Revising *Leishmania*'s life cycle

A study of blood-feeding female sand flies has shown how successive blood meals amplify *Leishmania* infections in the vector's gut and enhance transmission of the tropical disease leishmaniasis.

Vector-borne diseases, such as leishmaniasis, are all transmitted by arthropods of some kind, and in the case of *Leishmania* the vectors are female sand flies. When an infected sand fly bites the skin of a person or animal, the *Leishmania* parasites are injected into a new host, which can then lead to the development of disease. Feeding on a person or animal is also how vectors become infected, and these blood meals are important for development of the parasites inside the fly. Now, Valenzuela and colleagues[1] reveal how *Leishmania* infections in the sand fly gut are massively amplified following successive blood meals and identify a new stage in the life cycle that is responsible for this amplification. In addition to providing a key insight into the life cycle of a human pathogen, these observations are important for attempts to control disease by targeting the vectors, and for understanding the broader epidemiology of leishmaniasis.

Exploiting the blood-feeding habits of arthropods has been a successful transmission strategy evolved by numerous pathogens, including the various *Leishmania* species. However, this strategy is not without its challenges, as the various vectors all have their own immune systems and other defences against such pathogens[2]. To date, our understanding of *Leishmania* transmission mechanisms has been almost exclusively derived from laboratory studies in which sand flies are given an infected blood meal containing the parasites, and then studied over the subsequent days to follow the development of the infection, including by monitoring the transformation and differentiation of various parasite life-cycle stages in the sand fly gut. Ultimately, this progression leads to the differentiation of metacyclic promastigotes (metacyclogenesis), the name given to the highly motile flagellated infective life-cycle stage pre-adapted for survival in a vertebrate host, which is a mammal for most species of *Leishmania*[3] (Fig. 1). In this scenario, the timing of metacyclogenesis, the numbers of metacyclic promastigotes produced and their location in the sand fly gut are all critical parameters in determining the success of transmission. From this previous work, we know that it takes about 7-14 days for a transmissible infection to develop within the vector, depending on parasite and vector combination, and that production of promastigote secretory gel (PSG) to create a 'blocked fly' is crucial, as this forces infected sand flies to regurgitate parasites into the skin before they can take a second blood meal[4]. The gap in our knowledge has been what happens next, as most of the parasites are known to remain in the sand fly; only a minority of parasites are regurgitated, and the sand fly remains infected for life, but the fate of those remaining parasites and how they influence transmission are questions that have remained unanswered.

The key findings reported by Valenzuela and colleagues are that metacyclic promastigotes are capable of de-differentiation in the sand fly, enhancing population growth of parasites in a second blood meal, and leading to a sand fly with an even greater potential to transmit disease than following a first infected blood meal (Fig. 1). Previously, metacyclic promastigotes were generally considered capable of doing one thing only – if they were injected into a vertebrate host, they would then be taken up by phagocytic cells in the skin, such as neutrophils and macrophages, transforming into the amastigote stage of the life cycle, which then replicates as an obligate intracellular parasite of the phagolysosomal system[5]. Now, we know that the resources invested by the parasite in producing metacyclic promastigotes can have another important benefit, because those that are not transmitted by bite but remain in the gut can revert or de-differentiate into a leptomonad-like form that the authors call retroleptomonad promastigotes. Crucially, unlike metacyclic promastigotes,

these are replicative stages. Notably, leptomonad promastigotes are the known producers of PSG, so this seems likely to be feature of the new retroleptomonad promastigotes as well. In addition, the population of another life cycle stage is boosted – the haptomonad promastigotes that are anchored to the chitin surface of the anterior midgut by their flagella. These stages, together with the PSG, create the blocked fly that is essential for transmission. The authors show that the retroleptomonad promastigotes increase the parasite population substantially, generating infected sand flies with much higher numbers of metacyclic promastigotes and enhanced capacity for transmission. This boost is likely to be particularly important under natural conditions where, unlike in the laboratory, flies will become initially infected with very small numbers of parasites by feeding on an infected vertebrate host[6]. This new link in the life cycle is therefore potentially very important in understanding the epidemiology of leishmaniasis, a disease that affects millions of people worldwide[7,8].

