Battleground midgut: The cost to the mosquito for hosting the malaria parasite

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In eco-evolutionary studies of parasite–host interactions, virulence is defined as a reduction in host fitness as a result of infection relative to an uninfected host. Pathogen virulence may either promote parasite transmission, when correlated with higher parasite replication rate, or decrease the transmission rate if the pathogen quickly kills the host. This evolutionary mechanism, referred to as ‘trade-off’ theory, proposes that pathogen virulence evolves towards a level that most benefits the transmission. It has been generally predicted that pathogens evolve towards low virulence in their insect vectors, mainly due to the high dependence of parasite transmission on their vector survival. Therefore, the degree of virulence which malaria parasites impose on mosquito vectors may depend on several external and internal factors. Here, we review briefly (i) the role of mosquito in parasite development, with a particular focus on mosquito midgut as the battleground between Plasmodium and the mosquito host. We aim to point out (ii) the histology of the mosquito midgut epithelium and its role in host defence against parasite’s countermeasures in the three main battle sites, namely (a) the lumen (microbiota and biochemical environment), (b) the peritrophic membrane (physical barrier) and (c) the tubular epithelium including the basal membrane (physical and biochemical barrier). Lastly, (iii) we describe the impact which malaria parasite and its virulence factors have on mosquito fitness.

Introduction

In order to establish the severity of parasite virulence, it is important to understand the mechanics of the interaction between the host and parasite, especially the development of the parasite and its transmission success. Anopheles mosquitoes are the main vectors of human Plasmodium, the parasite responsible for causing malaria disease. The parasite sexual cycle starts in the midgut (MG): a digestive organ of the Anopheles mosquitoes [Aly et al., 2009]. The healthy female mosquito’s digestive organ is mainly focused on the blood digestion. However, both males and females also need basic nutrients for their survival and metabolism, predominantly plant sugars which are an important source of energy during the adult life, and the only food resource for males during their short lifespan [Clements, 2000]. The mosquito’s digestive organ is divided into three sections, named foregut (FG), MG and hindgut (HG) (Figure 1A). The main functions of FG are motility, storage and the location of the crop. The HG is a place for excretion which is connected to the rectum and anal canal; this part of HG has roles in ionic, osmotic and excretory regulation through Malpighian tubules. The

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Figure 1 | (A) Schematic image of topography of the *Anopheles* alimentary canal. Here, the ventral view of an *Anopheles* body is shown. The main topographical divisions of the gut contain: anterior midgut, midgut, Malpighian tube, anterior intestine and rectum. (B) Schematic drawing of topographically characterised cells in the mosquito midgut. (a) Brush border formed by the microvilli, (b) columnar epithelium, (c) regenerative cells, (d) epithelial digestive cells covered by microvilli.
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MG, which is the main focus of this article, is central to digestion and absorption. The MG is divided into two sub-regions, anterior and posterior MG (AMG and PMG) with distinct physiological functions [Richards, 1975]. Aside from its main physiological role, Anopheles MG plays especially significant role in the life cycle of malaria parasite [Aly et al., 2009]. To assist the rate of transmission of malaria, the parasite completes its complex development stages in the MG tissue (sporogony). Below we describe the interaction between parasite and mosquito MG, especially at the vector's MG histology, how the parasite's cycle within mosquito affects the mosquito's MG cells and how this interaction may be exploited in terms of parasite development, virulence and transmission success.

Role of mosquito in parasite development

Historically, Plasmodium that infects human is the most extensively studied parasite and majority of research has been focused on human–parasite interaction [Cook, 1997]. One of the crucial factors for malaria parasite survival is the mosquito host. Plasmodium survival depends on mosquito host, and breaching two of the vector's epithelia inside a vector [Vaughan, 2007]. Normally, the Plasmodium parasite (sporozoite stage) enters into human host when an infected female mosquito bites the human host. Generally, the human malaria parasite requires two hosts to complete its life cycle: an insect vector (mosquito) and a vertebrate host (human). The life cycle consists of three stages: infection of a human with sporozoites [Aly et al., 2009], asexual reproduction (symptomatic) [Clements, 2000] and sexual reproduction (asymptomatic) [Richards, 1975]. During gametogenesis, the parasite can differentiate into male (microgamete) and female (macrogamete) that are asymptomatic form of the parasite. When biting an infected person, the female anopheline mosquito ingests these mature gametocytes along with the blood meal. Subsequently, in the mosquito gut lumen, the nucleus of microgamete divides and produces eight nuclei; each nucleus fertilises a macrogamete leading to a zygote. Then the zygote develops into a motile ookinet that penetrates the epithelial cells of MG and develops into an oocyst. The oocyst undergoes several rounds of multiplication and replication resulting in the production of thousands to millions of sporozoites (sporogony). That is the end of the sexual reproduction stage or sporogony cycle [Barton and Ranford-Cartwright, 2005]. After rupture of the mature oocyst, the sporozoites are released into the hemocoel (body cavity of mosquito) and invade the salivary glands epithelia, thus completing its life cycle.

