Natural malaria infection reduces starvation resistance of nutritionally stressed mosquitoes

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Summary

1. In disease ecology, there is growing evidence that environmental quality interacts with parasite and host to determine host susceptibility to an infection. Most studies of malaria parasites have focused on the infection costs incurred by the hosts, and few have investigated the costs on mosquito vectors. The interplay between the environment, the vector and the parasite has therefore mostly been ignored and often relied on unnatural or allopatric Plasmodium/vector associations.

2. Here, we investigated the effects of natural avian malaria infection on both fecundity and survival of field-caught female Culex pipiens mosquitoes, individually maintained in laboratory conditions. We manipulated environmental quality by providing mosquitoes with different concentrations of glucose-feeding solution prior to submitting them to a starvation challenge. We used molecular-based methods to assess mosquitoes’ infection status.

3. We found that mosquitoes infected with Plasmodium had lower starvation resistance than uninfected ones only under low nutritional conditions. The effect of nutritional stress varied with time, with the difference of starvation resistance between optimally and suboptimally fed mosquitoes increasing from spring to summer, as shown by a significant interaction between diet treatment and months of capture. Infected and uninfected mosquitoes had similar clutch size, indicating no effect of infection on fecundity.

4. Overall, this study suggests that avian malaria vectors may suffer Plasmodium infection costs in their natural habitat, under certain environmental conditions. This may have major implications for disease transmission in the wild.

Key-words: avian malaria, Culex pipiens, host-parasite co-evolution, life-history traits, Plasmodium, resource limitation, trade-offs

Introduction

Organisms often face environments with limited amount of resources, giving rise to the well-known allocation trade-off between different life-history traits (Stearns 1992). Organisms have thus developed diverse strategies aiming to optimize fitness through differential allocation of available resources (Roff 1992; Stearns 1992).

Parasites live at the expense of their hosts and by definition alter host fitness through different mechanisms, such as resource depletion, tissue damage or even castration (Combes 2001). In turn, hosts have developed several strategies to decrease the costs of parasitism (reviewed in Sheldon & Verhulst 1996), ranging from immune to behavioural defences (Hart 1994; Wakelin 1996; Moore 2002). They may thus tolerate the parasite (sensu Restif & Koella 2004; Read, Graham & Raberg 2008) by modifying their life-history traits and reduce the loss of fitness associated with parasite burden (Forbes 1993; Perrin, Christe & Richner 1996). The nature of parasite-induced modifications on host life-history traits is a question of major importance in evolutionary biology (Møller 1997), epidemiology and the control of vector-borne diseases (Dye 1986) such as malaria (Schwartz & Koella 2001).

Some haematophagous dipteran insects act as vectors of several diseases and play a central role in the complex life cycle of malaria parasites such as Plasmodium spp. (Apicomplexa: Haemosporidae). Although most dipteran insects metabolize carbohydrate macronutrients gained from nectar feeding to optimize energy-demanding functions such as flight, reproduction and survival (Nayar & van Handel 1971; Foster 1995; Impoinvil et al. 2004; Stone & Foster 2012), vectors bite vertebrates for a blood

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meal to acquire the proteins that are indispensable for egg maturation. In order for a malaria parasite to successfully complete its life cycle, parasite gametocytes must first enter the vector during feeding. Vectors must then survive the incubation period of the parasite oocysts, which later develop into sporozoites, the transmissible life stages (Garnham 1966). Small changes in a vector’s survival, behaviour or parasite incubation time could therefore have strong consequences on parasite transmission and fitness (Kingsolver 1987; Koella 1999; Cohuet et al. 2010).

Previous studies investigating Plasmodium-induced costs on vector fitness have consistently reported a lower reproductive success in infected vectors (Hurd 2003; Araujo et al. 2011; but see Ferguson et al. 2003), while the effects of malaria parasites on vector survival have often lead to contrasting results (see Ferguson & Read 2002b for a meta-analysis). These discrepancies may be the result of complex interactions between parasite and host genotypes and environment.

