1971a. Gamma irradiation of eggs, larvae, pupae, and adults of *Culex pipiens quinquefasciatus* Say. Mosq. News 31:102–105.
Smittle, B. J., La Brecque, G. C., Patterson, R. S. and Carroll, E. E. 1971b. Comparison of fast neutrons and gamma rays in producing sterility in *Culex pipiens quinquefasciatus* Say. Mosq. News 31:477–478.
Tantawy, A. O., Abdel-Malek, A. A. and Wakid, A. M. 1967a. Studies on the eradication of *Anopheles pharoensis* by the sterile-male technique using Cobalt-60. IV. Mating behavior and its frequency in the sterilized mosquitoes. J. Econ. Ent. 60: 696–699. V. Mating competitiveness in radiosterilized males. *Ibid*:696–699.
Ward, R. A. and Scanlon, J. E. 1969. Conference on anopheline biology and malaria eradication. Misc. Publ. Ent. Soc. Amer. 7:196 pp.
Weidhaas, D. E., Schmidt, C. H. and Seabrook, E. L. 1962. Field studies on the release of sterile males for the control of *Anopheles quadrimaculatus*. Mosq. News 22:283–291.

ESTABLISHMENT OF A LABORATORY COLONY OF *ANOPHELES (MYZOMYIA) ANNULIPES* WALKER 1856

ELIZABETH C. KALUCY [1]

INTRODUCTION. *Anopheles annulipes* is the most common and widespread anopheline species in southern Australia. Many attempts to colonise it have failed, the refusal of the adults to copulate in the laboratory being the principal cause of failure of Hill (1919), Wallace (Lee and Woodhill 1944) and Mackerras and Lemerle (1949). Cannibalism among larvae affected Lee's attempt in 1935, and the refusal of adult females to take a blood meal hindered Rudeforth in 1950.

These difficulties and others were encountered in the present attempt, the purpose of which was to provide vector anophelines for a malaria research programme. They were overcome in various ways, particularly by the use of a technique of induced copulation (McDaniel and Horsfall, 1957).

COLLECTION OF MOSQUITOES. In coastal areas of New South Wales *A. annulipes* adults are scattered among so many suitable resting places in low vegetation and rock crevices that collection of adults was usually unsuccessful. They were collected once in a large humid drainpipe near a creek in which a few larvae were present; the progeny of these gravid females supplemented the larger collections of larvae made elsewhere over a long period.

The larvae live in clean shallow water, in both temporary and permanent ground pools; they were scarce in Sydney in 1967 but a reliable breeding site was found late in 1967 at the Nattai River near Mittagong, 80 miles from Sydney. In the summer months 500–1500 larvae could be collected in 2–3 hours from the filamentous green algae at the margins of the slow-flowing river.

LABORATORY CONDITIONS. All field-collected larvae and adults were taken to a 14′ x 16′ insectary, which was maintained at a temperature of 78±2° F, and a relative humidity of 70–90 percent. Two large windows facing west provided natural light which could be supplemented by fluorescent light.

REARING METHODS. Larvae were reared in white plastic trays measuring 4″ x 6″ x 2″, filled with river water to a depth of half an inch. River water gave con-

[1] C/O Professor R. H. Black, School of Public Health and Tropical Medicine, The University of Sydney, Sydney, N.S.W. 2006, Australia.

sistently satisfactory results for larval rearing, and could be used again after filtering through cotton wool with even better results, probably because of the presence of micro-organisms suitable for larval food. Distilled water was satisfactory if buffered to pH7 with a glycine or phosphate buffer. Tap water was not satisfactory, as there was heavy larval mortality probably due to contamination from water pipes, or to an excess of chlorine or fluorine ions. The forty larvae in each tray were fed daily by scattering a pinch of finely ground fish food on the surface of the water.

Pupae were transferred by pipette each day to a beaker of river water in a gauze-covered 24-oz. paper cup. When the adults emerged 40–44 hours after pupation they were provided with a cotton wool pad of sugar solution for sustenance until mating.

