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On the evolutionary ecology of mosquito immunity and the use of transgenic mosquitoes for malaria control

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Abstract

Mosquitoes that are genetically manipulated to encapsulate the malaria parasite *Plasmodium falciparum* are being considered a possible method to control malaria. Hopes for this have been raised by the identification of genes involved in the mosquito's encapsulation response and by advances in the tools required to transform mosquitoes. But will such genes be able to spread in natural populations? What will their impact be on the epidemiology of the disease? This article attempts to give answers to these questions by reviewing some of theoretical and empirical considerations underlying the evolutionary epidemiology of genetic manipulation and refractoriness.

Keywords: malaria control; genetic manipulation; mosquito immunity; refractoriness; evolutionary epidemiology

Introduction

Genetic manipulation is attracting considerable attention as a potential tool for malaria control (Aultman, Beaty and Walker 2001). The underlying idea is (i) to identify genes that render mosquitoes refractory against malaria, (ii) to transform mosquitoes with these genes, (iii) to release the mosquitoes into natural populations so that the genes can spread, and thus (iv) to block malaria transmission.

The first two of these steps appear to be in reach due to recent advances in molecular biology. Thus, the molecular knowledge concerning the mosquito's immune response against malaria parasites is increasing rapidly with studies concerning antimicrobial peptides, signalling pathways and pattern-recognition peptides (Barillas-Mury, Wizel and Han 2000; Dimopoulos et al. 2001). In parallel with these studies are genetic approaches that are beginning to localize and identify the genes involved in the immune response (Dimopoulos et al. 2000; Oduol et al. 2000). Several genes, for example, that determine the difference between a susceptible line and one selected to be refractory against the malaria parasite *Plasmodium cynomolgi* have been localized in the mosquito's genome (Gorman et al. 1997; Zheng et al. 1997).

Once relevant genes have been identified, they could be inserted into the mosquito's genome with modern transformation techniques. To date, the most promising approach is to transform mosquitoes with genes linked to transposons,

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several of which are currently available for *Anopheles* mosquitoes (Catteruccia et al. 2000). Indeed, one transposon (piggyBac) was used recently to transform *Anopheles stephensi* with a protein sequence making the mosquitoes partially refractory against infection by malaria (Ito et al. 2002).

It is, therefore, time to start thinking about the other steps on the way to malaria control, and indeed these thoughts are critically required before transgenic mosquitoes can be released. Under what conditions are resistance genes expected to spread? Will they be able to reduce transmission sufficiently to ameliorate the malaria problem? Here I argue that we can gain important insights into these questions by studying the evolutionary ecology of mosquito immunity and thus gain a deeper understanding of the ecological and epidemiological processes underlying the co-evolution of mosquito-malaria interactions in natural settings.

In dealing with natural systems, we must of course discuss issues related to the natural immune system of mosquitoes. Refractory mosquitoes, however, may also be created by transformation with genes or proteins that are not found in mosquitoes. Indeed, in the study mentioned above, where mosquitoes were transformed to be partially resistant against infection, the most efficient of many random protein sequences was used (Ito et al. 2002). Although such an approach is not explicitly considered here, there is no reason to expect that other processes will determine ultimate success of a control programme or that using novel proteins for genetic manipulation will invalidate our discussion.

The cost of refractoriness and the spread of refractory genes

One of the most striking aspects about the mosquito's immune response against malaria infection is the lack of an effective encapsulation response in natural populations. In one study in Tanzania, for example, less than 0.5% of the infected mosquitoes had encapsulated their parasites (Schwartz and Koella 2002). Although other aspects of the immune response may control malaria infection, the lack of encapsulation is surprising, as there seems to be ample selection pressure for increased refractoriness: malaria infection can diminish the reproductive success of mosquitoes by decreasing the mosquito's fecundity (Hogg and Hurd 1995a; 1995b; 1997and Figure 1a) and by increasing its mortality (Anderson, Knols and Koella 2000 and Figure 1b). Although the increased mortality of infected mosquitoes is still controversial (Ferguson and Read 2002) one should thus expect mosquitoes to have evolved mechanisms to kill the infecting parasites.

One reason for the lack of refractoriness would be that an evolutionary cost of the immune response outweighs its benefits, so that genes enabling an effective immune response would not be able to spread in a natural population. Immune responses are generally found to be costly, be it in vertebrates or in invertebrates. Thus, the encapsulation response reduces competitive ability in *Drosophila* (Kraaijeveld and Godfray 1997), an antibacterial response reduces survival in bumblebees (Moret and Schmid Hempel 2000), and an increased reproductive effort in damselflies (Siva-Jothy, Tsubaki and Hooper 1998) or in *Drosophila* (McKean and Nunney 2001) is associated with reduced immuno-competence and a reduction of the ability to clear a bacterial infection, respectively. Mosquitoes are no exception, and two types of costs of the immune response have been observed. First, mounting an effective immune response requires the maintenance of specific cellular and biochemical machinery, which may be expected to require resources that would otherwise be used for other functions such as growth or reproduction. A maintenance cost would be expressed as

