Elsevier Editorial System(tm) for Current Opinion in Insect Science

Manuscript Draft

Manuscript Number:

Title: Puppet master in a Tango: Immunity, Host Physiology, and Behaviour in Infected Vectors

Article Type: 20 Ecology 2017*

Corresponding Author: Dr. Lauren Cator,

Corresponding Author's Institution: Imperial College London

First Author: Courtney C Murdock, Ph.D.

Order of Authors: Courtney C Murdock, Ph.D.; Shirley Luckhart, Ph.D.; Lauren Cator

Abstract: When infection alters host behaviour such that pathogen benefits, the behaviour is termed a manipulation. There are several examples of this fascinating phenomenon in many different systems. Vector-borne diseases are no exception. In some instances, as the term implies, pathogens directly interfere with host processes to control behaviour (puppet masters). However, we argue that in many instances, and most especially for vector-borne pathogens, the host response to infection and host physiology is likely to play an important role in these phenotypes. We highlight the importance of considering host response and physiology from recent work on altered host-seeking in malaria parasite infected mosquitoes and argue that this general approach will provide useful insights across vector borne disease systems.

***Highlights (for review)**

Highlights (3-5 bullet points):

- There is evidence that infection alters the behaviour of disease vectors and the majority of these changes in behaviour have been interpreted as manipulation.
- Recent work in malaria has highlighted the potential importance of vector physiology and in particular the role of the vector immune response in infection behaviours.
- We discuss this recent work and highlight the importance of host physiology in understanding infection associated behaviours.

*Manuscript
Click here to view linked References

Puppet master in a Tango: Immunity, Host Physiology, and Behaviour in Infected Vectors

Courtney C. Murdock[1,2,3,4,5,6], Shirley Luckhart[7], and Lauren J. Cator[8]*

[1]Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens, GA 30602 U.S.A.

[2]Odum School of Ecology, University of Georgia, 140 E. Green Street, Athens GA 30602 U.S.A.

[3]Center for Tropical and Emerging Global Diseases, University of Georgia, 500 D.W. Brooks Drive, Athens GA 30602, U.S.A.

[4]Center for the Ecology of Infectious Diseases, Odum School of Ecology, University of Georgia, 140 E. Green Street, Athens GA 30602, U.S.A.

[5]Center for Vaccines and Immunology, College of Veterinary Medicine, University of Georgia, 501 D.W. Brooks Drive, Athens GA 30602, U.S.A.

[6]University of Georgia Riverbasin Center, University of Georgia, 203 D.W. Brooks Drive, Athens, GA 30602, U.S.A.

[7]Department of Medical Microbiology and Immunology, University of California, Davis

[8] Grand Challenges in Ecosystems and Environment, Department of Life Sciences, Silwood Park, Ascot, SL5 7PY, United Kingdom, l.cator@imperial.ac.uk

Abstract (100-120 words)

When infection alters host behaviour such that pathogen benefits, the behaviour is termed a manipulation. There are several examples of this fascinating phenomenon in many different systems. Vector-borne diseases are no exception. In some instances, as the term implies, pathogens directly interfere with host processes to control behaviour (puppet masters). However, we argue that in many instances, and most especially for vector-borne pathogens, the host response to infection and host physiology is likely to play an important role in these phenotypes. We highlight the importance of considering host response and physiology from recent work on altered host-seeking in malaria parasite infected mosquitoes and argue that this general approach will provide useful insights across vector borne disease systems.

Highlights (3-5 bullet points):

- There is evidence that infection alters the behaviour of disease vectors and the majority of these changes in behaviour have been interpreted as manipulation.
- Recent work in malaria has highlighted the potential importance of vector physiology and in particular the role of the vector immune response in infection behaviours.
- We discuss this recent work and highlight the importance of host physiology in understanding infection associated behaviours.

