

Short Communication

Brazil's first free-mating laboratory colony of *Nyssorhynchus darlingi*

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Abstract

Introduction: The lack of highly-productive *Nyssorhynchus darlingi* laboratory colonies limits some studies. We report the first well-established laboratory colony of *Ny. darlingi* in Brazil. **Methods:** Mosquitoes were collected from Porto Velho and were reared at the Laboratory of Fiocruz/RO. After induced mating by light stimulation in the F1 to F6, the subsequent generations were free mating. Larvae were reared in distilled water and fed daily until pupation. **Results:** In 11 generations, the colony produced a high number of pupae after the F5 generation. **Conclusions:** These results demonstrate the potential for permanently establishing *Ny. darlingi* colonies for research purposes in Brazil.

Keywords: *Nyssorhynchus darlingi*. Colony establishment. Brazil. Malaria vector.

Nyssorhynchus darlingi is the most significant malaria vector in the Amazon region and one of the most efficient malaria vectors in the world¹. However, important entomological aspects of *Ny. darlingi* remain unknown because continuous propagation of this species is difficult to achieve in the laboratory. The difficulty in developing colonies of some mosquito species is due to challenges in finding the right conditions for successful mating by non-artificial methods and selecting appropriate populations for their ability to mate in a restricted space². A free-mating laboratory colony of *Ny. darlingi* was established in Peru five years ago^{3,4}, but, until now, no free-mating laboratory colonies have been successfully established outside Peru.

Previous attempts to establish *Ny. darlingi* colonies have been beset with difficulties^{5,6,7}. In Brazil, no *Ny. darlingi* colony has ever been permanently established, and currently, no colonies are available for research purposes.

A highly productive laboratory colony of *Ny. darlingi* is an essential resource for malaria research in the Brazilian Amazon; this is especially true for research into malaria caused by *Plasmodium vivax*, which has been the predominant species causing malaria in Brazil since the mid-1990s⁸. In endemic regions of the Brazilian Amazon, experimental studies into the biology of *P. vivax*-*Nyssorhynchus* interactions have been limited by the lack of laboratory-reared *Ny. darlingi* mosquitoes.

In the few studies that have been conducted, *P. vivax*-*Ny. darlingi* interactions (in the form of direct feeds or membrane feeding assays) have only been observed in laboratory-reared F1 generations^{9,10,11}. Due to the importance of establishing such a mosquito colony in Brazil, a self-mating *Ny. darlingi* colony was initiated in March 2018 at the Entomology Laboratory of Fiocruz Rondônia. Colony initiation was achieved using an

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Received: 17 April 2019

Accepted: 31 May 2019

adapted version of the natural induction technique developed for *Ny. darlingi* in the Peruvian Amazon^{3,4}.

The colony was initiated using 320 adult females that were collected by BG-Malaria traps and protected human landing in a peridomicile environment (08°39.145' S/063°56.155' W), 31 km from Porto Velho City, Rondônia, Brazil. Approximately 800-2,000 three to five-day-old *Ny. darlingi* males and females (ratio 1:1) of each generation were placed in screened cages (61 × 61 × 61 cm) and fed honey-water solution (15%) *ad libitum*. Copulation induction was conducted on five to seven consecutive evenings, and each cage was exposed to a light beam (four cycles of 10 min on and 10 min off), the insectary temperature was reduced to 24 ± 1 °C at 6 p.m., and humidity was maintained at 80%. In the initial phases, these cycles were performed manually using a flashlight, but after the F3 generation, an automated system was developed and implemented. Following the final light cycle, female mosquitoes were fed with chicken blood for 15 min during the first 4-6 days of induction. Oviposition was induced by cutting one wing until natural oviposition occurred. Copulation induction was conducted for 5 days (up to the F6 generation), after which free-mating was confirmed and natural oviposition was observed in the F5 generation. Larvae were reared in pans (30.3 × 22.1 × 7.5 cm) containing approximately 200 larvae per pan, and larvae were fed with Tetra Marine Granules®.

Species identity (F1 to F4 generations, 10 specimens per generation) was confirmed by DNA barcoding¹² and the colony exhibited 99-100% identity with *Ny. darlingi* sequences from Peru (KP193458), Venezuela (KC555065), and Brazil (JF923693) available in GenBank.

