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The energetic budget of *Anopheles stephensi* infected with *Plasmodium chabaudi*: is energy depletion a mechanism for virulence?

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Evidence continues to accumulate showing that the malaria parasites (*Plasmodium* spp.) reduce the survival and fecundity of their mosquito vectors (*Anopheles* spp.). Our ability to identify the possible epidemiological and evolutionary consequences of these parasite-induced fitness reductions has been hampered by a poor understanding of the physiological basis of these shifts. Here, we explore whether the reductions in fecundity and longevity are the result of a parasite-mediated depletion or reallocation of the energetic resources of the mosquito. Mosquitoes infected with *Plasmodium chabaudi* were expected to have less energetic resources than uninfected mosquitoes, and energy levels were predicted to be lowest in mosquitoes infected with the most virulent parasite genotypes. Not only was there no evidence of a parasite-mediated reduction in the overall energetic budget of mosquitoes, but *Plasmodium* was actually associated with increased levels of glucose, a key insect nutritional and energetic resource. The data strongly suggest the existence of an increase in sugar feeding in mosquitoes infected with *Plasmodium*. We suggest different adaptive explanations for an enhanced sugar uptake in infected mosquitoes and call for more studies to investigate the physiological role of glucose in the *Plasmodium*–mosquito interaction.

Keywords: malaria; parasite virulence; vector–parasite interactions; resource allocation; feeding behaviour

1. INTRODUCTION

Models of malaria epidemiology and evolution are frequently based on the assumption that vector–parasite associations are benign (Anderson & May 1991; Gandon *et al.* 2001). However, evidence that the malaria parasites (*Plasmodium* spp.) reduce the survival (Ferguson & Read 2002*b*) and fecundity (Hacker 1971; Hacker & Kilama 1974; Freier & Friedman 1976; Hogg & Hurd 1995*a*,*b*, 1997) of their mosquito vectors (*Anopheles* spp.) continues to accumulate.

Our ability to identify the possible epidemiological and evolutionary consequences of these parasite-induced fitness reductions has been hampered by a poor understanding of the origin and physiological basis of these shifts. One explanation for the pathogenicity of *Plasmodium* to its vectors is that parasites damage vital organs such as the midgut or salivary glands (Sinden & Billingsley 2001), disrupt mosquito physiology (e.g. change the levels of salivary-gland proteins; Shandilya *et al.* 1999) and/or increase the risk of secondary infection by other pathogens (Seitz *et al.* 1987). An additional explanation, and one of particular interest for its potential adaptive implications, is that the reductions in fecundity and longevity could be the result of a depletion or reallocation of the energetic resources of the mosquito (Maier *et al.* 1987; Hurd 2001).

Energetic allocation is central to many theories concerning evolutionary and other life-history shifts (Chippindale *et al.* 1993). Energetic constraints imposed by parasite development have been implicated in the observed change in life-history parameters in several host–parasite systems (Toft 1991; Kearns *et al.* 1994; Sorensen & Minchella 1998). In malaria vectors, energy depletion would come about either as a result of a direct competition for resources between *Plasmodium* and the mosquito (Maier *et al.* 1987) or because infected mosquitoes require extra nutrients to compensate for parasite damage (e.g. tissue repair) or to fuel the mounting of a costly immune response (Ferdig *et al.* 1993; Ahmed *et al.* 2002).

In this study, we examine whether energetic constraints could account for the virulence of the rodent malaria *P. chabaudi* in its *A. stephensi* mosquito vector. Recent laboratory studies of this parasite have shown that infection reduces the longevity (Ferguson & Read 2002*a*) and fecundity (Ferguson *et al.* 2003) of mosquitoes. The magnitude of fitness reductions, however, varies strongly with parasite genotype (Ferguson & Read 2002*a*; Ferguson *et al.* 2003). The physiological basis of such genotype-specific virulence is unknown. Genetically different parasite strains may have different physiological requirements, or induce variation in mosquito resource use and/or nutrient uptake.

