16. Parasite-mediated enhancement of transmission by haematophagous insects

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Abstract

Blood-sucking arthropods are vectors for a variety of parasites and pathogens, including many that infect man and his domestic animals. Infective stages are imbibed during blood feeding and their life cycle is completed when they are transferred back to their vertebrate host during another blood-feeding episode. Transmission of vector-borne parasites is therefore dependent upon contact between hosts and haematophagous arthropods. In order for blood feeding to occur, a vector performs a series of behaviour patterns that are initiated in response to both endogenous and exogenous triggers; the latter including odour signals from the host. If the host is infected these signals may change in ways that makes them more attractive to the vector of the parasite. In addition, infection-induced lethargy in the host may make blood feeding less risky and blood that has fewer erythrocytes is easier to imbibe. When vectors are infected several aspects of blood-feeding behaviour may be changed in ways that increase host contact or facilitate feeding. Mosquitoes infected with rodent malarias have been shown to exhibit increased response to host odours, to have a lower blood volume threshold at which they cease feeding and to show increased feeding persistence in the face of host-defensive behaviour. Increased feeding persistence is also observed in *Leishmania*-infected sand flies, as is increased biting behaviour. The latter is due to blockage of the gut with promastigote secretory gel. Vector infections also alter mechanisms that combat host haemostasis, such as the mosquito anti-platelet aggregation factor, apyrase. This causes additional probing attempts when sporozoite infections are present. The evidence that some vector-transmitted parasites and pathogens increase transmission prospects by altering the blood-feeding process suggests that they are manipulating the host or the vector. Host contact determines the spread of infection thus information concerning parasite-induced changes in haematophagy needs to be incorporated into epidemiological models.

Keywords: vectors, parasites, transmission, haematophagy, blood feeding, behaviour manipulation

Introduction

The particular interest in the olfactory response of haematophagous insects witnessed in this volume is undoubtedly prompted by their capacity to transmit the causative agents of infectious diseases to humans and their domestic animals. The majority of these vector-borne infectious agents have complex lifecycles that require two or more hosts and one of the major problems associated with these parasitic lifestyles is the safe transmission from host to host. Parasites have evolved different strategies to solve the various problems associated with transmission, one of which is a reliance on insect vectors to ferry them between hosts. Insects that are used as vectors invariably visit vertebrates to feed upon their blood or other body fluids, thus a prerequisite for this form of transmission is that the parasite is in easy reach of an insect vector when it is feeding. The transmission stages of these parasites thus inhabit the blood or superficial skin layers (reviewed in Lehane 2005) and their vectors are usually females requiring a protein rich meal for egg production (but see Otranto *et al*. 2008). Not only does vertebrate blood provide a nutritious food source for haematophagous insects, it is also an ideal environment for the development of a

multitude of parasitic organisms ranging in size from arboviruses to helminths (for lists see Lehane 2005 and Otranto *et al.* 2008).

Vector insects were once regarded as flying syringes, doing little more than sucking up parasites in one host and injecting them into another. It is now recognised that complex molecular, biochemical and behavioural interactions occur between parasites and vectors. These interactions affect the fitness of the insect and the outcome of parasite transmission (Hurd 2003). The exception to this is when mechanical transmission occurs, during which pathogens are rapidly moved from insect to insect on contaminated mouth parts. One example is *Stomoxys nigra* Macquart, which transmit *Trypanosoma evansi* Steel in this way (Mihok *et al.* 1995, Sumba *et al.* 1998). The more general pattern is biological transmission, where the parasite may multiply in the insect vector, change into another developmental stage or both change and multiply (Hamilton and Hurd 2002, Lehane 2005). All of the developmental stages may occur within the midgut lumen, as is the case for *Leishmania* spp. infecting sand flies or *Trypanosoma cruzi* Chagas that multiply in the crop and midgut of the triatomine bug, *Triatoma infectans* Klug, and are deposited on the skin when the bug defecates during blood feeding. In many cases, however, the gut wall is traversed and parasites invade tissues such as the thoracic muscle in the case of the filarial worms *Wuchereria bancrofti* (Cobbold) and *Onchocerca volvulus* Bickel or the Malpighian tubules, as does *Dirofilaria immitis* (Leidy). Once these worms have moulted into L3 larvae they make their way to the mouthparts, ready for transmission back to a mammalian host during the vector's next blood meal. Although the malaria parasite, *Plasmodium*, passes through the midgut cells, it remains on the gut wall beneath the basal lamina undergoing sporogony to produce the infective sporozoites which migrate to the salivary gland and are transferred into the vertebrate host in the saliva.

Pathogens transmitted by haematophagous insects interact with their vertebrate hosts at many different levels, often causing severe morbidity or mortality. Many of the diseases that disproportionately affect the poor and marginalised populations of the world are caused by insect-transmitted parasites. These diseases include malaria, caused by protozoan parasites of the genus *Plasmodium*; sleeping sickness, caused by the flagellates *Trypansoma brucei rhodesiense* and *T. b. gambiense*; Chagas' disease, caused by *Trypanosoma cruzi*; leishmaniasis cause by *Leishmania* spp.; lymphatic filariasis caused by *Wuchereria bancrofti* or *Brugia malayi* Brug; river blindness caused by *Onchocerca volvulus* and dengue fever, caused by DEN viruses.