Introduction

Across taxa and transmission routes, there have been many documented cases of host behaviours that change with infection [1,2]. Currently, any change in host behaviour associated with infection that benefits the pathogen is broadly categorized as manipulation. This categorization is currently applied regardless of the mechanisms that lead to that change, the role of the host in these behaviours, or how the change in behaviour affects the fitness of the infected host.

The reasons for this generalization are partially historical. Previous definitions of manipulation have used evidence of pathogen adaptation as one of the criteria for distinguishing manipulation from other causes of behavioural change such as pathology [3]. However if an adaptation is simply defined as a phenotypic variant that results in the highest fitness among a specified set of variants in a given population [4], then all changes in host behaviour that lead to an enhancement of parasite transmission are considered adaptations. Even when a change in host behaviour is the result of pathology, it will be selected for if it enhances transmission. Thus, even if a change in behaviour is a consequence of host adaptive response or pathology, it can be classified as manipulation [3,5].

Calling all altered phenotypes that increase pathogen transmission success “manipulations” groups together behaviours with potentially divergent mechanisms into one phenomenon. The term manipulation also suggests an active role for the pathogen in host behavioural changes. Indeed, it casts the pathogen in the role of puppet master, dynamically pulling on the host’s strings. In reality the host can play a large role in these changes. As more recent work has started to uncover the elusive mechanisms of behavioural changes, this definition seems inadequate to capture the diversity of scenarios that lead to altered behaviour in infected individuals.

Clarifying mechanisms of altered phenotypes is particularly important for vector-borne diseases (VBDs). Unlike the classic manipulations leading to completely novel behaviours, such as those observed in *Cordyceps* infected ants [6] or crickets carrying hairworms [7], changes in infected vector behaviour are for the most part changes in the degree and timing of normal behaviours. Notably, many behavioural changes in vectors are associated with feeding related behaviour (Table 1). These feeding events are both a point of contact between infectious vectors and susceptible hosts and intimately intertwined with major vector life history events such as reproduction. In the case of VBDs, there are also important applied consequences of these changes and, therefore, interest in their mechanisms. The applied importance of knock-on effects for transmission means that even minimal changes in vector behaviour in the small proportion of the population responsible for transmission can have large implications for pathogen transmission and human health [8]. Finally, if manipulation is occurring we would like to know how pathogens alter

host behaviour, both to allow us to target infected individuals, but also because we would like to develop our own tools for manipulating vectors to decrease transmission.

There is a wide diversity of mechanisms that can lead to changes in the behaviour of infected individuals. Pathogens can alter behaviour directly by interacting with the host tissues [3,9]. This is an active process resulting from pathogen secretion of substances that act directly on the host nervous system [9]. Pathogens can also highjack host cells and tissues to express these modulators [10–13]. For example, evidence suggests that the parasite *Toxoplasma gondii* increases dopaminergic activity by directly producing an enzyme required for the synthesis of L-DOPA (a dopamine precursor) in its mouse host [14]. Increased dopamine levels have been associated with changes in mouse fear perception, which is thought to increase parasite transmission success [Reviewed by [15]]. These neuropharmacological manipulations produce many of the dramatic and novel behavioural phenotypes most commonly associated with manipulation [2]. There are currently no examples of this type of mechanism explaining potential manipulation in VBD systems. However, some vector-borne parasites can physically block or inhibit vector host functions to accomplish manipulation. For example, *Leishmania* parasites secrete a gel that blocks the feeding apparatus of sandflies [16]. Similarly, plague bacilli form a biofilm that blocks a portion of the flea midgut, resulting in repeated attempts at feeding and increased pathogen transmission [17]. The mechanisms utilized by parasites and pathogens in these instances fit comfortably into the current definition of manipulation because they demonstratively increase transmission and can be directly attributed to specific parasite traits.

