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LABORATORY COLONIZATION OF THE MALARIA VECTOR ANOPHELES CULICIFACIES[1]

by

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Although Anopheles culicifacies, the principal malaria vector for most of the Indian subcontinent, has been extensively investigated ecologically, it has not been studied genetically or physiologically because appropriate laboratory colonies were not available. Russell & Rao (1942) did maintain a colony in a large outdoor cage in the 1940's, but such a colony is not readily amenable to the experimental manipulation required for genetic investigation. During 1967-69 the Ross Institute of Tropical Hygiene, London, established and maintained a colony of A. culicifacies with egg batches originating from Maharashtra State, India. Mating occurred in cages of 30 cm on each side, but this was supplemented by artificial mating. The colony contained resistance to both DDT and dieldrin (Dr G. Davidson, personal communication 1976). The present attempts to colonize A. culicifacies, were initiated during the summer and fall of 1975.

Colonies were successfully established using females captured from cattle shed resting sites in three different villages: Sattoki (64 km south of Lahore on the Multan Road), Kot Baghicha (near Balloki Headworks, about 48 km south of Lahore), and Dhariwal (40 km south of Lahore). The colony from this last village was maintained for four generations but was subsequently lost. In each case, 500 or more wild-caught females were taken to the laboratory and kept in metal wire cages measuring 30 cm x 30 cm x 30 cm and were provided with a mouse at night for feeding. Eggs were collected daily in plastic petri dishes lined with filter paper on the sides and flooded with water to a depth of 3 mm. After hatching (36 to 48 hours), the larvae were transferred to enamel pans measuring 45 cm x 22 cm filled with 1 cm of water. The larvae were fed daily a finely ground mixture containing equal parts by weight of wheat germ, Kellogg's concentrate cereal, and brewer's yeast. Egg-to-adult viability was determined and found to vary widely from as low as 10% to as high as 90%; it averaged near 50% when 100-200 eggs which appear to be optimal density, were placed in each pan. Larvae pupated 8-15 days after hatching. Pupae were isolated and transferred to 500 ml plastic cups with lids. Adults were collected the next day and transferred to metal wire cages measuring 60 cm on all sides which were partially covered with wet towels as efficient egg production depended upon their use. Eggs were usually laid beginning on the sixth day of adult life and occasionally as early as the fourth day. The eggs were then collected and the process repeated, except that guinea pigs instead of mice were sometimes used at night as blood hosts.

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Insectary conditions were critical to success. The temperature was maintained between 28-30°C. The humidity was kept at 60% RH. Lighting was provided by fluorescent tubes as well as incandescent bulbs. Fifteen hours of "full daylight" was provided during which all the lights were used. At 21.30 hours, the fluorescent tubes were turned off, leaving only incandescent bulbs which were dimmed gradually over a period of 80 minutes by the use of a motor-driven powerstat transformer until there was total darkness. The process was reversed in the morning.

Insemination rates were evaluated in an effort to determine how A. culicifacies adapted to the laboratory environment. The first laboratory-reared generation of the Dhariwal colony (F_1-D), established with approximately 1000 pairs of first generation virgin adults, yielded 500 to 1500 eggs daily. Twenty-seven per cent. (n = 100) of the females were found to be inseminated. This result differed dramatically from that of the seventh laboratory generation of Sattoki stock, which was also established with approximately 1000 adult pairs. The Sattoki cage (F_7-S) yielded 4000 to 8000 eggs daily. The fourth laboratory generation of the Kot Baghicha colony, also established with 1000 adult pairs, had intermediate rates of egg production (1500 to 2000 eggs daily) and an intermediate insemination rate (38%, n = 50). The insemination rate appeared to increase, although not significantly ($\chi^2 = 1.9$, df = 1), as the mosquitos adapted to the laboratory environment. Even if the trend were significant, the increase from 27% to 38% of inseminated females is unlikely to account for all of the five-fold or more increase in egg production observed. Therefore, it appears likely that the fraction of blood-fed, inseminated females actually laying eggs increased as the mosquitos adapted to the laboratory.

Comparisons of the F_1-D and F_7-S male behaviour yielded interesting results. When F_7-S males were mated to F_1-D females, 52% of the females were inseminated compared to the 27% mated by the F_1-D males ($\chi^2 = 9.3$, df = 1). Females of F_7-S appear to mate more readily than F_1-D females given matings to non-adapted F_1-D males; but, laboratory adapted F_7-S males mate more aggressively than non-adapted F_1-D males given matings to F_1-D females. Consequently, mating behaviour of both sexes was involved in adaptation to the laboratory environment.

Comparison of the insemination rates in cages of different sizes indicated that mating occurred most readily in cages of 60 cm on each side (insemination rate, 38%; n = 50) and less readily in cages with a 30 cm base and 60 cm in height (insemination rate, 20%; n = 80), while mating in cages of 30 cm on each side was extremely rare (insemination rate, 2%; n = 43).

Recently it was discovered that the isolation of males and females for 48 h, allowing females a blood meal 24 h prior to their introducing into small wire cages measuring 30 cm on a side, significantly improved mating success. After maintaining four generations of small-cage reared mosquitos derived from the large-cage Sattoki colony, sub-samples of fourth generation adults and of adults from the large-cage Sattoki colony were dissected after being held 10 days in small wire cages. In the case of progeny from the large-case Sattoki colony, eight of 82 dissected females were inseminated as opposed to only one in 43 when males and females were not isolated prior to introducing new adults in small wire cages. The insemination rate of females in the fourth generation reared in small cages (14 of 62 females were fertilized) was significantly greater than the insemination rate of progeny from the large-cage Sattoki colony which were kept in a small wire cage ($\chi^2 = 4.5$, 1 df). Thus selection for efficient mating in smaller cages is progressing satisfactorily. However, inbreeding effects were and continue to be important judging from the large number of unusually small-sized larval and adult progeny from the small-cage populations. To overcome this problem, the sixth generation adults of small-cage reared mosquitos are being added to the fifth generation adults and selection in each of the five small cages is being carried out continuously and simultaneously for several months.

The author plans to use small-cage adapted mosquitos to initiate formal genetic studies of A. culicifacies.

SUMMARY

Anopheles culicifacies was successfully colonized twice in wire cages measuring 60 cm on each side. One of the colonies was selected for mating in smaller wire cages measuring 30 cm on each side. Successful mating was achieved and experiments indicated that adaptation to the laboratory conditions involved changes in mating behaviour of both sexes.

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RESUME

Deux colonies d'Anopheles culicifacies ont été établies avec succès en utilisant des cages grillagées de 60 cm de côté. L'une de ces colonies a été choisie pour obtenir l'accouplement dans des cages grillagées plus petites mesurant 30 cm de côté. La réussite de ces expériences montre que l'adaptation aux conditions de laboratoire entraîne des changements chez les deux sexes dans le comportement lors de l'accouplement.

REFERENCE

Russell, P. F. & Rao, T. (1942) On the swarming, mating, and oviposition behavior of Anopheles culicifacies, Amer. J. trop. Med., 22, 417-427

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