Summary of PhD thesis work

Title: A molecular approach to the effect of malaria infection on anopheline mosquito reproductive fitness

Malaria parasites are known to affect the fecundity of several species of mosquito vector. This is the first time that the mechanisms underlying this reduction have been investigated during the first and second gonotrophic cycles post-infection in the major malaria vector in Africa, *An. gambiae*. The rodent malaria parasite, *Plasmodium yoelii nigeriensis* was the species used in this study. The study investigated the effect of malaria infection on Vg gene transcription, Vg titre in the haemolymph and the vitellin content in the ovaries.

Fecundity (total number of eggs produced) and fertility (number of larvae hatched) were significantly reduced by 41.2% and 61.8% respectively. In the resulting offspring, there was no significant difference in the survivorship of the larvae. The sex ratio, wing length and symmetry, and the blood meal size of the F₁ adults were also unaffected, suggesting that parasite-induced reduction in fitness is not carried over to the succeeding generation.

The effect of the rodent malaria, *Plasmodium yoelii nigeriensis*, on Vg gene transcription of the major malaria vector in Africa, *Anopheles gambiae*, during the first and second gonotrophic cycles was also investigated. We demonstrated that Vg mRNA appeared 1-2h post-blood meal, peaked between 24 and 36h and terminated after 48h post-blood meal. Quantification of Vg mRNA, following Northern blotting, showed that no significant differences occurred between non-infected and infected female mosquitoes during the first gonotrophic cycle, when ookinetes were penetrating the mid gut. However, in the second gonotrophic cycle, when oocysts were developing, Vg mRNA transcription was down-regulated in infected mosquitoes at 2, 8, 12 and 24h post-blood meal. This suggests that down-regulation of Vg mRNA transcription may contribute to fecundity reduction.

During the first gonotrophic cycle, there was no significant difference in the Vg concentration between infected and non-infected mosquitoes up to 24h post-blood
meal. Thus confirming that the synthesis of yolk protein in the fat bodies was initiated normally. However by 30h there was a significant reduction in the Vg concentration. In the second gonotrophic cycle, initial concentrations of Vg were significantly reduced by infection, as predicted from mRNA measurements. However, by 24h Vg was accumulating in infected mosquitoes. This accumulation may occur due to impaired uptake in the ovaries of infected mosquitoes.

Vitellin (Vn) deposition occurred normally during the first gonotrophic cycle until 30h post-blood meal, after which significantly less Vn was detected. In the second gonotrophic cycle, a significant decrease in the Vn content in the ovaries of infected mosquitoes was detected throughout the experimental time points (4, 8, 12, 18, 24, 36 and 48h). It would appear that the malaria parasite disrupts all stages of vitellogenesis and that the effect is more pronounced when oocysts are developing than when the parasite first invades the vector.

We have used this vector to test the hypothesis that the operation of a surveillance or immune system against microorganisms and parasites can be costly to the reproductive success of the host. Blood-fed mosquitoes were challenged with an immune elicitor, lipopolysaccharide (LPS), and their resultant antimicrobial activity, accumulation of yolk protein in the ovary and egg production was monitored. Humoral activity against the Gram-positive bacterium Micrococcus luteus was induced by LPS injection in a dose responsive manner. LPS treatment also caused a concomitant significant reduction in the accumulation of protein in ovaries 24 h after injection and in the production of eggs during the same gonotrophic cycle. Unlike immune stimulation, reduction in reproductive fitness was not dose responsive. Oral administration of LPS also significantly reduced ovarian protein content although we could not detect the presence of anti-M. luteus activity in the gut tissue by using an inhibition zone assay. These findings indicate that immune stimulation imposed reproductive fitness costs on mosquitoes.