Environmental influence on the genetic basis of mosquito resistance to malaria parasites

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The genetic basis of a host's resistance to parasites has important epidemiological and evolutionary consequences. Understanding this genetic basis can be complicated by non-genetic factors, such as environmental quality, which may influence the expression of genetic resistance and profoundly alter patterns of disease and the host's response to selection. In particular, understanding the environmental influence on the genetic resistance of mosquitoes to malaria gives valuable knowledge concerning the use of malaria-resistant transgenic mosquitoes as a measure of malaria control. We made a step towards this understanding by challenging eight isofemale lines of the malaria vector *Anopheles stephensi* with the rodent malaria parasite *Plasmodium yoelii yoelii* and by feeding the mosquitoes with different concentrations of glucose. The isofemale lines differed in infection loads (the numbers of oocysts), corroborating earlier studies showing a genetic basis of resistance. In contrast, the proportion of infected mosquitoes did not differ among lines, suggesting that the genetic component underlying infection load differs from the genetic component underlying infection rate. In addition, the mean infection load and, in particular, its heritable variation in mosquitoes depended on the concentration of glucose, which suggests that the environment affects the expression and the evolution of the mosquitoes' resistance in nature. We found no evidence of genotype-by-environment interactions, i.e. the lines responded similarly to environmental variation. Overall, these results indicate that environmental variation can significantly reduce the importance of genes in determining the resistance of mosquitoes to malaria infection.

Keywords: *Anopheles stephensi*; *Plasmodium yoelii yoelii*; resistance; environment; malaria

1. INTRODUCTION

Understanding the genetic basis of a host's resistance to parasites represents a major pursuit for parasitologists, as it determines to a large extent natural patterns of infection (Lively & Apanius 1995). It is also a fundamental issue for evolutionary biologists, as variation in the genes underlying resistance and influencing the expression of disease enables parasite-mediated selection to occur (Hamilton 1980; Anderson & May 1982; Webster & Davies 2001). The extent of genetic variation for resistance to parasites thus has important evolutionary and epidemiological consequences (Sorci *et al.* 1997). Therefore, even in cases where it is clear that resistance has a genetic underpinning, it is important to understand whether and to what extent the level of resistance can be influenced by non-genetic factors such as the quality of the environment. Indeed, even slight environmental changes can influence differences among individuals and thus modify greatly the outcome of infection (Thomas & Blanford 2003). This might explain why the genetic component of resistance sometimes fails to predict disease patterns in the field (Scott 1991) or host responses to selection (Henter & Via

1995). Thus, the influence of the environment on the genetic resistance of hosts has considerable implications for host–parasite coevolution and for epidemiology.

The environment may alter the expression of genetic resistance if the extent of genetic variability of a host's resistance depends on the environmental conditions. Thus, for example, the heritability of many traits is higher in poor than in rich environments (Hoffman & Parson 1991), which can lead to discrepancies between laboratory and field estimates of heritability (Sorci *et al.* 1997). At the extreme, if environmental factors overwhelm the effect of the genotype, resistance alleles can be a poor predictor of disease patterns because an individual's resistance does not reflect the underlying genetic basis. In addition, parasite-mediated selection might be impaired, as natural selection can only occur if the heritable basis of resistance is not swamped by the environmental noise (Little & Ebert 2000). The environment may also influence the expression of resistance if the phenotype is determined by genotype-by-environment interactions, i.e. if different genotypes respond differently to variation of the environmental conditions (Thomas & Blanford 2003; Mitchell *et al.* 2005). This situation implies that natural selection on resistance may lead to different evolutionary results in different environments.

