COLONIZATION AND MAINTENANCE OF *ANOPHELES DEANEORUM* IN BRAZIL[1]

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ABSTRACT. *Anopheles deaneorum*, an important malaria vector and a member of the *Anopheles albitarsis* complex, was colonized by an artificial mating technique. Morphological and behavioral differences between *An. deaneorum* and *An. albitarsis* from Costa Marques, Rondonia, Brazil, are discussed. The essential methods and colonization techniques are described. Immature development and mortality rates were reduced when dried grass was added to larval rearing pans. Males frequently mate more than once using the force mating technique. However, insemination and larval eclosion rates decline as males are successively mated with unmated females.

INTRODUCTION

Anopheles (*Nyssorhynchus*) *albitarsis sensu lato*, a complex of polymorphic species, occurs from Central America to northern Argentina (Deane et al. 1946, Kreutzer et al. 1976, Rios et al. 1984). *Anopheles albitarsis s.l.* Lynch-Arribalzaga is believed to be a complex of at least 3 species in Brazil (Deane 1988) and in some regions is a confirmed vector of human malaria (Arruda et al. 1986, Klein and Lima 1990). *Anopheles deaneorum* Rosa-Freitas, a member of this complex, was colonized in 1988 to facilitate studies on taxonomy and susceptibility to *Plasmodium vivax* and *P. falciparum*. The colonization techniques are described below.

MATERIALS AND METHODS

A colony of *An. deaneorum* was established from females collected from human bait in Costa Marques (12°26'S, 64°14'W), Rondonia, Brazil, during 1988. Females were placed in screen-topped pint cartons and provided blood meals on human volunteers. Engorged females were provided a 5% sucrose solution and held for 2–3 days at 26 ± 2°C. Two methods were used to obtain eggs. First, on day 3 after a blood meal, gravid females were anesthetized with ethyl acetate and traumatized by removing one wing with forceps as described by Lanzara et al. (1988). Females were then placed individually in small plastic cups (5 × 3.5 cm) filled with 0.5 cm of filtered tap water. Females normally oviposited within 2–4 h following removal of the wing. Second, on days 2–3 after a blood meal, females were placed individually in screen-topped oviposition vials filled with approximately 2.0 cm of water. Females that oviposited were provided another blood meal and processed as above.

Filtered tap water was used for larval rearing. City water was obtained from the Guapore River and was often untreated and unfiltered. During some periods, the filtered tap water was a yellow-brown color.

Eggs were transferred from the plastic cups or oviposition vials in groups of 200–300 to small plastic larval rearing pans (3.5 × 11 × 21 cm), one-half filled with filtered water. The eggs were surrounded by a plastic drinking straw formed into a triangle to prevent them from becoming stranded on the sides of the rearing pans and desiccating. Approximately 0.5 ml of 5% solution, by weight, of 1 part finely ground wheat germ and 2 parts baby fish food was added to each pan containing 2- to 3-day-old eggs. On day 4 after oviposition, larvae were separated into groups of approximately 100 per rearing pan and fed a dry mixture 1 part finely ground wheat germ and 2 parts baby fish food added to the water surface. On days 8–9, larvae were transferred to larger plastic larval rearing pans (5 × 28 × 34 cm).

Pupae were transferred daily to small bowls and placed in screened cages (32 cm on each side). Emerging adults were provided a 5% sucrose solution in saturated cotton.

Three- to 4-day-old females were starved for 6 h and provided blood meals on human volunteers. Engorged females were force-mated with 2- to 6-day-old males (Ow Yang et al. 1963), put in screen-topped pint cartons and provided a 5% sucrose solution. Eggs were obtained by the 2 methods and larvae reared as described above.

Males were mated with females up to 4 times to determine the number of females they could successfully inseminate. The females were kept in separate cages and the eggs were obtained by removing a wing as described above. The num-

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ber of eggs oviposited and the number of larvae that hatched were recorded for each female. After oviposition, the spermathecae were removed and examined for sperm by light microscopy at 400×.

To determine if floating vegetation improved survival and reduced development time of mosquito larvae, grass was collected and allowed to air dry for 6 or more days. Three to 5 leaves of dried grass were added to 15 of the larval rearing pans on day 4 after oviposition. Larval development time and mortality of 15 paired replicates between the 2 rearing methods were compared.

