Molecular genetic analysis of *Anopheles* mosquitoes when challenged by *Plasmodium* parasites

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A thesis submitted to the Faculty of Health Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of

Doctor of Philosophy

Johannesburg, 2014

Abstract

Malaria is the most serious tropical infectious disease in humans, caused by parasites of the *Plasmodium* genus and transmitted by anopheline mosquitoes. The interaction between the parasites and vectors has become a focus for malaria research as it may present an alternative disease control method by enhancing anti-plasmodial factors within the mosquito to impede parasite development and transmission. *Anopheles gambiae* is the best studied African malaria vector and is often used with the murine malaria parasite, *Plasmodium berghei,* for investigating parasite-vector interactions in the laboratory setting. *Anopheles funestus* has not been studied and its interactions with *Plasmodium* were unknown, until now. Although the Vector Control Reference Laboratory routinely maintains *An. funestus* and a number of *An. gambiae* colonies, none have been infected with *Plasmodium* since their establishment. This study aimed to use *P. berghei* to determine the vectorial capacity of these colonies and to examine the involvement of the 2La paracentric chromosomal inversion and antimicrobial peptides during *Plasmodium* infection in *An. gambiae* and *An. funestus*, respectively.

Most of the *An. gambiae* complex colonies were susceptible to *P. berghei*, but the range of feeding and infection rates varied considerably. The infection rates for some of the older colonies were lower than previously documented. *Anopheles funestus* colonies were all viable

vectors and there was an inverse correlation between the insecticide resistance profile and parasite susceptibility. Increased detoxification enzyme activities may have been contributing to a greater degree of parasite elimination.

In *An. gambiae,* molecular karyotyping of the 2La inversion using PCR was validated against traditional cytogenetic techniques. The PCR was shown to be a reliable substitute for identifying the inversion. Using molecular karyotyping on 2La polymorphic colonies infected with *P. berghei*, it was found that infected females were more likely to carry the 2La inversion, indicating possible correlation between the inversion and susceptibility to parasites.

In *An. funestus*, the expression of antimicrobial pepetide genes during *P. berghei* infection was examined using real-time PCR. Although all three genes showed increased activity at certain points of the infection, none displayed significant anti-plasmodial properties. However, in the less parasite susceptible strain, expression of two genes was higher towards the end of the infection, which was not observed in the other strains. It is possible that the co-expression of both peptides has led to a decrease in parasite load in late infection, but given the multi-factorial nature of the parasite-vector interaction, further investigation is required.