**Brazilian mosquito life history project- Revised scope (4/12/18)**

Problem statement

Brazil has the second largest burden of malaria in Latin America1. The primary malaria vector in Brazil is *Nyssorhynchus darlingi*, widely distributed from 0°S-23°S across three major biomes, the Amazonia, Cerrado and Mata Atlântica 2,3. Temperatures have been projected to rise 2-4°C worldwide due to climate change4. Rising temperatures have been shown to increase the development rate of mosquitoes5–8 but it is unclear if this will be uniform across Brazilian populations and how this may affect life history traits relevant to malaria transmission.

This mosquito has been notoriously difficult to rear in the laboratory, with a colony recently established in Peru9. Due in part to this barrier, predictive ecological niche models have relied on current distribution 10 and presence-only records 11 and treat *N. darlingi* as one homogenous population responding to changes in temperature. In this study, we aim to investigate how differences in rearing temperature and population affect the life history traits of Brazilian *N. darlingi*.

Goals

1. Generate a linear regression model to identify relationship between temperature, state and the outcomes of larvae development, adult longevity, and wing length
   1. Bayesian
2. Genetic variation and phenotypic plasticity
   1. Compare localities
   2. Compare between families within localities
3. Investigate sexual differences
   1. Did males emerge before females (protandry)?
      1. ANOVA on time to emergence by temperature, sex and state
   2. Was sexual dimorphism observed based on wing lengths?
      1. ANOVA on wing length by temperature, sex and state

Study design

Paired replicate locality sites within 4 states of Brazil were visited for mosquito collections. Each state was chosen to represent three latitudes and three biomes. Progeny from single, unmixed families were reared across three constant temperature environments (20, 24, 28°C). First instar larvae from each family were divided between the three temperatures, with no more than 5 larvae per rearing well and no more than 15 larvae from each family at each temperature. Larvae, pupae and adults were tended to with daily recordings of development stage and death. Adults were water-starved and upon death, the left wing was removed and mounted to a glass slide for wing length measurements.

Limits of study

1. This study does not have a paired replicate in Rio de Janiero despite multiple attempts to find an additional location to collect *N. darlingi*.
2. Biome and latitude are confounded so the state level, which incorporates both elements, will be used. Neither biome or latitude combined with temperature fit the data better than state.
3. The lowest level of analysis should be at the locality level because the study design expected them to be replicates and while variation between them can be examined, identifying a cause/source of variation is beyond the scope of this project (perhaps in population structure of P females).
4. Growth rate can be examined but a lot of literature utilized weight (pupal, dry adult) rather than wing size despite wing size and weight have been found to be correlated.

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