Such ideas are particularly relevant for the resistance of mosquitoes to malaria parasites of the genus *Plasmodium*,

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as resistance (an important component of the parasite's basic reproductive number and thus its intensity of transmission) is the focus of current efforts to control malaria with genetically modified mosquitoes that would interrupt the development of *Plasmodium* parasites and thus block disease transmission (Beaty 2000; Aultman *et al.* 2001; Alphey *et al.* 2002). These efforts are based on the fact that resistance to malaria parasites is at least partly determined by the mosquito's genes, as was first demonstrated by the successful artificial selection of a mosquito strain that is refractory to infection by several malaria parasites (Collins *et al.* 1986) and has been confirmed by, for example, the observation of genetic variation for resistance in a natural West African population of mosquitoes (Niaré *et al.* 2002). The recent identification of several mosquito genes that affect the development of *Plasmodium* in a rodent model of malaria (Blandin *et al.* 2004; Osta *et al.* 2004; Michel *et al.* 2005), together with advances in the molecular tools that can transform mosquitoes (Coates *et al.* 1998; Catteruccia *et al.* 2000), has considerably raised the hope for the use of genetically manipulated mosquitoes in malaria control. Yet, before genetic manipulation becomes a realistic control method, several challenges need to be overcome (Riehle *et al.* 2003). In particular, a laboratory technology has to be transferred into field conditions, which are generally very different from the laboratory conditions in which transgenic mosquitoes are created (Scott *et al.* 2002). Indeed, if we choose to neglect social problems such as the acceptability of genetically modified mosquitoes, the efficacy of the control will largely depend on the efficacy of mosquito resistance genes in natural situations (Boëte & Koella 2002), i.e. the importance of non-genetic factors on resistance. Thus, a critical step in developing genetically manipulated mosquitoes for the control of malaria is to evaluate the environmental influence on the genes conferring resistance to malaria (Tabachnick 2003; Boëte 2005).

Here, we address this question by measuring the influence of environmental quality on the resistance of different genotypes of mosquitoes to infection by malaria parasites. A first step in this direction was the earlier demonstration that adult nutrition—blood and sugar feeding—influences the melanization response of *Anopheles stephensi* mosquitoes (Koella & Sorensen 2002), an immune defence that is determined genetically and that can potentially block the development of *Plasmodium* in the mosquito (Collins *et al.* 1986; Gorman *et al.* 1996). We extend this study by challenging several genetic backgrounds of the malaria vector *A. stephensi* with the rodent malaria parasite *Plasmodium yoelii yoelii* in different environmental conditions. Specifically, we used eight mosquito isofemale lines as different genetic backgrounds of the mosquito, and varied environmental quality by feeding adult mosquitoes with glucose solutions of different concentrations (2, 4 and 6%). Although it is not sure to what extent some African anopheline mosquitoes use sugar in natural situations (Beier 1996; Gary & Foster 2004), females of most mosquito species are known to use sugar as a necessary source of energy (Clements 1992; Foster 1995). In *A. stephensi* mosquitoes, sugar feeding has been shown to affect the virulence of malaria parasites (Ferguson & Read 2002) and the strength of at least one component of the immune

response (Koella & Sorensen 2002). We used a standard analysis of quantitative genetics to assess the effect of the mosquito's genotype (i.e. the isofemale line), the effect of the environment (glucose concentration) and the effect of the genotype-by-environment interaction (interaction between glucose and line) on the mosquito's resistance to malaria infection. Resistance was quantified with the proportion of infected mosquitoes that developed oocysts (infection rate) and with the number of oocysts (infection load) following an infective blood meal. In addition, we measured the influence of glucose concentration on the amount of heritable variation for resistance (estimated by the proportion of phenotypic variation explained by differences among the isofemale lines).

2. MATERIAL AND METHODS

(a) Mosquitoes

We created isofemale lines of the mosquito *A. stephensi* by maintaining the descendants of single females separately from other families for several generations, each generation being produced by mass mating of 25 individuals of each sex from the previous one. We maintained each line with an equal number (approx. 525) of larvae (randomly chosen after hatching) at each generation. The results presented here correspond to the fourth generation of eight lines. Each generation, females were allowed to blood-feed on a rabbit. Larvae were reared in 30 cm × 30 cm plastic trays filled with 3.5 l of tap water at a density of approximately 150 larvae per litre in an insectary with 24 h light. They were fed daily on a mixture of 92% dog biscuits and 8% yeast as food (0.08 mg per larva on day 0; 0.12 mg per larva on day 1; 0.16 mg per larva on day 2; 0.24 mg per larva on day 3; 0.32 mg per larva on day 4; 0.4 mg per larva on day 5; 0.48 mg per larva on day 6; 0.56 mg per larva on day 7; 0.64 mg per larva on day 8; 0.72 mg per larva on day 9; 0.8 mg per larva on the following days). In the fourth generation, adults that emerged 14 or 15 days after hatching were randomly placed into three groups, and each group was given access to cotton soaked with 2, 4 or 6% glucose solutions. Glucose solutions were changed every 2 days. Adult mosquitoes were kept in rearing cages according to the line and the glucose concentration in conditions of 12 light : 12 dark cycle, 23 ± 1 °C and 80 ± 5% humidity. Infections took place when mosquitoes were 4–5 days old.