Interestingly, the malaria parasite exploits the feeding behaviour of mosquito for its transmission from one host to another [Smith and Jacobs-Lorena, 2010]. The parasite manipulates its human host’s metabolism, enhancing the vector-attractive smell emission of the infected host [Emami et al., 2017, De Moraes et al., 2014, De Moraes et al., 2018], which in combination with the life history and feeding behaviour of the mosquito can increase its transmission success [Emami et al., 2019, Schwartz and Koella, 2001]. To complete the life cycle, Plasmodium needs to survive in the insect host, cross several physical barriers (the peritrophic matrix (PM), the MG epithelial cell later, basement membrane (BM) and salivary gland epithelium), and try to survive the mosquitoes immune response [Smith and Jacobs-Lorena, 2010].

Plasmodium bottleneck

Parasite transit in the mosquito body is a complicated and risky journey with a substantial mortality rate, as well as unravelling mechanisms, responses and signals which still require to explore [Barillas-Mury and Kumar, 2005]. The main aim of the parasite during this risky sojourn is to maximise its chances of reaching the human host as fast as possible. The first physical barrier for Plasmodium is the chitinous PM which is secreted by the MG epithelial cells after each blood meal. The second is MG epithelium, that is a single cell layer surrounded by a thin and sparsely reticulated muscular tissue and BM [Vlachou et al., 2006, Angrisano et al., 2012]. The BM is an extracellular protein sheet surrounding tissues of insects and animals which composed primarily of laminin, collagen IV and proteoglycans. There is a high homology in composition and function between the BM of vertebrates and invertebrates [Yurchenco and O’Rear, 1993].

During Plasmodium sporogony cycle, ookinete reaches the BM around 24—36 h post infectious feeding, and differentiates into a vegetative oocyst. The parasite oocyst acquires nutrition from mosquito
haemolymph to grow (∼5–50 μm in diameter) during the following 10—12 days [Rosenberg and Rungswiengse, 1991, Canning and Sinden, 1973, Nacer et al., 2008]. Sporozoites released into the haemolymph of mosquitoes. Parasite at this stage migrates, either by gliding motility while adhered to the BM or commonly the open circulatory flow of the haemolymph. They pass through the tubular heart of the mosquito to reach the salivary glands. Only 19% of sporozoites reach the salivary glands, all invasion occurs within 8 H at a rate about 200 sporozoite per hour, and sporozoite that fail to invade in this time window die and are degraded. The sporozoite degradation is a rapid immune process most efficient in region of high haemolymph flow (sporozoite can be found in various mosquito tissues: haemolymph ∼85%; heart chamber ∼74%; heart circulation ∼35%; cuticle ∼72%; muscle ∼68%; legs ∼65%; wings ∼59%; halters ∼24%; antennae ∼45%; proboscis ∼32%; ovaries ∼74%) [Hillyer et al., 2007]. The sporozoites cross the BM and epithelial cells of the salivary glands, and stay in the lumen of the glands until the mosquito injects it with the saliva when taking the next blood meal [Mueller et al., 2010, Rodriguez and Hernandez-Hernandez Fde, 2004].

Once parasites have successfully infected a mosquito, their development (sporogony) into the final human-infective stage (sporozoites) depends on the efficiency with which transitions between key parasite life cycle stages are made. During the first half of sporogony, ingested gametocytes form gametes, and then ookinetes which invade the mosquito MG and develop into oocysts (Figure 1A). This transition from gametocyte to oocysts results in high losses (Plasmodium bottleneck) [Alavi et al., 2003]; only 0.2% of ingested P. berghei gametocytes become viable ookinetes, and only 2—20% of them develop into mature oocysts [Alavi et al., 2003]. Similar losses have been documented through the macrogametocyte to oocyst stage in P. falciparum laboratory cultured gametocytes that were membrane fed to An. Gambiae [Beier et al., 1991]. Overall a net decrease of 2,754-fold was observed (an average of 40-fold decrease in transition from macrogametocyte to ookinete stage, and a 69-fold decrease in transition from ookinete to oocyst stage) in An. Gambiae mosquitoes infected with P. falciparum [Vaughan et al., 1992].

Sporogony is the longest replicative process in the life cycle of all Plasmodium species lasting 12—16 days depending on the parasite species. Given the average female mosquito survival of 30 days [McDonald, 1977, Clements and Paterson, 1981], parasite sporogony roughly corresponds to the half-life of the mosquito. A new cycle will start when the above-mentioned infected mosquito takes a blood meal from the next vertebrate host. Why are so few parasites successful in completing this vital cycle? These estimation and numeric evaluations call into the general assumption that the MG is the major battleground to parasite infection of the mosquito vector.

