OPERATIONAL AND SCIENTIFIC NOTES

ESTABLISHMENT OF A FREE-MATING COLONY OF *ANOPHELES BARBERI* WITH NOTES ON DEVELOPMENTAL RATES[1]

ROBERT S. COPELAND[2]

Vector Biology Laboratories, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556[3]

As part of an ongoing study of the tree hole mosquitoes of northern Indiana, an attempt was made to colonize *Anopheles barberi* Coquillett. Induced copulation has been used to maintain *An. barberi* for a few generations, but, invariably, these strains have been lost (Lorraine Taylor, personal communication). Previous attempts to establish a free-mating strain in this laboratory have also failed, but it was not known whether failure was due to the eurygamous nature of this species or because the number of caged adults was below some threshold density necessary for the initiation of mating. Several authors have reported that *An. barberi* larvae characteristically occur in small numbers in tree holes, rarely exceeding 10 or 12 (Jenkins and Carpenter 1946, Zavortink 1969). I have found, however, that local abundance of *An. barberi* is highly variable, and depends largely on tree hole type and time of collection. In northern latitudes, spring and early summer collections are small due to overwintering mortality. However, late summer and early fall collections from rotholes which contain water on a permanent or semi-permanent basis often yield large numbers of early instar larvae. For example, a 1.4 liter hole sampled on October 20, 1985 contained 365 larvae, of which 65% were 2nd instars. Thus, large numbers of larvae were obtainable and colonization could be attempted with a high density of young adults.

The colony was started with over 1,100 larvae collected during August 1985. Over 95% of this stock was from tree holes and tires located in St. Joseph County, IN (41.40° N). A small proportion originated from tree holes in the Forest Park Nature Center, Peoria, IL (40.43° N) and tires in Greenville, OH (40.06° N). Larvae were reared in tap water at 21°C in an insectary maintained at 85% RH and 21°C, with an 18 hr:6 hr L/D photoperiod. Larvae were fed *ad libidum* a mixture of 60% whole wheat flour, 25% brewer's yeast, 10% crude blood meal and 5% non-fat dried milk. Adults emerged into a 0.6 m^3 cage in the insectary, and were provided with honey-soaked absorbent wadding (Kendall Hospital Products Division, Boston, MA) and water-soaked cotton balls. Females were offered anesthetized (nembutal), unrestrained mice as a blood source. Oviposition sites were provided by black-painted beer cans. The cans were filled with tap water to within 3 cm of the top. Empty black cans were used by adults as preferred resting sites.

Females were cautious feeders and, in contrast to *Aedes* species, were unable to secure a bloodmeal by piercing parts of the body which are

Table 1. Developmental times (in days) for stages in the life cycle of *Anopheles barberi*.[a]

Stage	Rearing temperature[b] 19°C	24°C
Emergence to bloodmeal	4.0 (8.1)[c]	3.0 (9.6)
Bloodmeal to oviposition	6.5 (13.2)	4.0 (12.8)
Oviposition to hatch	6.0 (12.2)	3.2 (10.4)
1st instar	6.7 (13.6)	4.2 (13.4)
2nd instar	4.3 (8.8)	2.8 (9.0)
3rd instar	6.0 (12.2)	3.8 (12.2)
4th instar	9.7 (19.8)	6.8 (21.8)
Pupa	5.9 (12.0)	3.6 (11.5)
Total	49.1	31.2

[a] Times for the duration of larval and pupal stages represent the mean of five replicates of 50 larvae each. For each replicate, the length of each larval instar and the pupal stage was estimated as the number of days required for 50% of the individuals to molt to the succeeding stage. Times for other life cycle events were determined by observation of caged populations.

[b] Larvae were reared and adults were held in either of two insectaries (19°C or 24°C). Both insectaries were maintained at 85% RH with an 18 hr:6 hr L/D photoperiod.

[c] Percentage of life cycle duration.

[1] This work was supported by National Institute of Health Research Grant No. AI-02753.

[2] Current address: International Center of Insect Physiology and Ecology, P.O. Box 30772, Nairobi, Kenya.

[3] Send reprint requests to Dr. George B. Craig, Jr., Vector Biology Laboratories, Department of Biological Sciences, University of Notre Dame.

covered with hair. Preferred feeding sites were the ears, nose, mouth and feet. Large numbers of fertilized eggs were deposited by females of the founding generation. This level of production has been maintained to the present through 10 generations. Small samples of eggs from the F_5 and F_6 generations were observed for embryonation. Eighty-seven percent (325/374) of the eggs from the former and 84% (250/298) of those from the latter generation hatched. Mating has not been observed and is presumed to occur in darkness.

Individuals from this colony have been used for studies of photoperiodically-induced dormancy, cold hardiness and predation. In addition, generation times were determined for individuals reared at 19°C and 24°C under conditions of continuous food availability. Each day, food was added until a thin layer covered the water surface. Water was changed at the first appearance of a bacterial surface film. Data for the duration of discrete periods in the life cycle are presented in Table 1. The time required to complete a single generation was 31.2 days at 24°C and 49.1 days at 19°C.

REFERENCES CITED

Jenkins, D. W. and S. J. Carpenter. 1946. Ecology of the treehole breeding mosquitoes of nearctic North America. Ecol. Mon. 16:31–47.

Zavortink, T. J. 1969. Mosquito studies (Diptera, Culicidae). XIX. The treehole *Anopheles* of the new world. Contrib. Am. Entomol. Inst. (Ann Arbor) 5(2):1–35.