Once established in haematophagous insects, many vector transmitted parasites interact with their hosts in ways that result in a decrease in vector longevity and /or reproductive fitness (see reviews by Ferguson and Read 2002, Hurd 1993, Hurd 2003 and Moore 1993). In keeping with the theme of this volume, this chapter will focus on the point of contact between vector and vertebrate host and how this contact is affected by pathogens and parasites that may be present in host or vector.

For transmission to occur, a minimum of two contacts must be made, one to transmit the infectious organism to the vector and one to return it to a vertebrate host. Thus I will consider transmission from an infected host to an uninfected insect and from an infected insect back to a vertebrate; thereby considering transmission at both stages of the parasite's life cycle. The blood feeding that causes both of these contacts to occur can be broken down into a series of vector behaviour patterns namely; appetitive search, attraction to the host, landing, probing and feeding (Hamilton and Hurd 2002). There is sparse, but growing, evidence that several aspects of this sequence of events can be altered by infectious agents and that, although the mechanism underlying these changes may differ, the resulting consequences are similar. This chapter will present an overview

of parasite-induced changes in blood-feeding behaviour that affect each point of contact between vector and host. As much of this was reviewed in Hamilton and Hurd (2002) and Hurd (2003), and as space is limiting, the focus of this article will be upon detailing more recent studies.

The attractiveness of the odour of infected-hosts

Parasitic infection is known to modify the odour profile of host urine, exhaled breath or skin microbial flora (Braks *et al.* 1999, Hamilton and Hurd 2002, Kavaliers and Colwell 1995, Penn and Potts 1998). These odours can be detected by conspecifics and may act as chemical signals during social interactions (Kavaliers and Colwell 1995). In mice they play a role in avoidance behaviours and mate choice (Barthelemy *et al.* 2005, Kavaliers *et al.* 1998). It is conceivable that over evolutionary time selection pressures will have favoured parasites that exploit or manipulate host odour changes that influence the host-seeking behaviour of the respective vector, and thereby enhance their transmission.

The odour profile of individual hosts differ considerably (Qiu *et al*. 2006, Rebollar-Tellez *et al.* 1999) and haematophagous insects clearly distinguish between them, as reviewed by Clements (1999). Surprisingly, there are very few published studies that compare the response of vectors to parasite-infected hosts and most of these have examined the response of mosquitoes to malaria or arboviral-infected hosts (reviewed by Clements (1999)). Sand flies have been shown to be more attracted to *Leishmania*-infected dogs (Coleman *et al.* 1988) and, in a bioassay of the entrained odours of hamsters infected with *L. infantum* Nicolle, they were shown to be more attractive to the sand fly, *Lutzomyia longipalpis* (Lutz and Neiva), than uninfected hamster odour (O'Shea *et al.* 2002). Likewise, pilot studies with rodent malaria-infected mice showed a similar preferential attraction of uninfected anopheline mosquitoes to the odours of infected hosts, attractiveness increasing with parasitaemia. (reviewed by Hamilton and Hurd (2002)). The latter studies corroborated the earlier findings of Day and colleagues (Day *et al*. 1983) studying mice infected with rodent malarias. Increased attractiveness of a host will only benefit parasite transmission if stages infective to the vector are present. In the case of malaria parasites these are the gametocytes. Rodent malarias have been shown to cause the mouse host to be most attractive when gametocytes are circulating, suggesting that these hosts may receive more bites and thus infect more mosquitoes (Day and Edman 1983). A positive association was found between both asexual parasitaemia and gametocyte density and the proportion of mosquitoes feeding on *P. chabaudi*-infected mice at 14 days post-infection (when gametocytes are present in this species) (Ferguson *et al.* 2003). Mosquitoes four days post-infection with *P. chabaudi* I. Landau & A. Chabaud were also found to be more attracted to infected mice than uninfected ones when offered a second blood meal (Ferguson and Read 2004). However, chickens infected with *P. gallinaceum* Brumpt were found to be less attractive to *Aedes aegypti* (L.) than uninfected ones (Freier and Friedman 1976). Although this latter study did not state whether gametocytes were present, other experiments presented in the paper suggest their chickens would have had a parasitaemia of at least 28%. *Plasmodium gallinaceum* gametocytes are present in chickens with rising parasitaemias of 10+% (Warburg *et al.* 1992), thus in this species gametocyte presence does not seem to make the host more attractive and the authors ascribed the decrease in attractiveness of infected hosts to infection associated metabolic changes that lead to odour changes (Day and Edman 1983).