Vector tolerance or resistance to malaria infection has actually been shown to vary with genetic factors associated with the vectors (Ward 1963; Niare et al. 2002; Vézilier et al. 2012; but see O’Donnell & Armbruster 2010 and Vézilier et al. 2010), the parasite (Ferguson, Rivero & Read 2003; Ferguson et al. 2003) or both (Lambrechts et al. 2005; Harris et al. 2010). This may result in patterns of local adaptations between Plasmodium and vectors (Hume et al. 2007; Joy et al. 2008; Harris et al. 2012) and highlights the importance of accounting for the natural diversity of parasites and vectors occurring in the same location (Glaiizot et al. 2012; Lalubin et al. 2013).

Environmental conditions can also greatly influence host defences against infectious diseases and parasites. This is well documented for vertebrate (see e.g. Christie et al. 2001; Christie et al. 2003; Hunter 2005), plant (Scholthof 2007) and invertebrate hosts (Sadd 2011; Vale et al. 2011; see Sandland & Minchella 2003; Wolinska & King 2009 for reviews), but only a few studies have investigated the interaction between parasite resistance and environment quality in dipteran vectors (Lefèvre et al. 2013). Environmental stressors, such as drought or limited carbohydrate resources, have previously been shown to decrease vector immunocompetence (Koella & Sörens 2002) and to increase the susceptibility of several mosquito strains to malaria infection (Ferguson et al. 2003; Lambrechts et al. 2006; Aboagye-Antwi et al. 2010). In addition, malaria-induced mortality in vectors has been shown to vary with the coupled effect of parasite genotype and nutrient availability (Ferguson & Read 2002a).

Experimental studies investigating the costs of malaria infection have often relied on unnatural or allopatric vector–parasite combinations, and it is unclear whether results from such vector–parasite associations can be generalized (Tripet 2009). In recent years, however, our knowledge of natural avian malaria vector is increasing (Massey et al. 2007; Gager et al. 2008; Ishtiaq et al. 2008; Kimura, Darbro & Harrington 2010; Ejiri et al. 2011; Kim & Tsuda 2012; Ventim et al. 2012), making it possible to study the costs of infection in a natural system. Here, we took advantage of the recent characterization of a natural model system (Glaiizot et al. 2012), to conduct an experiment using avian malaria parasites and C. pipliens mosquitoes that co-occur in the same location, in Western Switzerland. We manipulated environmental quality by providing different glucose diet treatments to field-caught mosquitoes maintained in the laboratory. Our objectives were therefore to experimentally test the effects of diet and malaria infection on survival and fecundity of wild, naturally infected and uninfected C. pipliens females.

**Materials and methods**

**MOSQUITO COLLECTION AND GENERAL PROCEDURE**

Mosquitoes were sampled two to three times per week, from April to August 2011, at the edge of the forest of Doringny, on the campus of the University of Lausanne, Switzerland. This area has been previously characterized as a zone with high prevalence of avian malaria (see Christe et al. 2012; Glaiizot et al. 2012; van Rooyen et al. 2013a,b). In the early spring, 179 containers (50 × 30 × 25 cm) baited with baker’s yeast, a well-known attractant for Culex mosquitoes looking for an oviposition site (Madder, Surgeoner & Helson 1983; Reiter 1986; Guerstein et al. 1995), were placed to catch adult female mosquitoes.

Adult sampling was performed about twice a week, from April until the end of August (21 weeks). Each sampling date, two to four gravid mosquito traps (BioQuip, Rancho Dominguez, CA, USA) were mounted on containers before sunset. Traps were collected the next morning, after sunrise, and field-caught mosquitoes were transferred to the insectary (24 ± 0.5°C, 55 ± 5%, relative humidity and a 14 : 10 h light-dark cycle), where they were individually maintained in plastic vials (Sarstedt, 30 mL). Females were initially provided with a glucose-rich diet (ad libitum access to a 6% glucose solution), mimicking optimal natural conditions (see e.g. Impoinvil et al. 2004) and an oviposition substrate (2.5 mL of spring water) (Briegel 1990). Under these conditions, 75% of the females oviposited within 4 days after capture (mean ± SE: 4.6 ± 0.2 days). All fresh single-layered egg-rafts laid at the laboratory by the wild-caught female mosquitoes were collected and photographed with a binocular microscope at 25x magnification. The number of eggs per raft was then counted using the IMAGEJ software (National Institutes of Health, Bethesda, MD, USA) to determine the clutch size of individual mosquitoes. Females that did not oviposit in the laboratory were discarded from the experiment.