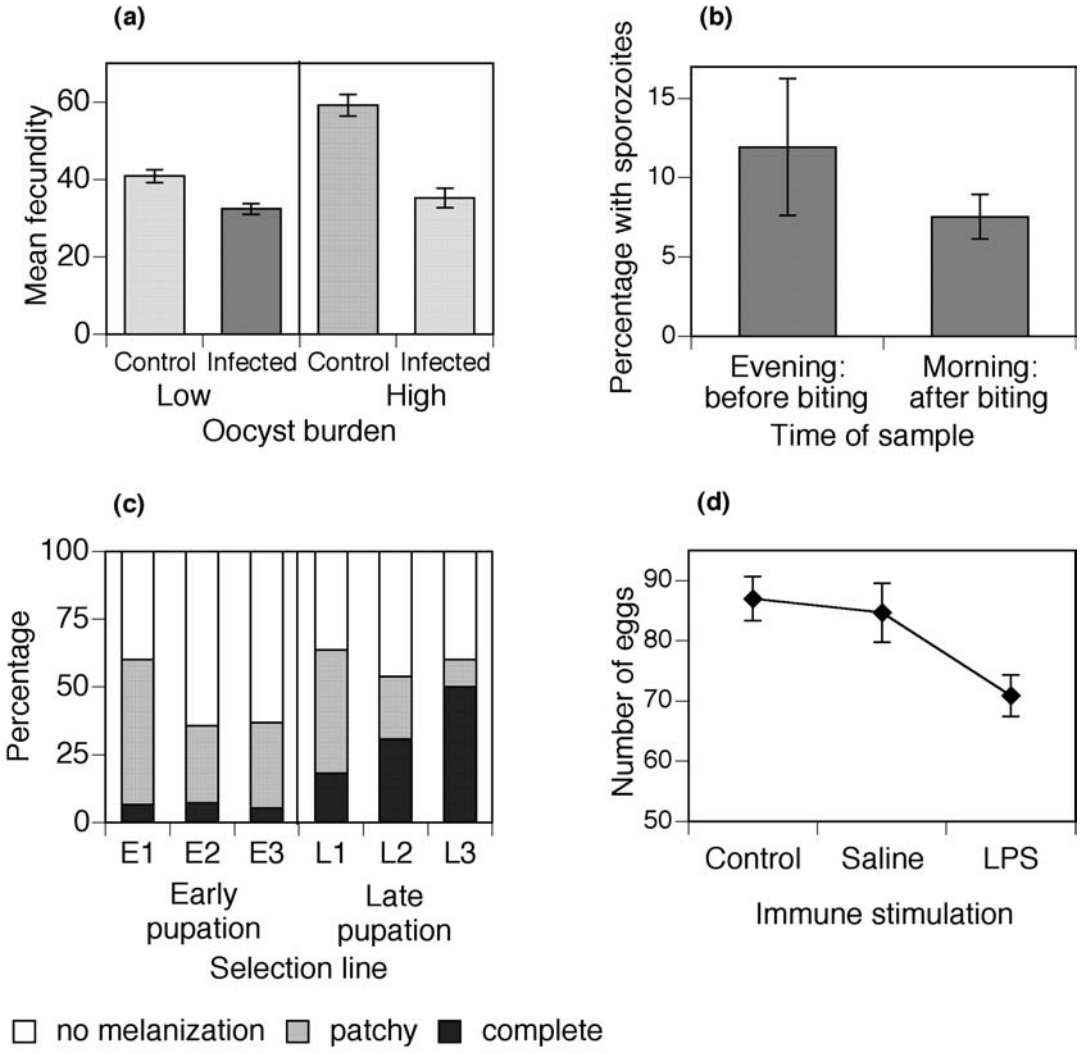

Figure 1. Costs and benefits of refractoriness. Benefits (a and b) are inferred from the detrimental effect of infection by malaria on the mosquito, which would be avoided or at least reduced if the mosquito were refractory. Two types of cost are considered: maintenance costs (c) that are associated with maintaining the machinery that enable an immune response, and activation costs (d) that are associated with activating and mounting the immune response. (a) Effect (in the laboratory) of low (mean 4.4 ± 0.4 oocysts per midgut) and high (> 75 oocysts per midgut) oocyst burdens of the parasite *P. yoelii nigeriensis* on the fecundity of *An. stephensi*. A different control was used for comparison of the mosquitoes differing in oocyst burdens. The bars show the mean number of eggs per mosquito and the vertical lines show the standard errors of the mean (modified from Hogg and Hurd 1995b). (b) Effect (in the field) of *P. falciparum* on the risk of feeding-associated mortality of *An. gambiae*. A sample of mosquitoes was caught early in the evening (before they started to bite), and a second sample of the same population was caught in the following morning (after they had completed their biting activity). The bars show the prevalence of sporozoites and the vertical lines show the standard errors of the percentage. A decrease in prevalence suggests that a higher proportion of sporozoite-infected than of uninfected mosquitoes died during the observation period of one night, i.e. that sporozoites increased mortality rate (modified from Anderson, Knols and Koella 2000). (c) Maintenance cost: effect of selecting *Aedes aegypti* during 10 generations for early or late pupation on the mosquito's ability to melanize an inoculated Sephadex bead. The percentage of mosquitoes that were able to melanize a bead completely, that had an intermediate (i.e. patchy) response, and that were not able to mount a response are shown for each of the six selection lines (from Koella and Boëte 2002). (d) Activation cost: effect of

inoculating female *Anopheles gambiae* with LPS (to stimulate the antibacterial immune response) or saline on egg production (from Ahmed et al. 2002).

a genetic correlation between the effectiveness of the immune response and other traits that determine fitness. This has been observed in the yellow-fever mosquito *Aedes aegypti*, where selection for early or late pupation brought with it a correlated response in the effectiveness of encapsulating negatively charged CM-25 Sephadex beads: the lines with the earliest pupation had the weakest encapsulation response (Koella and Boëte 2002 and Figure 1c). Second, mosquitoes may be expected to pay for activating the immune response. Such an activation cost has been observed for the antibacterial immune response (stimulating the immune response with LPS leads to decreased fecundity (Ahmed et al. 2002and Figure 1d) and for the encapsulation response (stimulating the immune response with negatively charged Sephadex beads leads to decreased fecundity of *An. Gambiae* (Schwartz and Koella 2002)).