The majority of studies of rodent malarias are made in the laboratory, using host / parasite / vector models that do not occur in nature and would not have co-evolved. If increased host attractiveness at a time of peak infectivity also occurs in malaria-infected humans then the increase in biting rate and hence increased parasite transmission that this may cause would have important implications

for disease epidemiology. This is predicted for example in the simulations made by Kingsolver (1987) using increasing-preference and switching behaviour models that took account of non-random feeding behaviours. For example, increasing consistent host preference for infected hosts was predicted to make it easier to maintain a stable infection, relative to the random choice model (Kingsolver 1987). A very elegantly designed study undertaken in the field in western Kenya was able to confirm that non-random feeding on malaria-infected humans does occur (Lacroix *et al*. 2005). Twelve groups of three children between 3 and 5 years old were initially exposed to *Anopheles gambiae* Giles *s.s*. mosquitoes. One child in each group was uninfected, one was naturally infected with the asexual stages of *P. falciparum* Welch (that are not transmissible to mosquitoes) and the third child harboured gametocytes. None of these children had symptomatic malaria. The same children were then given anti-malaria treatment and again exposed to mosquitoes when parasites had been cleared from the blood. In the first assay, gametocytaemic children were twice as attractive as other children, but this increased attractiveness disappeared once the infection had been cleared. Thus increased attractiveness was a function of the presence of transmission stages of the parasite alone, and not due to an intrinsic attractiveness of this group of children. In contrast, a field study undertaken in Papua New Guinea failed to detect any increase in the attractiveness of *Anopheles punctulatus* Dönitz to malaria or filarial-infected individuals (Burkot *et al*. 1989).

It is likely that other vector-transmitted parasites also increase host attractiveness as has been shown in a field studiy that detected greater attraction of *Glossina pallidipes* Austen towards oxen infected with *T. congolense* Broden than uninfected or *T. vivax* Grassi and Feletti-infected oxen (Baylis and Mbwabi 1995b). However there are surprisingly few studies that have examined this issue, even though it is of such importance for disease epidemiology. Other practical aspects that may follow from studies of host odour changes are the potential for diagnosis of infectious diseases using odour profiles and the value of using infection-specific odours as baits to lure haematophagous insects away from their targets.

Host temperature and attractiveness

Alteration in long range attraction to infected hosts is likely to be the result of changes in odour profile, however, at close range host temperature may also come into play. Are vectors attracted to hosts with elevated temperatures? Increased body temperature is associated with many parasitic infections making this an attractive proposition, but this hypothesis is not supported by empirical evidence, in fact quite the contrary. Studies of arbovirus-infected chickens, Rift Valley Fever virus-infected lambs and rodents infected with malaria all failed to make an association between increased feeding and elevated host-temperatures. Indeed, mice infected with rodent malaria experience depressed rather than elevated temperatures, yet are more attractive to *Ae. aegypti* and *Culex quinquefasciatus* Say (reviewed by Clements 1999). Although these studies did not demonstrate a role for infection-induced temperature elevation in altering the attractiveness of infected hosts, the relationship between host temperature and parasite transmission may not be obvious. For instance, the increased attractiveness of pregnant women to mosquitoes has been ascribed to elevation of body temperature and an increased release of volatile substances (Lindsay *et al*. 2000). This may, in part, explain the increased vulnerability to malaria experienced by pregnant women (Ansell *et al*. 2002).

Defensive behaviour of infected hosts

Animals react to insect blood feeding with defensive behavioural responses that are likely to interrupt the vector feeding process and lead to the taking of multiple meals (Klowden and Lea 1979). This increases the chance that a vector will feed on several different hosts, including some infected ones. However, the pathology associated with some vector transmitted diseases induces host lethargy thus decreasing defensive behaviour, making it easier for the vector to fully engorge. For example, *Plasmodium* infection causes lethargy in infected mice, which limits their defensive behaviour; the timing of the onset of lethargy varying depending upon the species of rodent malaria. The vulnerability of infected mice to mosquito biting was shown to increase in synchrony with increased lethargy (Day *et al*. 1983). The advantage of feeding on a defenceless host seems obvious, yet few examples of increased blood feeding on lethargic hosts exist, as discussed by Clements (1999) and Moore (1993). This could be a consequence of the lack of investigations that would demonstrate it. Alternatively, it may not be as advantageous for the vector as it first seems. If a parasite decreases the fitness of its vector then an easy to come by, but infective, meal will have its own costs. Selection pressured that mitigate against favouring infected hosts when foraging may be operating. Additionally, in terms of transmission success, the times at which transmission stages of the parasite are present may not coincide with disease symptoms that induce pathology, as seen for instance in *P. falciparum* infection, where gametocytes are largely present in asymptomatic people.

Even when defensive behaviour fails, vertebrates are not the passive victims of the blood feeding attempts of other organisms. They have a battery of internal defence mechanisms to combat this theft of blood. These are reviewed by Lehane (2005) and include haemostasis mechanism such as coagulation and platelet aggregation that control bleeding at the wound site. As is to be expected, an arms race has ensued to produce counter measures to these defences. Thus the saliva of haematophagous arthropods in a complex mix of biologically active proteins that assist the feeding mechanism by resisting host haemostasis, including anticoagulants, immunomodulators, vasodilators and anti-platelet compounds. It is becoming evident that many of these molecules have been usurped by vector transmitted parasites to enhance their transmission prospects.

