Journal of the American Mosquito Control Association, 15(1):72–73, 1999

COLONIZATION OF *CULEX NIGRIPALPUS* THEOBALD (DIPTERA: CULICIDAE) BY STIMULATION OF MATING USING MALES OF OTHER MOSQUITO SPECIES

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ABSTRACT. *Culex nigripalpus* Theobald (Diptera: Culicidae) was recolonized successfully by cohabiting *Aedes taeniorhynchus* males or males of other mosquito species starting in a large outdoor cage under natural light–dark cycles and temperatures and ending in a 1-ft.^{3} cage under artificial light–dark cycles at 24°C without added stimulation.

KEY WORDS *Culex nigripalpus, Culex salinarius, Culex restuans,* male *Aedes taeniorhynchus,* colonization, mating stimulation

Culex nigripalpus Theobald is an important vector of diseases of humans and is a major pest of livestock in rural south-central Florida. It is incriminated as a vector of St. Louis encephalitis in humans, eastern equine encephalitis in horses, dog heartworm (*Dirofilaria immitis*) in dogs, wild turkey malaria (*Plasmodium hermani*) avian pox virus of wild turkeys in wild turkeys, and avian malaria (*Plasmodium elongatum*) in hawks and owls (Nayar 1982, Nayar et al. 1998). Even though this species was colonized in the mid-1960s (Haeger 1963, Provost 1969) and was maintained at the Florida Medical Entomology Laboratory (FMEL) in Vero Beach, FL, for about 20 years before the colony was lost, few details have been published on its colonization. In the earliest attempts to colonize *Cx. nigripalpus* in the laboratory, Haeger (1963) observed low sperm transfer during mating under artificial conditions in the laboratory. Subsequently, he obtained significant insemination from *Cx. nigripalpus* copulations when he provided the stimulus of swarming and mating *Aedes taeniorhynchus* in the same cage (Provost 1969). Later, Haeger and O'Meara (1970) were able to introduce wild genes into an established *Cx. nigripalpus* colony by mating colony females with wild males. Earlier, Nielsen and Haeger (1960) showed that, in mosquitoes, male swarming and mating frequently occur simultaneously, especially under laboratory conditions, even though swarming is not required for mating. These studies suggested that, in mosquitoes, male swarming may be an important factor in stimulating mating under artificial caged conditions. A new colony of *Cx. nigripalpus* has recently been established in our laboratory by cohabiting males of *Ae. taeniorhynchus* or of other species of mosquitoes in the cage. This paper presents a procedure for establishing and maintaining a laboratory colony of *Cx. nigripalpus.*

Egg rafts of wild *Cx. nigripalpus* were collected in black plastic pans (12 in. wide × 18 in. long × 9 in. high) containing oak-leaf infusion at the FMEL. The wild egg rafts were hatched, first-instar larvae were identified a few hours after a hatch (Dodge 1966), and larvae were reared to the adult stage in the laboratory by the method of Nayar (1968). Simultaneously, *Ae. taeniorhynchus* adults from a laboratory colony were reared in the laboratory by the method of Nayar (1967). Only *Ae. taeniorhynchus* males, separated from females, were used to stimulate copulation of *Cx. nigripalpus* adults.

Newly emerged adults of *Cx. nigripalpus* and newly emerged males of *Ae. taeniorhynchus* were released in cages for mating trials. Adult mosquitoes were provided a 10% sucrose solution in cotton wicks. After they were allowed to mate for 8–10 days, the adult mosquitoes were fed blood overnight on restrained chickens. Ten days after bloodfeeding, groups of 100 females were placed in 1-pint plastic cups with lids and provided with oak-leaf infusion for egg laying overnight. Egg rafts were collected the next day and put in individual tubes containing water and a small amount of liver powder and yeast (1:3) mixture as a source of food. Egg rafts that hatched were counted and recorded.

In the first series of trials, the wild adults of *Cx. nigripalpus* (2,000+) and males of *Ae. taeniorhynchus* (2,000+) were released at emergence into a large, screened, wood-framed, walk-in cage (6 ft. long × 6 ft. wide × 8 ft. high) on a cement floor under a Lexan-paneled roof enclosure under a canopy of oak trees providing natural light–dark cycles and temperature. Additional humidity was provided when the relative humidity of the air dropped below 70%. Sixteen percent of the egg rafts collected from these females hatched. In subsequent 4 trials, cage-mated *Cx. nigripalpus* progeny were mixed with adults reared from wild egg rafts to populate the cage adequately for mating in the above manner outdoors. About 60% of the egg rafts hatched during these trials. In the final trial in the large cage outdoors, only cage-mated (colony) progeny of *Cx. nigripalpus* were used, and 80% of the egg rafts hatched. After this trial outdoors, mating was attempted in 3-ft.^{3} cages indoors in a room with large

windows for natural light–dark cycles and maintained at 24°C. In 2 trials in 3-ft.^{3} cages, *Cx. nigripalpus* adults were stimulated to mate by the addition of *Ae. taeniorhynchus* males. In these trials, 18% and 21% of the egg rafts hatched. At 24°C, many of the *Cx. nigripalpus* survived up to 2 months.

Colonized *Cx. nigripalpus* were adapted to a 1-ft.^{3} cage during the next 4 trials. In all these trials, *Ae. taeniorhynchus* males were added to cages to stimulate mating. Egg rafts collected from these females showed increases in egg hatching from 8% in the first 2 trials to 40% in the 3rd trial and to 76% in the 4th trial. At present, we are maintaining our laboratory colony of *Cx. nigripalpus* in 1-ft.^{3} cages in a bio-room maintained at 24°C with light–dark cycles of 12 h, and *Ae. taeniorhynchus* males are no longer added to stimulate mating.

We point out here that, during the process of laboratory colonization of this species, some loss in genetic variability may have occurred, even though it has not yet been demonstrated. However, genetic variability of this species can be substantially increased by incorporation of wild genes by mating colonized females with wild males, as was previously demonstrated by Haeger and O'Meara (1970).

Other species of newly emerged males, such as *Aedes albopictus* and *Anopheles quadrimaculatus,* added to cages with newly emerged *Cx. nigripalpus* also stimulated mating and a higher percentage of egg raft hatching was found than without their addition. These studies show that the addition of *Ae. taeniorhynchus* males or males of other species of mosquitoes apparently stimulate swarming of *Cx. nigripalpus* to the extent that they mate with caged females in the laboratory. We have successfully colonized *Cx. nigripalpus, Culex salinarius,* and *Culex restuans* by this technique.

ACKNOWLEDGMENTS

This is Florida Agricultural Experimental Station, Journal Series R-06286. We thank Jamie A. Jennings for her technical assistance in conducting this study.

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