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Infection barriers and responses in mosquito–filarial worm interactions

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As a function of size, migration trajectory through the body and developmental site, filarial worm parasites inflict significant damage on the mosquito host. Some mosquitoes are equipped with physical and physiological barriers that confer a refractory state to parasite infection. In a susceptible host, parasites migrate to a developmental site and achieve an intracellular existence; during this process, worms elicit canonical mosquito immune response elements, particularly melanization and antimicrobial peptide (AMP) production. It is clear now that the response to infection also involves mitigating stress and manipulation of host cell machinery to delay necrosis. This review focuses on mechanisms of refractoriness and resistance to *Brugia malayi*, *Brugia pahangi*, and *Dirofilaria immitis*, with emphasis on infection in the mosquito, *Aedes aegypti*.

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Introduction

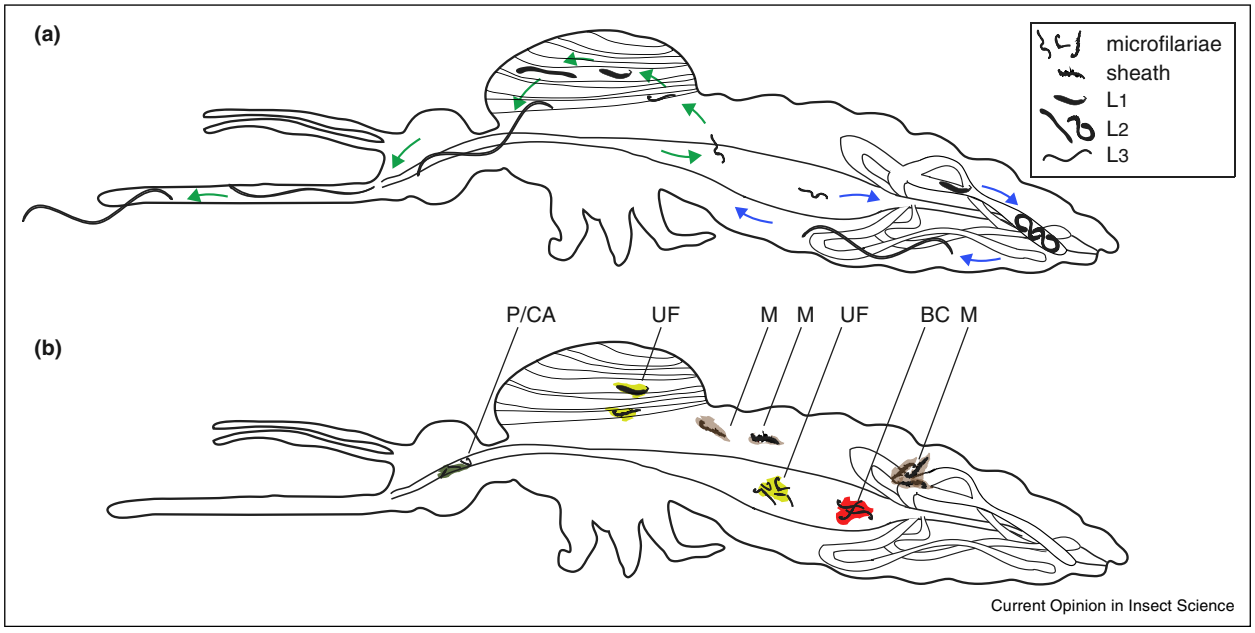
The filarial worms are unique among the pathogens transmitted by mosquitoes in that they are much larger in size than viruses (~80 nm) or *Plasmodium* parasites (~10 μM) upon entry into the body of the mosquito; the mosquito-borne nematodes are approximately 200 μM in length at the point of uptake and over a millimeter in length in the infectious stage. As a function of size, migration trajectory through the body and developmental site, these parasites inflict significant damage on the intermediate mosquito host. Furthermore, during the process of migration and even during intracellular development, these large parasites are overt elicitors of canonical mosquito immune response elements, particularly melanotic encapsulation. More recently, it has become clear that the response to filarial worm infection also involves significant metabolic stress and potentially manipulation of host cell machinery to prevent necrosis before the parasite achieves the infectious stage.

This review focuses on infection responses to three species of parasite that have been the subject of significant research effort; these include *Brugia malayi* and *Brugia pahangi*, an agent of Lymphatic Filariasis (LF) and closely related model species (respectively), and *Dirofilaria immitis*, the causative agent of dog heartworm. When possible, infection responses to *Wuchereria bancrofti* will be discussed because this parasite causes 90% of the burden of LF disease.