(b) Infections

Plasmodium yoelii yoelii (strain 17X, clone 1.1) was used to infect four- to six-week-old female Swiss IOPS OF₁ mice (Charles Rivers). In order to increase the number of reticulocytes, a volume of phenylhydrazine (Sigma) equivalent to 100 mg kg⁻¹ was injected intraperitoneally into each mouse. Three days later, mice were inoculated with 10⁶ parasites in NaCl 0.9% (Cooper) from the same stock inoculum. On a daily basis, thin smears of tail blood were taken, fixed with methanol, and stained 45 min in 10% Giemsa (Merck) in phosphate buffer (pH 7.2). Parasitaemia (percentage of infected red blood cells) and gametocytaemia (percentage of red blood cells infected with gametocytes) were evaluated by microscopic inspection of blood films. Mosquitoes were fed on the mice 4 days after mouse infection, when the mice had sufficiently high gametocytaemia (more than 0.2%). Mice were anaesthetized with a 50 µl injection of 16.7%, Rompun 2% (Bayer) and 33.3% Ketamin

1000 (Virbac) in NaCl 0.9% (Cooper). Four different mice were used in total, and each mouse was used to feed two of the eight lines. Mosquitoes were deprived of glucose for 8–10 h before blood feeding. They were immobilized in a cold chamber at 8 °C and transferred to small plastic tubes according to line and glucose concentration just before the feed. Tubes were placed randomly two by two below a mouse, and mosquitoes were allowed to take a blood meal for 20 min. After the feed, unfed and partially fed mosquitoes were discarded, and 25 fully fed mosquitoes of each line and glucose combination were returned to their rearing cages and supplied with glucose solution according to the initial treatment. Dead mosquitoes were counted daily from the blood meal to the eighth day after the blood meal. For each line and glucose combination, twenty-five 4–5 days old mosquitoes were also fed on an uninfected blood source as controls, and their mortality was monitored daily. Most of the results presented here correspond to control mosquitoes blood-fed on a rabbit, but we ensured that there was no qualitative difference by using an uninfected mouse. Eight days after the infective blood meal, 10 mosquitoes of each line and glucose combination were randomly collected and dissected. Their midguts were observed microscopically in 0.9% NaCl (Cooper) in order to assess oocyst prevalence and load. For each mosquito we measured the wing length from the tip (excluding the fringe) to the distal end of the allula (with a precision of 0.05 mm) as an indication of adult body size. If both wings could be measured, we used the mean of the two lengths. All experimental animals were maintained according to European Union guidelines.

(c) Statistics

To estimate the effects of the mosquito's genotype, the environment and their interaction on the level of resistance, we analysed infection success as a function of line, glucose and their interaction in two steps. First, we analysed the proportion of infected mosquitoes (infection rate) with a nominal logistic analysis. Second, we analysed the number of oocysts (infection load) in infected mosquitoes with an analysis of variance (ANOVA), where we used the square root of the number of oocysts in order to satisfy the assumptions of the statistical tests (in particular, normality of the residuals). In both analyses, the mouse and its interaction with glucose were included as potential confounders. As different lines were fed with each mouse, line was nested within mouse. Mouse and line were considered as random nominal factors, while glucose was considered as a fixed nominal factor. Note that we did not consider glucose concentration as a continuous factor, because we had no *a priori* expectation of the relationship of the infection to glucose, and, in particular, we did not want to assume a linear relationship.

To evaluate the influence of the environment on the amount of heritable variation for resistance, we estimated the contribution of the isofemale line to the phenotypic variation of resistance for each glucose concentration. To do so, we used a variance component analysis of the number of oocysts in infected mosquitoes (transformed using the square root and corrected for the differences among mice by using the residuals) to calculate the percentage of the variance that was explained by the isofemale line relative to the variance due to random factors. Line was considered as a random nominal factor.