Histology of mosquito’s MG epithelium
MG of the hematophagous insects is the primary organ responsible for blood digestion, and nutrient absorption, ion exchange, metabolite synthesis and release of neurohormones [Clements, 2000]. Morphological aspects of the MG organisation have been described for mosquitoes at different life stages or under different feeding conditions [Bertram and Bird, 1961, Bauer et al., 1977, Houk, 1977, Houk and Hardy, 1982, Okuda et al., 2005] (Figure 1A). In mosquitoes, the MG plays a significant role in fluid and nutrient acquisition, excretion and digestion as well as in the synthesis and secretion of mucus, digestive enzymes and PM [Hecker, 1977]. Mosquito’s MG epithelium is mainly composed of three functionally types of cells: the columnar cells or digestive cells, the regenerative cells and the endocrine cells [Nuttall and Shipley, 1903, Brown et al., 1985]. The AMG leads digestion and absorption of sugar content from food, whereas the PMG promotes the synthesis of the PM and digestive enzymes, storage of the ingested bloodmeal and transport, absorption of blood-digesting metabolites. The MG comprises a single columnar epithelium resting on a basal lamina, which is externally surrounded by a complex muscle network of well-organised circular and longitudinal fibres [Brown et al., 1986, Billingsley, 1990, Onken and Moffett, 2015] (Figure 1A).

In a healthy MG
The columnar cells are the most abundant cells in the MG. Their width is around 9 μm wide. They are
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characterised by microvilli, basal labyrinth, an abundant rough endoplasmic reticulum and lysosome containing acid phosphatases. These cells have different kinds of junction: in the apical half, there are continuous junctions like zonula continua, permitting transit of the epithelium by nutrients or solutes; in the basal half, there are desmosomes (maculae adherents) with gap junctions to connect the cells and enabling the structural balance of the tissue [Reinhardt and Hecker, 1973]. The architecture of columnar cells makes them specialised in absorption and digestion. Their microvilli and basal labyrinth increase the surface of the cell to absorb nutrient efficiently. They also assist in release of metabolites in the haemolymph [Reinhardt and Hecker, 1973]. The digestion ability is mainly characterised by lysosome and rough endoplasmic reticulum. The metabolic activity of these organelles increases after a blood-meal due to the synthesis of digestive enzymes [Rossignol et al., 1982] like esterase [Geering and Freyvogel, 1974] and α-glucosidase [Billingsley and Hecker, 1991] which also are present in the lumen of the MG. Proteolytic enzymes such as trypsin, aminopeptidase and so on are mostly secreted to digest the bloodmeal. The role of enzymes synthesis and secretion explains the presence of dense-cored secretory vesicles located in the apical cytoplasm [Hecker, 1977]. There is also variation in the columnar cells based on their location in the MG [Clements, 2000]. The cells located at the AMG have microvilli and more developed basal labyrinth and a smooth endoplasmic reticulum abundant compared to posterior part. It seems that the anatomical architecture is optimised for the absorption of nutrients, especially basic carbohydrates hydrolysed by the saliva enzymes [Marinotti et al., 1996, Moreira-Ferro et al., 1998]. Moreover, the columnar cells of this region are probably responsible for quickly transferring the nutrients to the haemolymph. This region contains smooth endoplasmic reticulum to metabolise the nutrients. However, the cells of the PMG of the females have more abundant rough endoplasmic reticulum which forms ‘whorls’, whose abundance varies between species [Bertram and Bird, 1961]. However, this whorl anatomical architecture seems to be specific of female mosquitoes, and it possibly has a role in blood digestion. Unlike the cells of the AMG, the PMG cells contain lipid or carbohydrate deposits [Hecker, 1977, Schneider et al., 1986]. In male mosquitoes, these cells are smaller, and they contain less rough endoplasmic reticulum and a less expansive basal labyrinth [Hecker, 1977]. These differences strongly suggest that the PMG is the location which mainly responsible for the blood digestion and absorption, which is not necessary in most male mosquitoes.

Recently, it has been reported that the monolayer of columnar epithelial cells in the MG epithelium divide into two cell populations: microvillar epithelial cells and basal cells. The microvillar epithelial cells can be further subdivided into light and dark cells, based on their affinities to toluidine blue and their electron density [Baia-da-Silva et al., 2019] (Figure 1B). Like a typical epithelial tissue, there are some regenerative cells, on the basal side of the MG epithelium. These cells are small with a large nucleus, and have a few organelles whose function remains to be characterised. They develop microvilli and basal labyrinth [Hecker, 1977], like columnar cells. The purpose of those cells is the renewal of epithelium in a classic cycle, to replace the apoptotic cells for instance, in both AMG and PMG. However, their involvement in tissue repair seems to be influenced by various mechanisms which still need further investigations. The insect MG’s epithelium can also contain some goblet cells too; but in mosquitoes, those cells seem to have a role during larval stage but not in adult stage [Wassim et al., 2014]. The purpose of those goblet cells in larvae is to reinforce the development of the mosquito into an adult stage (Figure 1B).