**SURVIVAL EXPERIMENT**

Females that oviposited within the same day (N = 870) were uniformly assigned to one of the two treatment groups; they were fed for seven days either with a 6% glucose solution (optimal diet group) or with a 2% glucose solution (nutritional stress). After the seven days of treatment, surviving females were deprived of glucose and provided only with water to avoid desiccation. We measured starvation resistance as the daily survival of individual mosquitoes during this starvation challenge. One hundred and nine individuals were excluded from these analyses because they...
died during the treatment period of seven days before the starvation challenge.

The wings of all dead individuals were cut-off and glued on a slide covered with a lamella. We used a digital video camera (Leica DFC 290 HD, Bannockburn, IL, USA) mounted on a binocular microscope to measure wing lengths of all dead individuals. The camera was connected to a computerized data acquisition system (Leica application suite) allowing us to determine the distance from the fringe of the alula to the peripheral tip of the R3 vein (Mpho, Holloway & Callaghan 2000), to the nearest 0.01 mm. Wing length3 was used in the statistical analysis as a proxy for body size (van Uitregt, Hurst & Wilson 2012).

**MOLECULAR DIAGNOSIS OF INFECTION**

DNA from the mosquitoes’ thorax samples was extracted by using the DNeasy tissue extraction kit combined with the BioSprint 96 workstation (QIAGEN, Valencia, CA, USA), according to the manufacturer’s instructions. A nested-PCR protocol was used to amplify a portion (524-bp long) of the mitochondrial cytochrome b gene (mtDNA cyt b) of the parasite (see Bensch et al. 2000; Waldenström et al. 2004 for further detailed explanations of the method).

The use of PCR-based methods to screen mosquito thorax samples for malaria infection does not distinguish among Plasmodium development stages and may thus result in the false-positive detections by successfully amplifying mtDNA cyt b fragment from abortive Plasmodium sporozoites or from parasites that are remnant in the thoracic midgut of fed mosquitoes (Valkiunas 2011). Resistant mosquitoes hosting abortive sporozoites may even show up positive with PCRs up to day 17th post infection (Valkiunas et al. 2013). To avoid false-positive infections in our data, mosquitoes were caught while looking for an oviposition site, which occurs about 5 up to 10 days after the blood meal (Vinogradova 2000), when blood digestion has been fully completed. Moreover, field-caught mosquitoes were laboratory maintained for an additional period of 23 days in average, without access to blood.

**STATISTICAL ANALYSES**

Statistical analyses were performed using R 2.15.2 (R Development Core Team 2012) and JMP 9.0 (SAS Institute Inc., Cary, NC, USA).

Multiple regression analyses by Cox proportional-hazards models were used to examine the effects of malarial infection on C. pipiens starvation resistance (N = 761). Starvation resistance of individual females (dependent variable) was modelled as a function of diet treatment (Diet: glucose-rich or glucose-poor diet), time (months of capture, April to August), infection status (infected or uninfected), body size and second-order interactions. The unknown age of wild-caught mosquitoes was assumed to be randomly distributed into the different treatment groups. To determine the explanatory power of each fitted parameter, likelihood ratio tests (LRT) were conducted following a standard backward procedure. Non-significant interactions (P > 0.05) were then dropped out from the full model (Crawley 2007). *Post hoc* contrasts were conducted by aggregating the data by factor levels, and between-group differences were tested by Kaplan–Meier analyses along with log-rank tests. To account for multiple comparisons, we adjusted the level of significance α using a Bonferroni correction (α = 0.05/k = 0.012 where k = 4 is the number of comparisons).

To investigate whether Plasmodium-induced changes in starvation resistance were mediated by the alteration of the vectors’ reproductive output, we sequentially adjusted the minimal model of starvation resistance by adding clutch size as a covariate (Ferguson & Read 2002a; Vézilier et al. 2012). Levels of significance were determined using a LRT.

We used a two-way ANOVA to determine whether body size estimates differ between healthy and parasitized female C. pipiens and between months of capture.

We used a two-way ANCOVA to analyse the effect of body size (continuous covariate), infection status, time and all second-order interactions on clutch size of individual female mosquitoes (N = 870). Differences between groups were tested with paired-t tests.