Table 1. Analysis of variance of the number of oocysts in infected mosquitoes. (The square root of the number of oocysts was analysed as a function of mosquito line, glucose concentration and their interaction. Mouse was included as a confounding factor. As different lines were fed on different mice, line was nested within mouse.)

source	d.f.	sum of squares	F	p
mouse	3	30.4	0.162	0.917
glucose	2	63.9	16.3	<0.001
line (within mouse)	4	251	32	<0.001
glucose × mouse	6	4.45	0.378	0.893
line × glucose (within mouse)	8	24.3	1.55	0.143
error	187	367		

To estimate the effects of mosquito genotype, environment and genotype-by-environment interactions on parasite-induced mortality, we compared the proportion of mosquitoes that died within 8 days after an infected or an uninfected blood meal with an ANOVA of the arcsine-transformed mortality rate as a function of mosquito line, glucose concentration and infection status of the blood meal. Mosquito line was considered as a random factor, infection status and glucose were considered as fixed factors. We did not include the mouse as a confounder, as initial forward and backward analyses showed that it had no influence on mortality.

In all analyses, we included up to two-ways interactions between factors. All analyses were performed with the software JMP v. 5.0.1.2 (<http://www.jmpdiscovery.com>).

3. RESULTS

We assessed the presence and the number of *P. yoelii yoelii* oocysts by dissecting 10 mosquitoes for each combination of the eight mosquito lines and the three glucose concentrations (i.e. 240 mosquitoes in total). The mean wing lengths in isofemale lines ($\pm$ s.e.) varied between 2.71 ± 0.018 and 2.73 ± 0.016 mm, and they did not differ among glucose treatments (one-way ANOVA: $F_{2,237} = 0.014$, $p = 0.986$) or between lines (one-way ANOVA: $F_{7,232} = 0.297$, $p = 0.954$). Overall, 88% of the mosquitoes were infected, and the infection rate did not differ among glucose treatments ($\chi^2_2 = 2.61 \times 10^{-4}$, $p = 0.999$) or mosquito lines ($\chi^2_4 = 6.01$, $p = 0.198$). By contrast, the infection rate depended on the mouse used to feed the mosquitoes ($\chi^2_3 = 9.84$, $p = 0.02$). The mean infection rate per mouse ranged from 78.3 to 95% and was not significantly correlated to gametocytaemia ($r^2 = 0.272$, $p = 0.479$), which ranged from 0.29 to 0.41% among mice. Among infected mosquitoes, the variation in infection load ranged from 1 to 86 oocysts per midgut (mean: 15.9, median: 12). While neither the mouse used to feed the mosquitoes nor the interaction between mouse and glucose concentration significantly accounted for this variation, differences among lines were substantial (table 1). Averaged across glucose concentrations, the mean infection load ranged from 4 to 26.3 oocysts per line, and the median ranged from 3 to 26 oocysts per line.

In addition to the effect of the isofemale line, the concentration of the glucose solution used to maintain the mosquitoes significantly influenced the infection load

Figure 1. Genetic and environmental components of infection load. The mean numbers of oocysts in infected mosquitoes and their standard errors are given for eight isofemale lines fed on 2% (white bars), 4% (grey bars) and 6% (black bars) glucose solutions. The lines are ranked along the x-axis according to their mean number of oocysts (averaged across glucose concentrations). The numbers of oocysts have been transformed using the square root and corrected for the mice used to feed the mosquitoes (the residual gives the difference to the average for a given mouse).

(table 1). While the number of oocysts, averaged across lines, was similar for mosquitoes fed on 2 and 6% glucose (mean: 11.4, median: 7 and mean: 13.3, median: 8, respectively), it was significantly higher in mosquitoes fed on 4% glucose (mean: 22.5, median: 19). This pattern was observed for each isofemale line (figure 1). Although differences between glucose treatments appeared to be slightly smaller for the most resistant lines compared to the least resistant ones (figure 1), the interaction between line and glucose did not have a statistically significant effect on the number of oocysts (table 1).

