Laboratory Colonization of *Anopheles aquasalis* (Diptera: Culicidae) in Belém, Pará, Brazil

ANA N. M. DA SILVA,[1] CARLA C. B. DOS SANTOS,[1] RAIMUNDO N. L. LACERDA,[1] EDVALDO P. SANTA ROSA,[1] RAIMUNDO T. L. DE SOUZA,[1] DEOCLECIANO GALIZA,[1] IZIS SUCUPIRA,[1] JAN E. CONN,[2, 3] AND MARINETE M. PÓVOA[1]

J. Med. Entomol. 43(1): 107–109 (2006)

ABSTRACT *Anopheles aquasalis* Curry, a coastal malaria vector with a Neotropical distribution, was collected from Belém, Pará state, Brazil, and 500 adults per cage were maintained at the Instituto Evandro Chagas insectary at 26–30°C and 80–90% RH, where they fed on a 10% domestic sugar solution and blood from white mice. Oviposition of the parental generation (P) occurred in fresh water in dark cups introduced into mosquito cages. After eclosion, 100 larvae per pan were reared in artesian well water (salinity 0.04 g liter^{-1}) and fed ground fish food until pupation. After force mating in the F_1 generation, the eight subsequent generations were free mating. Mean larval mortality was <1%, and the mean developmental time from eclosion to emergence was 7.7 d (F_1), 7.6 d (F_2), 8 d (F_3), and 7.5 d (F_4). The maximum daily production of pupae (from the fourth generation on) occurred on day 6 postoviposition.

KEY WORDS *Anopheles aquasalis*, Brazilian Amazon, malaria vector, colonization

Anopheles aquasalis Curry, a Neotropical species with a coastal distribution as far north as Nicaragua and as far south as southeastern Brazil on the Atlantic coast and Ecuador on the Pacific coast (Faran 1980, Rubio-Palis and Zimmerman 1997) is the only member of the *Nyssorhynchus* subgenus that normally breeds in saltwater. Its involvement in malaria transmission is significant in coastal areas from Venezuela (Berti et al. 1993) to southeastern Brazil (Fleming 1986, reviewed in Lounibos and Conn 2000). In Belém, it was first incriminated as a malaria vector by Galvão et al. (1942), and its status was recently reevaluated and confirmed by Póvoa et al. (2003).

Colonies of mosquitoes have provided basic information on taxonomic (Forattini 1962), genetic, and hybridization studies (Coluzzi 1964) and have been useful in assessing comparative susceptibility to *Plasmodium* parasites (González-Ceron et al. 1999, Rodriguez et al. 2000). The lack of successful free-mating in some anopheline colonies has been a limiting factor to colony maintenance (Klein et al. 1990, but see Horosko et al. 1997, Villarreal et al. 1998, de Carvalho et al. 2002). The current study presents the first successful colonization of several generations of free-mating *An. aquasalis* in freshwater, in Amazonian Brazil.

Materials and Methods

Rearing Conditions for Colony Establishment. Thirty months was needed to determine all the appropriate rearing conditions for colony establishment, including exposure to sunlight, type of sugar for adult feeding, and type of animal used for blood feeding. Both sunlight during the morning and fluorescent lights (during daylight) were used for larval rearing. We reared larvae at a density of 100 larvae per clear plastic circular pan (diameter 23 cm, height 8.5 cm). For adult feeding, a 10% solution of honey and a 10% solution of domestic sugar were tried.

The animals tested for the bloodmeal source for adult females included suckling, juvenile, and adult hamsters, white suckling and adult mice, chicks and hens, and adult quail.

Natural oviposition and very occasionally induced oviposition (removal of one wing) were used for the P generation only. Oviposition from F_1 on was induced using small black cups containing a minimal amount of the water in which the pupae from the previous generation had emerged. The time from oviposition to hatch was 1 to 2 d.

During larval development, the water was only changed after the accumulation of considerable debris either on the water surface or on the bottom of the pan. Mortality of larvae using our method was extremely low (<1%). For the F_4 and F_5 generations, larval mortality was 0.6 and 0.5%, respectively.