We infected mosquitoes with two different parasite genotypes and their mixture, treatments that are known to generate variable levels of virulence in *A. stephensi* (Ferguson & Read 2002*a*; Ferguson *et al.* 2003), and measured the levels of the three key insect energetic and nutritional resources: sugar, glycogen and lipids (Gillot 1980; Clements 1992; Nijhout 1994; Rivero & Casas 1999). In addition, the levels of protein, an important structural component but only rarely burned as fuel (Clements 1992), were also measured. These resources were analysed at two different time points during the parasite's development:

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(i) when the parasite was growing in the mosquito midgut as an oocyst (*ca*. 7–8 days after infection); and
(ii) when the transmissible sporozoite stage had invaded the mosquito salivary gland (*ca*. 14 days after infection).

We made two specific predictions with respect to the physiological basis of *P. chabaudi*-induced fitness reductions in mosquitoes:

(i) we expected mosquitoes infected with *Plasmodium* to have less energetic resources than control (uninfected) mosquitoes; and
(ii) we expected the energetic budget of mosquitoes to be negatively correlated with parasite genotype-specific virulence (reduction in mosquito fecundity and/or survival).

In addition, we tentatively expected shifts in the energetic budget of mosquitoes between the oocyst stage and the sporozoite stage of the parasite's development. Although evolutionary theory predicts that parasite virulence should be minimal during the oocyst stage (Schwartz & Koella 2001), oocysts are metabolically demanding (Maier *et al*. 1987) and thus are likely to impose a drain on the mosquito's resources. Thus, we may expect energetic depletion to be more severe in the oocyst stage than in the sporozoite stage of the parasite's development. We discuss the potential adaptive significance of our results and suggest how physiological studies of resource use and uptake in infected mosquitoes are useful to understanding the basis and probable evolution of mosquito–*Plasmodium* interactions.

2. MATERIAL AND METHODS

(a) *Mosquito rearing and infection*

Anopheles stephensi larvae were reared under standard insectarium conditions at 27 °C, 70% humidity and a 12 L : 12 D photo-period. Eggs were placed in plastic trays (25 cm × 25 cm × 7 cm) filled with 1.5 l of distilled water. To reduce variation in adult size at emergence, larvae were reared at a fixed density of 500 per tray. Larvae were fed on Liquifry for 5 days and then on ground TetraFin fish flakes. On days 10–13, groups of 250 pupae were randomly taken from the rearing trays and placed in one of 13 mesh cages (16 cm × 16 cm × 16 cm). The adults that emerged (approximately 160–240 per cage) were fed *ad libitum* on a 10% glucose solution supplemented with 0.05% para-aminobenzoic acid (PABA).

Two clones of *P. chabaudi*, known as CR and ER, were used (from the World Health Organization's Registry of Standard Malaria Parasites, University of Edinburgh; Beale *et al.* 1978). Previous studies have shown that, while ER is relatively benign in mosquitoes, CR reduces their longevity when access to sugar is restricted and a combination of CR and ER (henceforth CR/ER) reduces both their longevity and their fecundity when sugar is provided *ad libitum* (Ferguson & Read 2002*a*; Ferguson *et al.* 2003). Mice (c57BL/6J) were infected with 10^6 parasites of CR ($n = 3$), 10^6 parasites of ER ($n = 3$), or 10^6 parasites of a 1 : 1 mix of CR and ER (CR/ER, $n = 3$). Four uninfected mice were maintained as controls (total = 13 mice). From the fifth day of infection onwards, thin blood smears were taken on a daily basis. Mosquito feeds took place 14 days after mouse infection, when all infected mice had sufficiently high gametocytaemia (a proportion of red blood cells infected with gametocytes of greater than 0.1%). On the day of the feed, the number of red blood cells in a 2 µl blood sample (RBC) and gametocyte density (RBC × gametocytaemia) were recorded.

