# Interactions Between Parasites and Insects Vectors

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*This review stresses the importance of studies that will provide a basic understanding of the pathology of parasite-infected vector insects. This knowledge should be a vital component of the very focussed initiatives currently being funded in the areas of vector control. Vector fecundity reduction is discussed as an example of such pathology. Underlying mechanisms are being investigated in a model system,* Hymenolepis diminuta- *infected* Tenebrio molitor *and in* Onchocerca-*infected blackflies and* Plasmodium-*infected* Anopheles stephensi. *In all cases, host vitellogenesis is disrupted by the parasite and, in the tapeworm / beetle model, interaction between the parasite and the endocrine control of the insect's reproductive physiology has been demonstrated.*

Key words: vector-pathology - fecundity reduction - *Hymenolepis diminuta* - *Onchocerca* - *Simulium* - *Plasmodium* - *Anopheles stephensi* - insect vitellogenesis

It is now recognised that conventional methods used for controlling vector insects are failing and novel strategies for vector and disease control are being sought. Of particular importance are two initiatives; the development of vaccines based on transmission-blocking immunity, and the production of genetically engineered vectors that are resistant to infection. Rational approaches to these strategies originate from studies of insect physiology, biochemistry and molecular biology. These programmes should also be based on a detailed understanding of the interactions that occur between parasites and insects, at all of these levels.

Aspects of insect pathology that have been studied include some of the events associated with ookinete invasion of the mosquito midgut, infection associated changes in haemolymph and whole body amino acid concentrations, alteration of glucose metabolism, changes in flight performance and probing and feeding behaviour (Maier et al. 1987). Investigations of the insect humoral and cellular responses to parasites (Ham 1992) and studies of the structure and functioning of the digestive tract (Billingsley 1994) in noninfected and infected mosquitoes are also being made. However, much of this knowledge is rudimentary.

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An aspect of vector pathology that has received little attention is that of host reproduction. Reduced reproductive output is known to occur in insects parasitized by a variety of protozoans and helminths (Hurd 1990, 1993) However, there is a paucity of information concerning the underlying mechanism. Three models are being used by the author to investigate this parasite / host interaction. In each case the process of vitellogenesis is affected by the parasite.

In the majority of insects, the major yolk protein, vitellin, is not synthesised in the ovaries, but in the fat body. It is transported in the haemolymph as vitellogenin. After passage between the cells of the follicular epithelium, it is sequestered by oocytes undergoing vitellogenesis. In most insects, juvenile hormone stimulates ovarian competence for vitellogenesis and induces morphological changes in the follicular epithelium, including enlargement of follicle cells and development of inter-follicular spaces, (patency). At the same time juvenile hormone controls vitellogenin synthesis in the fat body. In many Dipteran species, although juvenile hormone appears to play a role, ecdysteroids are also involved in the control of vitellogenesis (Hagedorn 1989, Dhadialla & Raikhel 1994).

Most of our current knowledge of mechanisms underlying parasite-induced fecundity reduction in insects comes from studies of the reproductive physiology of *Hymenolepis diminuta* (Cestoda; the rat tapeworm) -infected *Tenebrio molitor* (Coleop-

tera). Egg production in this beetle is continuous and asynchronous. Initiation of oviposition was delayed and egg production reduced in infected females (Hurd & Arme 1986a). Host reproductive curtailment was shown to be the result of impaired yolk sequestration in infected females (Hurd & Arme 1986b) and follicles from beetles infected with developing metacestodes contained significantly less vitellin than follicles of the same size from non-infected females (Webb & Hurd, personal observations). This was not, however, the result of nutrient deprivation as vitellogenin was present in abundance in the haemolymph and titres were significantly elevated in insects by day twelve post-infection (Hurd & Arme 1984).

The development of patency in the ovarian follicular epithelium was retarded in infected beetles, and this may account for the observed reduction in vitellogenin uptake (Hurd & Arme 1987). Patency and many other events controlled by juvenile hormone titres, were down-regulated in parasitized beetles; however, hormone synthesis, circulation and degradation were not affected by infection (Hurd & Weaver 1987, Hurd et al. 1990). Recent findings suggest that the binding of juvenile hormone to microsomal fractions of follicles was significantly reduced in females containing developing metacestodes. Results of studies of binding kinetics indicated the presence of a competitive inhibitor of this juvenile hormone binding (Webb & Hurd, personal observations). The source of this putative inhibitor (parasite or host) is currently being investigated.

It would thus appear that, in the tapeworm / beetle association, a parasite may be able to modulate host reproductive output in a subtle way, via an interaction with the natural endocrine control mechanisms. Studies of the mechanisms underlying fecundity reduction in diptern vectors are not so advanced as those outlined above.

In autogenous vector insects, egg production is initiated by blood feeding. Development of oocytes is synchronous, and oviposition marks the end of a gonotrophic cycle. Thus vitellogenesis is intrinsically linked to disease transmission via the blood meal. In addition, fat body production of other molecules associated with egg production is initiated by blood feeding (Raikhel 1992).