**Results**

The significant interaction between infection status and diet (LRT: χ² = 4.86, P = 0.028, see Table 1) indicates that parasitized and unparasitized vectors reacted differently to the diet treatment. Avian Plasmodium is detrimental for nutritionally stressed vectors (Fig. 1 log-rank test, infected vs. uninfected: χ² = 13.5, d.f. = 1, P < 0.001) but not for females fed on a glucose-rich diet (Fig. 1 log-rank test, infected vs. uninfected: χ² = 0.0, d.f. = 1, P = 0.845).

The effect of the diet treatment on C. pipiens starvation resistance also varied with time, as shown by the significant interaction between diet and time (Table 1). Starvation resistance of the female mosquitoes reared on the glucose-rich diet slightly increased throughout the season (effect of the glucose-rich diet: χ² = 20.98, P < 0.001; Mean starvation resistance (days) ± SE: April: 14.56 ± 0.88, May: 14.65 ± 0.55, June: 18.65 ± 0.95, July: 15.16 ± 0.86, August: 16.90 ± 0.90), whereas mosquitoes fed on a glucose-poor solution had significantly lower starvation resistance from spring to summer months (effect of the glucose-poor diet: χ² = 55.44, P < 0.001; Mean starvation resistance (days) ± SE: April: 13.45 ± 0.71, May:

<table>
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<th>Effect tested</th>
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<th>d.f.</th>
<th>P-value</th>
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Survival of malaria-infected mosquitoes

Fig. 1. Cumulative survival rate of Culex pipiens female mosquitoes showing the coupled effects of infection status and diet. Malarial infection status is indicated by the colour of the symbols, infected mosquitoes by filled symbols and uninfected by open symbols. Glucose-rich (GR) and glucose-poor (GP) diet treatments are represented by circle and squares, respectively. The error bars represent the lower and the upper bounds of the 95% confidence interval around the means daily survival in each experimental class. N is the number of mosquitoes monitored during the starvation challenged initiated at day zero.

11.98 ± 0.35, June: 10.55 ± 0.58, July: 7.28 ± 0.61, August: 9.61 ± 0.69.

Sequential adjustment of the minimal model of starvation resistance by clutch size did not significantly improve the model (LRT: effect of clutch size: \( \chi^2 = 0.42, \text{d.f.} = 1, P = 0.518 \)).

Body size was not significantly different between infected and uninfected mosquitoes \( (F_{1,755} = 1.97, P = 0.160) \) but significantly decreases from spring to summer months (mean body size (mm\(^3\)) ± SE: April: 67 ± 2, May: 69 ± 1, June: 62 ± 1, July: 51 ± 1, August: 47 ± 1; \( F_{4,755} = 61.54, P < 0.001 \)).

Clutch size was significantly positively correlated with body size \( (F_{1,854} = 689.45, P < 0.001; \text{Intercept} \pm \text{SE} = 79.55 \pm 7.98, \text{slope} \pm \text{SE} = 3.02 \pm 0.12 \). Clutch size was also significantly affected by time \( (F_{4,854} = 39.79, P < 0.001 \) and by the interaction between time and body size \( (F_{4,854} = 4.05, P = 0.003 \). This significant interaction resulted in C. pipiens females laying bigger clutches throughout the months of capture despite their decreasing body size (Least square means of clutch size (egg counts) adjusted for body size ± SE: April: 190.2 ± 9.3, May: 263.8 ± 5.1, June: 283.3 ± 6.4, July: 286.0 ± 6.5, August: 292.3 ± 7.9). Clutch size was, however, not significantly affected by infection status \( (F_{1,854} = 1.61, P = 0.204 \), neither by the interaction between time and infection status \( (F_{4,854} = 0.41, P = 0.801 \), nor by the interaction between wing size and infection status \( (F_{1,854} = 0.48, P = 0.487 \).

Discussion

In this study, we used a naturally co-occurring Plasmodium – vector system to show that malaria infection reduces starvation resistance of field-caught nutritionally stressed C. pipiens females. Mosquito fecundity was not affected by the parasite, and there was no evidence of a trade-off between survival and reproduction.