Mosquitoes were deprived of glucose for 24 hours before the feed. Blood feeds were carried out by placing one anaesthetized mouse on top of a randomly chosen cage for 20 min. Immediately after the feed, 35–40 fully engorged females were taken from the cage and placed individually into 30 ml plastic tubes covered with mesh. Food was henceforth provided in the form of a cotton pad soaked in a 10% glucose solution (with 0.5% PABA) placed on top of each tube. This cotton pad was replaced daily throughout the remainder of the experiment.

Mosquitoes were kept in the tubes for 3 days to allow all haematin (a by-product of the decomposition of haemoglobin) to be excreted. Blood meal size was indirectly estimated from the amount of haematin excreted (as in Briegel *et al.* 1978). For this purpose, on the day of collection (third day after infection), all excreted haematin was dissolved in 1 ml of a 1% $LiCO_3$ solution. The absorbance of the resulting mixture was read at 387 nm, using $LiCO_3$ solution as a blank, and compared with a standard curve made with porcine serum haematin (Sigma-Aldrich). Solutions that were within the error range of the $LiCO_3$ blanks (absorbance of not more than 0.01) were eliminated from the analysis and the mosquito was classified as a non-feeder.

After the 3 day haematin-collection period, the females were moved to new 30 ml tubes containing *ca*. 3 ml of water to allow oviposition. On day 7, half of the mosquitoes from each cage were randomly selected and killed with chloroform. One wing was removed from each and measured along its longest axis as an index of body size, and their midguts were dissected to determine oocyst load (see § 2b). The remaining mosquitoes were left in the tubes to allow the parasites to develop to sporozoite stage. On day 14 the remainder of the mosquitoes were killed with chloroform and measured as above, and their salivary glands were dissected to determine whether sporozoites were present (see § 2b).

(b) *Mosquito dissections*

Mosquitoes were dissected under a binocular microscope in 100 µl of 0.01 M phosphate-buffered saline (PBS). After dissection, the midguts were transferred to a new slide with a pin, placed under a cover slip and observed under a compound microscope to assess parasite presence and burden (number of oocysts per gut). Mosquitoes collected at the sporozoite stage (day 14) were also dissected in 100 µl of PBS. The salivary glands were transferred to a new slide, crushed and observed under a compound microscope to determine whether there were sporozoites in the gland (sporozoite density was not estimated). In both stages, the bodies of mosquitoes fed on uninfected mice were dissected and treated in the same way as the rest. For the CR, ER and CR/ER treatments, the mosquitoes where at least one oocyst or sporozoite was found were classified as 'infected', and the rest were classified as 'uninfected'. The dissected bodies of the females in each cage were then divided into two groups. Half of the females were randomly allocated to the quantification of lipids, glycogen and sugars (which can be carried out on the same specimen) (see § 2c) and half to the quantification of proteins (see § 2d).

(c) *Quantification of lipids, sugars and glycogen*

The dissected bodies of the females allocated to the quantification of lipids, sugars and glycogen were transferred individually to Pyrex glass tubes (7.3 cm in length, 1 cm in diameter) and crushed with a glass rod. The saline solution in which each female was dissected was recovered with a pipette and added to the tube. We added 100 µl of sodium sulphate (which adsorbs glycogen) and 750 µl of a 1 : 2 chloroform–methanol solution (which dissolve lipids and sugars, respectively) to the tube, which was then covered and left to react at room temperature for 24 hours. Subsequent analysis was carried out in blocks of 24–25 randomly chosen tubes. For each analysis block a blank tube was prepared following exactly the same procedure without adding the crushed mosquito body.