Our knowledge of mosquito vitellogenesis is extensive, but we know very little about the effect of parasites on mosquito reproductive physiology. Vitellogenesis is poorly understood in most other dipteran vectors and the effect of parasites on vitellogenesis in Diptera had not been investigated. Thus, studies of the mechanisms underlying parasite-induced fecundity depletion in two dipterans have recently been initiated.

Blackflies can act as vectors for filarial nematodes of the genus *Onchocerca,* some of which are the causative agents of onchocerciasis or "river blindness". In both African and UK host/parasite associations, infected blackflies produced fewer eggs than noninfected counterparts (Ham & Gale 1984). Using a British simuliid, *Simulium ornatum*, we have demonstrated that ovarian uptake of vitellogenin was affected by the presence of microfilariae as early as 24h post-infection. Blackflies were infected by intrathoracic injection and control, sham injected and infected blackflies were fed on the same blood source. Thus, in this model system, we were able to isolate the effect of variation in blood meal from that of the developing parasite. Inoculation of heat killed microfilariae did not affect vitellogenesis and we were unable to demonstrate a significant dose effect; one nematode was sufficient to induce a significant reduction in ovarian protein content (Renshaw & Hurd 1994).

Total haemolymph protein content and vitellogenin titres (as determined by ELISA) were significantly depressed 6h post-infection. However, later in the infection, protein haemolymph titres were elevated by 30%, the major component being vitellogenin. We thus observed a similar scenario to that described above, when *T. molitor* was infected with *H. diminuta.*

*In vitro* culture of fat bodies from flies at various stages post-infection demonstrated that synthesis of vitellogenin was significantly reduced at 8h and 24h post-infection. We believe that, in *Onchocerca* infected blackflies, fecundity depletion may initially result from a deficient supply of yolk protein to the ovaries as a result of reduced fat body synthesis in infected females. Individual follicles may fail to initiate vitellogenesis, may enter a state of arrested development or may be resorbed. The accumulation of vitellogenin seen in the haemolymph 24h post-infection may be caused by a mismatch between fat body synthesis and the requirements of the reduced number of developing oocytes or it may be the result of resorption of vitellin from partially developed follicles.

In the case of mosquitoes, initiation of vitellogenin gene transcription in the fat body begins within 1h of blood feeding (Racioppi et al. 1986) and transcription peaks at 24h post blood-meal in *Aedes aegypti* (Raikhel 1992). Thus vitellogenin gene transcription coincides with the time of malaria parasite invasion. In addition, three or four more blood meals will be taken whilst malaria parasites are developing in the mosquito. Promoter sequences of vitellogenin genes could represent very useful tools to aid the expression of foreign, anti-parasite genes in mosquitoes as transcription is also initiated when oocysts are developing and when sporozoites are produced. It is thus extremely important to have a full understanding of any interactions that occur between parasites and vector vitellogenesis.

Reduction in reproductive output has also been associated with *Plasmodium gallinaceu-* infected *Aedes aegypti* (Freier & Friedman 1976). However, until recently, no detailed studies have been made of anopheline mosquitoes. We have established that egg production was reduced in *Anopheles stephensi* DUB infected with the rodent malaria parasite, *Plasmodium yoelii nigeriensis* N67. This fecundity reduction occured when females where fed on mice exhibiting high (45%) parasitaemias but containing no gametocytes (ie. non infective) and to a much greater extent (over 40% reduction in egg production) when fed on mice containing gametocytes but with parasitaemias of only 10-15%. Measurement of blood meals showed that this reduction in egg production was not due to differences in blood meal size (Hogg & Hurd 1994). Mosquitoes infected with developing oocysts, and subsequently fed on the same mouse as control females, also produced significantly fewer eggs on the second and third gonotrophic cycle. Furthermore, parasite burden was not correlated with fecundity and infections of 1-10 oocysts induced the same significant depletion as those of 50+ (Hogg & Hurd personal observations).

Studies of protein content of ovaries from infected mosquitoes revealed a significant decrease in uptake 20h post blood-feeding and at all stages examined after that. Ovarian protein profiles obtained by SDS PAGE showed that the concentration of the major yolk protein, vitellin, was reduced in ovaries from *Plasmodium* infected mosquitoes (Hogg & Hurd personal observations).

As with our studies of microfilariae-infected blackflies, we have observed that the presence of a malaria parasites downregulates the process of vector vitellogenesis very early in infection. In both cases, this initially occurs when parasite biomass is very small relative to the host and is not due to parasite associated changes in blood meal size. In addition, parasite burden does not significantly alter the reduction in egg production. Evidence thus points to the existence of an intimate interaction between parasites and vectors. This may operate at the molecular level, as we are beginning to reveal in the *Hymenolepis diminuta /Tenebrio molitor* model. However, much work still needs to be done before we will be able to understand the underlying mechanisms and assess their potential significance to future vector control methods.

Once organisms become infected with parasites, their metabolic processes may become profoundly altered. Reduction in reproductive output is just one of many ways in which vector insects respond to these infections. It is thus naive to proceed with the quest for a transgenic mosquito that is refractory to infection without a concomitant investigation of the pathology of malaria-infected mosquitoes. Sadly, these studies do not feature highly in the very focussed approach to vector control strategies that is currently being pursued.

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