Despite the identification of this new stage in the life cycle of the parasite, some interesting questions remain. For example, how different the new retroleptomonad promastigotes are to the leptomonad promastigotes of the first infected blood meal is not yet clear. They clearly have a different developmental origin, but more work is needed to determine if they are biochemically and functionally different to justify the use of a new name. It is also clear from this study that infected sand flies that take a second blood meal have significantly increased transmission potential, but we do not know how many sand flies live long enough in the wild to take up additional blood meals or transmit disease after such events. Under laboratory conditions, there is substantial fly mortality, much of it associated with egg-laying (one of the technical challenges of this work), and under natural conditions these vectors almost certainly fare much better. Longevity in the wild will vary amongst species and be heavily influenced by environment, so while we can be confident that they will live longer in the wild[9], how much longer and the impact of that on transmission of leishmaniasis remains to be determined. Interestingly, anecdotal evidence is that wild-caught infected sand flies do tend to have very heavy infections similar to those reported in the second blood meal of the Valenzuela study. Following this work, we can now consider the possibility that most infections are transmitted by flies at their second or later blood meal. In the wild, only a very small minority of sand flies are ever infected[10], so improving the transmission capacity of those infected sand flies is clearly of great selective benefit to the parasite.

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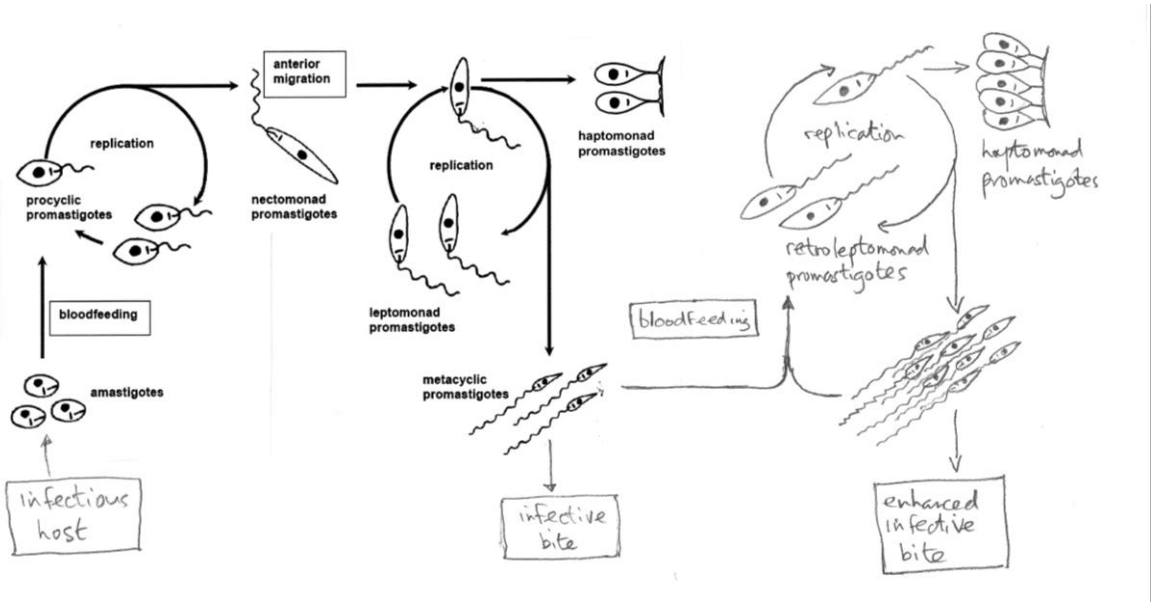

Figure 1. Revised life cycle of *Leishmania* in the sand fly[1,3]. Two replicative phases occur in the first blood meal, involving procyclic and leptomonad promastigotes and leading to the differentiation of metacyclic promastigotes, the infective stage. The new replicative cycle involving retroleptomonad promastigotes is shown on the right, leading to a substantially increased population of metacyclic promastigotes and haptomonad promastigotes, both contributing to enhanced transmission.