Coalition Politics: Linking Malaria Transmission to Mosquito Reproduction (8 words max)

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Abstract

Female Anopheline mosquito reproduction is intimately linked to the *Plasmodium* sporogonic cycle, whereby malaria parasites ostensibly compete for the same resources required for mosquito egg development. However, in a recent study, Werling and colleagues (*Cell* 2019; 177:315-325) uncovered a parasitic strategy supporting co-existence exploiting mosquito nutrients without affecting mosquito fitness and reproductivity.

Main Text

Malaria is a devastating parasitic disease with 219 million cases and 435,000 death worldwide in 2017 [1]. Malaria is caused by apicomplexan parasites of the *Plasmodium* genus, which during their complex life cycle transition between a mammalian host and a mosquito vector. During the blood feed of a female, *Anopheles* mosquito on a *Plasmodium*-infected individual, gametocytes are taken up with the blood meal into the midgut. Gametocytes generate gametes that once fertilised quickly develop into motile ookinetes which cross the mosquito midgut epithelium settling on its basal side (within 16-30h after blood feeding). Ookinetes then transform into sessile oocysts, producing thousands of individual sporozoites within 10-14 days. Following oocyst rupture sporozoites migrate to the salivary glands where they reside ready to initiate a new infection when the mosquito feeds again.

For the female mosquito the sole purpose of the blood feed is to acquire essential nutrients required for production of its eggs (**Figure 1A**). Following a blood meal, mosquito egg development (vitellogenesis) within the ovaries is initiated through a cascade of hormones released from the mosquito brain [2]. This in turn triggers the synthesis of the steroid hormone ecdysone (E) in the ovaries. Hydroxylation of E into its active form, 20-hydroxyecdysone (20E), in the mosquito fat body then promotes the synthesis of nutrient transport proteins and nutrient uptake within the ovaries, enabling the maturation of 100-150 eggs per mosquito. *Plasmodium* oocysts also exploit these nutrients, linking mosquito reproduction with parasite proliferation (**Figure 1A**). Maybe not

unexpectedly, previous studies have pointed mostly towards a negative impact of *Plasmodium* infection on mosquito fitness and survival [3,4]. However, the exact relationship between resource exploitation by the parasite and potential costs for mosquito fitness and fecundity remained elusive.

A recent study by Werling and colleagues [5] sheds unexpected light on the link between reproductive fitness of the mosquito vector and *Plasmodium* infection. Rather surprisingly, the authors uncovered a positive relationship between the number of eggs produced by a female *Anopheles gambiae* mosquito and the number of *P. falciparum* oocysts in the same individual. Furthermore, transgenic mosquitoes engineered to produce a greatly reduced number of eggs also had significantly less *P. falciparum* oocysts, confirming the positive relationship noted above. The authors demonstrated that this positive correlation was dependent on 20E signalling and therefore, retained when the 20E signalling pathway was disrupted through silencing of the 20E nuclear receptor EcR (dsEcR), resulting in reduced egg production and fewer oocysts (**Figure 1B**). The effect on egg number was also independent of infection with uninfected and infected dsECR mosquitoes producing similar egg numbers. However, the fewer parasites in dsEcR mosquitoes grew faster and, at day 10 after an infected blood meal (pIBM), had produced larger oocysts than those in the control group. This unexpected, faster parasite growth also resulted in increased transmission potential, with dsEcR mosquitoes displaying a higher likelihood of having salivary glands colonized by sporozoites at day 10 pIBM and reducing what is called the Extrinisic Incubation Period (EIP) of the sporozoite. These EIP-enhanced sporozoites were competent, performing comparably to their control counterparts in an *in vitro* infection assay.

In search for the potential source of the observed faster parasite growth, the authors investigated the lipid level in dsEcR midguts. Several lipid species, as well as the level of the lipid transporter lipophorin (Lp), were significantly increased upon 20E disruption. Tellingly, when Lp and the 20E signaling pathway were silenced simultaneously, faster parasite growth was abolished. Additionally, in wild-type mosquitoes the 20E level negatively correlated with oocyst size, which was again lost upon Lp silencing. When lipids were experimentally induced to accumulate in midguts through TAG lipase silencing, the oocysts grew more quickly. Overall, these results point towards an unexpected effect of 20E-signalling on parasite developmental speed, likely resulting from lipid availability mediated by Lp. This is also in line with previous findings, where Lp has been found inside developing *Plasmodium* oocysts [6]. It would also explain why parasites grow faster if mosquito egg production is impaired linking the activity of the 20E pathway to parasite growth benefitting from the excess of nutrients not destined for egg development. Currently, the authors have not

established why oocyst numbers are reduced when egg development is impaired but speculate that immune (TEP1 independent) or somatic processes could be involved in a trade-off over resource allocation balancing immunity with egg production.