One such example is seen in ticks. Unlike the brief encounter that blood-sucking insects have with their hosts, ticks engorge on vertebrates for several days, engendering the recruitment of inflammatory cells such as neutrophils to the bite site in an attempt to disrupt tick feeding. These efforts are countered by an array of salivary gland proteins (Nuttall and Labuda 2003). One such is the antioxidant Salp25D, expressed in the midgut and salivary glands of the tick *Ixodes scapularis* Say (Das *et al.* 2001). This tick is a vector for several pathogens including the spirochete *Borrelia burgdorferi* (Johnson), causative agent of Lyme disease. By silencing salivary gland *salp25D* using dsRNA injection, Narasimhan and colleagues (Narasimhan *et al.* 2007) showed that, although engorgement was not affected, acquisition of a spirochete burden in the midgut was significantly reduced by the first 24 h of feeding, compared to control ticks. In contrast, transmission of *Borrelia* from infected ticks to mice was not impaired. This study further demonstrated that Salp25D is likely to protect the incoming spirochete from the damaging hydroxylradicles generated at the bite site. Thus transfer of this pathogen from the vertebrate host to the tick is enhanced by the activity of a salivary gland protein used by the vector to produce an anti-inflammatory response. The spirochete is, in effect, piggy backing on a mechanism involved in normal feeding behaviour.

Blood feeding on an infected host

As we have seen, blood feeding is a dangerous strategy for insects to pursue, as the act of piercing or biting the host engenders defensive actions on the part of the host that are likely to be lethal to the vector. The majority of haematophagous arthropods thus visit their feeding site only briefly and the morphology and mechanics of their highly specialised feeding apparatuses have evolved to efficiently penetrate the host tissue and obtain a meal in as short a time as possible. Blood is a viscous fluid and flows up the food canal as a result of the negative pressure applied by the pharangeal pump and/or the cibarial pump, both located in the head capsule. Models used to describe the flow of blood during vector feeding suggested that the radius of the food canal has the largest influence over feeding time (Daniel and Kingsolver 1983), however, the Daniel and Kingsolver model has been challenged, as discussed by Clements (1999). Terminal diameters of feeding canals are not much greater than the diameter of single red blood cells and it would appear that the size may be limited by the need to pierce the skin efficiently and without detection. This is discussed in detail by Lehane (2005).

Host blood quality

In addition to the mechanics of the insect's feeding apparatus, the nature of the blood will influence feeding success. The proportion of erythrocytes present (haematocrit) will affect the viscosity of the blood and hence its rate of flow, its nutritious value (as much of blood protein is contained in the red blood cells) and, in the case of parasites that infect erythrocyte, the level of parasitaemia. Chronic parasitic infections such as malaria can induce long term reductions in haematocrit which may affect vector feeding success. This was demonstrated in a study of mice infected with the rodent malaria, *P. yoelii nigeriensis* (Killick-Kendrick). Mice exhibited a drop in haematocrit (measured as packed cell volume (PCV)) as days-post-infection and parasitaemia rose. Early in infection, when PCV has only dropped 1-3%, mosquitoes allowed to feed on anaesthetised infected mice were able to imbibe significantly larger amounts of haemoglobin (a proxy measurement for red blood cells) than mosquitoes feeding on control mice. However, as parasitaemia increased and PCV fell below 6% of the control mice the haemoglobin taken by mosquitoes feeding on infected mice was significantly reduced (Taylor and Hurd 2001). Thus, initially, the predicted decrease in viscosity as PCV falls appears to enhance erythrocyte uptake. The infective, gametocyte stages of *P. y. nigeriensis* are present early in infection when haematocrit has fallen only slightly. It is thus likely that their transmission to mosquitoes will be enhanced by a small drop in PCV.

In addition to increasing erythrocyte uptake, the speed at which blood was initially imbibed was also demonstrated to increase when the yellow fever mosquito, *Ae. aegypti,* fed on *P. chabaudi* infected mice or hamsters infected with the Rift Valley Fever virus compared with their uninfected counterparts (Shieh and Rossignol 1992). This was due to a reduction in the time taken to locate blood during probing which, the authors proposed, may be due to an infection-induced reduction in the number of host platelets.

Both the reduction in probing time and the increased erythrocyte uptake seen when mosquitoes feed on slightly anaemic blood may be advantageous. It will enhance the chances that mosquitoes take enough protein for egg production before host-defensive behaviour is initiated. However, mosquitoes feeding on very anaemic blood will need to seek further feeding opportunities, thereby increase risky host contact. If the advantage of obtaining a quick meal outweighs the disadvantage of incurring an infection, then mosquitoes may favour feeding on slightly anaemic

hosts. Nevertheless, it must be noted that associations used in these study do not occur in the field and it would be necessary to repeat these findings in a naturally occurring host/parasite/vector system before the value of enhanced feeding on an anaemic host could be hypothesised to enhance transmission. Whether these trade-offs operate in favour of human parasite transmission in a malaria endemic area, where slight anaemia is likely to be commonplace, has yet to be tested.

The affect of host haematocrit on blood feeding has also been investigated in tsetse flies. The consumption of ox blood by *G. pallidipes* decreases as PCV exceeded 30% (Baylis and Mbwabi 1995a). However, although tsetse fly feeding was greater on cattle infected with *T. congolense* and *T. vivax*, this was not attributed to host anaemia but to vasodilation caused by *T. congolense* attaching to the wall of the host microvasculature (Moloo *et al*. 2000).