Quantification of lipids, glycogen and sugars (glucose) on the same specimen was carried out using a colorimetric technique developed for mosquito analysis (for a detailed description of the technique see Van Handel (1985*a*,*b*, 1988)). Briefly, sample tubes were centrifuged and the chloroform–methanol supernatant was separated into two fractions: one for lipid analysis and the other one for sugar analysis. For lipid determination, the solvent was evaporated completely in a heating block and sulphuric acid was added to the tubes, which were then reheated to convert the unsaturated lipids to water-soluble sulphonic acid derivatives (Van Handel 1985*b*). These develop a deep pink colour after addition of a vanillin–phosphoric acid reagent, which is read in a spectrophotometer at OD_{525}. Lipid concentrations were obtained from a standard curve made with vegetable oil.

For sugar determination, the solvent was evaporated in a heating block and the residue was then heated with anthrone–sulphuric acid reagent (Van Handel 1985*a*). The heat breaks down body sugars into their glucose units and the anthrone binds to them, turning the mixture green. Tubes are then read at OD_{625} against the blank, and sugar concentrations are obtained from a standard curve made with glucose. Finally, the precipitate in the original tube, containing the glycogen, was first washed with methanol to eliminate residual sugars and then heated with anthrone and read at OD_{625} against the blank. As with the sugars, concentrations were obtained from a standard glucose curve.

(d) *Quantification of proteins*

The dissected bodies of the females allocated to the quantification of proteins were placed in a 1.5 ml Eppendorf tube and crushed with a glass rod, and 100 µl of a saline solution (0.15 M NaCl) and 0.001% Triton X-100 (Sigma-Aldrich) was added. The blanks consisted of saline solution and Triton solution but no crushed mosquito. Tubes were left for 5 days at 4 °C for the Triton to dissolve the proteins in the body.

Protein analysis was carried out using the Bradford dye-binding microassay procedure (Bradford 1976). For this purpose, 80 µl of the sample was extracted into a plastic tube (7.5 cm in length, 1 cm in diameter), to which 720 µl of physiologic water and 200 µl of Bradford reagent (Bio-Rad Laboratories, Munich, Germany) were added. After 15 min, samples were read in a spectrophotometer (Jenway-6300) at OD_{595}. Protein concentrations were obtained from a standard curve based on bovine serum albumin (Sigma-Aldrich).

(e) *Statistical analysis*

The data were analysed with the SAS statistical package (SAS Institute, Inc. 1997). The energetic value of sugars and glycogen was calculated as 16.74 J mg^{-1} and that of lipids as 37.65 J mg^{-1} (Clements 1992). Analyses were done both on total energetic reserves (summed value of lipids, glycogen and sugars) and separately for each of the different resources (glucose, lipids, glycogen and protein). Each resource response variable was modelled as a function of parasite genotype (control, ER, CR or CR/ER), mouse (nested within genotype), body size (wing length), chemical analysis block and blood meal size (haematin mass). The maximal model was simplified by sequentially eliminating non-significant terms and interactions. After the minimal adequate model (the model including only significant terms and interactions) was obtained, its appropriateness was tested by inspecting a plot of the residuals against the fitted values. The significant values given in the text are for the minimal model, while non-significant values are those obtained before the deletion of the variable from the model.

3. RESULTS

The average energetic resources available to mosquitoes were 7.00 J (± 0.19 s.e., $n = 119$) on day 7 (during the oocyst stage of parasite development) and 7.14 J (± 0.31 s.e., $n = 76$) on day 14 (sporozoite stage). Contrary to expectations, the energetic content of mosquitoes fed on a control host was no different from that of those fed on a *Plasmodium*-infected host at either day 7 ($F_{1,76} = 0.01$, n.s.) or day 14 ($F_{1,66} = 0.70$, n.s.). Neither were there differences between the total energy contents of mosquitoes across parasite treatments (control, ER, CR, CR/ER) at either sampling date (day 7: $F_{3,86} = 1.21$, n.s.; day 14: $F_{3,56} = 1.31$, n.s.; table 1). However, on day 7, mosquitoes that fed on the CR- and CR/ER-infected mice contained *ca.* 50% more glucose in their bodies than mosquitoes in either the control or the ER treatments (figure 1). This difference, which was very significant ($F_{3,105} = 4.06$, $p < 0.01$), was not explained by differences in blood meal size ($F_{1,79} = 0.07$, n.s.). By contrast, the levels of glucose found on day 14 showed no differences between the four different treatments ($F_{3,76} = 0.31$, n.s.; figure 1) and were generally lower than those found on day 7 ($F_{1,184} = 4.32$, $p < 0.05$).

