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COLONIZATION AND LABORATORY BIOLOGY OF *AEDES NOTOSCRIPTUS* FROM BRISBANE, AUSTRALIA

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ABSTRACT. Methods are described for the laboratory colonization of *Aedes notoscriptus* from Brisbane, Queensland, Australia. To initiate colonization, efforts were made to duplicate the natural environment of this species, including the use of a bromeliad as a swarm marker and oviposition substrate. The colony stabilized after the F_8 with eclosion rates >50%, and an average production of 5,220 adults since the F_9. The fecundity of *Ae. notoscriptus* averaged 29.4 ± 10.5 eggs (range 14–57). The average development times from egg hatch to adult were 11 and 20 days under typical summer (20.5–28.9°C) and winter (10.1–21.2°C) conditions in Brisbane, respectively. This is the 1st published report of the colonization of *Ae. notoscriptus.*

KEY WORDS *Aedes notoscriptus,* colonization, Ross River virus, Barmah Forest virus, Australia, mosquito

INTRODUCTION

Aedes notoscriptus (Skuse) has been incriminated on epidemiologic grounds as a suspected arbovirus vector in Australia; Ross River virus (RR) has been isolated from wild-caught females from various regions of Australia (Whelan and Weir 1993; Russell 1995; Ritchie et al. 1997; Ryan et al., in press), high adult abundance has concurred with the incidence of urban RR epidemics (Ritchie et al. 1997) and the domestic and anthropophilic behavior of this species suggest sufficient contact with humans for arbovirus transmission. Consequently, questions concerning the importance of *Ae. notoscriptus* as an urban vector, particularly of RR, highlighted the need for laboratory studies of its biology.

Until recently, the unavailability of a laboratory colony, plus the uncertainty of field-collected material being available in sufficient quantities, have limited detailed biological and vector competence studies. Past attempts at continuous colonization of *Ae. notoscriptus* have been unsuccessful, principally because of the failure of this species to mate under caged conditions (Standfast et al. 1966, Foot 1970). Limited success was reported by Standfast et al. (1967) when a laboratory colony, initially dependent on forced mating, persisted for several generations as a free-mating colony; however, specific information on rearing procedures was not reported. This paper details the methods used for the 1st successful colonization of *Ae. notoscriptus* and measures some basic biological parameters (fecundity, development time, survival) in the laboratory.

MATERIALS AND METHODS

The *Ae. notoscriptus* colony was established in March 1995 from mosquito larvae collected from a tire pile at Closeburn (~30 km north of Brisbane) and from domestic sources in western Brisbane. The larvae were transported to the Queensland Institute of Medical Research insectary, and 200 were placed in plastic trays (42 × 36 × 6 cm; Ilford, United Kingdom) containing 4 liters of distilled water. Larvae were fed finely ground K9® fish food (Friskies Pet Care, Noble Park, Victoria, Australia), supplemented twice weekly with a mixture of 80% Wardley® gold fish flakes (Wardley Corp., Secaucus, NJ), 10% dried liver powder (Sigma Chemical Co., St. Louis, MO), and 10% dried yeast powder (Soland Health Foods, Brisbane, Australia). Pupae were collected daily and placed in small plastic containers within a 1-mm mesh screened cage (50 × 50 × 50 cm). The interior of the cage was darkened by placing black plastic over the back and sides of the cage. Emerging adults were supplied with cotton soaked in 15% sucrose solution and sliced apple. Females were offered a restrained guinea pig as a blood source every 7 days. Larvae and adults were maintained under insectary conditions (28°C, 70% relative humidity, and a photoperiod of 11:11 h light:dark with a 1-h dawn and dusk period).

For oviposition, a bromeliad (*Vriesea* hybrid 'Red of Rio') potted in a 10-cm plastic pot was lined with a strip of filter paper and placed in a plastic base containing water and a small pinch of larval food (Fig. 1). Eggs were conditioned by slow drying of the filter paper for 2–3 days under insectary conditions. To induce hatching (Judson 1960), eggs were submerged in deoxygenated water. After stimulation, all hatching occurred within 24–36 h. The number of eggs oviposited on the filter paper was counted weekly under a dissecting microscope. Egg hatch was measured by counting 1st-stage larvae.