The importance of correct parasite location in the host

Many vectors exhibit peak biting activity at specific times of the day. For example, *An. gambiae* are nocturnal feeders with host landing building up from 22:00 h to 04:00 h and continuing until just before day break. In contrast, *An. funestus* Giles show a peak feeding activity around 6:00 h (reviewed by Clements (1999). Some vector- transmitted parasites have a circadian rhythm that matches that of their vector. They are present in the peripheral blood vessels during the period that their vector is most likely to bite. This phenomenon is particularly evident in species of filarial nematodes. It is known as 'microfilarial periodicity' and has been discussed by Lehane (2005). Of particular interest is the difference in periodicity shown by two strains of *W. bancrofti*. In one strain microfilariae congregate in the lungs during the day and are only found in the peripheral blood vessels at night; coinciding with the biting pattern of their vector *Cx. quinquefasciatus*. In contrast, the strain transmitted by the day-biting vector, *Ae. polynesiensis* (Marks), are subperiodic and can also be found circulating during the day (Hawking 1962). Parasite periodicity, known as the 'Hawking phenomenon', has also been reported to occur in several species of malaria (Gautret and Motard 1999), but could not be demonstrated following a field study of biting behaviour of *An. gambiae* feeding on *P. falciparum*-infected children in Kenya (Githeko *et al.* 1993). The significance of parasite periodicity as a means of enhancing transmission has not been fully determined but, especially in situations where parasitaemia is low, it could enhance the chances that transmission stages will be imbibed by a suitable vector and, in the case of *Plasmodium*, in numbers that maximise the chances of fertilisation.

Parasites are also known to congregate in locations that vectors have access to. *Lutzomyia longipalpis* (Lutz & Neiva) preferentially probes on skin lesions rich in *Leishmania mexicana* (Biagi) amastigotes, the infective stage to the vector (Coleman and Edman 1988). Developing gametocytes of *P. falciparum* are sequestered in the microcirculature whilst developing, thus they are only exposed to potential transmission when they are mature. There is also evidence that suggests that circulating gametocytes are aggregated, rather than having a homeogeneous distribution. This aggregation is reflected in infection patterns in mosquitoes and may help to facilitate the chance of macrogametocyte fertilisation in the midgut (Pichon *et al.* 2000).

The blood-feeding behaviour of infected vectors

Arthropod haematophagy consists of a series of physiological and behavioural steps beginning with an appetitive search, and proceeding through activation and orientation, attraction, landing, probing or biting and finally imbibing blood (Clements 1999, Hamilton and Hurd 2002, Lehane 2005). The initiation and completion of all of these stages is influenced by exogenous

and endogenous factors including, in some cases, the presence of parasites. Although parasite transmission will only occur during the latter two behavioural phases, all of the preliminary events are crucial. We can surmise that changes to any of them that result in more host contact will increase the prospects of parasite transmission, once the parasite has developed into the life cycle stage that is transmitted back to the vertebrate host (Hurd 2003, Lefevre *et al.* 2006). Various vector-transmitted parasite, including bacteria, protozoans and nematodes do indeed alter one or more stages of blood-feeding behaviour and, in a few cases, this has been shown to increase transmission chances. A few such examples have been well documented in the reviews quoted in this chapter and here the focus will be upon vector/parasite associations that have recently been explored in greater depth.

Appetitive search and attraction

Once parasites have reached the infective stage of their development, any host contact on the part of their vector will provide an opportunity for transmission. We can therefore argue that changes in vector behaviour that enhance appetitive searches and make the vector more responsive to hosts odours will favour parasite transmission back to the vertebrate, thereby completing its life cycle. Unfortunately, there are few studies that specifically measure these response in vectors infected with transmissible stages of parasites. Comparisons need to be made between vectors with immature and mature parasite stages. Increased attraction at an inappropriate time would not benefit the parasites and may just be a response, for instance, to food robbery caused by the parasite increasing a hunger response rather than manipulated behaviour (as seen in *T. cruzi*-infected triatomines (Schaub 2006)).

Those studies that have specifically investigated the effect of infection on host attractiveness have focused on mosquitoes. For example, an increase in the olfactory response of *P. gallinaceum* sporozoites-infected *Ae. aegypti* to host odours has been reported (Rossignol *et al.* 1986). Additionally, the proportion of *An. stephensi* infected with *P. chabaudi* that took a second blood meal from an anaesthetised mouse rose from 0.39 to 0.56 if they were infected with oocysts and this change in behaviour was not influenced by the burden of infection (Ferguson and Read 2004). The significance of the latter finding is, however, unclear as increased host contact at this stage of infection would not benefit parasite transmission and the experimental design did not make it possible to clarify whether the behaviour change was actually due to a change in response to host odours.

Feeding persistence and the role of host-defensive reactions

As has already been discussed, in a natural situation haematophagy is often interrupted by host-defensive responses that can result in the death of the insect. Feeding persistence is the repeated attempt to feed, despite these interruptions.