Despite these well-characterized examples, the majority of behavioural changes in vectors lack any evidence of direct manipulation by pathogens [18]. The lack of evidence for direct manipulation mechanisms for many of these phenotypes suggests that some mechanisms are indirect and that vector physiology may play an important role in shaping these behaviours. Across infections, pathogens have been shown to alter behaviour indirectly by interacting with diverse tissues resulting in host-mediated changes in behaviour [9]. Some of these include psychoneuroimmunological changes in host behaviour, which derive from ancient bidirectional connections between the immune and nervous systems [13]. For example, it has been speculated that neuro-inflammation in response to infection, rather than direct parasite interference, is responsible for altered behaviours in infected crustaceans [19]. The behavioural phenotypes that derive from perturbations in these networks are often difficult to separate from generalized sickness behaviours [9].

Case study: Malaria as a manipulator, but not as we know it?

A variety of changes in the behaviour of malaria parasite infected mosquitoes have been reported [18–20]. Generally, during parasite development host-seeking propensity and sensitivity to host odours seem to be depressed, while feeding efficiency is elevated (Table 1). Once the parasite reaches its infectious stage, a behavioural switch occurs in which infectious females are more likely to feed and less efficient when they do so. These changes in behaviour are predicted to increase transmission in two ways. First, feeding and reproduction are relatively risky activities and decreased feeding during parasite development theoretically could increase transmission by increasing the number of females that survive the extrinsic incubation period of the parasite [8]. Second, decreased feeding efficiency associated with infectious parasites improves the likelihood that females will transmit parasites to hosts and bite multiple hosts in attempting to engorge successfully.

Given that these changes in vector behaviour should benefit the parasite, they are termed a manipulation. Until recently, mechanisms explaining observed changes in host-seeking, probing, and host feeding were limited. Changes in feeding efficiency and probing behaviour associated with infection have been associated with decreased apyrase activity in the salivary glands in some mosquito-malaria parasite combinations [21]. There are also documented changes in the vector head proteome during sporozoite infection, but it remains unclear if and how these changes in protein expression and enzyme activity are caused by parasites [21]. For example, it may be that parasites inhibit production of apyrase directly or mechanically damage the salivary gland and its secretion machinery during invasion of this tissue [22].

Until very recently, it was unclear by what mechanism infection was altering host-seeking of infected mosquitoes. Our work and the work of others demonstrated that changes in the sensitivity of the mosquito peripheral nervous system to host odours underlie changes in host-seeking [23,24]. The host response to non-specific immune challenge may also play an important role in regulating these “manipulated” host-seeking phenotypes. Specifically, when female mosquitoes were challenged with heat-killed *Escherichia coli*, we observed changes in behaviour magnitude,

pattern, and timing [24] that were similar to those observed during infection with malaria parasites. Hence, our observations indicated that changes in host-seeking were not specific to infection with malaria parasites or to infection. Rather, the magnitude of the change in feeding was correlated with the dose of heat-killed *E. coli*, the magnitude of the resulting immune response, and the timing of the immune challenge relative to the blood meal [25]. In this context, pre-existing infection-induced trade-offs among digestion, immunity, reproduction and vector behaviour may benefit parasite development.

Functional trade-offs have been proposed to drive other infection associated behaviours. For example, down regulation of feeding during infection or illness-induced anorexia resulting from immune and neural connections [26,27] may be adaptive by increasing host survival during infection [28,29] and reducing trade-offs between energy-consuming digestion and immunity [27,29,30]. The timing of infection is intimately linked with several important life history traits for the female mosquito. Life history traits affect the basic survival and reproductive schedules of organisms (e.g., age-specific birth rates, growth rates, age-specific death rates, size at birth, quantity of offspring produced, etc.)[31]. Further, life history theory assumes that organismal investment into one trait – for example, reproduction – is a zero-sum game that decreases potential investment in other traits such as survival, growth, or future reproduction. Organisms that are most successful, in turn, maximize life time fitness by optimizing differential investment across a suite of life history demands [31]. Thus, a female mosquito must optimally allocate resources toward somatic effort (e.g. self-maintenance – nutritive reserves and immunity) and reproductive effort (e.g. egg production – quantity and quality of eggs) after taking an infectious blood meal. We hypothesized that functional trade-offs between reproduction and digestion or between reproduction and immunity could, therefore, drive the altered feeding phenotypes of malaria parasite infected mosquitoes.