There are around 500 endocrine cells in the MG of mosquito. Their size ranges from 2 to 6 μm, so they are smaller than the columnar cells. The endocrine cells can be distinguished because of their lack of basal labyrinth and the presence of dense-cored secretory vesicles in the basal half cytoplasm, unlike the secretory vesicles in the columnar cells located in the apical half cytoplasm [Clements, 2000]. These cells have microvilli when they are in contact with the lumen and are named ‘open cells’. In contrast, ‘closed cells’ are not in contact with the lumen and do not carry microvilli [Brown et al., 1985]. Density of endocrine cells in the MG varies with their position. A higher number of them are located in the posterior section of the MG compared with the lower number which are located in the anterior part of the MG. It has been also reported that the endocrine cells in the insects family are mainly responsible for the
secretion of hormones with different roles, such as development, reproduction and immune-defence [Stefano et al., 1989, Stefano et al., 1989], as well as diuresis [Patel et al., 1995]. The control of food digestion is connected to the concentration of pancreatic polypeptide-like (FMRFamide) metabolites, secreted by the endocrine cells in the MG [Brown et al., 1986] (Figure 1B).

In *Plasmodium*-infected MG
As described above, the mosquito MG tissue has direct contact with wide variety of external factors such as the microbiota, the complex mix of dietary components from different host blood meal, microorganisms that are ingested with the meal, by products of digestion, pathogens and toxins. Because the FG and HG are sclerotised, it is the MG which is the main battleground against the parasite infection (Figure 2).
et al., 1994, Shahabuddin et al., 1995]. However, some studies reported that the endoperitrophic space of MG lumen is main barrier for making mosquito refractory to particular parasite strain such as An. albimanus – P. falciparum clone 3D7A [Baton and Randford-Cartwright, 2012, Gonzalez-Ceron et al., 2007]. Apparently, it was observed that ookinete failed to migrate from blood meal and invade the MG epithelium, and were destroyed within the endoperitrophic space before the PM was attained. Almost >99% of ookinetes forming within the blood meal of An. albimanus failed to reach the PM. They claimed the lumen is the site of mosquito refractoriness in particular malaria-parasite interaction.

A number of factors might reduce ookinete densities within the lumen, including MG proteases, any microbial flora and mosquito immune responses [Moreno-Garcia et al., 2014]. Digestive proteases secreted from MG epithelium following bloodmeal ingestion can cause ookinete destruction [Gass, 1977, Gass, 1979, Gass and Yeates, 1979]. It is possible that temporal differences in the peak of digestive protease activity in various mosquitoes are responsible for the increased loss of ookinetes in particular vector-parasite combination.

In recent decades, substantial number of investigations have been done on mosquito MG-associated bacteria (MAB) in laboratory and wild Anopheles mosquitoes [Briones et al., 2008, Dong et al., 2006, Favia et al., 2007]. Some studies have reported that MAB influence the ability of parasite to develop to the oocyst stage in the mosquito gut tissue, and if the natural MAB affected by antibiotic treatments, the mosquitoes were more susceptible to Plasmodium infection and parasite transmission [Gendrin et al., 2015]. Reconstitution of the bacterial flora results in infections at the same level as untreated control mosquitoes [Dong et al., 2009]. Bacteria produce compounds with potential antimalarial properties (reviewed in [Moraes et al. 2008]. For example, an Enterobacter bacterium isolated from wild mosquitoes in Zambia produces reactive oxygen intermediates that kill developing parasites in the MG lumen, inhibiting the Plasmodium infection [Cirimotich et al., 2011]. Gram-negative bacteria show various levels of parasite inhibition at the early stages, suggesting diverse mechanisms of bacteria-mediated parasite inhibition exist (reviewed in [Cirimotich et al., 2011].

Bacteria may have an indirect role in parasite interference through the activation of immune response in the MG. The immune deficiency (IMD) innate immune pathway is stimulated by presence of G-bacteria and appears to be the primary immune pathway activated in the mosquito MG, and it has been shown to control P. falciparum infection intensities through the expression of anti-Plasmodium effector molecules in multiple anopheline species [Garver et al., 2009, Meister et al., 2009]. These effector molecules control bacterial populations, establishing a direct link between antibacterial and anti-Plasmodium immunity in MG [Dong et al., 2009, Dong et al., 2006, Frolet et al., 2006, Meister et al., 2005]. Although there is a potential for using bacteria as biological malaria control strategy, the absolute mechanism of parasite inhibition remains unknown. It is also unclear whether immune responses are active in the MG lumen and can induce anti-parasitic effectors within the endoperitrophic space of the MG lumen.

Finally, the abundance of key energetic resources within mosquitoes, such as lipids, sugars and proteins, could influence parasite development [Atella et al., 2006, Atella et al., 2009], virulence and transmission by determining the availability of nutritional substrates for uptake by the developing parasites [Rivero and Ferguson, 2003, Emami et al., 2017]. Environmental (e.g. temperature and pH) and biochemical (e.g. concentration of amino acids like valine, histidine and methionine in mosquito haemolymph) factors may also influence the outcome of sporogony in mosquito vectors, but much remains unknown about if and how these factors combine to influence malaria parasite-mosquito vectors interaction (reviewed in [Sreenivasamurthy et al., 2013, Tripet et al., 2008] (Figure 2).