It is the balance between such costs and benefits of being refractory that determine the spread of refractoriness in natural populations. Predicting the spread requires theoretical modelling of the relevant processes. A recent model, which combines basic population genetics of mosquitoes with the epidemiology of malaria, makes two predictions (see Figure 2a). First, if the cost of refractoriness is sufficiently low, refractoriness increases with the intensity of transmission but never sufficiently to eliminate malaria from the population. Second, if the cost exceeds a threshold, refractoriness cannot spread (Boëte and Koella 2002).

What does this tell us about the potential for driving genes into natural populations? We can modify the model to account for genetic manipulation, assuming that refractoriness genes are linked to a transposon. The effect of a transposon that is most important in terms of genetic manipulation is that it causes a segregation bias; it drives itself and the genes linked to it to offspring with a probability higher than the 50% of Mendelian segregation. Earlier population-genetic models showed that a transposon (together with the genes linked to it) will spread to fixation if the transposon creates sufficient segregation bias (Kiszewski and Spielman 1998; Ribeiro and Kidwell 1994). Our more detailed model (see Appendix), which combines population-genetic with epidemiological processes, gives the same conclusion: refractoriness can be fixed even if the cost of refractoriness is considerable (Boëte and Koella 2002 and Figure 2b). One should note, however, that this conclusion might be overly optimistic, as the model assumes that refractoriness remains linked to the transposon. Any cost of refractoriness, however, would lead to selection pressure for the transposon to be dissociated from refractoriness (leading to the loss of the isolated gene) or that mutations (for example the introduction of a stop-codon) would render the refractory gene useless.

Such precautions aside, it seems that there is room for optimism: once refractoriness genes are known, it is likely that they can be driven to fixation given sufficiently efficient transposons. Unfortunately, the currently used transposons of *Aedes* and *Anopheles* appear to show Mendelian inheritance (Catteruccia et al. 2000), so that the search for more efficient transposons is necessary (see also O'Brochta elsewhere in this volume).

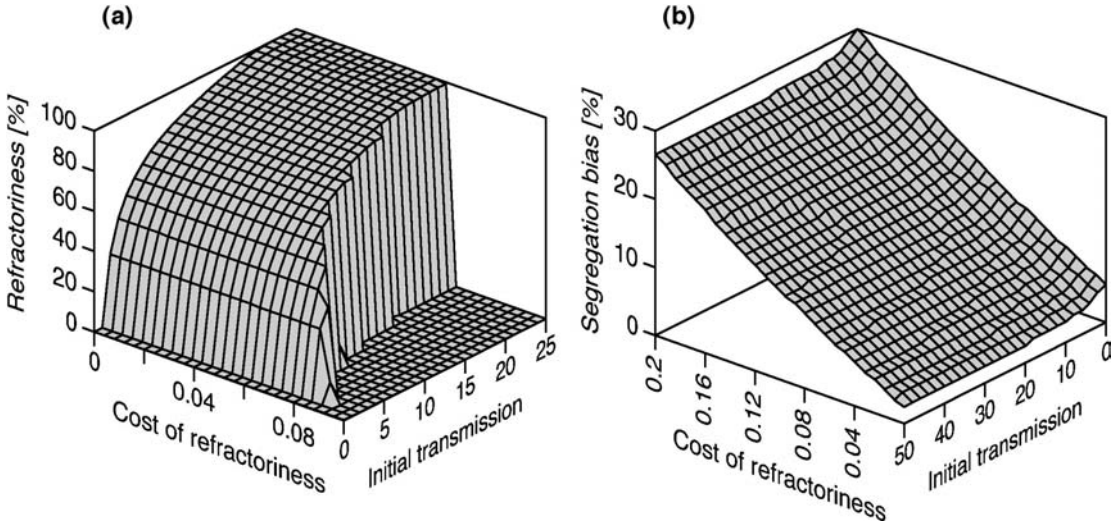

Figure 2. Theoretical predictions concerning the spread of refractoriness, as functions of the evolutionary cost of refractoriness and the intensity of transmission in a population (before a gene coding for refractoriness has spread). (a) Spread of refractoriness in the absence of a transposon as a genetic drive mechanism. (b) Efficacy of the genetic drive mechanism (i.e. the segregation bias) that is required for the linkage group (refractoriness-transposon) to spread to fixation. For details of the model and of the parameters used, see Boëte and Koella (2002). Note that for clarity, the two panels differ in the scales and the directions of the axes.

Lack of refractoriness despite effective immune system?

But perhaps the lack of refractoriness in natural populations is not due to the lack of an effective immune response. As mentioned above, in one study in Tanzania, less than 0.5 % of infected, field-collected *An. gambiae* encapsulated their parasites. In the same cohort of mosquitoes, in contrast, about 90% of the individuals encapsulated negatively charged Sephadex beads (Schwartz and Koella 2002). This suggests that it is not the cost of refractoriness that limits the spread of refractory genes. In other words, it is not a lack of effective immune function per se, but some other mechanism that limits the expression of refractoriness. Three possibilities for such mechanisms are discussed here.