INDUCED COPULATION. The technique used was similar to that described by Horsfall (1964). Male mosquitoes at least 2 days old were anaesthetised with chloroform and glued to a glass slide ventral side uppermost; when the effect of the anaesthetic had worn off the head and legs were removed. A female mosquito was lightly anaesthetised, held with a fine vacuum pipette attached dorsally to the thorax, and brought to the male so that the female genital opening contacted the male phallosome at an angle of 45 degrees. The male's claspers released the female after about 12 seconds of copulation, and she was put in a 6-oz. gauze-covered paper cup to recover.

Some males would copulate many times; but dissection of 21 series of 5 females mated to a single male showed that only two males fertilized more than three females. Therefore males were used only twice unless it was necessary to use them more often.

FEEDING AND OVIPOSITION. On the morning after copulation mated females were offered a blood meal from human fingers or a rabbit's ear; they fed best while the insectary was dark and undisturbed by activity, and when kept without sugar solution the night before feeding.

Females were transferred on the day after blood feeding to 24-oz. paper cups containing a petri dish of river water. Oviposition generally occurred about 60 hours after feeding; the females were then offered another blood meal, and the eggs were left in the petri dish to hatch, care being taken to ensure that they were not stranded on the sides of the cup by movement or evaporation.

Hatching began about 40 hours after oviposition; when the larvae were 1 or 2 days old they were put into white plastic trays.

PROGRESS OF COLONISATION. During 1967 progress was very slow due to the scarcity of larvae resulting from small collections and high larval mortality. The use of unsuitable rearing water, and inadequate feeding before the use of fish food was started, were the main reasons for the mortality, and in these unfavourable conditions bacterial and fungal infections occurred readily.

Natural copulation was highly unlikely when there were frequently less than 10 adults at any one time, and the sex ratios were distorted by these low numbers. Gaining experience in the technique of induced copulation was also difficult with so few adults. Females which were mated by induced copulation did not readily take a blood meal, and rarely a meal large enough to result in ovary development. No eggs were laid until June 1967, although the presence of sperm and fully developed eggs was demonstrated in many dead females by dissection. Since this indicated that the containers provided for oviposition did not stimulate the oviposition responses of all the females, a greater variety of oviposition containers was provided. Eight batches of eggs were laid by three females during June, forming the first generation of a line that existed for only three generations, with nine batches of eggs in the second, and four batches in the third generation. Only 21 pupae formed in the third generation; the female

adults were difficult to mate and feed, and none of them oviposited.

The existence of this line showed that breeding of *A. annulipes* in the laboratory was possible with the use of induced copulation. In order to establish a colony, it would be necessary to collect a large number of adults or larvae, from which individuals able to survive and breed in laboratory conditions could be selected.

A high proportion of larvae collected from the Nattai River in the last quarter of 1967 died before pupation as a result of emergence of parasitic nematodes; the remaining healthy larvae matured, were mated with difficulty, fed with difficulty, and died without ovipositing.

In the first quarter of 1968 conditions at the Nattai River were ideal for breeding of *A. annulipes;* several thousand nematode-free larvae were collected on each trip. During February about 12 adult *A. annulipes* were collected from a dark humid drainpipe over a creek at Castle Hill on the outskirts of Sydney. About 1500 larvae were reared from the eggs laid in the laboratory by these gravid females.

All adults available during the next few months were used for induced copulation. Mosquitoes originating from the Nattai River were crossed with those from Castle Hill wherever possible to reduce inbreeding and increase the variability of the progeny. Providing the mated females with the full blood meal essential for ovary development was most time-consuming at first but gradually the proportion of females willing to feed increased. Females became less selective about oviposition sites after several generations; initially sand or alga was essential in the water of the oviposition container, but later generations would oviposit on clean water. Table 1 shows the figures for mating, feeding and oviposition from January until July 1968. The number of egg batches shown is less than the actual number, since three or four females were placed in each oviposition container in order to save space, and the eggs laid were counted as one batch. The size of egg batches laid by isolated females varied between 12 and 200 eggs, the average size of the first batches being 112, and of the second batches being 133.