Adult Collection (P Generation) and Egg Production. The colony was started with female *An. aquasalis* collected from humans (by using informed consent

[1] Programa em Malaria, Seção de Parasitologia do Instituto Evandro Chagas, Belém, Pará, Brazil.
[2] Department of Biology, University of Vermont, Burlington, VT 05405.
[3] Current address: Griffin Laboratory, The Wadsworth Center, New York State Department of Health, Albany, NY 12159.

0022-2585/06/0107–0109$04.00/0

forms approved by the Instituto Evandro Chagas [IEC] Ethical Committee on Human Subjects) in 2000 between 1800 and 2100 hours at the Emilio Goeldi Zoobotanical Park in Belém, Pará state, Brazil. The wild-caught females (P generation) were maintained in standard size cages (20 by 20 by 20 cm) in the insectary at IEC on 10% domestic sugar until they were identified using the morphological key of Gorham et al. (1967). Male maintenance was better using 10% honey compared with 10% domestic sugar. Insectary conditions were as follows: 26–30°C, 80–90% RH, and photoperiod of 12-h fluorescent light combined with daylight, followed by 12-h darkness. There was no crepuscular period. After identification, females were transferred to double-mesh standard cages and fed to repletion on anesthetized white adult mice. On day 3 postblood feeding, small black cups (diameter 5 cm, height 4 cm) lined with filter paper (to prevent egg desiccation) containing ≈30 ml of well water were introduced into each cage for oviposition. Artesian well water from the IEC with a salinity of 0.04 g liter^{-1} was stored in containers for a maximum of 3 d before use to allow dissolved oxygen to escape. Free mating did not take place in the laboratory until after forced-mating of the F_1 generation (as in Arruda et al. 1982).

Larval and Pupal Rearing. After eclosion, larvae were placed in clear round plastic pans (diameter 23 cm, height 8.5 cm) in ≈700 ml of well water at a density of 100 larvae per pan. The pans were placed near the windows in the insectary for exposure to sunlight. First and second instars were fed 200 mg of finely ground Tetramin tropical fish food per container sprinkled on the water surface one to two times per day. Third and fourth instars also were fed 200 mg per container, two to three times per day until pupation. The decision to feed two versus three times per day was determined by water turbidity. Pupae were transferred to disposable plastic cups containing well water and then placed into empty cages until adult emergence.

Adults (F_1 and Subsequent Generations). Five hundred adults per cage were fed exclusively on 10% domestic sugar on cotton pads until 6 d days after the last pupae had been placed into each cage, and after insemination had been verified for each cage by dissection of a small sample of female spermathecae to detect live sperm. Adult females were then placed on anesthetized adult white mice for 30 min for a bloodmeal. This took place either early morning or late afternoon, after the domestic sugar pads had been removed for 12 h. On the third day postbloodmeal, small black cups with well water were introduced as described above for oviposition.

The maximum daily production of pupae (≈50% of the total number/cage) took place on day 6 postoviposition. The mean time from eclosion to pupation was as follows: 7.7 d for F_1, 7.6 d for F_2, 8 d for F_3, and 7.5 d for F_4. The adults normally emerged in 1 to 2 d, and males emerged before females.

Results and Discussion

Using these simple, reproducible methods, we maintained a colony of *An. aquasalis* through nine generations. Galvão et al. (1944) described a free-mating colony of *An. aquasalis* maintained for five generations in Rio de Janeiro state, Brazil, by using time-consuming techniques that are difficult to duplicate (e.g., an infusion of fish food of unknown concentration for larvae) that resulted in considerable contamination in the rearing pans and high larval mortality. They also used saltwater for larval rearing and had low, irregular egg production. Like Galvão et al. (1944), we did not systematically observe the time of copulation inside the cages. However we suggest that copulation began ≈2000 hours when we noted that the males all began to fly.