Lack of recognition

The mosquito's immune system might not recognize the parasite as foreign, so that no immune response is activated. Indeed, the fact that parasites incorporate a substance secreted by mosquitoes (laminin) into the cell wall of their oocysts may enable them to hide from the mosquito's immune system (Adini and Warburg 1999). Thus, the requirement for creating refractory mosquitoes would be to find genes that enhance the mosquito's ability (or sensitivity) to recognize malaria parasites, rather than genes that enhance the efficacy of the immune response per se. This requirement, however, may be made more difficult by the possibility that the recognition of the parasite may require a precise matching of the parasite's and the mosquito's genotype. An indication of such genotype-by-genotype interactions comes from a selection experiment, where mosquitoes selected to encapsulate the parasite *P. cynomolgi* became completely refractory against isolates of *P. falciparum* from the New World, but were less likely to encapsulate isolates from the Old World (Collins et al. 1986 and Figure 3a). Indeed, genotype-by-genotype interactions are quite common in

parasite-host interactions (Barrett 1985) and underlie, for example, the interactions between the encapsulation response of *Drosophila* against parasitoids (Kraaijeveld and Godfray 2001).

If immunity were genotype-specific, genetic manipulation would have to transform mosquitoes with several allelic variants of a recognition gene, thus enabling the mosquito to recognize all genotypes of the parasite. While this might be possible if there is sufficient cross-recognition among genotypes, mutation and genetic recombination among different variants can be expected to create new and unrecognized variants in a relatively short time. Indeed, one of the evolutionary raisons d'être of sex is generally believed to be the creation of rare genotypes that will succeed in the co-evolutionary race between hosts and parasites (Hamilton, Axelrod and Tanese 1990). In other words, we would have to evolve our technology along with the parasites to transform the mosquitoes continually with the novel immune-recognition proteins that natural selection cannot provide.

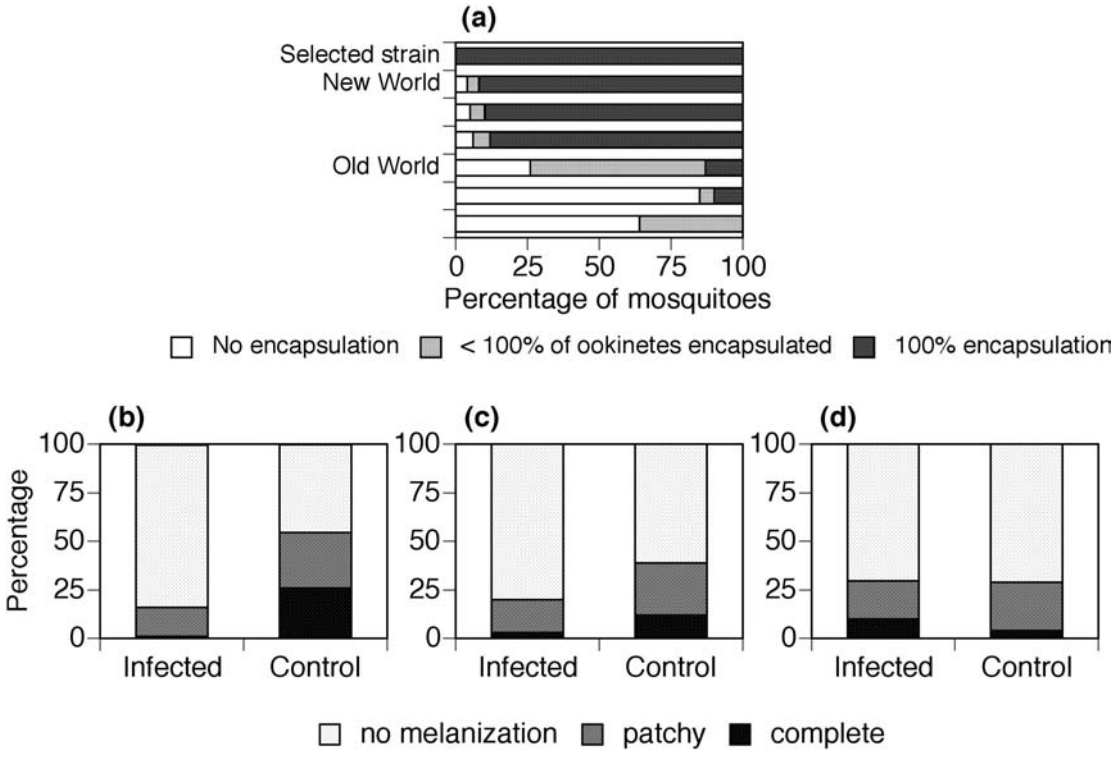

Figure 3. Potential reasons, induced by the parasite's evolutionary response to refractoriness, for reduced efficacy of a mosquito's immune response and refractoriness against malaria parasites. (a) Genotype-by-genotype interactions. After selection for refractoriness against an isolate of the malaria parasite *P. cynomolgi* (top bar), the 'refractory' mosquitoes were tested against several isolates of *P. falciparum* from the New World and the Old World with varying success. The bars show the mean percentage of the oocysts encapsulated by each mosquito. Modified from Collins et al. (1986). (b-d) Immuno-suppression by the parasite. Each panel shows the proportion of *Ae. aegypti* infected with *P. gallinaceum* and uninfected controls that melanize a bead to different degrees (no, patchy and complete melanization of the surface of each bead). (b) Response against beads inoculated 24 hours after infection (or blood-feeding), i.e. when the parasite is in its late ookinete stage. At this stage, the parasite is most sensitive to encapsulation. (c) Inoculation after 48 hours, i.e. at an early oocyst stage. (d) Inoculation after 96 hours, i.e. at a later oocyst stage. At this stage, the oocyst appears to hide from the mosquito's immune system by having incorporated laminin into the wall of the oocyst (from Boëte, Paul and Koella 2002).

