production procedures for *Culicoides guttipennis*. Mosq. News 26:(3)429-433.
- JONES, R. H. 1957. The laboratory colonization of *Culicoides variipennis* (Coq.). J. Econ. Entomol. 50:(1)107-108.
- . 1960. Mass production methods for the colonization of *Culicoides variipennis sonorensis*. J. Econ. Entomol. 53:(5)731-735.
- SMITH, L. M., and LOWE, H. 1948. The black gnats of California. Hilgardia. 18:157-183.
- TEMPELIS, C. H. 1966. Personal communication.
- WHITSEL, R., and SCHOEPPNER, R. F. 1965 and 1966. *Leptoconops torrens* Tns. research progress. San Mateo County (Cal.) Mosquito Abatement District. Mimeos, 26, 18 pp.
- WIRTH, W. W. 1965. Personal communication.