Overall, the results by Werling and co-workers elegantly expand the findings of another recently published study [7], which postulated the model of non-competitive parasitic resource exploitation minimises fitness costs for the mosquito while maximising parasite proliferation. This link between 20E signalling, egg production and oocyst growth would allow the parasite to adapt its development to the host's status, optimizing its own fitness without harming the vector. *Plasmodium* parasites can grow faster if the female mosquito produces fewer eggs or generates more oocysts in a mosquito with more eggs (**Figure 1B**). Considering the potential use of gene drive systems aiming to reduce the reproductive capacity of mosquitoes or especially heighten their immune status as a vector control strategy, this could have unexpected consequences favouring faster sporozoite production and transmission [8]. The nutritional status of the mosquito in the field may also become important: Werling's mosquitoes were all optimally fed. The outcome of a life-time of sub-optimal blood feeding might well be to promote parasite growth, frequency of blood feeding and thereby, transmission. Another open question which will require future work is to establish the mechanism behind 20E dependent lipid accumulation and parasite growth in the midgut. How can the parasite adjust its growth pattern in a 20E dependent manner? It will be fascinating to see whether *Plasmodium* uses similar mechanisms as recently discovered for the mammalian host to sense the nutritional status of the vector and to adjust its proliferation rate accordingly [9]. Furthermore, it would be important to expand the findings of this study to other mosquito-*Plasmodium* combinations as well as to investigate their impact in other vector-borne pathogens.

References

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Figures

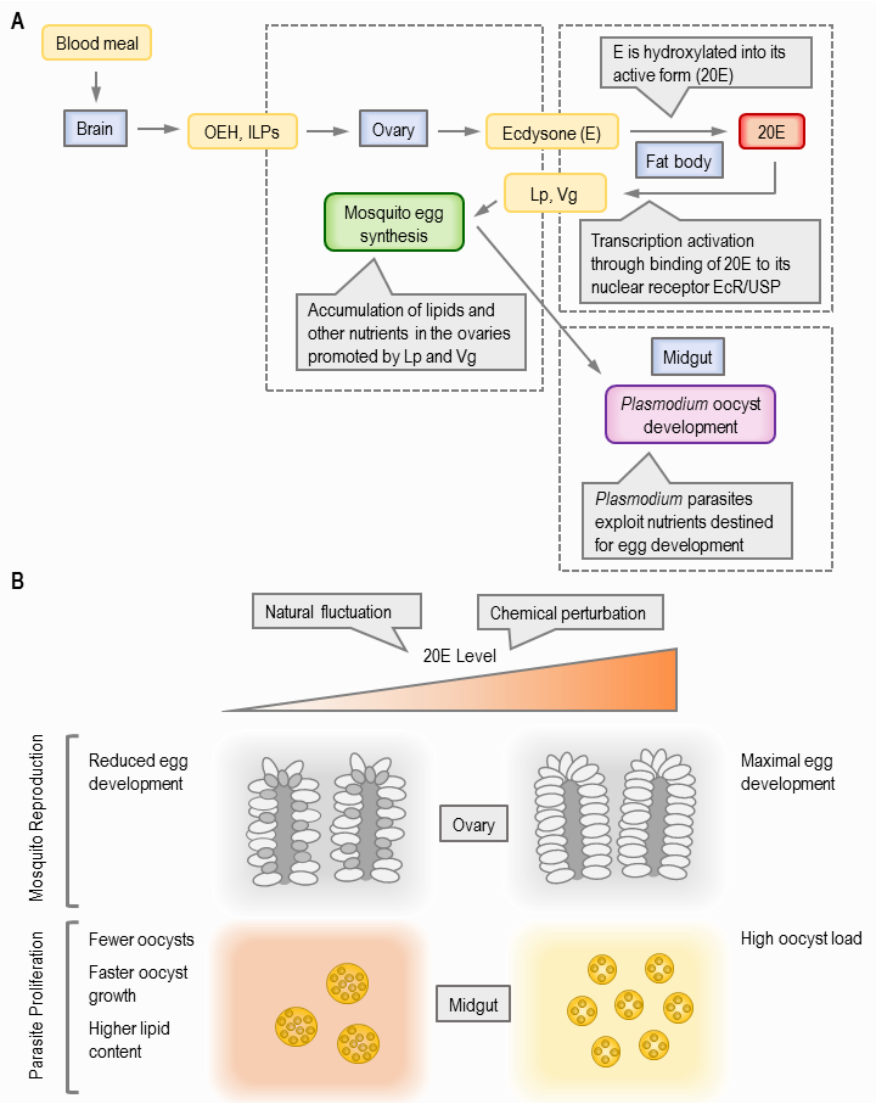

Figure 1. 20E Signalling Coordinates Mosquito Reproduction and *Plasmodium* Proliferation.

(A) Following the blood meal of a female mosquito a vast signalling cascade is activated, leading to the accumulation of 20E in the mosquito fat body. 20E triggers the synthesis of the nutrient transporter Lp and Vg, which accumulate in the ovaries and promote the uptake of lipids and other nutrients required for egg development. In *Plasmodium* infected mosquitoes, developing parasites in the midgut utilize mosquito lipid transporter (e.g. Lp) for proliferation and exploit nutrients destined for egg production. 20E, 20-hydroxyecdysone; E, Ecdysone; EcR, ecdysone receptor; ILP, insulin-like peptides; Lp, lipophorin; OEH, ovarian ecdysiotropic hormone; USP, ultraspiracle; Vg, vitellogenin.

(B) Variation in 20E level (via natural fluctuation or experimental manipulation) effect egg numbers and parasite growth. When 20E level are low, mosquitoes produce fewer eggs and *Plasmodium* parasites generate less oocysts compared to higher 20E level. Additionally, low 20E level lead to an accumulation of lipids in the midgut, promoting faster oocyst growth.