Bionomic and Genetic Characterization of *Anopheles gambiae* from Ghana

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Abstract

Malaria vector control relies principally on the use of insecticides. However, the increasing incidence of insecticide resistance threatens to undermine the effectiveness of this approach, necessitating resistance management strategies. Multiple insecticide resistance is becoming common in West Africa including Ghana. In particular, a population of the major malaria vector *Anopheles gambiae,* from the Ahafo region of Ghana, shows resistance to all classes of insecticide currently available for use in public health. Insecticide resistance is a primary adaptive characteristic of epidemiological importance although other adaptive traits such as staggered larval time-to-hatch may also be important. Typically, *An. gambiae* oviposits in small, temporary, sun-lit water bodies and eggs generally hatch 2-3 days post-oviposition. However, staggered distribution of hatching has previously been shown and was also observed in a newly colonized strain (GAH) from Ahafo. The broad aims of this project were therefore to assess and characterize multiple insecticide resistance in *An. gambiae* from Ahafo as well as to quantify and describe staggered time-to-hatch in a laboratory colony of this population in terms of its adaptive significance and pleiotropic effects on resistance.

WHO insecticide susceptibility bioassays conducted on wild-caught samples from Ghana and colonized material (GAH) indicated resistance to all insecticide classes. The presence of known metabolic resistance mechanisms as well as target-site insensitivity mutations were detected in GAH and are likely representative of the wild population from which the colony was derived. Staggered time-to-hatch was further investigated by monitoring hatching in GAH as well as in families reared from wild-caught females from Ghana and the Republic of the Congo. In addition, GAH was selected for early and late time-to-hatch and cross-mating experiments were conducted to determine the genetic heritability of this trait. The proportion of late hatching increased following selection, indicating that there is a genetic component involved in time-to-hatch. Staggered distribution of hatching was observed both in GAH and

wild families, some of which were exclusively early or late hatching. The effect of egg disturbance on time-to-hatch was also explored and was found to be necessary for optimal hatching. The association of insecticide resistance with selection for time-to-hatch was investigated and resistance profiles of the time-to-hatch selected strains differed significantly, suggesting a link between time-to-hatch and the expression of insecticide resistance, possibly as a consequence of pleiotropy. To further understand the mechanisms behind time-to-hatch, metabolic rates of early and late hatching strains were measured and embryonic development was qualitatively observed in the GAH time-to-hatch selected strains. It was found that embryos developed fully within four days regardless of subsequent hatch time and that some eggs are then able to delay hatching in a state of late embryonic diapause.

It is concluded that delayed hatch in *An. gambiae* is an adaptation to maximize reproductive output in an unstable aquatic environment. That this trait associates with resistance to insecticides suggests that enhanced phenotypic variation may be produced as a consequence of pleiotropy, providing a greater platform for resistance selection and resulting in increased ability of *An. gambiae* to adapt to an unstable environment.