Natural avian malaria infection negatively affected the starvation resistance of mosquitoes. This result suggests that malaria-infected vectors may incur physiological costs reducing survival under challenging environmental conditions only. The malaria infection costs for the vectors may arise from the immune system activation that has been shown to be energetically costly, leading for example to reproductive costs (Schwartz & Koella 2004; Ahmed & Hurd 2006). Direct damages of the vector’s tissue caused by Plasmodium may also impair the somatic processes of the mosquitoes. For instance, growing Plasmodium oocysts within the mosquitoes’ midgut are known to disrupt the epithelial cells of the midgut wall (Ziever & Dvorak 2000; Sinden & Billingsley 2001). Plasmodium sporozoites can also damage mosquitoes’ salivary glands (Sterling, Aikawa & Vanderberg 1973), which might increase vector susceptibility to bacteria or other diseases (Vaughan & Turell 1996). Our results support thus the hypothesis that malaria infection is generally costly for the vectors (see Ferguson & Read 2002b for a meta-analysis), which may select for parasite avoidance, for example, by discriminating between healthy and parasitized birds (Lalubin et al. 2012).

Our finding that environment quality, manipulated with different glucose diet quality, mediates the malaria infection costs for the vectors is in agreement with previous studies conducted on mosquitoes artificially infected with rodent malaria (Ferguson & Read 2002a; Ferguson et al. 2003). Lambrechts et al. (2006) also found, using an Anopheles stephensi/Plasmodium yoelii yoelii association, that the mortality rate of malaria-infected mosquitoes increased under low glucose conditions. Providing mosquitoes with ad libitum access to a glucose-rich solution in laboratory experiments does not necessarily reflect the conditions where the malaria infection costs are expressed (Ferguson & Read 2002a). Evidence that sugar feeding is limiting for mosquitoes in their natural habitat is increasing (Schlein & Müller 1995; Gu et al. 2011), and hence,
the flowering phenology likely affects the sugar resources available to the mosquitoes and therefore their vectorial capacity (Stone, Jackson & Foster 2012).

The premature death of a malaria-infected vector during the development of Plasmodium oocysts may strongly impair the disease spread in the susceptible host population (Cohuet et al. 2010). However, in our experimental setting, malaria-infected mosquitoes died more than 3 weeks after the capture, presumably while harbouring the sporozoite life stage of the parasite in their salivary glands. In that case, the epidemiological consequences of a reduced starvation resistance are not straightforward: a lower survival probability may induce a higher host-seeking activity for blood meal (Koella, Sørensen & Anderson 1998; Anderson, Knols & Koella 2000) to mature and lay as much eggs as possible before death. Such a compensatory response of the vectors may in turn be beneficial for parasite transmission (Lefèvre et al. 2008). On the other hand, a higher host-seeking activity of the vectors may also increase the risk of being killed by predators or by behavioural defences of the vertebrate hosts (Anderson, Knols & Koella 2000; Darbro & Harrington 2007).

The age of mosquitoes used in our experiments was unknown, and one could argue that parasitized females died earlier because they were older than uninfected ones. Indeed, the probability for a mosquito to be infected may increase with the number of blood meals eaten with the consequence that older mosquitoes are more prone to be parasitized. Age might thus be a confounding factor in our design with parasitized females being on average older than healthy ones. However, individuals were randomly affected to the diet treatment groups (2 and 6% glucose-feeding solution). Older, more parasitized females of both treatment groups would be less resistant than younger, healthier individuals. In our experiments, both parasitized and healthy females follow the same survival curve in the glucose-rich group, suggesting no age effect. One cannot exclude, however, other hidden interactions between age and one or several factors in our experimental setup.

We found no effect of malaria infection on fecundity. This contradicts results of previous studies that have shown a negative effect of malaria on vector fecundity (Ahmed & Hurd 2006; Araujo et al. 2011; Vézilier et al. 2012; see Hurd, Hogg & Renshaw 1995; Hurd 2003 for reviews on fecundity reduction). Interestingly, the rodent malaria parasite Plasmodium chabaudi has been reported to enhance the fecundity of A. stephensi (Ferguson et al. 2003). The authors of this study suggested that some infection-related changes in blood chemistry might increase blood quality in mosquitoes. In our experimental set-up, infected females might have also avoided the reproductive costs imposed by Plasmodium through increased blood uptake by biting multiple bird hosts before capture (Koella, Sørensen & Anderson 1998; Koella 1999). Alternatively, they also could have increased sugar uptake during the time period between the capture and the oviposition date, where they were offered a glucose-rich diet. Finally, it could be that female C. pipiens compensated for Plasmodium-induced reduction in survival, by shifting their resource allocation towards fecundity at the expense of their starvation resistance. Such hosts’ defence strategies have been reported across diverse taxa of parasitized organisms (see Sorct, Clobert & Michalakis 1996; Adamo 1999; Schmid-Hempel 2011). Because diet treatment occurred after females have laid their eggs, our experiment was not designed to accurately evaluate the potential trade-off between fecundity and survival.