Contrary to the results of the glucose analysis, glycogen, lipid and protein levels were unaffected by parasite treatment on both day 7 (lipid: $F_{3,91} = 0.90$, n.s.; glycogen: $F_{3,82} = 0.21$ n.s.; protein: $F_{3,79} = 0.08$, n.s.) and day 14 (lipid: $F_{3,62} = 0.80$, n.s.; glycogen: $F_{3,71} = 0.28$ n.s.; protein: $F_{3,61} = 0.17$, n.s.). Blood meal size did not have a significant effect on the abundance of any of the nutritional resources at day 7 (lipid: $F_{1,91} = 0.02$, n.s.; glycogen: $F_{1,94} = 4.04$, n.s.; protein: $F_{1,74} = 0.00$, n.s.) or on the levels of glycogen ($F_{1,71} = 0.15$, n.s.) or protein ($F_{1,64} = 2.17$, n.s.) at day 14. The amount of blood consumed was, however, significantly negatively correlated with the level of lipids at day 14 ($F_{1,76} = 8.31$, $p < 0.01$).

To explore further the nature of the increase in glucose at day 7 in mosquitoes allocated to the CR and CR/ER treatments, we carried out a subsequent analysis where we distinguished between mosquitoes that had been exposed to the parasite (i.e. those that fed on ER-, CR- or CR/ER-infected mice) but remained uninfected (i.e. no oocysts found on dissection) and those that became infected (49.2% of the total). The rationale behind this analysis was to determine whether the decrease in glucose was caused by a change in the quality and/or quantity of

Table 1. Summary of the mean ± s.e. amounts of energetic resources (lipids, glucose and glycogen) and total energetic content obtained from control mosquitoes and mosquitoes exposed to ER-, CR- or ER/CR-infected blood at two different times (days 7 and 14, corresponding to the oocyst and sporozoite stages of parasite development, respectively).
(Values are given both in μg and in the energetic equivalent (J).)

	control		ER		CR		ER/CR	
	day 7	day 14	day 7	day 14	day 7	day 14	day 7	day 14
lipids (μg)	97.3 ± 4.7	110.7 ± 10.1	92.8 ± 6.3	110.9 ± 7.9	93.9 ± 7.2	123.6 ± 13.8	92.9 ± 4.8	170.7 ± 19.1
(J)	(3.7 ± 0.2)	(4.2 ± 0.4)	(3.5 ± 0.2)	(4.2 ± 0.3)	(3.5 ± 0.3)	(4.7 ± 0.5)	(3.5 ± 0.2)	(6.4 ± 0.7)
glucose (μg)	101.9 ± 17.1	69.3 ± 11.4	99.2 ± 15.5	60.9 ± 10.8	129.6 ± 21.0	59.7 ± 7.0	160.5 ± 30.0	81.0 ± 9.9
(J)	(1.7 ± 0.3)	(1.2 ± 0.2)	(1.6 ± 0.3)	(1.0 ± 0.2)	(2.2 ± 0.3)	(1.0 ± 0.1)	(2.7 ± 0.5)	(1.3 ± 0.3)
glycogen (μg)	82.2 ± 8.7	82.3 ± 12.5	83.9 ± 8.6	74.3 ± 10.5	93.7 ± 8.7	74.5 ± 9.8	85.7 ± 7.5	80.1 ± 9.9
(J)	(1.4 ± 0.1)	(1.4 ± 0.2)	(1.4 ± 0.1)	(1.2 ± 0.2)	(1.6 ± 0.1)	(1.2 ± 0.2)	(1.4 ± 0.1)	(1.3 ± 0.2)
total energetic content (J)	6.8 ± 0.3	6.9 ± 0.6	6.6 ± 0.35	6.5 ± 0.4	7.1 ± 0.4	6.5 ± 0.7	7.6 ± 0.4	8.6 ± 0.7