Immuno-suppression

The parasite may be able to suppress the mosquito's immune response, This would indeed come as no surprise, as immuno-suppression has evolved in several parasites and parasitoids of insects. The best-known examples of immuno-suppression are found in several dipteran and hymenopteran parasitoid species, whose larvae develop within their insect host (Strand and Pech 1995). These parasitoids inject a poly-DNA virus or a protein into the insect host (Beckage 1998; Vinson 1990), which shuts down the insect's immune system (Rizki and Rizki 1990). Immuno-suppression is also found in several bacteria/nematode complexes (e.g. *Heterorhabditis/ Photorhabdus*) that parasitize insects. The nematode penetrates the insect, releases immuno-suppressive substances into the insect's haemocoel, and then releases its bacteria. The bacteria, uninhibited by the insect's immune response, replicate extensively within the insect, thereby providing the nematode with nutrition (Boemare et al. 1997; Wang and Gaugler 1999).

Similar immuno-suppressive processes may be involved in the malaria-mosquito interaction, as mosquitoes infected by malaria parasites encapsulate negative Sephadex beads less effectively than uninfected controls, in particular during the parasite's late ookinete and early oocyst stage (Boëte, Paul and Koella 2002and Figure 3b-d), the period when the parasite is most sensitive to the mosquito's encapsulation immune response (Collins et al. 1986; Gouagna et al. 1998). Note that there is no suppression at later stages, when the parasites have incorporated laminin into the cell wall of their oocysts to hide from the mosquito's immune system (Adini and Warburg 1999). It is not yet clear whether the lack of an immune response is due to active suppression of the immune mechanism by the parasite or a by-product of, for example, differences in the blood-meal between infected and uninfected hosts. In an evolutionary and epidemiological sense, however, this does not matter. What matters is that infection by malaria parasites in some way renders the immune response less effective.

It is even less clear whether the efficacy of 'immuno-suppression' has a genetic basis. But if it does, one may fear that any increase in the efficacy of the mosquito's immune response due to genetic manipulation will be compensated by an increase in the efficacy of immuno-suppression. If this is the case, efforts to boost the general efficacy of the immune response may be wasted. A greater research effort is needed on mechanisms and genes that counteract the parasite's immuno-suppressive action.

Environmental effects

The genetic basis of refractoriness may be overridden by environmental or other factors. It is indeed clear that the immune response of mosquitoes is modified by environmental quality, as it is for most invertebrates (Brey 1994). If, for example, larvae are reared in crowded or undernourished conditions, the emerging adults have a weak encapsulation immune response. Thus, in one experiment using the strain of *An. gambiae* previously selected to be refractory (Collins et al. 1986), reducing food from 100% to 25% of a standard diet reduced the proportion of mosquitoes that melanized more than half of the surface of inoculated Sephadex beads from 75% to 36% (Suwanchaichinda and Paskewitz 1998 and Figure 4a). Similarly, if adults are stressed by temperature (Suwanchaichinda and Paskewitz 1998) or by the lack of nutrition, be it blood or sugar (Chun, Riehle and Paskewitz 1995; Schwartz and Koella 2002), the encapsulation immune response is weakened (Figure 4b).

Furthermore, the immune response of mosquitoes weakens with age (Boëte, Paul and Koella 2002; Chun, Riehle and Paskewitz 1995 and Figure 4c). Thus, in one

natural population of *An. gambiae* the efficacy of the immune response of blood-fed females decreases from close to 100% just after emergence to about 75% four days after emergence (Schwartz and Koella 2002). The epidemiological problem associated with the lower efficacy of immunity in the older mosquitoes is that in the same area about 25% of the mosquitoes become infected during a night of blood-feeding (Lyimo and Koella 1992). Therefore, most mosquitoes will become infected when they are more than 5 days old, i.e. when the immune response is no longer completely effective.