Christensen and Severson defined susceptible mosquitoes as those that support complete development of the parasite, resistant mosquitoes as those in which an active immune response interferes with parasite development, and refractory mosquitoes as those where there is a physiologic incompatibility between parasite and mosquito [1]. Only a few species of mosquitoes successfully support the development of filarial worms, and susceptibility varies significantly between strains within a species. Natural vectors of filarial parasites primarily include mosquitoes in the genera *Aedes*, *Anopheles*, *Culex*, and *Mansonia*, but the majority of research done on mosquito–filarial worm interactions utilizes *Aedes aegypti*. This species is easily propagated and maintained in the laboratory and was the subject of early classical genetics studies that defined susceptibility to *Brugia* and *Wuchereria* as a heritable trait that was later delineated into two quantitative trait loci (see reviews [2,3]); in fact, one of the original mosquito strains, selected for susceptibility to *B. malayi*, provided the material that was used 45 years later in the *Ae. aegypti* genome sequencing effort [4]. *Armigeres subalbatus* also has been the subject of intense research on the immune response of mosquitoes to filarial worms because this mosquito is naturally refractory to *B. malayi* by virtue of a strong melanotic encapsulation response [5,6].

In a susceptible host, *W. bancrofti*, *B. malayi*, and *B. pahangi* migrate to and develop in the indirect flight muscles; in contrast, *D. immitis* develops in the Malpighian tubules so never leaves the alimentary tract (Figure 1a). In either developmental destination, parasites achieve and maintain an intracellular state, wherein they metamorphose from the microfilaria (mf) to the infectious L_3 stage — a process that necessitates a physical transformation to transition from mf to sausage-shaped first stage larvae (L_1), and two subsequent

Figure 1



(a) Parasite migration and development in a susceptible mosquito host. *W. bancrofti*, *B. malayi* and *B. pahangi* move through the midgut to the hemocoel and into the indirect flight muscles and transition through life stages as shown by green arrows. *D. immitis* moves into the midgut and migrates to the Malpighian tubules without leaving the alimentary tract, then transitions through life stages as shown by blue arrows. **(b)** Physical, physiologic, and immunobiological barriers to filarial worm development in the mosquito host. The pharyngeal and/or cibarial armature (P/CA) mechanically damage the mf cuticle; melanization (M) occurs in the hemocoel and occasionally in developmental sites for either *Brugia* spp or *D. immitis*. Unknown factors (UF) cause subcuticular damage and death to developing *Brugia* spp. parasites in the gut of *Cx. pipiens*. *B. malayi* reach the musculature in *Ae. aegypti* (RED strain), transition to the L_1 stage, then fail to develop further due to unknown factors (UF).

molting events. In the L_3 stage, parasites exit the developmental site, move to the head and migrate down the proboscis to be transmitted to the next host during a subsequent blood meal. Under optimal temperature conditions, development takes approximately 10–12 days, and parasites increase in size 4–6 times [3]. This is not a benign process; worms inflict debilitating or even lethal damage on the mosquito hosts by disrupting normal cellular and physiologic processes in the flight muscles or Malpighian tubules.

Physical and physiologic determinants of a refractory state

In refractory species, each of the organs and tissues that parasites encounter in a mosquito potentially presents a physical or physiologic barrier to further development and, thereby, a first line of defense against infection (see Figure 1b). Microfilariae enter the fascicle with a blood meal and pass into the midgut, passing through the cibarial and pharyngeal pumps that function to suck blood into the gut; these pumps are lined with spines that can fatally damage worms at the point of uptake so that parasites are digested in the midgut and excreted (see [7]). Parasites that reach the midgut unscathed are subjected to additional potential physiologic barriers in the midgut lumen. Mosquitoes in the *Culex pipiens* complex are primary vectors of *W. bancrofti* in many parts of the world. Despite exhibiting susceptibility to *W. bancrofti*, these mosquitoes are largely refractory to infection with the related parasites, *B. malayi* and *B. pahangi*. Unidentified factors in the midgut inflict subcuticular damage to *Brugia* species mf and significantly alter parasite motility and survival [8]. *Cx. pipiens* is otherwise physiologically compatible with *Brugia* parasites because mf that bypass the midgut environment, via direct injection into the hemocoel, develop to the infectious stage [8].

In most cases, mf that reach the midgut unscathed rapidly traverse the epithelial layer to arrive either in the hemocoel (*Wuchereria* and *Brugia* species) or the Malphigian tubules (*D. immitis*). The peritrophic matrix is not a barrier to development of filarial parasites, because it is not completely formed for at least 5 (*Ae. aegypti*) and up to 48 hours (*An. gambiae*) after a blood meal (see [9,10]). Furthermore, disruption of PM formation in *Ae. aegypti* has no effect on the development of *B. pahangi* [9]. Coagulation of the blood meal can hinder mf migration across the midgut because in a coagulated blood bolus, parasites are trapped and digested so do not reach the