Moreover, the effect of the line on the number of oocysts (i.e. an estimation of the genetic component of phenotypic variation) was strongest in mosquitoes fed on 4% glucose. When analysed separately for each glucose concentration, the variation of the number of oocysts due to differences between lines was very significant in each case ($p < 0.001$). However, while the line explained 32.3 and 34.9% of the total variance in mosquitoes fed on 2 and 6% glucose solutions, respectively, it explained 47.3% of the variance in mosquitoes fed on 4% glucose. This can be observed graphically, since within-line variances (related to the vertical lines) are similar in all glucose concentrations, while the variation among lines was greatest at 4% glucose (figure 1).

Mortality following an infected or uninfected blood meal was monitored for 25 mosquitoes of each combination of line and glucose treatment (i.e. for 1200 mosquitoes in total). On average, 23% of the mosquitoes fed on an infected mouse died before the eighth day following the blood meal. This mortality was considerably higher than the average mortality during the same period in control mosquitoes of the same lines that fed on a rabbit (7.3%) or an uninfected mouse (7%). The mosquito line influenced the average mortality rate and, in particular, influenced the effect of the infection status of the blood meal on mortality (table 2). In other words, the rank order of the mortality of a given line depended on whether it had fed on infected or uninfected blood (figure 2). Notably, in infected mosquitoes mortality rates were higher for mosquitoes fed on 2% glucose (33.9%) than for mosquitoes fed on 4% (17.4%) or 6% glucose (17.7%) (figure 2). While this effect of glucose on mortality rates

Table 2. Analysis of variance of the post-blood meal mortality. (The arcsine-transformed mortality rate was analysed as a function of mosquito line, glucose concentration and the infection status of the blood meal.)

source	d.f.	sum of squares ($\times 10^3$)	<i>F</i>	<i>p</i>
line	7	255	23.1	<0.001
glucose	2	202	63.8	<0.001
infection status	1	858	543	<0.001
line $\times$ glucose	14	34.1	1.54	0.214
line $\times$ infection	7	321	29.1	<0.001
glucose $\times$ infection	2	30.6	9.7	0.002
error	14	22.1

Figure 2. Genetic and environmental components of parasite-induced mortality. Mortality that occurred during 8 days following an infected or an uninfected blood meal is given for mosquitoes fed on (a) 2%, (b) 4% and (c) 6% glucose solutions in eight isofemale lines. Each pair of connected symbols represents a different mosquito line.

was also observed in uninfected controls (10.3, 5.9 and 5.8% in 2, 4 and 6% treatments, respectively), the interaction between glucose concentration and infection status of the blood meal (table 2) shows that infected mosquitoes suffered more than uninfected ones in conditions with low glucose.

4. DISCUSSION

We confirmed that the resistance of the mosquito *A. stephensi* to its malaria parasite *P. yoelii yoelii* has a genetic basis, and went one step further by suggesting that the genetic component underlying infection load may

differ from the genetic component underlying infection rate. Moreover, we showed that the environment can affect the expression of the genetic resistance that determines infection load. Indeed, the mean number of oocysts and, in particular, its genetic variance (relative to the variance due to non-genetic factors) depended on the concentration of the glucose solution used to feed adult mosquitoes. There was no evidence of genotype-by-environment interactions, i.e. the isofemale lines responded to different glucose concentrations in similar ways.

It is well established that the resistance of mosquitoes to malaria parasites is partly determined by the mosquito's genes (Collins *et al.* 1986), and the identification of these genes is currently carried out with powerful molecular approaches combining genomics and reverse genetics (Vlachou *et al.* 2005). Our eight *A. stephensi* isofemale lines differed in oocyst numbers but not in the proportion of infected mosquitoes, indicating that the genetic basis for being infected differed from the genetic basis for the intensity of infection. Nevertheless, it is remarkable that, in our mosquito colony, we found a strong genetic variation for the resistance that determines infection load despite the lack of direct selection pressure during the 40 years or so since the colony was established. Given that maintaining resistance genes is usually costly (Yan *et al.* 1997), one might have predicted that resistance to *Plasmodium* would have been lost. The fact that this was not the case suggests that the genetic basis underlying resistance to malaria is subjected to other evolutionary constraints, e.g. genetic correlations between different immune responses (Lambrechts *et al.* 2004) or resistance mechanisms to other pathogens that are more prevalent in a laboratory situation.