RESULTS AND DISCUSSION

Two members of the *An. albitarsis* complex, *An. deaneorum* and *An. albitarsis* collected in Costa Marques, Rondonia, Brazil, exhibited different morphological characteristics. *Anopheles deaneorum* has recently been described (Rosa-Freitas 1989). In Costa Marques, both *An. albitarsis* and *An. deaneorum* are sympatric. The adults can easily be separated by differences in the wing spot pattern and wing scale color (Fig. 1). In general, *An. deaneorum* wing scales are yellow, the prehumeral dark spot (PHD) (nomenclature based on Harbach and Knight 1980) is often absent and the subcostal pale spot (SCP) is always larger than the dark spot on the cubitus (Cu) basal to the mediocubital crossvein (mcu). The wing scales of *An. albitarsis* collected in Costa Marques are white on the anterior veins, pale yellowish white on the remainder and the subcostal pale spot is smaller than the dark spot on the cubitus basal to the medicubital crossvein.

The *An. deaneorum* colony has been maintained in the laboratory more than 25 generations. Galvao et al. (1944), Galvao and Grieco (1943), and Barretto and Coutinho (1943) re-

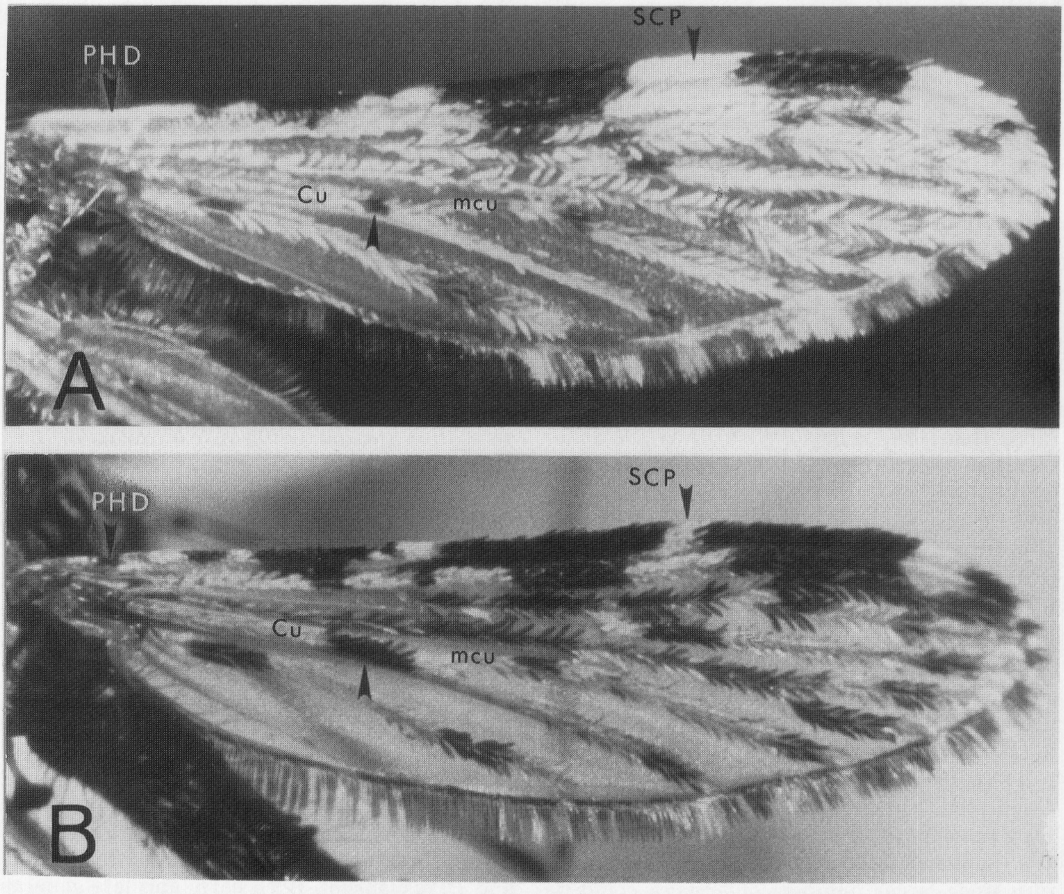

Fig. 1. Wings of *Anopheles deaneorum* (A) and *An. albitarsis* (B) from Costa Marques, showing differences in the presence of the prehumeral dark spot (PHD) and length of the subcostal pale spot (SCP) when compared to the dark spot on the cubitus (Cu) basal to the mediocubital crossvein (mcu).