In the face of host-defensive responses, mosquitoes infected with sporozoites of the rodent malaria *P. y. nigeriensis* are significantly more persistent than uninfected mosquitoes in their feeding attempts when interrupted before any blood is imbibed. This is a complete reversal of the reduction in feeding persistence that occurs when the parasites is developing on the midgut wall, before it reaches the infective stage (Anderson *et al*. 1999). Stage specific alteration of biting behaviour was also seen by Koella and colleagues, investigating the size of blood meal that would inhibit further blood feeding following interruption. They showed that *Ae. aegypti* infected with oocysts of *P. gallinaceum* had a lower threshold value for blood meal size and were thus less likely

to re-feed than uninfected mosquitoes. In contrast, the presence of sporozoites increased the threshold volume required to inhibit host-seeking behaviour success (Koella *et al*. 2002).

In addition to these laboratory investigations, several studies have examined the feeding behaviour of malaria-infected mosquitoes in field situations. Not only are peak feeding times of anopheline mosquitoes changed (reviewed in Hamilton and Hurd (2002) and Hurd (2003)), infected mosquitoes have been shown to engorge more fully (Koella and Packer 1996, Koella *et al*. 1998, Maxwell *et al*. 1998) suggesting that more tenacious feeding behaviour also occurs in naturally infected mosquitoes.

A similar increase in blood-feeding persistence has also been observed to occur in sand flies infected with the metacyclic promastigote forms (infective to mammalian hosts) of *L. infantum* or *L. mexicana*. Sand flies were allowed to feed on an anaesthetised mouse but feeding was artificially interrupted by brushing the antennae of the flies. Rogers and Bates found a positive correlation between feeding persistence and the number of metacyclic promastigotes per fly (Rogers and Bates 2007). In both cases only the infective-sand fly stage caused this behavioural change.

Changes in biting and probing behaviour

In addition to an increase in feeding persistence, vector biting or probing behaviour has been shown to be affected by several vector/parasite associations including malaria/mosquitoes, *Leishmania*/sand flies, *Trypanosoma*/tsetse flies and *Yersinia pestis/*fleas. Of these, recent studies of the changes in biting behaviour induced by *Leishmania* infections are beginning to elucidate mechanisms underlying behavioural changes that have been recognised for over a century and have been reviewed by Hurd (2003), Ready (2008) and Rogers and Bates (2007).

Sand flies become infected with amastigote forms of *Leishmania* following an infected blood meal and the parasites complete their entire developmental process within the sand fly gut. The final phase takes place in the stomodeal valve where they transform into motile metacyclic promastigote forms that are infective to mammals. Transmission occurs when these promastigotes are regurgitated within a gel-like material that fills the cardia and stomodeal valve, causing a blockage. This promastigote secretory gel (PSG) is formed by the parasites and has been shown to enhance cutaneous infections when deposited onto the skin with sand fly saliva (Rogers *et al*. 2004, Titus 1998). A major component of PGS is a filamentous glycoprotein, proteophosphoglycan (fPPG) (Ilg *et al.* 1996), which forms the 3D matrix of the plug that blocks the gut (Stierhof *et al*. 1999) and keeps the valve open. The production of PSG has been shown to occur is several *Leishmania*-sand fly combinations (Stierhof *et al*. 1999). It has been suggested that the plug facilitates regurgitation and, by restricting blood flow into the fly, prolongs feeding time and causes flies to bite more, a concept known as 'the blocked fly hypothesis'.

Sand flies harbouring infective stages of *Leishmania* take longer to feed on an anaesthetised mouse than uninfected flies, but do not probe more often (Rogers and Bates 2007). These authors concluded that the interruption of feeding as a result of host-defensive behaviour (which is not occurring when the host is anaesthetised) and the subsequent persistence in attempts to feed is crucial to engendering multiple feeding attempts, and may be more important than the 'blocked fly hypothesis'. They suggest that the presence of fPPG may inhibit the functioning of the mechanoreceptors in the foregut that detect blood flow, thereby altering the hunger state or increasing the threshold blood volume at which feeding is deceased.

If this behavioural change is to be advantageous to the parasite, it must increase the basic reproductive number (R_0) of the infection by increasing the chance that an infectious vector will feed on more than one host. Rogers and Bates (2007) showed that infected sand flies were more likely to feed on multiple hosts in a laboratory setting.

Promastigote secretory gel is one of the few examples of a product produced by parasites that, via its action in the vector, appears to manipulate blood-feeding behaviour in a way that increases parasite fitness by enhancing transmission. Rogers and Bates (2007) argue that this is an example of true manipulation as it fulfils the criteria set out by (Poulin 1995) and discussed further by Thomas and colleagues (Thomas *et al.* 2005). Briefly, it is an example of a complex change in behaviour that achieves the purpose of increasing transmission chances, it fits its purpose as increased feeding behaviour only occurs once the infective stages are present and it occurs in several sand fly/*Leishmania* combinations. PGS could thus be regarded as a manipulator molecule.