Incorporating this view of host physiology into our exploration of altered host-seeking behaviour led us to ask which cellular pathways might regulate life history trade-offs during parasite development in the mosquito host. Our studies pointed to the insulin/insulin like growth factor signalling (IIS) pathway and to action of endogenous mosquito insulin-like peptides (ILPs) in the midgut. In particular, ILP activation of IIS appears to connect midgut-specific control of parasite infection with changes in host-seeking behaviour. For example, similar patterns of *ILP3* and *ILP4* transcript expression were evident in the midgut following challenge with malaria parasites or with heat killed *E. coli,* with sequential under- and over-expression of these peptides coincident with temporal changes in feeding behaviour associated with “manipulation”. Further work has established that distinct ILP3- and ILP4-dependent biochemical shifts in intermediary metabolism and mitochondrial function regulate parasite infection and changes in mosquito blood feeding [32]. ILP3 induces an orexigenic response perhaps through increased synthesis of excitatory neurotransmitters, resulting in increased nutrient intake and a high energy, biosynthetic state that can reduce parasite infection. In contrast, ILP4 induces an energy deficient, low biosynthetic state that increases parasite infection. In response to ILP4, flight is reduced, fat deposits are mobilized for energy, and food intake is increased at the second blood meal to restore energy levels. Here, energy consuming-biosynthesis is associated with *increased* resistance to infection. Further, both ILP3 and ILP4 reduce expression of known anti-parasite immune genes, indicating that ILP-specific effects on metabolism rather than host immunity per se explain the observed effects on parasite infection. ILP3-dependent biosynthesis clearly does not benefit parasite development, but to what extent ILP4-mediated energy deficiency degrades non-immune host defenses to increase parasite infection is not yet clear. Collectively, these observations indicate that investigation of the role of host physiology in infection-associated behaviour, rather than hunting for manipulator molecules, has facilitated a broader understanding of the behaviour of malaria parasite-infected mosquitoes.

Taking this approach to malaria associated phenotypes could provide answers to other lingering questions about experimental evidence for altered feeding in infected mosquitoes. For example, in a recent study using field derived mosquito colonies and parasites there was no change in host-seeking response with infection [33]. Traditionally, it would be challenging to explain why some combinations (usually unnatural laboratory combinations) exhibit altered phenotypes, while coevolved natural mosquito-parasite combination do not. Approaching these phenotypes as shifts in host physiology provides potential explanations for this discrepancy. For example, different parasite-mosquito combinations may elicit different metabolic responses toward infection (e.g. rodent vs. human parasites) or different levels of response toward infection [34]. If altered phenotypes are in part a result of host functional trade-offs we would expect the altered phenotype to be sensitive to these parameters. Further, like other components of behaviour that influence vector life history these phenotypes are likely to be highly dependent on the condition and environment of the vector instead of being fixed by a manipulator.

Suggestions for moving forward

For many years we have thought of parasites as puppet masters. However, many infection associated behavioural phenotypes appear to be coevolved traits with a large role for the host to play. Instead of thinking of these changes in behaviour as fixed host or parasite traits, it may be more appropriate to consider them an extension of vector and parasite life history trade-offs. A parasite maximizes its fitness in a host under immune challenge and a host maximizes its fitness while being challenged. These goals are not necessarily at odds and they may not require any "manipulations" from either party. The emergent phenotype is the result of an interaction between parasite and host physiology. Parasites may be pulling the strings, but many times the host pulls back. If the parasite is a puppet master, it is in a tango with its marionette.