The peritrophic membrane (physical barrier)
Blood feeding triggers structural modification, formation and polymerisation of a thick noncellular, chitinous PM which is secreted by MG epithelium cells. The PM surrounds the blood meal bolus and forms a physical barrier for pathogens such as bacteria and parasites (reviewed in [Lehane, 1997]). The PM depth is critical for Plasmodium invasion. For example, in Aedes aegypti MG where the thickness of PM is increased, the infectivity of the P. gallinaceum is reduced [Billingsley and Rudin, 1992]. The PM barrier
can be disrupted by ookinete secretion of chitinases [Huber et al., 1991]. However, the An. gambiae–P. berghei model showed that the PM development is significantly insufficient under laboratory infection experiments, and majority of ookinetes penetrate the MG prior to full PM formation. The suggested reason for this retarded PM is the lower temperatures (∼20.5°C) necessary for mice malaria parasite sporogony cycle [Vanderberg and Yoeli, 1966]. The mechanism by which the PM is instructed, and the structural complexity or consolidation at the time of the parasite maturation differ between anopheline and aedine species (Figure 2).

**The tubular epithelium**

This is the battleground for the parasite. The process of ookinete invasion is thought to produce severe damage to the MG as the ookinete traverses multiple cells during its journey to the basal lamina. Invaded cells undergo dramatic cytoskeletal changes and increase the production of reactive oxygen and nitrogen species that trigger apoptosis and cell death [Han et al., 2000]. During this time, ookinetes face a highly toxic extracellular environment. To ensure its survival, the ookinete must rapidly escape before it is damaged or before the damaged cell is extruded from the MG epithelium. Evasion requires either invading neighbouring naive cells or exiting the epithelium to reach at its final extracellular destination between the basal side of the epithelium and the basal lamina. Although it remains unclear how ookinete invasion triggers programmed cell death, this is likely a general response to remove damaged epithelial cells and may not be specific to parasite invasion [Baton and Ranford-Cartwright, 2007, Baton and Ranford-Cartwright, 2005, Okuda et al., 2007] (Figure 2).

It has been proposed that the apoptosis of cells and their extrusion from epithelium forces ookinetes to migrate laterally to a neighbouring cell to avoid being discarded. This hypothesis is the so-called ‘time bomb’ theory [Han et al., 2000]. Another proposed theory, referred to as the ‘cellular treadmill’ suggests that ookinetes only move from the apical surface to the basal membrane of the epithelial cell, not laterally. But since neighbouring cells fill the gap left behind by an apoptotic cell, an ookinete should invade these cells as well [Baton and Ranford-Cartwright, 2005].

*Plasmodium* invasion is a density dependent process. Under laboratory conditions, *An. stephensi–P. berghei* oocyst production efficiency begins to reduce when there are over 355 ookinetes per mosquito [Sinden et al., 2007]. Therefore, within a certain threshold, the lower the parasite burdens in the mosquito, the greater the transmissibility of the disease. This phenomenon is ascribed to the fact that the parasite might damage the mosquito, and greater energy is redirected to the immune response mounted against it [Tripet et al., 2008]. Accordingly, the lesser damage caused, the greater the fitness for all interacting parties [Churcher et al., 2010].

Parasite numbers expand during the second half of sporogony when sporozoites multiply in oocysts, before being released into the haemolymph and infect mosquito salivary glands. Ross originally reported that approximately 1000 sporozoites could develop in a *P. falciparum* oocyst [Ross and Thomson, 1910]. Further studies with Asian vector *An. dirus* revealed mean sporozoite counts of 3688 from *P. vivax* oocysts and 3385 from *P. falciparum* oocysts, respectively [Rosenberg and Rungsiwongse, 1991]. However, a much higher count of 9555 sporozoites per oocyst was found in a wild-caught *An. funestus* in Northern Tanzania [Pringle, 1965], reflecting the high variability in sporozoite development between settings, parasite and vector species. In the laboratory, a linear relationship between oocyst density and salivary gland sporozoite densities has been documented, with the majority of oocyst-infected mosquitoes developing sporozoite infections [Vaughan et al., 1992]. The situation in wild-caught mosquitoes may differ as sporozoites failed to enter the salivary glands in 43.5% and 10% of oocyst-infected *An. gambiae* sampled from two villages, respectively [Lombardi et al., 1987]. However, above a certain minimum threshold, sporozoite numbers are unlikely to limit mosquito transmission potential. This is because the morphological experiments have shown that the majority of *P. falciparum*-infected *An. gambiae* and *An. stephensi* mosquitoes transmit only 1–25 sporozoites during feeding [Beier et al., 1991], so the number of sporozoites produced by even one small oocyst (<1000 sporozoites) greatly exceeds the number required to infect every host a mosquito bitten in their short lifetime.