For most Neotropical *Anopheles*, egg-laying takes place at dawn (de Carvalho et al. 2002). In a study of *An. aquasalis* oviposition behavior in Trinidad and Tabago, Chadee and Mohammed (1996) observed that oviposition began at 2200 hours and ended at 0600 hours, with a well-defined peak from 2400 to 0400 hours. Although we did not monitor the time of oviposition in our colony, we are certain that it took place overnight, because most mornings we found a large number of eggs in oviposition cups.

In other studies of anopheline colonization in Brazil (Klein et al. 1990, Horosko et al. 1997), larval mortality rates were 10% and 20–30%, respectively, compared with our rate of <1%. However, it should be noted that the species colonized were distinctive, although all were in the *Nyssorhynchus* subgenus. Nevertheless, our techniques differed from those of the aforementioned studies, perhaps contributing to the low larval mortality.

It has been known since the earliest description of the bionomics of *An. aquasalis* (Curry 1932) that this species commonly breeds in saltwater. Despite this preference, both Galvão et al. (1944) and Deane et al. (1948) successfully reared *An. aquasalis* in water with zero salinity. Natural breeding in freshwater has been reported in Brazil (Rachou 1958) and in Venezuela (Berti et al. 1993). In Rio de Janeiro, a colony of *An. aquasalis* is being successfully maintained using 10% saltwater for the larval stage (de Carvalho et al. 2002). Probably these differences reflect the tolerance of *An. aquasalis* to a range of salt concentrations, resulting in its ability to breed in diverse habitats. We suggest that its coastal distribution/salt water tolerance has evolved as a means of avoiding competition with other anophelines for food during the larval stage (Grillet 2000). The colonization of *An. aquasalis* at the Instituto Evandro Chagas has already had practical results: we successfully used the F_3 generation in comparative susceptibility experiments between *An. aquasalis* and *Anopheles darlingi* Root to *Plasdodium vivax* variants (da Silva 2000).

Acknowledgments

Financial support was provided by the Evandro Chagas Institute project "Susceptibilidade natural e experimental do

Anopheles aquasalis (Diptera: Culicidae) e colonização em laboratório." The protocol for this study was reviewed and approved by the Research Board of the Evandro Chagas Institute.

References Cited

Arruda, M. E., R. I. Rios, P. C. Arantes, A. C. Oliveira, and L. P. Nascimento. 1982. Manutenção em laboratório de *Anopheles albitarsis* e *Anopheles aquasalis* por copulação induzida. Mem. Inst. Oswaldo Cruz 77: 89–91.

Berti, J., R. Zimmerman, and J. Amarista. 1993. Adult abundance, biting behavior and parity of *Anopheles aquasalis* Curry, 1932, in two malarious areas of Sucre state, Venezuela. Mem. Inst. Oswalod Cruz 88: 363–369.

Chadee, D. D., and R. T. Mohammed. 1996. Diel rhythms of oviposition and fecundity of *Anopheles aquasalis* (Diptera: Culicidae) under laboratory conditions. J. Med. Entomol. 33: 686–688.

Coluzzi, M. 1964. Maintenance of laboratory colonies of *Anopheles* mosquitoes. Bull. World Health Organ. 31: 441–443.

Curry, D. P. 1932. Some observations on the *Nyssorhynchus* group of the *Anopheles* (Culicidae) of Panama. Am. J. Hyg. 15: 566–572.

da Silva, A.N.M. 2000. Susceptibilidade natural e expierimental do *Anopheles aquasalis* (Diptera: Culicidae) e colonização em laboratório. M.S. thesis. Universidade Federal do Pará, Belém, Pará, Brazil.

Deane, L. M., O. R. Causey, and M. P. Deane. 1948. Notas sobre a distribuição e a biologia dos anofelinos das regiões nordestina e amazônica do Brasil. Rev. Serv. Espec. Saúde Púb. 1: 827–966.

de Carvalho, S.C.G., A. J. Martins Junior, J.B.P. Lima, and D. Valle. 2002. Temperature influence on embryonic development of *Anopheles albitarsis* and *Anopheles aquasalis.* Mem. Inst. Oswaldo Cruz 97: 117–120.