This shift in perspective has important implications for how we should pursue identification of mechanisms. Specifically, we need to go beyond hunting for manipulator molecules and take more unbiased approaches to understanding how host-parasite interactions influence multiple physiological systems (stress, immunity, reproduction, digestion) to produce the behavioural changes associated with parasite infection. This approach will not only better inform our investigation of the mechanisms of manipulation, but will also provide useful insights into the regulation and coordination of multiple life history traits.

Table 1. Examples of vectors that exhibit altered feeding behaviours when infected with pathogens they transmit. There are many ways that parasites alter vector physiology. Here we specifically focus on changes in host feeding behaviour as opposed to other behavioural changes (e.g., dispersal), host physiological processes (e.g., immunity) or host life history traits (e.g., survival). These behaviours have been grouped into feeding efficiency (probing, regurgitation, ingestion, engorgement), host-seeking (response to host stimuli), and host-attack persistence (the probability that a vector attempts to bite multiple hosts or a single host multiple times). In some examples, direct mechanisms have been identified (yes or "Y"). In others, while the mechanisms have not been identified ("N"), they are known to be specific to infection with a particular pathogen as opposed to a general response to immune challenge. In still other cases, the response has been found to be non-specific and to involve host physiology. In these cases it is unclear whether indirect manipulation or simply a host response is responsible for altered behaviour. In a surprising number of cases, including economically and medically important pathogens, the specificity or role of the host in infection associated changes in feeding behaviour has not been investigated (indicated as "?").

Study	Vector/Pathogen	Feeding Behaviours Altered By Infection	Potential Direct Mechanism Identified?	Specific to Infection with VB Pathogen?	Role of vector physiology?
[17]	Fleas/Plague	Feeding Efficiency	Y	Y	N
[16,35,36]	Sandflies/*Leishmania*	Feeding Efficiency	Y	Y	N
[37,38]	Tsetse Flies/ *Trypanosoma*	Feeding Efficiency and Host-attack Persistence	Y	Y	N
[29–32]	Mosquitoes/Malaria Parasites	Feeding Efficiency	Y	Y	N
[43]	Mosquitoes/Malaria Parasites	Host-attack Persistence and Feeding Efficiency	N	N	?
[23–25]	Mosquitoes/Malaria Parasites	Host-seeking (however see [33])	N	N	Y
[44]	Mosquitoes/Malaria Parasites	Host-attack Persistence	N	Y	?
[45]	Mosquitoes/Filarial Parasites	Host-seeking	N	N	?
[46]	Mosquitoes/Dengue Virus	Feeding Efficiency	N	?	?
[47]	Mosquitoes/Dengue Virus	Host-seeking	N	?	?
[47]	Mosquitoes/Dengue Virus	Host-attack Persistence	N	?	?
[48]	Mosquitoes/LaCrosse Virus	Feeding Efficiency	N	?	?
[49]	Midges/Vesicular Stomatitis Virus	Feeding Efficiency	N	?	?
[50]	Aphids/Barley Yellow Dwarf Virus	Host reference	N	?	?
[51]	Thrips/Tomato Spotted Wilt Virus	Feeding Efficiency	N	?	?
[52]	White Flies/Tomato Spotted Wilt Virus	Feeding Efficiency	N	?	?

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (grant number AI073745 and AI073745 to SL) and the Biotechnology and Biological Sciences Research Council (grant number BB/N013573/1 to LJC).

REFERENCES

1. Hughes DP, Brodeur J, Thomas F: *Host Manipulation by Parasites*. Oxford University Press; 2012.

2. Moore J: *Parasites and the behavior of animals*. Oxford University Press, USA; 2002.

3. Thomas F, Adamo S, Moore J: **Parasitic manipulation: where are we and where should we go?** *Behav. Processes* 2005, **68**:185–199.

4. Reeve HK, Sherman PW: **Adaptation and the goals of evolutionary research**. *Q. Rev. Biol.* 1993, **68**:1–32.

5. Lefèvre T, Roche B, Poulin R, Hurd H, Renaud F, Thomas F: **Exploiting host compensatory responses: the "must"of manipulation?** *Trends Parasitol.* 2008, **24**:435–439.