This colony of *A. annulipes* was maintained by induced copulation for 25 generations until the research programme ceased temporarily in August 1969; another colony of this species has since been established by the Malaria Research Laboratory. Once the colony was established, maintenance was straightforward and all essential tasks could be performed in a few hours each day. Fifty females could be mated by induced copulation in 2 hours, including the time needed to prepare the males. Feeding and oviposition no longer presented problems as intensive selection led to the development of a strain well adapted to the particular conditions of the laboratory. However, it was important to keep the size of the colony well above the minimum necessary for breeding, to allow for occurrences such as the breakdown of humidifying equipment which could cause considerable mortality.

SUMMARY. *Anopheles annulipes* was successfully colonised in Sydney during

TABLE 1. FEEDING, MATING AND OVIPOSITION FIGURES

Month	No. mated	No. fed	% Fed mated	No. egg batches	% Egg batches fed females
Jan.	487	152	31.2	12	7.9
Feb.	292	87	21.9	30	46.9
Mar.	260	107	38.5	30	30.0
Apr.	315	278	66.6	102	36.7
May	581	491	63.2	155	31.6
June	280	269	81.0	100	37.2
July	410	370	65.0	160	43.3

the period 1967–1969. Difficulties in the establishment of this colony are reported and the rearing methods found successful are described.

ACKNOWLEDGMENTS. This work was carried out while the author was an officer in the Malaria Research Laboratory of the Royal Australian Army Medical Corps, at the School of Public Health and Tropical Medicine in the University of Sydney. I am indebted to Associate Professor D. J. Lee for a great deal of advice and assistance in this project.

References Cited

Hill, G. F. 1919. Half-yearly reports. Report of the entomologist from 25 Feb to 31 Dec 1919. Australian Inst. of Trop. Med.

Horsfall, W. R. 1964. Induced copulation of mosquitoes. WHO/EBL/28.64.

Lee, D. J. and Woodhill, A. R. 1944. Anopheline mosquitoes of the Australasian region. University of Sydney Monograph No. 2.

McDaniel, I. N. and Horsfall, W. R. 1957. Induced copulation in Aedine mosquitoes. Science, 125, 745.

Mackerras, M. J. and Lemerle, T. H. 1949. Laboratory breeding of *Anopheles punctulatus punctulatus*, Donitz. Bull. Ent. Res. 40, 27–41.

PARASITISM OF *ANOPHELES ANNULIPES* WALKER BY A MERMITHID NEMATODE

ELIZABETH C. KALUCY [1]

INTRODUCTION

Numerous examples of the parasitism of mosquitoes by mermithid nematodes have been reported (Jenkins, 1964). The only previous Australian observation was made in Townsville, Queensland, where 9 out of 13 third and fourth instar *Anopheles annulipes* larvae were found to be infected with an unidentified mermithid nematode (Laird, 1956).

OCCURRENCE

In 1967 and 1968 *A. annulipes* were collected in considerable numbers from the Nattai River near Mittagong, New South Wales. A large proportion of the larvae collected in November and December 1967, never reached maturity because they were killed by the emergence of nematode parasites, frequently during the journey back to Sydney. The coiled nematodes were visible lying ventrally in the thoracic and abdominal haemocoel of infected third and fourth instar larvae, which were slightly swollen ventrally and moved sluggishly.

The incidence of parasitism was measured on returning to the laboratory by counting the number of live parasitised larvae and the number of larvae killed during the journey by emerging nematodes. Table 1 shows the rate of infection on four successive collection days.

Conditions for the breeding of *A. annulipes* became increasingly favourable after December, resulting in greater numbers of larvae in January. Approximately 1500 larvae, most of them first and second instar, were collected on both 4 Jan. and 9 Jan.; the proportion of these larvae infected with mermithids could not be determined directly as the larvae were too small for the parasites to be visible, but very few parasites later emerged from

[1] % Professor R. H. Black, School of Public Health and Tropical Medicine, The University of Sydney, Sydney, N.S.W. 2006, Australia.