Table 1. Percentage of female *Anopheles deaneorum* inseminated, mean number of eggs and percent eggs hatched when males are used in multiple matings.

Successive male matings	Number females mated	Percent females inseminated[1]	Percent females oviposit	Mean eggs oviposited	Percent eggs hatched
1	55	80	70	108	43
2	53	43	67	91	26
3	51	35	74	114	17
4	51	23	66	110	11

[1] Females were dissected on day 2 after mating or after oviposition.

ported that *An. albitarsis* from Rio de Janeiro mated in screened cages in the laboratory. However, male *An. deaneorum* did not mate naturally with females when maintained in screened cages 32 cm or 48 cm on each side and, therefore, were force-mated using the technique described by Ow Yang et al. (1963). Insemination and copulation rates for males mated with females at ambient temperatures of 26°C in the laboratory were low. However, *An. deaneorum* males readily copulated with females of either *An. deaneorum* or *An. albitarsis* at ambient temperatures of 23°C or less and venting cool air from the air conditioner toward the work bench.

The modified oviposition technique described by Lanzara et al. (1988) was used to obtain eggs. Usually between 80–90% of the traumatized females oviposited within 2–4 h. Females also oviposited in individual screened vials with moist paper as described by Galvao et al. (1944) for *An. albitarsis domesticus* Galvao and Damasceno and in pans of water when held in screened cages, but this required more time and larger numbers of artificially mated females to obtain sufficient eggs to maintain the colony.

Using the force-mating technique, *An. deaneorum* males could frequently be mated with more than one female. W. Collins (personal communication) reported that males were frequently used twice to maintain a colony of *An. albitarsis* from Colombia. To determine the value of using males more than once, we force-mated males up to 4 times with unmated females (Table 1). The insemination rate was reduced from 80% (first mating) to 43%, 35% and 23% for females mated with males used in the second, third and fourth matings, respectively. The amount of sperm transferred on each successive mating was apparently reduced since fewer eggs hatched on each successive mating. These results show that while some males inseminate females on the third and fourth mating, there is little value to use a male for more than 2 females. We frequently used males twice in the laboratory since larger numbers of females could be mated in less time.

Mortality for larvae reared in pans without dried grass was 35%. The addition of dried grass

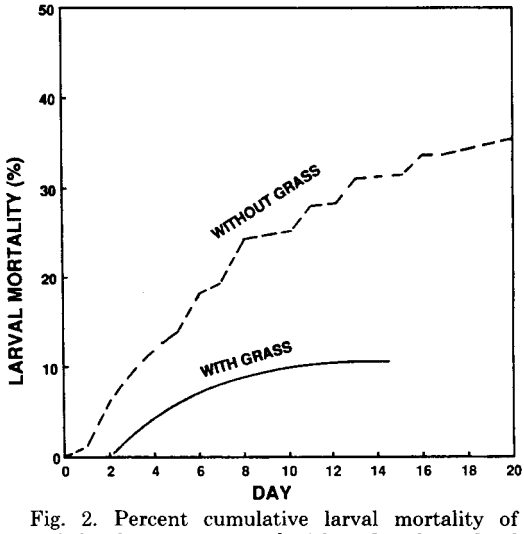

Fig. 2. Percent cumulative larval mortality of *Anopheles deaneorum* reared with and without dried grass in the laboratory.

reduced larval mortality to 10% (Fig. 2). In addition, the development period for immatures reared with dried grass was reduced (Fig. 3). By day 11, 50% of the larvae had pupated in pans with dried grass and only 10% of the larvae had pupated in pans lacking grass. Larval mortality was not associated with any particular stage of development. High larval mortality in some groups was associated with water quality. High mortality rates occurred in F_1 progeny of other species of anophelines as well during periods of poor water quality.

Anopheles deaneorum males, when force-mated, readily inseminate females of both *An. deaneorum* and *An. albitarsis* from Costa Marques. However, *An. albitarsis* males rarely inseminated females of either species and attempts to colonize *An. albitarsis* by artificial mating techniques has thus far failed.