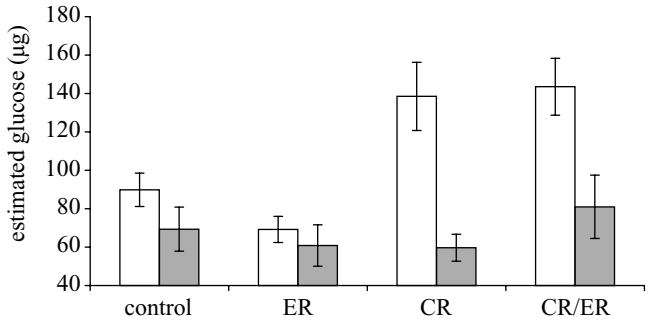

Figure 1. Mean amount of glucose (μg) in mosquitoes dissected on day 7 (during the oocyst stage of parasite development, white bars) and day 14 (sporozoite stage, dark bars) for each of the treatments (control, ER, CR and a 1 : 1 mixture of CR and ER). Figures are the values predicted by the minimal adequate model. Bars represent standard errors.

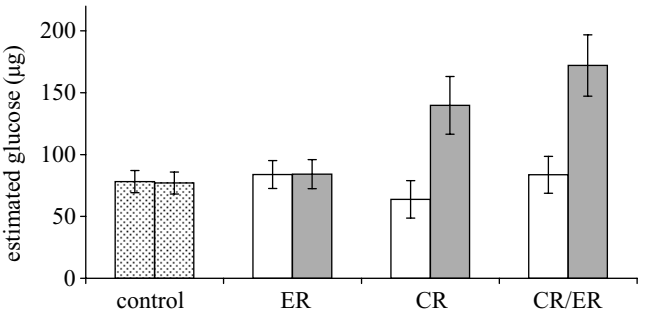

Figure 2. Comparison of the mean amounts of glucose (μg) in females dissected on day 7. Control females (dotted bars) were compared with females that were exposed to each of the parasite genotypes (ER, CR and a 1 : 1 mixture of CR and ER) but did not become infected (i.e. no oocysts found on dissection, white bars) and with females that did become infected (at least one oocyst found on dissection, dark bars). Figures are the values predicted by the respective minimal adequate models. Bars represent standard errors.

infected blood consumed, or by the presence of the parasite itself. We carried out a first analysis where we defined the presence or absence of oocysts in the gut as a binary explanatory factor: 'infected' or 'uninfected' (this analysis excluded unexposed, i.e. control, mosquitoes). Oocyst presence, regardless of parasite genotype, was a very significant predictor of glucose resource level ($F_{1,64} = 8.33$, $p < 0.01$). Further analyses were then carried out separately on infected and uninfected mosquitoes. Mosquitoes exposed to but not infected by the parasite contained similar amounts of glucose ($F_{3,61} = 0.50$, n.s.), lipid ($F_{3,54} = 0.91$, n.s.), glycogen ($F_{3,54} = 0.42$, n.s.) and protein ($F_{3,45} = 0.37$, n.s.) to non-exposed (i.e. control) mosquitoes, irrespective of the parasite genotype. By contrast, mosquitoes exposed to *and* infected by the CR and CR/ER genotypes contained significantly higher amounts of glucose than unexposed mosquitoes and mosquitoes infected by the ER genotype ($F_{3,61} = 3.62$, $p < 0.01$; figure 2). No differences were, however, found in their levels of lipid ($F_{3,62} = 0.14$, n.s.), glycogen ($F_{3,61} = 0.42$, n.s.) or protein ($F_{3,44} = 0.21$, n.s.) or in their total energetic content ($F_{3,60} = 1.29$, n.s.).