In addition to the effect of genes, the environmental factor (glucose concentration) accounted for a substantial variation in infection loads in our experimental design. Thus, mosquitoes fed on 4% glucose produced about twice as many oocysts as mosquitoes fed on 2 or 6% glucose. While the genetic component explained 35.4% of the total phenotypic variance, the environmental factor was responsible for 11.7% of this variation. It should be noted that we considered only three levels of a single environmental factor, and did not take into account others that may impact on mosquito resistance, such as temperature (Thomas & Blanford 2003) or larval nutrition (Suwanichaichinda & Paskewitz 1998). Thus, a substantial part of the variation in oocyst numbers observed in naturally infected mosquitoes might be due to an environmental component.

Moreover, we found that the expression of heritable variation for the resistance that determines infection load differed according to the quality of the environment. Thus, the effect of the isofemale line on the phenotypic variation in oocyst numbers was about 50% higher in mosquitoes fed on 4% glucose than in mosquitoes fed on 2 or 6% glucose. In other words, genetic differences between lines were differentially expressed according to glucose concentration. This has important evolutionary and epidemiological implications, as the amount of genetic variation for resistance is a critical determinant of the response of hosts to parasite-mediated selection, and thus their adaptive potential (Fisher 1958). Depending on its relative importance to the genetic component, and its influence on the expression of genetic variance, the environment

might thus alter the rate of response of mosquito resistance to selection in nature.

In contrast, the effect of glucose concentration on infection loads was similar among mosquito genotypes, so there was no evidence of genotype-by-environment interactions. Although the scale of variation in infection loads between glucose treatments seemed to be greater for the least resistant lines, and although the ranking of lines differed slightly according to glucose concentration, our analysis suggests that these effects could have been due to random factors. This indicates that a mosquito genotype that is most resistant in a given environment is also expected to be most resistant in other environmental conditions.

One striking aspect of our results was that infection load was highest at the intermediate value of glucose concentration. We speculate that this results from the combination of two distinct processes. On the one hand, the efficacy of the mosquito immune system is expected to increase with the quality of dietary resources, as it has been observed, for example, for the melanization immune response of *A. stephensi* mosquitoes (Koella & Sorensen 2002). If the immune mechanisms underlying resistance to malaria react similarly, this would lead to a decline in oocysts numbers with increasing glucose concentration. On the other hand, parasite-induced mortality is likely to be highest at high parasite loads (as suggested by Hogg & Hurd 1995), and in particular so when the concentration of glucose, an important dietary resource (Clements 1992; Foster 1995), is lowest. Indeed, mortality was highest for infected mosquitoes fed on the lowest glucose concentration. If, within this group, it is indeed the mosquitoes with the highest infection loads that were most likely to die, the surviving mosquitoes (i.e. those that were assayed for infection) would have harboured, on average, the least intense infections.

In conclusion, our results suggest that environmental variation can greatly reduce the importance of genes in determining the resistance of mosquito to malaria infection, at least for resistance mechanisms that determines infection load. Over the past two decades, numerous studies on the molecular determinants of *Plasmodium* development in mosquitoes have led to the generation of malaria-resistant transgenic anophelines and proposals of their use as a malaria control measure (Christophides 2005). Our observations suggest that the true usefulness of such transgenic mosquitoes can only be obtained once the influence of the highly variable environmental factors to which they will be subjected in nature has been investigated.

The authors thank S. Bertani, N. Dogna, F. Gonnet-Gonzalez, I. Landau and T. Voza for their assistance during the study, and two anonymous referees for valuable comments on an earlier version of the manuscript.