The saliva of haematophagous insects contains a cornucopia of molecules that, in one way or another, assist the blood-feeding process including anaesthetics, anti-coagulation factors, anti-platelet factors and vasodilators than combat the haemostatic defences of the host. Our current knowledge of the nature and wide range of these molecules is reviewed in detail by Lehane (2005). Many species contain an enzyme, apyrase, which inhibits the action of the platelet-aggregation factor ADP by converting it to AMP and orthophosphate. The presence of apyrase at the site of a blood vessel wound made by a probing insect will thus inhibit the sealing of a lanced blood vessel. This would allow a haematoma to form and the insect to locate blood more quickly and feed more efficiently, and for longer. This has been aptly demonstrated in mosquitoes where feeding time has been related to the quantity of apyrase in the glands of different anopheline mosquitoes (Ribeiro *et al.* 1985). More recently, Boisson and colleagues (Boisson *et al*. 2006) confirmed the role of apyrase in efficient mosquito feeding using gene silencing to reduced the expression of the apyrase gene *AgApy*. Saliva from mosquitoes injected with *ds-AgAyp* exhibited reduced enzyme activity and was less efficient at inhibiting platelet activity. *ds-AgAyp*-injected females took twice as long probing before obtaining a blood meal. The authors suggested that their results indicate that there may be redundant apyrase activity and also that products of the *AgApyLike*1 and *Ag9* genes may also be involved in inhibiting platelet aggregation.

Malaria sporozoites are transmitted to the vertebrate host during the probing phase of feeding (Rodriguez and Hernandez-Hernandez 2004). Salivary gland infections are associated with an increase in the time vectors spend probing. Median blood-location time for *Ae. aegypti* infected with the avian malaria, *P. gallinaceum*, thus increases three-fold compared to uninfected mosquitoes. This is probably as a result of a four fold decrease in the activity of salivary apyrase, even though the volume of saliva produced is constant (Rossignol *et al.* 1984). A similar increase in probing behaviour has been detected in malaria-infected anopheline mosquitoes, as reviewed recently (Hamilton and Hurd 2002; Hurd 2003). One study provided evidence that sporozoite infections cause multiple feeding episodes that lead to enhanced transmission. Using microsatellite markers to identify the source of blood meals in the midguts of mosquitoes feeding in a field situation, Koella and colleagues (Koella *et al.* 1998) were able to demonstrate that 22% of *P. falciparum*-infected *An. gambiae* had fed on more than one person during the night, compared with only 10% of uninfected females. Multiple feeding on different hosts had previously been detected in *Ae. aegypti* infected with *P. gallinaceum* (Kelly and Edman 1992). However, an alternative interpretation of these observations is that mosquitoes that become infected may have a propensity to multiple feeding.

Malaria sporozoites inhabit the distal lobes of mosquito salivary glands, the same location in which apyrase is produced (Sterling *et al.* 1973). Surprisingly we still do not know the mechanism causing the parasite-associated reduction in apyrase. Is it caused by damage incurred as the sporozoites migrate through the glands or do the sporozoites secrete an inhibitor that suppresses transcription or inhibits translation? The former would indicate that transmission is enhanced by an accidental by-product of pathology the latter that this may be another example of vector manipulation.

A final example comes not from mosquitoes but from fleas infected with plague bacilli. In nature, *Y. pestis*, the causative agent of plague, circulates amongst rodent species generally causing little overt disease symptoms. Violent outbreaks resulting in high mortality periodically occur amongst susceptible rodent populations and can increase the risk of human infection. Plague transmission occurs via flea bites. Within the flea gut, ingested bacteria are found in a mass of coagulated blood that blocks the proventriculus. The blockage results in the regurgitation of plague bacilli into the mammalian host during feeding. A coagulase- and fibrinolysin associated (*pla*) gene encoded on the bacilli plasmid, pKYP1, has been associated with coagulase activity that facilitates the formation of the proventricular mass (Cavanaugh 1971). Mcdonough and co-workers (Mcdonough *et al.* 1993) have shown that fleas infected with pla^+ bacteria had higher mortality than that caused by pla^- bacteria and that mortality was not linked to bacterial load. They suggest that the *pla* gene product may disrupt the flea's digestive process leading to blockage, starvation, and early death. Increased feeding attempts early in this process will enhance plague transmission. Although further studies are needed to substantiate these suggestions, the *pla* gene can be regarded as a promising manipulator molecule candidate with which *Y. pestis* may modify its vector's feeding behaviour to suit its own ends.

Are parasites manipulating vectors?

Much has been written about host manipulation by parasites. There are many incredible examples that are favourites amongst teachers and students alike (Moore 2002). In comparison with the fate of crickets infected with hair worms, or snails with trematode sporocysts in their tentacles, changes in vector feeding persistence or probing behaviour are far from dramatic. Despite this, the co-evolutionary implications of host and vector behavioural changes are similar. Many of the changes we see can be expected, and in a few cases have been shown, to lead to an increase in vector transmission that may be accompanied by a decrease in vector fitness due to the greater danger associated with increased host contact. Do these unequal consequences for the symbiotic partners imply that the parasite is manipulating its host? Alternatively, is the behavioural change a by-product of pathology caused by the infection or are we seeing attempts by the vector to compensate for the presence of the parasite? Here is not the place to detail the pros and cons of these theories, which have been much discussed (most recently by Lefevre *et al.* (2006) and Lefevre and Thomas (2008), and until we fully understand the mechanisms that govern them, it is difficult to do much more than speculate.