Mosquito MG cell epithelial cells invaded by ookinetes mount defence responses, such as expres-
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Activation of high levels of nitric oxide synthase (NOS) [Han et al., 2000, Luckhart et al., 1998]. These epithelial responses and the rate of nitration is a major determinant of Plasmodium survival and harmful for the parasite [Han and Barillas-Mury, 2002, Kumar et al., 2004]. In laboratory conditions, mosquito immunity is an important determinant of Plasmodium infection after ookinete invasion of the MG epithelium (reviewed in [Christophides et al., 2004]). The main anti-Plasmodium immunity signalling pathways are Toll (in German means amazing), IMD, Janus kinase (JNK) and signal transducers and activators of transcription (JAK-STAT) pathways.

Ookinetes during passage through the mosquito MG are exposed to components of the mosquito complement-like system present in the mosquito hemolymph (the serum-like fluid in the circulatory system of insects). The thioester-containing protein 1 (TEP1) is similar to vertebrate-containing protein C3 and circulates as a stable complex associated with other proteins of the leucine-rich repeat family, LRIM1, APL1 and CLIP-domain serine protease homolog SPCLIP1 [Povelones et al., 2009, Povelones et al., 2013, Fraiture et al., 2009]. The Toll and Imd pathways target the ookinete stage and promote activation of mosquito TEP1 complement like system [Crompton et al., 2014]. TEP1 binds to the surface of some parasites and is a critical component of a lytic complex [Blandin et al., 2004]. However, because all ookinetes come in contact with TEP1 circulating in the hemolymph, it has been difficult to understand why TEP1 binds to the surface of some ookinetes and triggers lysis, while other parasites are spared.

These pathways are activated when they recognise pathogen-associated molecular patterns, that activate NF-κB which leads activation of Relish (Rel1) and (Rel2) in Toll and Imd pathways, respectively. These pathways are crucial for the entry of antimicrobial peptides to nucleus such as defensins, cecropins, attacin and gambicin, which have anti-Plasmodium activity [Meister et al., 2005]. Rel1 and Rel2 are negatively controlled by Cactus and Casper, respectively. TEP1, Anopheles Plasmodium-responsive leucine-rich repeat protein (APl1), leucine-rich repeat protein (LRRD7) and fibrinogen immunolec tin 9 (FBN9) are also controlled by the Imd pathway. MG Rel2 immuno-enhanced An. stephensi demonstrated resistance to parasite infection and has been nominated for parasite control strategies [Clayton et al., 2014, Dong et al., 2011]. Activation of both Toll and Imd pathways induce the expression of AgDscam isoforms that have species-specific anti-Plasmodium response. The genes which mediate these pathways and tissues are still unknown [Crompton et al., 2014].

An. gambiae heme peroxidase (HPX2) and NADPH oxidase 5 (NOX5) identified as key enzymes induced in ookinete-invaded MG cells that, together with NOS, mediate protein nitration [Oliveira Gde et al., 2012]. The HPX2/NOX5 system potentiates NO toxicity and is required for mosquitoes to mount an effective anti-Plasmodium response. Epithelia nitration and TEP1-mediated lysis work sequentially and epithelial nitration serves as an opsonisation-like system that promotes activation of the mosquito complement cascade. In An. gambiae, the JNK pathway regulates the expression of two enzymes, HPX2, NOX5, in combination with TEP1 and FBN9 in haemocytes that promote TEP1-mediated lysis [Garver et al., 2013]. The JNK pathway also involves in anti-Plasmodium defence; however, the activation of this pathway is not clearly demonstrated [Clayton et al., 2014]. The STAT pathway targets parasites after MG invasion, when they changed into the oocysts (reviewed in [Crompton et al., 2014]. The genes involve in STAT1/AgSTAT-B and STAT2/AgSTAT-A mediate anti-P. falciparum immunity. AgSTAT-A induce transcriptional activation of NOS, which enhance reactive NO and transcription of suppressors of cytokine signalling, which affect the development of parasite. The exact role of AgSTAT-B has not been characterised yet.

Anoplophorin and apolipoprotein D precursors and fibrinogen-related proteins also play role in anti-Plasmodium defence [Clayton et al., 2014]. Antibodies have also negatively effect on ookinete motility, penetration of the MG wall, formation of parasite oocysts [Dennison et al., 2015]. It has been reported that an immune-modulatory peroxidase (IMPer) is secreted by MG cells of A. gambiae and, together with dual oxidase (Duox), catalyses the formation of a di-tyrosine network on the luminal surface of MG epithelial cells [Jaramillo-Gutierrez et al., 2010]. This network prevents activation of gut immune responses by immune elicitors from commensal microbes. The di-tyrosine network also enhances Plasmodium parasite.
transmission because it allows parasites to develop in the MG lumen without activating NOS expression. Disruption of this barrier by silencing either IMPer or Duox results in strong and effective pathogen-specific immune responses [Kumar et al., 2010].