Faran, M. E. 1980. Mosquitos studies (Diptera: Culicidae). XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Anopheles.* Contrib. Am. Entomol. Inst. 15: 1–215.

Fleming, G. 1986. Biologia y ecologia de los vectores de la malaria en las americas. PAHO/WHO, PNSP/86-72.

Forattini, O. P. 1962. Entomologica medica, vol. 1. São Paulo, Fac. Hig. Saúde Púb., São Paulo, Brazil.

Galvão, A.L.A., R. G. Damasceno, and A. P. Marques. 1942. Algumas observações sobre a biologia dos anofelinos de importância epidemiológica de Belém Pará. Arq. Hig. Saúde Púb. 12: 51–110.

Galvão, A.L.A., R. R. Corrêa, and S. J. Grieco. 1944. Alguns dados sobre a manutenção de colônias de *Nyssorrhynchus* em laboratório. Arq. Hig. Saúde Públ. São Paulo 9: 85–103.

González-Ceron, L., M. H. Rodriguez, J. C. Nettel, C. Villarreal, K. C. Kain, and J. E. Hernández. 1999. Differential susceptibilities of *Anopheles albimanus* and *Anopheles pseudopunctipennis* to infections with coindigenous *Plasmodium vivax* variants VK210 and VK247 in southern Mexico. Infect. Immunol. 67: 410–412.

Gorham, J. R., C. J. Stojanovich, and H. G. Scott. 1973. Clave ilustrada para los mosquitos anofelinos de Sudamerica Oriental. Mosq: Syst. 5: 97–156.

Grillet, M. E. 2000. Factors associated with distribution of *Anopheles aquasalis* and *Anopheles oswaldoi* (Diptera: Culicidae) in a malarious area, northeastern Venezuela. J. Med. Entomol. 37: 231–238.

Horosko, S. III, J.B.P. Lima, and M. B. Brandolini. 1997. Establishment of a free-mating colony of *Anopheles albitarsis* from Brazil. J. Am. Mosq. Control Assoc. 13: 95–96.

Klein, T. A., J.B.P. Lima, and A. T. Tang. 1990. Colonization and maintenance of *Anopheles deaneorum* in Brazil. J. Am. Mosq. Control Assoc. 6: 51–53.

Lounibos, L. P., and J. E. Conn. 2000. Malaria vector heterogeneity in South America. Am. Entomol. 46: 238–249.

Póvoa, M. M., J. E. Conn, C. D. Schlichting, J.C.O.F. Amaral, M.N.O. Segura, A.N.M. da Silva, C.C.B. Dos Santos, R.N.L. Lacerda, R.T.L. de Souza, D. Galiza, et al. 2003. Malaria vectors, epidemiology and the re-emergence of *Anopheles darlingi* in Belém, Pará, Brazil. J. Med. Entomol. 40: 379–386.

Rachou, R. G. 1958. Os anofelinos do Brasil. Comportamento das espécies vetoras de malária. Rev. Bras. Malar. Doen. Trop. 10: 145–181.

Rodriguez, M. H., L. González-Ceron, J. E. Hernández, J. C. Nettel, C. Villarreal, K. C. Kain, and R. A. Wirtz. 2000. Differential prevalences of *Plasmodium vivax* phenotypes VK210 and VK247 associated with the distribution of *Anopheles albimanus* and *Anopheles pseudopunctipennis* in Mexico. Am. J. Trop. Med. Hyg. 62: 122–127.

Rubio-Palis, Y., and R. H. Zimmerman. 1997. Ecoregional classification of malaria vectors in the neotropics. J. Med. Entomol. 34: 499–510.

Villarreal, C., J. I. Arredondo-Jíminez, M. H. Rodriguez, and A. Ulloa. 1998. Colonization of *Anopheles pseudopunctipennis* from Mexico. J. Amer. Mosq. Control Assoc. 14: 369–372.

Received 26 April 2005; accepted 29 April 2005.