It is traditionally assumed that malaria infection costs should occur at the expense of fecundity instead of survival as reduction in fecundity does not impair malaria transmission (Schwartz & Koella 2001). In line with this hypothesis, Vézilier et al. (2012) reported a decrease in fecundity associated with enhanced starvation resistance in Plasmodium-infected C. pipiens. Using a rodent malaria model system, it has been further shown that Plasmodium oocysts both enhance sugar intake and alter metabolic pathways of the vectors (Zhao et al. 2012). These Plasmodium-induced changes could result in a higher energy storage for infected mosquitoes (Rivero & Ferguson 2003; but see Mack, Samuels & Vanderberg 1979; Gray & Bradley 2006) enhancing thus mosquito survival and parasite transmission. Significant physiological differences, however, occur between anophelines, aedines and culicines mosquitoes (reviewed in Klowden 2007), and crossed-validation of results on different malaria model system is needed to allow one generalizing vectors’ response to Plasmodium infection.

Overall, the different results obtained across studies may lie in the complex interactions between environment and parasite and host genotypes. In the present study, we looked at wild-caught C. pipiens naturally infected with Plasmodium, whereas Vézilier et al. (2012) used C. pipiens reared in laboratory conditions through several generations. Furthermore, these mosquitoes were fed and infected on domestic canaries (Serimus canaria) that were experimentally infected with allopathic field isolates of Plasmodium isolated from the house sparrows Passer domesticus (Vézilier et al. 2012). Long-term maintenance in artificial condition of Plasmodium through intraperitoneal injection of infected blood on naive hosts as well as local adaptation that may occur in these complex interactions may select for different levels of virulence and explained the different outcomes observed between studies (see e.g. Cohuet et al. 2006). Differences in phenotypic traits may also influence the evolution of different life-history strategies in mosquitoes (McCann et al. 2009) and therefore their response to malaria infection. Indeed, C. pipiens were much smaller and laid smaller clutches in the study of Vézilier et al. (2012) than the ones in our study (mean wing size (mm) ± SE: Montpellier 3.36 ± 0.01; Lausanne 3.91 ± 0.01; clutch size ± SE: Montpellier 155 ± 2; Lausanne 264 ± 3). Larger mosquitoes may for instance develop higher number of oocysts as a result of...
higher blood intake (Lyimo & Koella 1992) and thus suffer higher mortality than smaller individuals. Alternatively, small mosquitoes may have less energy reserve allocated to their defence against the parasite (Suwanichitchinda & Paskewitz 1998). Another possibility to explain the discrepancies between studies may lie with the timing and length of the starvation challenge relative to the developmental stage of the parasite within the vectors. Plasmodium oocysts must keep their vector alive to achieve their development, whereas Plasmodium sporozoites aim to be transmitted to the vertebrate hosts through the mosquito bites.

Finally, the strength of the nutritional treatment increased throughout the date of mosquito collection, independently of the mosquito infection status. This was accompanied by a seasonal increase in vectors' fecundity when taking into account body size. Part of these variations in vectors' reproduction and starvation resistance may lie in the interplay of seasonally changing environmental factors, such as temperature, rainfall or resource availability which could have affected larval growth and/or supplied adult mosquitoes with different levels of stress prior to their capture (Triplet, Aboagye-Antwi & Hurd 2008; Aboagye-Antwi & Triplet 2010; Murdock et al. 2012).

In conclusion, we provide further evidence that Plasmodium infection can be costly for their mosquito vectors under high-nutrient-stressed environmental conditions. A further step would be to investigate the genetic mechanisms that underpin Plasmodium-induced changes in a vector’s behavioural or life-history traits. New advances in molecular biology (Holt et al. 2002; Arensburger et al. 2010; Palinauskas et al. 2012) will offer powerful tools to identify candidate genes of Plasmodium and mosquito vectors that determine the diversity of vector phenotypes (de Roode & Read 2003) and to understand how the environment mediates the expression of the genes of interest. This will shed light on the complex nature of parasite-induced costs on vectors.

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