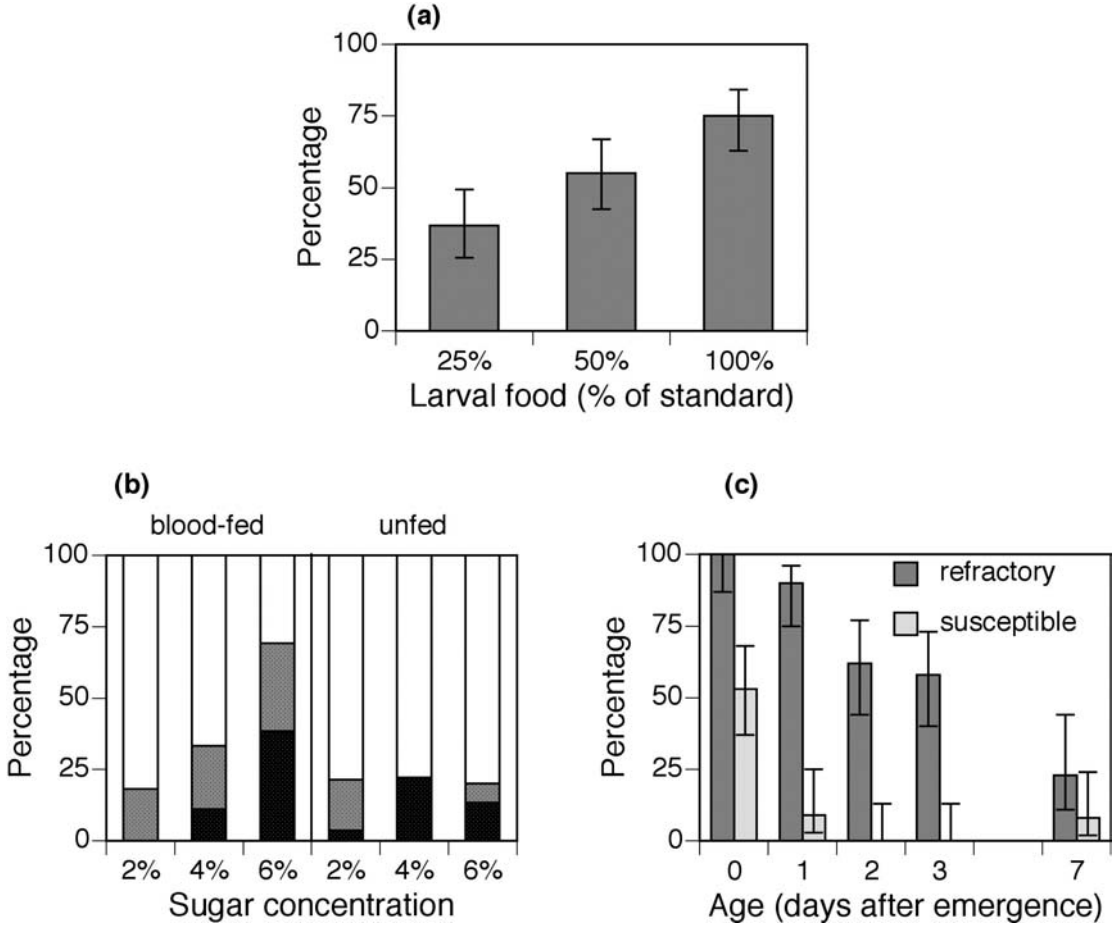

Figure 4. Potential reasons, due to environmental effects, for reduced efficacy of a mosquito's immune response and refractoriness against malaria parasites. In (a) and (c) the bars show the percentage of mosquitoes that melanized at least 50% of the bead's surface and the vertical lines show the 95% confidence interval of the percentage. In (b) the bars show the percentage of mosquitoes that completely melanized the bead, that had patchy melanization (i.e. that left unmelanized areas on the bead) or that had no visible melanization. (a) Effect of larval food (in percentage of a standard diet) on a refractory line of *An. gambiae* (after Suwanchaichinda and Paskewitz 1998). (b) Effect of sugar and blood-feeding on 4-day-old *Ae. aegypti* (from Koella and Sørensen 2002). (c) Effect of age after emergence on a refractory and susceptible line of *An. gambiae* (after Chun, Riehle and Paskewitz 1995).

Effect on malaria burden

These considerations give a less optimistic view of the possibility of controlling malaria with genetic manipulation of mosquito immunity. The problems are, on the one hand, that non-genetic factors might decrease the efficacy of the refractory genes

and, on the other hand, that the parasite's counter-response might leave some of the parasites unrecognized or that it suppresses the refractory response. Therefore, it seems unlikely that insect immunity refractory genes will lead to completely refractory mosquitoes that can resist all infections. In other words, the best that any control programme based on genetic manipulation can do is to reduce the number of susceptible mosquitoes, but not eliminate them. But reducing the number of susceptible mosquitoes – whether this is due to mosquito control or to the release of genetically manipulated mosquitoes – has already been shown by Macdonald to be inefficient control strategy that would have little effect on the epidemiology of malaria in areas of intense transmission (Macdonald 1957). The model used above to describe the spread of refractory genes confirms Macdonald's prediction: even if refractory genes were fixed, the prevalence of malaria in areas of moderate to intense transmission would decrease substantially only if the efficacy of the immune response in resisting infection were close to 100% (Figure 5), an efficacy that is so challenging that it is unlikely to be reached.

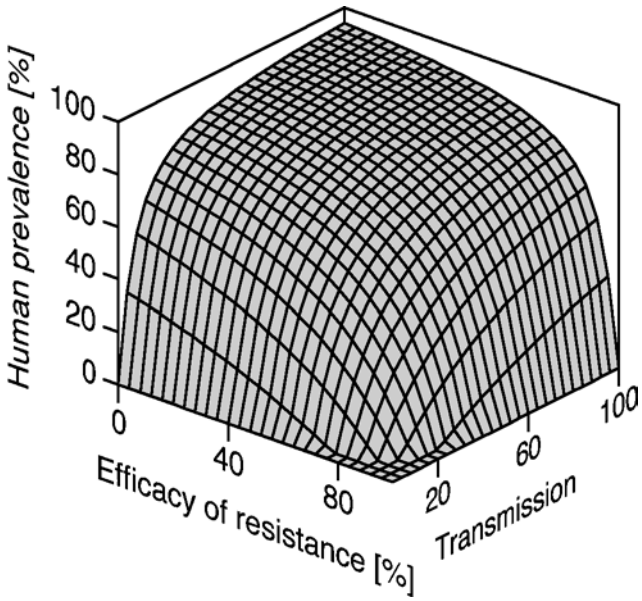

Figure 5. Theoretical predictions concerning the effect of fixation of a gene coding for refractoriness on the prevalence of malaria, as a function of the intensity of transmission (before a gene coding for refractoriness has spread) and the efficacy of the refractoriness (the proportion of infections that are cleared). For details of the model and parameters, see Boëte and Koella (2002).

Conclusion

In conclusion, one may fear that, even if we can boost the mosquitoes' immune response against malaria, the refractoriness will be too ineffective to have much influence on the prevalence of disease. I conclude, however, with two more constructive remarks. First, while it is unlikely that genetic manipulation alone will work in areas with intense transmission, simultaneous control strategies could reduce the intensity of transmission to a level where refractory mosquitoes are likely to have a major impact. Second, while we have shown that boosting the immune system is not enough for malaria control, our focus on the complexities of the immune system and its interactions with the environment have identified key points that should be

considered in designing more efficient genetic-manipulation strategies. What, for example, are the environmental conditions in natural populations and to what extent do they influence the immune system? Can we find genes that specifically boost the immune response in old mosquitoes (just as there are genes that slow the ageing process of *Drosophila*)? What is the mechanism of immune-suppression, and are there ways of preventing it? Answers to such questions, and thus effective malaria control with genetically manipulated mosquitoes, cannot be reached by laboratory studies alone, but must integrate molecular biology with field and theoretical studies of the evolutionary ecology of refractoriness.