6. Andersen SB, Gerritsma S, Yusah KM, Mayntz D, Hywel-Jones NL, Billen J, Boomsma JJ, Hughes DP: **The life of a dead ant: the expression of an adaptive extended phenotype**. *Am. Nat.* 2009, **174**:424–433.

7. Thomas F, Schmidt-Rhaesa A, Martin G, Manu C, Durand P, Renaud F: **Do hairworms (Nematomorpha) manipulate the water seeking behaviour of their terrestrial hosts?** *J. Evol. Biol.* 2002, **15**:356–361.

8. Cator LJ, Lynch PA, Thomas MB, Read AF: **Alterations in mosquito behaviour by malaria parasites: potential impact on force of infection**. *Malar. J.* 2014, **13**:164.

9. Adamo SA: **Parasites: evolution's neurobiologists**. *J. Exp. Biol.* 2013, **216**:3–10.

10. Pryor SC, Elizee R: **Evidence of opiates and opioid neuropeptides and their immune effects in parasitic invertebrates representing three different phyla: *Schistosoma mansoni, Theromyzon tessulatum, Trichinella spiralis***. *Acta Biol. Hung.* 2000, **51**:331–341.

11. Biron DG, Ponton F, Marché L, Galeotti N, Renault L, Demey-Thomas E, Poncet J, Brown SP, Jouin P, Thomas F: **"Suicide" of crickets harbouring hairworms: a proteomics investigation**. *Insect Mol. Biol.* 2006, **15**:731–742.

12. Hoover K, Grove M, Gardner M, Hughes DP, McNeil J, Slavicek J: **A gene for an extended phenotype**. *Science* 2011, **333**:1401–1401.

13. Shelley Adamo: **The strings of the puppet master: how parasites change host behavior**. In *Host Manipulation by Parasites*. . Oxford University Press; 2012:36–52.

14. Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA: **The Neurotropic Parasite *Toxoplasma gondii* Increases Dopamine Metabolism**. *PLOS ONE* 2011, **6**:e23866.

15. Kaushik M, Lamberton PHL, Webster JP: **The role of parasites and pathogens in influencing generalised anxiety and predation-related fear in the mammalian central nervous system**. *Horm. Behav.* 2012, **62**:191–201.

16. Rogers ME, Bates PA: **Leishmania manipulation of sand fly feeding behavior results in enhanced transmission**. *PLoS Pathog* 2007, **3**:e91.

17. Bacot AW, Martin CJ: **LXVII. Observations on the mechanism of the transmission of plague by fleas**. *J. Hyg. (Lond.)* 1914, **13**:423–439.

18. Lefèvre T, Thomas F: **Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases**. *Infect. Genet. Evol.* 2008, **8**:504–519.

19. Helluy S, Thomas F: **Parasitic manipulation and neuroinflammation: Evidence from the system *Microphallus papillorobustus* (Trematoda) - Gammarus (Crustacea)**. *Parasit. Vectors* 2010, **3**:38.

20. Cator LJ, Lynch PA, Read AF, Thomas MB: **Do malaria parasites manipulate mosquitoes?** *Trends Parasitol.* 2012, **28**:466–470.

21. Lefevre T, Thomas F, Schwartz A, Levashina E, Blandin S, Brizard J-P, Le Bourligu L, Demettre E, Renaud F, Biron DG: **Malaria *Plasmodium* agent induces alteration in the head proteome of their *Anopheles* mosquito host**. *Proteomics* 2007, **7**:1908–1915.

22. Hurd H: **Manipulation of medically important insect vectors by their parasites**. *Annu. Rev. Entomol.* 2003, **48**:141–161.

23. Smallegange RC, van Gemert G-J, van de Vegte-Bolmer M, Gezan S, Takken W, Sauerwein RW, Logan JG: **Malaria infected mosquitoes express enhanced attraction to human odor**. *PloS One* 2013, **8**:e63602.