The *An. deaneorum* colony was primarily established for studies of susceptibility to *P. vivax* and *P. falciparum*. Both early and late generations of *An. deaneorum* were susceptible to laboratory infections of *P. vivax* and *P. falciparum*.

In summary, colonization of *An. deaneorum*

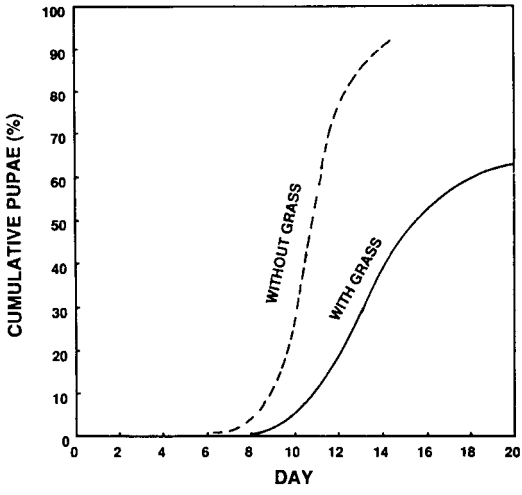

Fig. 3. Percent cumulative pupae of *Anopheles deaneorum* reared with and without dried grass in the laboratory.

was facilitated by 1) using lower ambient temperatures during copulation to increase the insemination rate, 2) mating each male with 2 females, and 3) using dried grass in larval rearing pans to reduce larval mortality and larval development time.

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REFERENCES CITED

Arruda, de M. E., M. B. Carvalho, R. S. Nussenzweig, M. Maracic, A. W. Ferreira and A. H. Cochrane. 1986. Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. Am. J. Trop. Med. Hyg. 35:873–881.

Barreto, M. P. and J. O. Coutinho. 1943. Criacao de algumas especies de anofelinos brasileiros. Rev. Brasil. Biol. 3:317–323.

Deane, L. M. 1988. Malaria studies and control in Brazil. Am. J. Trop. Med. Hyg. 38:223–230.

Deane, L. M., O. R. Causey and M. P. Deane. 1946. I. An illustrated key by adult female characteristics for the identification of 35 species of Anophelini, with notes on the malaria vectors (Diptera, Culicidae). Am. J. Hyg. Monogr. Ser. 18, pp. 1–16.

Galvao, A. L. A., R. R. Correa and S. J. Grieco. 1944. Alguns dados sobre a manutencao de colonias de *Nyssorhynchus* em laboratorio. Arch. Hig., S. Paulo 9:83–102.

Galvao, A. L. A. and S. J. Grieco. 1943. Acasalamento do *Anopheles* (*N.*) *albitarsis domesticus* em laboratorio. Rev. Paulista Med. 23:256.

Harbach, R. E. and K. L. Knight. 1980. Taxonomists' glossary of mosquito anatomy. Elsevier Publ., Amsterdam.

Klein, T. A. and J. B. P. Lima. 1990. Seasonal distribution and biting patterns of *Anopheles* mosquitoes in Costa Marques, Rondonia, Brazil. J. Am. Mosq. Control Assoc. (Accepted for publication).

Kreutzer, R. D., J. B. Kitzmiller and M. G. Rabbani. 1976. Cytogenetically distinguishable sympatric and allopatric populations of the mosquito *Anopheles albitarsis*. Acta Amazonica 6:473–481.

Lanzara, G. C., S. K. Narang, S. E. Mitchell, P. E. Kaiser and J. A. Seawright. 1988. Hybrid male sterility in crosses between field and laboratory strains of *Anopheles quadrimaculatus* (Say) (Diptera: Culicidae). J. Med. Entomol. 25:248–255.

Ow Yang, C. F., F. L. Sta Maria and R. H. Wharton. 1963. Maintenance of a laboratory colony of *Anopheles maculatus* Theobald by artificial mating. Mosq. News 23:34–35.

Rios, R. I., L. P. Nascimento and A. C. de Oliveira. 1984. Complexo *Anopheles* (*Nyssorhynchus*) *albitarsis* e *A. albitarsis domesticus* (Diptera: Culicidae). Rev. Brasil Biol. 44:461–465.

Rosa-Freitas, M. G. 1989. *Anopheles* (*Nyssorhynchus*) *deaneorum*: a new species in the *Albitarsis* complex (Diptera: Culicidae). Mem. Inst. Os. Cruz. Rio de J. 84:535–543.