REFERENCES

- Alphery, L. *et al.* 2002 Malaria control with genetically manipulated insect vectors. *Science* **298**, 119–121. (doi:10.1126/science.1078278)
- Anderson, R. M. & May, R. M. 1982 Coevolution of hosts and parasites. *Parasitology* **85**, 411–426.
- Aultman, K. S., Beaty, B. J. & Walker, E. D. 2001 Genetically manipulated vectors of human disease: a practical overview. *Trends Parasitol.* **17**, 507–509. (doi:10.1016/S1471-4922(01)02094-3)

- Beaty, B. J. 2000 Genetic manipulation of vectors: a potential novel approach for control of vector-borne diseases. *Proc. Natl Acad. Sci. USA* **97**, 10 295–10 297. (doi:10.1073/pnas.97.19.10295)
- Beier, J. C. 1996 Frequent blood-feeding and restrictive sugar-feeding behavior enhance the malaria vector potential of *Anopheles gambiae* s.l. and *An. funestus* (Diptera: Culicidae) in western Kenya. *J. Med. Entomol.* **33**, 613–618.
- Blandin, S., Shiao, S. H., Moita, L. F., Janse, C. J., Waters, A. P., Kafatos, F. C. & Levashina, E. A. 2004 Complement-like protein TEPI is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* **116**, 661–670. (doi:10.1016/S0092-8674(04)00173-4)
- Boëte, C. 2005 Malaria parasites in mosquitoes: laboratory models, evolutionary temptation and the real world. *Trends Parasitol.* **21**, 445–447. (doi:10.1016/j.pt.2005.08.012)
- Boëte, C. & Koella, J. C. 2002 A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control. *Malar. J.* **1**, 3. (doi:10.1186/1475-2875-1-3)
- Catteruccia, F., Nolan, T., Loukeris, T. G., Blass, C., Savakis, C., Kafatos, F. C. & Crisanti, A. 2000 Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature* **405**, 959–962. (doi:10.1038/35016096)
- Christophides, G. K. 2005 Transgenic mosquitoes and malaria transmission. *Cell. Microbiol.* **7**, 325–333.
- Clements, A. N. 1992 *The biology of mosquitoes*. London, UK: Chapman & Hall.
- Coates, C. J., Jasinskiene, N., Miyashiro, L. & James, A. A. 1998 Mariner transposition and transformation of the yellow fever mosquito, *Aedes aegypti*. *Proc. Natl Acad. Sci. USA* **95**, 3748–3751. (doi:10.1073/pnas.95.7.3748)
- Collins, F. H., Sakai, R. K., Vernick, K. D., Paskewitz, S., Seeley, D. C., Miller, L. H., Collins, W. E., Campbell, C. C. & Gwadz, R. W. 1986 Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science* **234**, 607–610.
- Ferguson, H. M. & Read, A. F. 2002 Genetic and environmental determinants of malaria parasite virulence in mosquitoes. *Proc. R. Soc. B* **269**, 1217–1224. (doi:10.1098/rspb.2002.2023)
- Fisher, R. A. 1958 *The genetical theory of natural selection*. New York, NY: Dover.
- Foster, W. A. 1995 Mosquito sugar feeding and reproductive energetics. *Annu. Rev. Entomol.* **40**, 443–474. (doi:10.1146/annurev.en.40.010195.002303)
- Gary Jr, R. E. & Foster, W. A. 2004 *Anopheles gambiae* feeding and survival on honeydew and extra-floral nectar of peridomestic plants. *Med. Vet. Entomol.* **18**, 102–107. (doi:10.1111/j.0269-283X.2004.00483.x)
- Gorman, M. J., Cornel, A. J., Collins, F. H. & Paskewitz, S. M. 1996 A shared genetic mechanism for melanotic encapsulation of CM-sephadex beads and a malaria parasite, *Plasmodium cynomolgi* B, in the mosquito, *Anopheles gambiae*. *Exp. Parasitol.* **84**, 380–386. (doi:10.1006/expr.1996.0126)
- Hamilton, W. D. 1980 Sex versus non-sex versus parasite. *Oikos* **35**, 282–290.
- Henter, H. J. & Via, S. 1995 The potential for coevolution in a host-parasitoid system. I. Genetic variation within an aphid population in susceptibility to a parasitic wasp. *Evolution* **49**, 427–438.
- Hoffman, A. A. & Parson, P. A. 1991 *Evolutionary genetics and environmental stress*. Oxford, UK: Science Publications.