24. Cator LJ, George J, Blanford S, Murdock CC, Baker TC, Read AF, Thomas MB: **"Manipulation" without the parasite: altered feeding behaviour of mosquitoes is not dependent on infection with malaria parasites**. *Proc. Biol. Sci.* 2013, **280**:20130711.

25. **Cator LJ, Pietri JE, Murdock CC, Ohm JR, Lewis EE, Read AF, Luckhart S, Thomas MB: **Immune response and insulin signalling alter mosquito feeding behaviour to enhance malaria transmission potential**. *Sci. Rep.* 2015, **5**:11947.
The magnitude of the behavioural change in mosquito host-seeking behaviour was correlated with the dose of heat-killed *E. coli*, the magnitude of the resulting immune response, and the timing of the immune challenge relative to the blood meal. The insulin/insulin like growth factor signalling (IIS) pathway and action of endogenous mosquito insulin-like peptides (ILPs) in the midgut were implicated as mediating interactions among digestion, immunity, reproduction.

26. Dantzer R: **Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity**. *Eur. J. Pharmacol.* 2004, **500**:399–411.

27. Adamo SA: **Bidirectional connections between the immune system and the nervous system in insects**. In *Insect Immunology*. Academic Press; 2008:129–149.

28. Murray MJ, Murray AB: **Anorexia of infection as a mechanism of host defense**. *Am. J. Clin. Nutr.* 1979, **32**:593–596.

29. Ayres JS, Schneider DS: **The Role of Anorexia in Resistance and Tolerance to Infections in Drosophila**. *PLoS Biol.* 2009, **7**.

30. *Sullivan K, Fairn E, Adamo SA: **Sickness behaviour in the cricket *Gryllus texensis*: Comparison with animals across phyla**. *Behav. Processes* 2016, **128**:134–143.
This study uses a comparative approach and a trade-off framework to investigate the relationship between an illness induced anorexia, predator avoidance, and energy conservation.

31. Stearns S: *The Evolution of Life Histories*. Oxford University Press; 1992.

32. *Pietri J, Pietri E, Potts R, Riehle M, Luckhart S: ***Plasmodium falciparum* suppresses the host immune response by inducing the synthesis of insulin-like peptides (ILPs) in the mosquito *Anopheles stephensi***. *Dev. Comp. Immunol.* 2015, **53**.
Further work has established that distinct ILP3- and ILP4- dependent biochemical shifts in intermediary metabolism and mitochondrial function regulate parasite infection and changes in mosquito blood feeding.

33. Vantaux A, Hien de S, François D, Yaméogo B, Dabiré KR, Thomas F, Cohuet A, Lefevre T: **Host-seeking behaviors of mosquitoes experimentally infected with sympatric field isolates of the human malaria parasite *Plasmodium falciparum*: no evidence for host manipulation**. *Front. Ecol. Evol.* 2015, **3**.

34. Dong Y, Aguilar R, Xi Z, Warr E, Mongin E, Dimopoulos G: ***Anopheles gambiae* immune responses to human and rodent *Plasmodium* parasite species**. *PLoS Pathog.* 2006, **2**:e52.

35. Killick-Kendrick R, Leaney AJ, Ready PD, Molyneux DH: **Leishmania in phlebotomid sandflies. IV. The transmission of *Leishmania mexicana amazonensis* to hamsters by the bite of experimentally infected *Lutzomyia longipalpis***. *Proc. R. Soc. Lond. B Biol. Sci.* 1977, **196**:105–115.

36. Schlein Y, Jacobson RL, Messer G: **Leishmania infections damage the feeding mechanism of the sandfly vector and implement parasite transmission by bite**. *Proc. Natl. Acad. Sci. U. S. A.* 1992, **89**:9944–9948.

37. Jenni L, Molyneux DH, Livesey JL, Galun R: **Feeding behaviour of tsetse flies infected with salivarian trypanosomes**. *Nature.* 1980, **283**: 383-385.