Parasite fitness can be expressed as R_0 which, for microparasites, can be defined as the number of secondary infections that arise from a primary infection. Following the seminal work of Ross and Macdonald (Macdonald 1957), models developed to take into account factors that affect R_0 were developed by Anderson and May (1979, 1992) and have since been modified and refined by many authors interested in the epidemiology of infectious disease.

Host contact is probably the most important factor limiting the spread of vector transmitted diseases and this concept, known as vectorial capacity, is incorporated into many of these models. Saul (2003) developed two deterministic models, one for stable transmission and one for epidemic situations. They were based on the cyclic feeding model he and his colleagues had previously developed (Saul *et al.* 1990) rather than the a continuous feeding model refined from Ross' model by Macdonald (1957). Saul's model assumed that mosquitoes will not feed for a few days after becoming engorged; whilst the respective gonotrophic cycle is completed (cyclical feeding). His model showed that search-related mortality of mosquitoes will have an important impact on malaria transmission. The sensitivity of malaria transmission to mosquito mortality has been also recognised by others including Smith and McKenzie (2004). One parameter of Saul's model (Saul 2003) is the 'constant attraction rate constant'. Saul recognised that this will, in fact, be dependent upon factors such as host emission of attractants and host availability to vectors (e.g. are they sleeping under bednets?). Saul's model was used to explore the effectiveness of using 'bait' animals to lure vectors away from biting humans and predicted that insecticide treatment of livestock will have an appreciable effect on the human inoculation rate (Saul 2003).

The classical models did not recognise that the parasite itself may have a role to play in affecting host contact. We have seen that there is evidence that parasitic infections of both mammalian hosts and arthropod vectors alter feeding behaviour in ways that increase host contact; that infected hosts are more attractive than uninfected ones and that mosquito biting is heterogeneous. The assumptions that mosquitoes, for instance, genuinely feed at random and that infected mosquitoes make the same host contacts as uninfected ones is probably erroneous. Smith *et al*. (2007) discuss classical and neoclassical models that estimate R_0 for malaria and reconsider R_0 in finite population with heterogeneous biting. They recognise the importance of R_0 as an important metric with which to plan malaria control programmes and that, amongst other measures, it may be more productive to target interventions towards those individuals who are bitten most, than those who are bitten least. Clearly it is germane to incorporate many more specific features of the parasite's life cycle and its effect on vector feeding behaviour into mathematic models that are to be of use for planning control strategies.

Conclusions

Parasite-induced changes in vector haematophagy have been recognised for over a century. However the overriding impression that I have gained whilst preparing this chapter is that, relative to the number of parasites transmitted by vectors, the documented cases of parasites changing vector feeding behaviour is very small and, in particular, the epidemiological impacts of these changes have been neglected. Investigations have mostly focused on malaria and *Leishmania* infections and we are certainly in no position yet to assume that this is a common strategy that parasites employ to enhance transmission. Hopefully, the burgeoning of studies of olfaction in vector-host interactions will provide further opportunities to incorporate more parasites into these investigations.

Although observations of biting behaviour in a laboratory setting are useful, they are severely limited as to the information they can give us about the consequences for parasite transmission in the field. Studies performed in field settings with natural hosts that can display normal defensive behaviours must be performed to evaluate laboratory findings, although ethical issues associated with investigating human infections must be considered. The few investigations of this kind that have been performed to date are exemplars to be followed.

Parasitic infection can alter several host behaviour patterns simultaneously and these changes can themselves alter as parasites mature. Experiments need to be planned to isolate individual aspects of feeding behaviour and determine which are affected, and when. Only then can we can decide whether, for instance, changes in feeding persistence have more influence on parasite transmission than changes in biting or probing.

In addition, to gaining an insight into the degree to which vector feeding behaviour can affect disease transmission, a knowledge of the mechanisms underlying these changes will help us to evaluate the evolutionary implications of behavioural change (Lefevre *et al.* 2006). As the genome sequences of more and more vectors and parasites are published and we move further into the post genomic era, investigation of protein profiles and gene transcription of nervous and other tissues that may govern changes in odour perception, flight behaviour, landing, probing and imbibing blood is becoming feasible. Some promising headway has recently been made by Lefevre and colleagues who have examined changes in the head proteome of tsetse flies infected with trypanosomes and of malaria-infected mosquitoes (Lefevre *et al.* 2007a,b).

The recent expansion of studies on olfactory behaviour into a wide range of disease vectors and the advanced technologies for more precise understanding of these behaviours, as described in the preceding chapters, is likely to stimulate incorporation of pathogens and parasites in these studies. If this happens we will be able to use knowledge such as this to determine who is manipulating whom, to understand the implication for parasite transmission and to apply our understanding to the planning of novel disease control strategies such as the deployment of transgenic technology to alter feeding behaviour and divert vectors away from anthropophagy.

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