A final analysis was carried out to determine whether the increased sugar levels in oocyst-infected mosquitoes were dependent on the level of infection. Sugar levels, however, were not dependent on the number of oocysts found in the midgut ($F_{1,35} = 0.97$, n.s., for the main effect of oocyst number, and $F_{2,35} = 0.42$, n.s., for the interaction between parasite genotype and oocyst number).

4. DISCUSSION

Plasmodium chabaudi made no difference to the energy levels of infected mosquitoes. The overall resource levels of mosquitoes infected with the most virulent genotype (CR) or genotype combination (CR/ER) of the parasite were no different from those of control mosquitoes or mosquitoes infected with an avirulent genotype (ER). Energy depletion cannot therefore account for the reduction in fecundity and longevity observed in mosquitoes infected with virulent genotypes (Ferguson *et al.* 2003). Furthermore, not only was there no evidence of a parasite-mediated loss in the overall energetic budget of mosquitoes, but *Plasmodium* was actually associated with increased levels of sugars, a key insect nutritional and energetic resource. Specifically, mosquitoes infected with the oocyst stages of the CR and CR/ER genotypes con-

tained up to 50% more sugars (glucose) than control mosquitoes or mosquitoes infected with an avirulent parasite genotype (ER). This apparent contradiction (same total energetic content despite a 50% increase in glucose) is simply the result of sugars having a low energetic content relative to lipids (see § 2e).

Sugars are a key ready-to-use source of metabolic energy and the sole energy source for flight in mosquitoes (Clements 1992). Free sugars such as glucose are either the product of the breakdown of stored molecules (such as glycogen and proteins; Clements 1999; Nijhout 1994) or ingested *de novo* either with the blood meal (Foster 1995) or from flower or extra-flower nectar and honeydew (Yee & Foster 1992; Foster 1995; Holliday Hanson *et al.* 1997; Burket *et al.* 1999; Clements 1999; Takken & Knols 1999). There are thus three different possibilities for the origin of the excess sugar found in mosquitoes infected with the virulent genotypes of *P. chabaudi*:

(i) a reallocation of resources within the mosquito as a response to the infection;
(ii) qualitative or quantitative differences in the glucose content of the ingested blood; or
(iii) differences in the amount of sugar ingested after infection.

Shifts in resource allocation in response to parasite infection are well documented, although they are often inferred from shifts in life-history traits, such as fecundity and longevity (Sorensen & Minchella 1998; Hurd 1998, 2001; Hurd *et al.* 2001), and are only rarely measured directly (Kearns *et al.* 1994; Brown *et al.* 2000). In our experiment, we tested for nutrient reallocation by measuring the levels of other resources in the body that are known to produce glucose as a result of their catabolism: glycogen and protein (Nijhout 1994; Clements 1999). We did not find a significant reduction in the levels of glycogen or protein concomitant with the increased levels of glucose in the CR- and CR/ER-infected mosquitoes, and thus the possibility of resource reallocation was dismissed. Thus, the only explanation for our results is that the increased levels of glucose were acquired *de novo*.

The sugar content of blood is low (Foster 1995) particularly in *Plasmodium*-infected hosts (Elased & Playfair 1994, 1996; Elased *et al.* 1996), and blood is consumed to acquire the proteins necessary for egg production rather than for its caloric value (Clements 1992). No differences were found in the quantity of blood consumed by mosquitoes allocated to the four different treatments. The increase in glucose was observed only in mosquitoes that fed on blood infected with CR or CR/ER and subsequently developed oocysts. The glucose levels of mosquitoes that fed on the same infected blood but did not develop oocysts were no different from those of the controls. The observed increase in glucose is therefore not a by-product of qualitative changes in the blood of mice infected with the parasite.