38. Van Den Abbeele J, Caljon G, De Ridder K, De Baetselier P, Coosemans M: ***Trypanosoma brucei* modifies the tsetse salivary composition, altering the fly feeding behavior that favors parasite transmission**. *PLoS Pathog* 2010, **6**:e1000926.

39. Wekesa JW, Copeland RS, Mwangi RW, others: **Effect of *Plasmodium falciparum* on blood feeding behavior of naturally infected *Anopheles* mosquitoes in western Kenya.** *Am. J. Trop. Med. Hyg.* 1992, **47**:484.

40. Rossignol PA, Ribeiro JM, Spielman A, others: **Increased intradermal probing time in sporozoite-infected mosquitoes.** *Am. J. Trop. Med. Hyg.* 1984, **33**:17.

41. Li X, Sina B, Rossignol PA: **Probing behaviour and sporozoite delivery by *Anopheles stephensi* infected with Plasmodium berghei**. *Med. Vet. Entomol.* 1992, **6**:57–61.

42. Ponnudurai T, Lensen AHW, Van Gemert GJA, Bolmer MG, Meuwissen JHE: **Feeding behaviour and sporozoite ejection by infected *Anopheles stephensi***. *Trans. R. Soc. Trop. Med. Hyg.* 1991, **85**:175–180.

43. Anderson RA, Koella JC, Hurd H: **The effect of Plasmodium yoelii nigeriensis infection on the feeding persistence of *Anopheles stephensi* Liston throughout the sporogonic cycle**. *Proc. R. Soc. Lond. B Biol. Sci.* 1999, **266**:1729–1733.

44. Anderson RA, Knols BGJ, Koella JC, others: ***Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae* sl**. *Parasitology* 2000, **120**:329–333.

45. *Gleave K, Cook D, Taylor MJ, Reimer LJ: **Filarial infection influences mosquito behaviour and fecundity**. *Sci. Rep.* 2016, **6**:36319.
Changes in mosquito host-seeking were specific to both the stage and intensity of infection with filarial worms. Exposed females responded similarly to controls at the time point associated with worm development, but a different response in the later test period. This suggests that parasite development is required to alter host-seeking in this system.

46. Sylvestre G, Gandini M, Maciel-de-Freitas R: **Age-dependent effects of oral infection with dengue virus on *Aedes aegypti* (Diptera: Culicidae) feeding behavior, survival, oviposition success and fecundity**. *PLoS One* 2013, **8**:e59933.

47. Maciel-de-Freitas R, Sylvestre G, Gandini M, Koella JC: **The influence of dengue virus serotype-2 infection on *Aedes aegypti* (Diptera: Culicidae) motivation and avidity to blood feed**. *PloS One* 2013, **8**:e65252.

48. Jackson BT, Brewster CC, Paulson SL: **La Crosse virus infection alters blood feeding behavior in *Aedes triseriatus* and *Aedes albopictus* (Diptera: Culicidae)**. *J. Med. Entomol.* 2012, **49**:1424–1429.

49. Bennett KE, Hopper JE, Stuart MA, West M, Drolet BS: **Blood-feeding behavior of vesicular stomatitis virus infected *Culicoides sonorensis* (Diptera: Ceratopogonidae)**. *J. Med. Entomol.* 2008, **45**:921–926.

50. Ingwell LL, Eigenbrode SD, Bosque-Pérez NA: **Plant viruses alter insect behavior to enhance their spread**. *Sci. Rep.* 2012, **2**:578.

51. Stafford CA, Walker GP, Ullman DE: **Infection with a plant virus modifies vector feeding behavior**. *Proc. Natl. Acad. Sci.* 2011, **108**:9350–9355.

52. Moreno-García M, Córdoba-Aguilar A, Condé R, Lanz-Mendoza H: **Current immunity markers in insect ecological immunology: assumed trade-offs and methodological issues**. *Bull. Entomol. Res.* 2013, **103**:127–139.