- Hogg, J. C. & Hurd, H. 1995 *Plasmodium yoelii nigeriensis*: the effect of high and low intensity of infection upon the egg production and bloodmeal size of *Anopheles stephensi* during three gonotrophic cycles. *Parasitology* **111**, 555–562.
- Koella, J. C. & Sorensen, F. L. 2002 Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*. *Med. Vet. Entomol.* **16**, 316–320. (doi:10.1046/j.1365-2915.2002.00381.x)
- Lambrechts, L., Vulule, J. M. & Koella, J. C. 2004 Genetic correlation between melanization and antibacterial immune responses in a natural population of the malaria vector *Anopheles gambiae*. *Evolution* **58**, 2377–2381.
- Little, T. J. & Ebert, D. 2000 The cause of parasitic infection in natural populations of *Daphnia* (Crustacea: Cladocera): the role of host genetics. *Proc. R. Soc. B* **267**, 2037–2042. (doi:10.1098/rspb.2000.1246)
- Lively, C. M. & Apanius, V. 1995 Genetic diversity in host-parasite interactions. In *Ecology of infectious diseases in natural populations* (ed. B. T. Grenfell & A. P. Dobson), pp. 421–449. Cambridge, UK: Cambridge University Press.
- Michel, K., Budd, A., Pinto, S., Gibson, T. J. & Kafatos, F. C. 2005 *Anopheles gambiae* SRPN2 facilitates midgut invasion by the malaria parasite *Plasmodium berghei*. *EMBO Rep.* **6**, 891–897. (doi:10.1038/sj.embor.7400478)
- Mitchell, S. E., Rogers, E. S., Little, T. J. & Read, A. F. 2005 Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution* **59**, 70–80.
- Niaré, O. et al. 2002 Genetic loci affecting resistance to human malaria parasites in a West African mosquito vector population. *Science* **298**, 213–216. (doi:10.1126/science.1073420)
- Osta, M. A., Christophides, G. K. & Kafatos, F. C. 2004 Effects of mosquito genes on *Plasmodium* development. *Science* **303**, 2030–2032. (doi:10.1126/science.1091789)
- Riehle, M. A., Srinivasan, P., Moreira, C. K. & Jacobs-Lorena, M. 2003 Towards genetic manipulation of wild mosquito populations to combat malaria: advances and challenges. *J. Exp. Biol.* **206**, 3809–3816. (doi:10.1242/jeb.00609)
- Scott, M. E. 1991 *Heligmosomoides polygyrus* (Nematoda): susceptible and resistant strains of mice are indistinguishable following natural infection. *Parasitology* **103**, 429–438.
- Scott, T. W., Takken, W., Knols, B. G. & Boëte, C. 2002 The ecology of genetically modified mosquitoes. *Science* **298**, 117–119. (doi:10.1126/science.298.5591.117)
- Sorci, G., Moller, A. P. & Boulinier, T. 1997 Genetics of host-parasite interactions. *Trends Ecol. Evol.* **12**, 196–200. (doi:10.1016/S0169-5347(97)01056-2)
- Suwanchaichinda, C. & Paskewitz, S. M. 1998 Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize sephadex beads. *J. Med. Entomol.* **35**, 157–161.
- Tabachnick, W. J. 2003 Reflections on the *Anopheles gambiae* genome sequence, transgenic mosquitoes and the prospect for controlling malaria and other vector borne diseases. *J. Med. Entomol.* **40**, 597–606.
- Thomas, M. B. & Blanford, S. 2003 Thermal biology in insect-parasite interactions. *Trends Ecol. Evol.* **18**, 344–350. (doi:10.1016/S0169-5347(03)00069-7)
- Vlachou, D., Schlegelmilch, T., Christophides, G. K. & Kafatos, F. C. 2005 Functional genomic analysis of midgut epithelial responses in *Anopheles* during *Plasmodium* invasion. *Curr. Biol.* **15**, 1185–1195. (doi:10.1016/j.cub.2005.06.044)
- Webster, J. P. & Davies, C. M. 2001 Coevolution and compatibility in the snail-schistosome system. *Parasitology* **123**, S41–S56. (doi:10.1017/S0031182001008071)
- Yan, G., Severson, D. W. & Christensen, B. M. 1997 Costs and benefits of mosquito refractoriness to malaria parasites: implications for genetic variability of mosquitoes and genetic control of malaria. *Evolution* **51**, 441–450.