Preliminary evaluation using infected human blood in artificial membrane assays confirmed that *Ny. darlingi* in the colony (F5, F6, F7, F10, F11, F12) are susceptible to infection by *P. vivax*. Oocysts ranged from 1 to 692 per mosquito (mean ± SD = 69.3 ± 90.2), and sporozoites ranged from 200 to 43,440 per mosquito (mean ± SD = $8,580.5 \pm 8,462.1$).

At present, our colony has been maintained for 13 generations over the course of 11 months. Pupae production has been high since the F5 generation, and copulation induction and oviposition are no longer necessary (**Figure 1**), which indicates the development of a stenogamic colony. A drop in pupae production in the F8 and F9 generations occurred as a result of the holiday period in Brazil (December and January). Pupae survival rates range between 83.3 and 97.1%, which indicates that the mosquitoes have adapted to the laboratory conditions.

These results demonstrate the potential for permanently establishing *Ny. darlingi* colonies for research purposes in all parts of Brazil. With colony mosquitoes, experiments will no longer be limited to the Brazilian Amazon, where *P. vivax* patients are often used for *ex vivo* infection experiments.

Our *Ny. darlingi* colony has allowed us to study unknown aspects of this important vector's biology, such as circadian cycle behavior, the role of *P. vivax* asymptomatic parasite carriers, the role microbiota play in *Ny. darlingi* infection, and the ability of potential drugs to block malaria transmission.

Going forward, future generations of the colony will continue to be used for *P. vivax* infection experiments, and we also hope to analyze genetic variation between each generation and between future colonies.

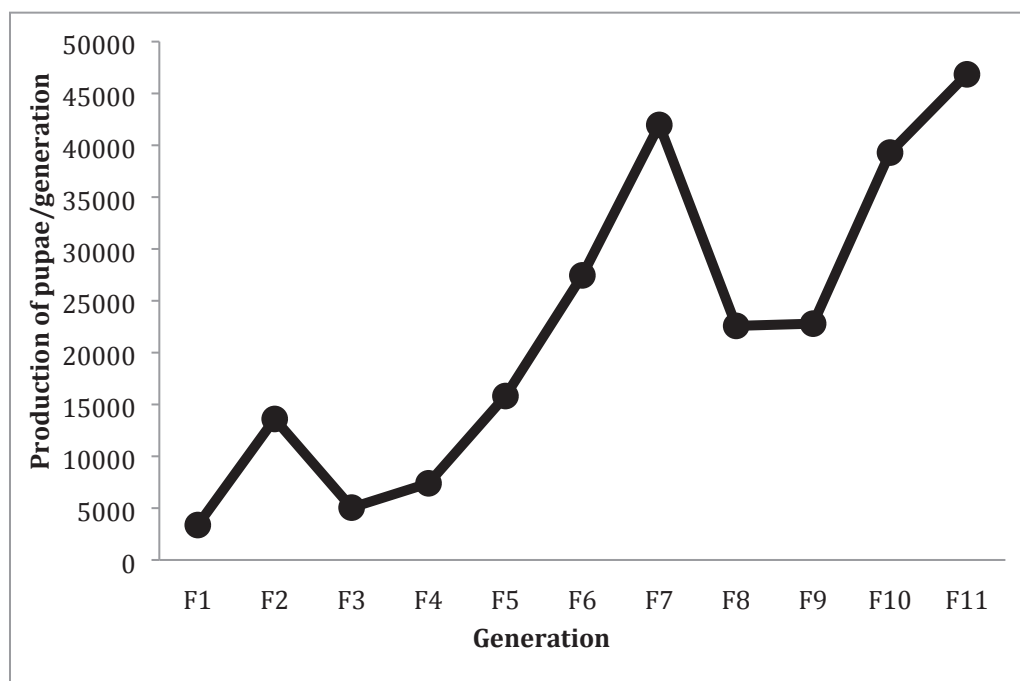


FIGURE 1. Pupae production of *Nyssorhynchus darlingi* reared for 11 generations in the Fiocruz Rondônia insectary in Porto Velho, Brazil.

Our main purpose in creating this colony has been to create a resource that will give the entire scientific community access to a malaria vector that is of great significance in the Amazon.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dr. Dionicia Gamboa (Universidad Peruana Cayetano Heredia, Lima, Peru), Dr. Manuela Herrera (Universidad Peruana Cayetano Heredia, Lima, Peru), and Dr. Joseph Vintz (Yale School of Medicine, USA) for their collaboration.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Financial Support

This research was supported by FIOCRUZ/RO, Fundação de Amparo à Pesquisa do Estado de Rondônia (Fapero), grant N02/2014, and in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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