To evaluate fecundity, 30 visibly bloodfed 2- to 3-day-old females were each placed into a separate oviposition cage (30 × 22 × 22 cm), identical in structure and layout to the colony cage. After 7 days, the number of eggs oviposited by each female were counted under a dissecting microscope. In addition, the number of eggs retained in the ovaries of each female was determined by dissecting their

Fig. 1. Aluminum cage (50 × 50 × 50 cm) for adult mosquitoes showing the potted bromeliad (*Vriesea* hybrid 'Red of Rio') used as a swarm marker and oviposition substrate.

ovaries and recording the number of stage V ova (Clements 1992).

The development and survivorship of immature *Ae. notoscriptus* was observed under typical summer (20.5–28.9°C temperature range, and a photoperiod of 14:10 h light : dark) and winter (10.1–21.2°C temperature range, and a photoperiod of 10:14 h light : dark) environmental conditions in Brisbane, Queensland (Bureau of Meteorology, Brisbane, Australia). Following Russell (1986), 3 replicates of 200 1st-stage larvae were reared in plastic trays (18 × 12 × 6 cm) filled with 200 ml

Table 1. Production of adults of *Aedes notoscriptus* in the laboratory through 13 generations.

Generation	Eggs		Adults	Rearing success (%)[1]
	Laid	Hatched (%)		
Parental[2]			3,975	
F_1	2,293	96 (4.2)	82	85.4
F_2	300	12 (4.0)	11 (+2,962)[3]	91.7
F_3	3,238	469 (14.5)	441 (+2,311)[3]	94.0
F_4	4,126	2,317 (56.2)	1,862	80.4
F_5	2,682	1,059 (39.5)	987	93.2
F_6	2,772	710 (25.6)	681	95.9
F_7	5,566	1,668 (30.0)	1,401	84.0
F_8	5,258	2,457 (46.7)	2,297	93.5
F_9	6,987	4,109 (58.8)	3,865	94.1
F_{10}	9,274	5,434 (58.6)	5,133	94.5
F_{11}	6,068	4,010 (66.1)	3,571	89.1
F_{12}	14,000	7,172 (51.2)	6,907	96.3
F_{13}	9,552	6,862 (71.8)	6,627	96.6

[1] Rearing success = (number of adults produced/number of hatched eggs) × 100.
[2] Originated from field-collected larvae and pupae from Closeburn and Brisbane, Queensland, Australia.
[3] Additional field-collected larvae and pupae were added to colony.

of distilled water. Larvae were fed as described above, at rates of 0.2, 0.3, 0.4, and 0.6 mg per larva on days 0, 1, 2, and 3, respectively, and then 0.6 mg on subsequent days. The numbers of larvae successfully pupating each day were counted into 200-ml screened cups for emergence, kept separate by replicate number and date. Pupae were monitored daily for mortality and adult emergence, and the development time in days to 50 and 90% pupation (P_{50} and P_{90}) and sex of the adults were recorded.

RESULTS

Egg hatch was highly variable until the F_8, and thereafter stabilized at eclosion rates of >50% (range = 51.2–71.8%) (Table 1). Mortality from immatures to emergence was low with a mean rearing success of 91.4% from F_1 to F_{13} (range = 80.4–96.6%) (Table 1). In contrast, adult mortality was high in the parental and F_1 generation, necessitating the addition of field-collected larvae in the F_2 and F_3. The average adult production was 970 from F_1 to F_8, but thereafter increased to 5,220 from F_9 to F_{13} (Table 1). By 1999, the colony had gone through an estimated 70 generations.

The mean (± SD) number of eggs laid was 16.9 ± 28.0 (range 1–143), with oviposition occurring 3–4 days after a single blood meal (median 3 days = ovarian cycle). However, several females surviving 7 days (20 of 26) retained mature stage V eggs in their ovaries after oviposition; ranges of 1–10, 11–20, 21–30, 31–40, and > 41 eggs were found in 4, 6, 6, 3, and 1 females, respectively. This would indicate that the overall fecundity of *Ae. notoscriptus,* including oviposited and retained eggs, averaged 29.4 ± 10.5 (range 14–57).