Many of the organ system of mosquito are homologous to those in human. Previously this comparison has been reviewed in fly, *Drosophila melanogaster* [Buchon et al., 2014]. As a final hallmark, the mosquito MG can briefly be compared with the stomach and small intestine of human; however, a major difference can be noticed. Indeed, the order of the digestive organs in the human body cannot be transposed to their equivalents in mosquito’s gut (Table 1). The AMG’s epithelium has characteristics that resemble the small intestine’s epithelium, whereas the PMG’s epithelium can be compared with the stomach. In human, the stomach is located before the small intestine; there is a difference in the order, which can be explained by the alimentation of mosquitoes, particularly sugar alimentation. For example, the nectar sugars do not need complex coordination of enzymes and time to be hydrolysed, and they can be easily absorbed. Part of this digestion is initiated by the saliva, and followed by the corp. The AMG is mainly optimised for absorption. Therefore, the MG can absorb the carbohydrate rapidly. The PMG is the place of the main enzyme secretion, especially in females, enables digestion of complex blood carbohydrates and other metabolites, particularly the protease secretion. PMG digestion needs more time due to the complex multiple structure metabolites and compounds. The architecture of the MG is adapted to the mosquito alimentation. To sum up, it can be worthwhile to notice that muscles are located around the digestive system in both human and mosquito’s body. In both species, there are two types of muscles: the intern muscle is circular, whereas the extern muscle is longitudinal. Those muscles enable the motility in the digestive system by contractions. For two very different species, there are many common features, from the organisation of the epithelium and the cells to the muscles around the digestive system. All in all, the digestive system has an old function which the basic have been conserved during evolution.
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How virulent is malaria parasite to mosquitoes?

Empirical studies have provided contradictory results about whether malaria parasites are virulent to their vectors or not. Virulence in vector–pathogen interaction studies is interpreted as the negative effects caused by the parasite on the mosquito host’s survival and fecundity [Cohuet et al., 2010]. Virulence in terms of reduced mosquito survival due to infection has been the most thoroughly investigated, as this fitness trait has great impact on malaria parasite transmission. Some laboratory studies of rodent and avian malaria parasites have indicated that malaria infection reduces mosquito vector survival [Maier et al., 1987, Ferguson and Read, 2002, Dawes et al., 2009, Gad et al., 1979, Klein et al., 1986]. However, other studies, including those with human parasites and their natural vector species, have found no impact of parasite infection on vector survival [Chege and Beier, 1990, Hogg and Hurd, 1997]. We can hypothesise that the differing outcomes reported in these studies may be due to variations in study design, with virulence being substantially more likely to be observed in studies of unnatural vector–parasite associations that have not co-evolved together [Ferguson and Read, 2002]. Laboratory investigations on transgenic mosquitoes presented the same variability in their outcome. Some studies showed transgenic strains exhibited significantly reduced survival [Irvin et al., 2004]. In contrast, other studies demonstrate that transgenic mosquitoes were as fit as wild-type controls [Moreira et al., 2004, Marrelli et al., 2007, Massonnet-Bruneel et al., 2013]. It has been postulated that the lower fitness observed for homozygous transgenic mosquitoes in some studies could either be due to (i) insertional mutagenesis and/or negative effects of transgene products or (ii) inbreeding and the harmful effects of homozygous recessive genes [Marrelli et al., 2006].

Parasite virulence as reflected by a reduction in mosquito fecundity appears to be much more widespread and consistent than effects on mosquito longevity. Most studies that have looked for an impact of malaria parasites on mosquito fecundity have reported a negative effect, mainly reduction in egg numbers [Carwardine and Hurd, 1997, Jahan and Hurd, 1997, Ferguson et al., 2003]. In addition to these effects on mosquito survival and reproduction, malaria parasites may influence other indirect determinants of mosquito fitness (e.g. larval hatch rate, flight ability).