The high levels of glucose found in mosquitoes from the CR and CR/ER treatments must therefore have arisen from increased glucose intake subsequent to infection. Furthermore, since this increase in glucose was observed only in oocyst-infected mosquitoes, the increased sugar feeding was, directly or indirectly, the result of infection by the parasite. The modification of the blood-feeding behaviour of mosquito vectors by the malaria parasite has been well established (Wekesa *et al.* 1992; Koella *et al.* 1998; Anderson *et al.* 1999) and can be clearly advantageous for the parasite (Anderson *et al.* 1999). Is the change in sugar-feeding behaviour also parasite driven, or is it a host response to parasitism?

We propose three different ways in which a manipulation of the mosquito's sugar-feeding behaviour could be beneficial to the oocyst stages of the parasite. First, there is evidence that sugar availability reduces the frequency of host biting in the laboratory, possibly because sugar-loaded mosquitoes are constrained in their ability to obtain blood meals owing to space limitations in the abdomen (Foster & Eischen 1987; Straif & Beier 1996). Host biting is known to incur mortality risks for mosquitoes, owing to the defensive behaviour of the vertebrate hosts (Day & Edman 1983), and this risk has been shown to be disproportionately high in sporozoite-infected mosquitoes (Anderson *et al.* 2000). Increased sugar feeding could thus benefit the parasite by reducing mosquito mortality rates during the non-transmissible stages of the infection. Second, parasites may manipulate the sugar-feeding behaviour of mosquitoes to meet the high glucose requirements of the oocysts. Schiefer *et al.* (1977) reported a study in which isolated oocyst-infected midguts used up to eight times the amount of glucose metabolized by non-infected midguts. Unfortunately, this often-cited study was not published, so the suggestion that oocyst-infected midguts have high glucose requirements remains possible but unconfirmed. Finally, a tantalizing possibility is that *Plasmodium* induces enhanced glucose uptake to neutralize the mosquito's immune system. In many organisms, a glucose overload impairs the production of nitric oxide (Prabhakar 2000; Golderer *et al.* 2001; Kimura *et al.* 2001), a molecule that in *A. stephensi* is an important line of defence against *Plasmodium* development (Luckhart *et al.* 1998; Luckhart & Rosenberg 1999; Han *et al.* 2000). The role of glucose in NO production in mosquitoes and the possibility that parasites could manipulate sugar intake to fight off the immune system of the host are intriguing and should be explored further.

It is also possible that enhanced sugar feeding could be the mosquito's response to parasitism. By increasing the consumption of sugars, which are important precursors of all carbon-based chemical compounds and an essential resource for maintenance in mosquitoes (Clements 1999), infected females could minimize or compensate for the harm caused by the parasite, such as damage to midgut epithelial cells and competition for host metabolic products (Maier *et al.* 1987). In a recent laboratory experiment, however, mosquitoes infected with a CR/ER mixture showed reduced longevity (Ferguson & Read 2002*a*) and fecundity (Ferguson *et al.* 2003) when sugar was provided *ad libitum*, but not when sugar was restricted, which does not seem to support this hypothesis.

In conclusion, although we found no evidence of energy depletion as a mechanism for virulence in the *P. chabaudi*–*A. stephensi* system, our results suggest that parasitism, specifically by virulent genotypes, affects the rate of mosquito resource acquisition (sugar intake). Further investigation of the physiological role of sugar in *Plasmodium*-infected mosquitoes, and its interactions with parasite

genotype, is required to evaluate adaptive hypotheses for the phenomenon. Ultimately, whether increased sugar feeding is adaptive for the parasite or for the mosquito, or simply a by-product of the infection, will be determined by which party's fitness is enhanced by this behaviour. Resolution of this issue will provide insight into the adaptive nature of vector–parasite interactions and their epidemiological consequences.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.