Appendix: structure of model

Our model describes the spread of refractoriness in populations by combining population-genetic with epidemiological ideas (Boëte and Koella 2002). Epidemiological equations are used to calculate the mosquito's fitness as a function of the cost and benefit of refractoriness. The population-genetic equations then use the mosquito's fitness to calculate the change of the frequency of the refractory allele, which in turn defines the prevalence of infection in the human population. The human prevalence feeds back onto the mosquito's fitness, because the conditional cost (i.e. the cost that is expressed when the immune system responds to an infection) and the benefit of refractoriness increase with the probability that the mosquito is infected. Details of cost and benefit of refractoriness can be found in the main text. The combination of epidemiological and population-genetic equations gives a negative feedback that is not found in standard population-genetic approaches (see Figure I).

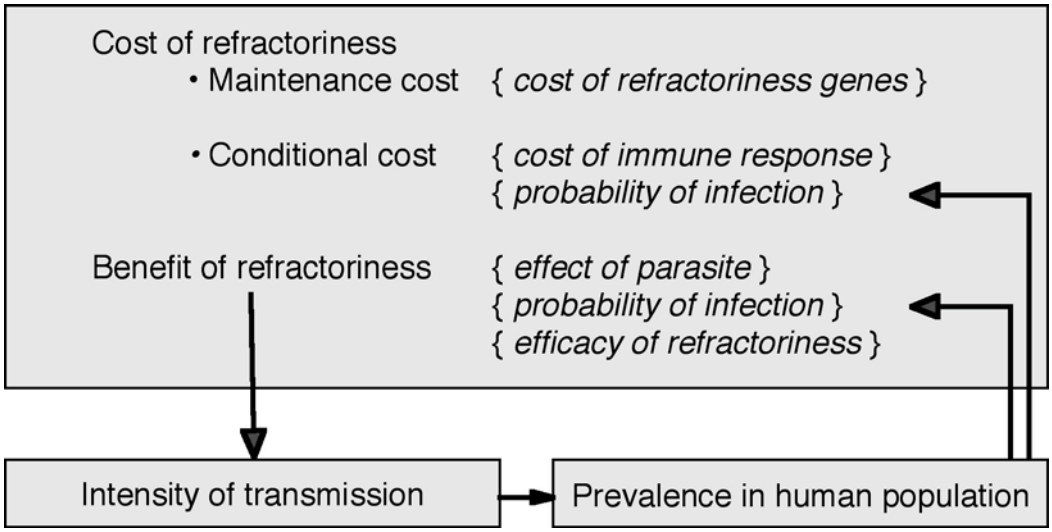

Figure I. Schematic description of the epidemiological feedback in a population-genetic model describing the spread of refractoriness. For equations and parameters, see Boëte and Koella (2002).

References

Adini, A. and Warburg, A., 1999. Interaction of *Plasmodium gallinaceum* ookinetes and oocysts with extracellular matrix proteins. *Parasitology,* 119 Part 4, 331-336.

Ahmed, A.M., Baggott, S.L., Maingon, R., et al., 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. *Oikos,* 97 (3), 371-377.

Anderson, R.A., Knols, B.G.J. and Koella, J.C., 2000. *Plasmodium falciparum* sporozoites increase feeding-associated mortality of their mosquito hosts *Anopheles gambiae* s.l. *Parasitology,* 120 Part 4, 329-333.

Aultman, K.S., Beaty, B.J. and Walker, E.D., 2001. Genetically manipulated vectors of human disease: a practical overview. *Trends in Parasitology,* 17 (11), 507-509.

Barillas-Mury, C., Wizel, B. and Han, Y.S., 2000. Mosquito immune responses and malaria transmission: lessons from insect model systems and implications for vertebrate innate immunity and vaccine development. *Insect Biochemistry and Molecular Biology,* 30 (6), 429-442.

Barrett, J., 1985. The gene-for-gene hypothesis: parable or paradigm. *In:* Rollinson, D. and Anderson, R. M. eds. *Ecology and genetics of host - parasite interactions : papers presented at an International Symposium organized by the Linnean Society of London and the British Society for Parasitology, held at Keele University, 12 - 13 July 1984*. Academic Press, London, 266. Linnean Society symposium series no. 11.

Beckage, N.E., 1998. Modulation of immune responses to parasitoids by polydnaviruses. *Parasitology,* 116 Suppl. S, S57-S64.

Boemare, N., Givaudan, A., Brehelin, M., et al., 1997. Symbiosis and pathogenicity of nematode-bacterium complexes - Review article. *Symbiosis,* 22 (1-2), 21-45.

Boëte, C. and Koella, J.C., 2002. A theoretical approach to predicting the success of genetic manipulation of malaria mosquitoes in malaria control. *Malaria Journal,* 1 (1), 1-7. [http://www.malariajournal.com/content/1/1/3]

Boëte, C., Paul, R.E.L. and Koella, J.C., 2002. Reduced efficacy of the immune melanization response in mosquitoes infected by malaria parasites. *Parasitology,* 125 Part 2, 93-98.

Brey, P.T., 1994. The impact of stress on insect immunity. *Bulletin de l'Institut Pasteur, France,* 92 (2), 101-118.

Catteruccia, F., Nolan, T., Loukeris, T.G., et al., 2000. Stable germline transformation of the malaria mosquito *Anopheles stephensi*. *Nature,* 405 (6789), 959-962.