Recently, it has been demonstrated experimentally and with mathematical models that there are links between metabolism and life history traits of vector and parasite, and their potential in shaping evolution of malaria virulence and transmission success [Costa et al., 2018]. Regarding transmission, the distance flown by An. stephensi mosquitoes infected with sporozoites was significantly lower than for uninfected mosquitoes [Schiefer et al., 1977]. Thus, there is good reason to predict that malaria parasites exert some degree of virulence on their mosquito vectors, even though the nature and magnitude with which this manifested is variable. Using a broader definition of parasite virulence, several studies in laboratory rodent models have shown that malaria parasite virulence varies between parasite genotypes [Ferguson et al., 2003], and may also be moderated by host genotype [Lambrechts et al., 2005], environmental conditions such as nutrient availability (glucose) [Lambrechts et al., 2006] and mosquito age [Dawes et al., 2009]. Parasites can also force fitness cost in vector in relation to its pathology and tissue invasion. Approximately 24—48 h after ingestion of an infectious blood meal, malaria parasite ookinetes penetrate the mosquito MG epithelial cells and this invasion can lead to the death and extrusion of invaded epithelial cells from MG wall [Han et al., 2000, Baton and Ranford-Cartwright, 2004]. The removal of damaged cells from the mosquito MG epithelium presumably has a cost although this has not yet been formally demonstrated [Baton and Ranford-Cartwright, 2007]. Temporal metabolic regulation also plays a critical role in host–parasite interactions in correlation with host immune responses. A number of environmental and biological factors have been hypothesised to influence the successful development of malaria parasites through the sporogonic cycle. The first is competition for nutritional resources within mosquitoes. Malaria parasites must extract several nutritional resources from mosquitoes to complete their sporogony, including components of the mosquito haemolymph which are incorporated into growing oocysts [Warburg and Miller, 1992]. There is some evidence that parasites obtain glucose from the haemolymph of mosquito vectors [Atella et al., 2006, Atella et al., 2009, Mack et al., 1979]. Furthermore, mosquitoes infected
with rodent malaria oocysts consumed more glucose than non-infected mosquitoes [Rivero and Ferguson, 2003, Mack et al., 1979] and the oocyst density of An. stephensi infected with P. yoelii yoelii was positively correlated with concentration of glucose in the sugar water provided to them [Lambrechts et al., 2006]. Some evidence of resource competition during sporogony comes from observation of density-dependent oocyst development. These studies have shown that oocyst size (an indicator of the number of sporozoites developing within them) is relatively uniform when the number of oocysts on the mosquito MG is low [Lombardi et al., 1987], but is variable at high oocyst densities [Sinden and Strong, 1978]. This has been interpreted as evidence of competition for mosquito resources at high parasite densities, with the rapid growth of some oocysts suppressing the growth of others.

Parasite virulence to mosquitoes could also be driven by the depletion of key mosquito energetic resources because of parasite growth. There is evidence that oocysts developing in the mosquito MG take a significant amount of digested lipids from mosquitoes [Atella et al., 2006, Atella et al., 2009]. Theoretical model combined with experimentally evaluated data determined that Plasmodium exploits the main mosquito lipid transporter (Lipophorin) for lipid delivery to actively proliferating oocysts after completion of the vector reproductive cycle. The time shift in oocyst maturation may benefit the parasite development, transmission and virulence by allowing a timely allocation of resources needed for host reproduction and for the parasite proliferation. Lipid restriction during sporogony blunted sporozoite’s mitochondrial membrane potential, and consequently reduced metabolic activity of transmissible sporozoites [Costa et al., 2018]. To sum up, these results indicated that lipid metabolism in vector is a crucial regulator within vector Plasmodium proliferation, transmission and virulence.

Furthermore, it has been reported that concentrations of amino acids such as valine, histidine, methionine, arginine, proline and asparagine significantly decreased in oocyst infected mosquitoes with P. relictum [Ball and Chao, 1976]. However, whether malaria parasites could reduce mosquito energetic reserves to the point where their fitness is compromised is unclear. If nutrient competition is the primary cause of malaria parasite virulence to mosquitoes, it would be predicted that mosquito fitness reduction increases with parasite burden. However, the mortality of very heavily infected mosquitoes has been frequently found to be similar to that of uninfected mosquitoes in many laboratory studies as reviewed above. Another cause of variation in parasite virulence to mosquitoes could be vertebrate blood factors such as red blood cell structure, haemoglobin level, red cell density which influence the resource value of infectious blood meals to mosquitoes. Although such a phenomenon has been hypothesised, to date there have been no definitive experimental investigations of the role of host haematological factors in promoting or mitigating parasite virulence to mosquito vectors.

Concluding remarks

This review has focused on the how activity of the mosquito MG affects the survival of the mosquito and the parasite. The MG is the centre of the delicate balance between optimal transmission, parasite fitness and the evolution of parasite virulence within mosquito host. Some of the key factors determining host–pathogen interaction are well known, like the trade-off between virulence across different host species, variation in host species quality and patterns of transmission, but as yet no complete picture of the phenomena is available. Determining how complex host–parasite interactions impacts the evolution of host–parasite relationships will require the development of cross-disciplinary studies linking theoretical, experimental and field investigations. The effects of natural and artificial perturbation of vector–parasite metabolism on malaria sojourn are necessary to predict potential switches between parasitic and competitive vector–parasite relationships. Central to all this knowledge will be evaluation of the multiple trait and responses within the mosquito body to determine how trade-off between them may constrain evolution and dictate parasite transmission success. A comprehensive understanding of the healthy mosquito vector MG will provide fundamental information about vector of important parasites, such as malaria. This knowledge could assist in vector control strategies aiming at parasite manipulation of the vector and/or disrupting the parasite life cycle by transmission blocking techniques.
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Author contribution
All authors listed have made a direct and intellectual contribution to the work and approved the manuscript for publication.

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