Six developmental attributes were examined for *Ae. notoscriptus* reared under summer and winter environmental conditions typical for Brisbane (Table 2). Survivorship from 1st instar to adult emergence was more than 90% for both summer and winter conditions. Development to pupation (P_{50} and P_{90}) was prolonged under winter conditions to average 12.4 ± 1.5 days and 15.0 ± 1.0 days, respectively. The male to female sex ratios were similar for summer (1.0:1.1) and winter (1.0:1.3) con-

Table 2. Survival[1] and larval development of *Aedes notoscriptus* in the laboratory under typical summer (20.5–28.9°C) and winter (10.1–21.2°C) environmental conditions in Brisbane, Queensland, Australia.

Attribute[2]	Mean ± SD[3]	
	Summer conditions	Winter conditions
Survivorship, L1 to P	0.93 ± 0.02 A	0.91 ± 0.03 A
P_{50} (days)	6.00 ± 1.00 A	12.40 ± 1.53 B
P_{90} (days)	8.30 ± 1.16 A	15.00 ± 1.00 B
Survivorship, P to A	0.99 ± 0.01 A	0.99 ± 0.01 A
Survivorship, L1 to A	0.93 ± 0.02 A	0.91 ± 0.03 A
Sex ratio (♂/total)	0.48 ± 0.01 A	0.43 ± 0.01 A

[1] Proportion of stage surviving.
[2] L1, 1st larval instar; P = pupae; A = adult; P_{50} = development time to 50% pupation of surviving larvae; P_{90} = development time to 90% pupation of surviving larvae.
[3] Row means followed by the same letter are not significantly different (*t*-test, df = 4, $P < 0.05$).

ditions; however, males emerged earlier than females. The average development times from egg hatch to adult were 11 days (larval stage, 8 days; pupal stage 2–3 days) and 20 days (larval stage, 15 days; pupal stage, 4–5 days) for summer and winter conditions, respectively.

DISCUSSION

The results demonstrate the complete adaptation of *Ae. notoscriptus* to laboratory conditions, with free mating, reproduction, and colony replacement occurring under insectary conditions. To initiate colonization, efforts were made to duplicate the natural environment of this species. In nature, *Ae. notoscriptus* preferentially selects larval habitats that are protected by dense vegetation, ranging from natural containers (e.g., tree holes, plant axils, bamboo stems, and rock pools) to artificial containers (e.g., tires, tins, bottles, and water storage tanks) (Lee et al. 1982). Preference for oviposition sites with organically rich water has also been noted in the field (Foot 1970), as has the predilection of adults for dark resting sites (Lee et al. 1982).

In view of these biological preferences, the outer surface of the cage was lined with black plastic to provide shaded oviposition and resting sites. Natural oviposition substrates were provided by the use of a potted bromeliad and plastic base. To further increase the attractiveness of the bromeliad as an oviposition site, a small pinch of larval food was added to increase the organic content of the distilled water. The potted bromeliad also provided males with a natural swarm marker and swarming behavior and mating were observed to occur over the bromeliad during the simulated dusk period.

Initially, the mating success of the colony was modest. However, in contrast with previous colonization attempts (Standfast et al. 1966, 1967; Foot 1970), egg hatch rates suggest that free mating occurred from the F_1. As the colony adapted to laboratory conditions, mating success rates increased to sufficient levels to sustain the colony. Reisen (1985) suggested that selection in the laboratory for a particular mating type may be a consequence of colonization. *Aedes notoscriptus* could possibly display many types of mating behavior in the field and 1 of these types may be preadapted to laboratory conditions. Such a strain could thus successfully mate in the laboratory and, through selection, would become increasingly adapted to the laboratory environment.

Oviposition strategies differ among mosquito species. Field observations for the container-breeding *Aedes aegypti* (L.), *Aedes albopictus* Skuse, and *Aedes polynesiensis* Marks suggest that eggs are distributed among several oviposition sites (Rozeboom et al. 1973, Reiter et al. 1995). Considering that 76.9% of ovipositing females retained some ova, *Ae. notoscriptus* may also utilize multiple oviposition sites.

The successful colonization of *Ae. notoscriptus* has facilitated laboratory vector competence studies. *Aedes notoscriptus* from Brisbane are susceptible to and capable of transmitting Ross River, Barmah Forest, and Rift Valley Fever viruses (Turell and Kay 1998; Watson and Kay 1998, 1999). This species also has low level susceptibility to oral infection with dengue virus types 1–4 but it is unlikely to be an important vector of dengue (Watson and Kay 1999).

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