Chun, J., Riehle, M. and Paskewitz, S.M., 1995. Effect of mosquito age and reproductive status on melanization of Sephadex beads in *Plasmodium*-refractory and -susceptible strains of *Anopheles gambiae*. *Journal of Invertebrate Pathology,* 66 (1), 11-17.

Collins, F.H., Sakai, R.K., Vernick, K.D., et al., 1986. Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science,* 234 (4776), 607-610.

Dimopoulos, G., Casavant, T.L., Chang, S.R., et al., 2000. *Anopheles gambiae* pilot gene discovery project: Identification of mosquito innate immunity genes from expressed sequence tags generated from immune-competent cell lines. *Proceedings of the National Academy of Sciences of the United States of America,* 97 (12), 6619-6624.

Dimopoulos, G., Muller, H.M., Levashina, E.A., et al., 2001. Innate immune defense against malaria infection in the mosquito. *Current Opinion in Immunology,* 13 (1), 79-88.

Ferguson, H.M. and Read, A.F., 2002. Why is the effect of malaria parasites on mosquito survival still unresolved? *Trends in Parasitology,* 18 (6), 256-261.

Gorman, M.J., Severson, D.W., Cornel, A.J., et al., 1997. Mapping a quantitative trait locus involved in melanotic encapsulation of foreign bodies in the malaria vector, *Anopheles gambiae*. *Genetics,* 146 (3), 965-971.

Gouagna, L.C., Mulder, B., Noubissi, E., et al., 1998. The early sporogonic cycle of *Plasmodium falciparum* in laboratory-infected *Anopheles gambiae*: an estimation of parasite efficacy. *Tropical Medicine and International Health,* 3 (1), 21-28.

Hamilton, W.D., Axelrod, R. and Tanese, R., 1990. Sexual reproduction as an adaptation to resist parasites (a review). *Proceedings of the National Academy of Sciences of the United States of America,* 87 (9), 3566-3573.

Hogg, J.C. and Hurd, H., 1995a. Malaria-induced reduction of fecundity during the first gonotrophic cycle of *Anopheles stephensi* mosquitoes. *Medical and Veterinary Entomology,* 9 (2), 176-180.

Hogg, J.C. and Hurd, H., 1995b. *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 Part 5, 555-562.

Hogg, J.C. and Hurd, H., 1997. The effects of natural *Plasmodium falciparum* infection on the fecundity and mortality of *Anopheles gambiae* s.l. in north east Tanzania. *Parasitology,* 114 Part 4, 325-331.

Ito, J., Ghosh, A., Moreira, L.A., et al., 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature,* 417 (6887), 452-455.

Kiszewski, A.E. and Spielman, A., 1998. Spatially explicit model of transposon-based genetic drive mechanisms for displacing fluctuating populations of anopheline vector mosquitoes. *Journal of Medical Entomology,* 35 (4), 584-590.

Koella, J.C. and Boëte, C., 2002. A genetic correlation between age at pupation and melanization immune response of the yellow fever mosquito *Aedes aegypti*. *Evolution,* 56 (5), 1074-1079.

Koella, J.C. and Sørensen, F.L., 2002. Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*. *Medical and Veterinary Entomology,* 16 (3), 316-320.

Kraaijeveld, A.R. and Godfray, H.C.J., 1997. Trade-off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Nature,* 389 (6648), 278-280.

Kraaijeveld, A.R. and Godfray, H.C.J., 2001. Is there local adaptation in *Drosophila*-parasitoid interactions? *Evolutionary Ecology Research,* 3 (1), 107-116.

Lyimo, E.O. and Koella, J.C., 1992. Relationship between body size of adult *Anopheles gambiae* s.l. and infection with the malaria parasite *Plasmodium falciparum*. *Parasitology,* 104 (Pt. 2), 233-237.

Macdonald, G., 1957. *The epidemiology and control of malaria*. Oxford University Press, London.

McKean, K.A. and Nunney, L., 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America,* 98 (14), 7904-7909.

Moret, Y. and Schmid Hempel, P., 2000. Survival for immunity: The price of immune system activation for bumblebee workers. *Science,* 290 (5494), 1166-1168.

Oduol, F., Xu, J.N., Niare, O., et al., 2000. Genes identified by an expression screen of the vector mosquito *Anopheles gambiae* display differential molecular immune response to malaria parasites and bacteria. *Proceedings of the*

National Academy of Sciences of the United States of America, 97 (21), 11397-11402.

Ribeiro, J.M. and Kidwell, M.G., 1994. Transposable elements as population drive mechanisms: specification of critical parameter values. *Journal of Medical Entomology,* 31 (1), 10-16.

Rizki, R.M. and Rizki, T.M., 1990. Parasitoid virus-like particle destroy *Drosophila* cellular immunity. *Proceedings of the National Academy of Sciences of the United States of America,* 87, 8388-8392.

Schwartz, A. and Koella, J.C., 2002. Melanization of *Plasmodium falciparum* and c-25 sephadex beads by field-caught *Anopheles gambiae* (Diptera: Culicidae) from southern Tanzania. *Journal of Medical Entomology,* 39 (1), 84-88.

Siva-Jothy, M.T., Tsubaki, Y. and Hooper, R.E., 1998. Decreased immune response as a proximate cost of copulation and oviposition in a damselfly. *Physiological Entomology,* 23 (3), 274-277.

Strand, M.R. and Pech, L., 1995. Immunological basis for compatibility in parasitoid-host relationships. *Annual Review of Entomology,* 40, 31-56.

Suwanchaichinda, C. and 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. *Journal of Medical Entomology,* 35 (2), 157-161.

Vinson, S.B., 1990. How parasitoids deal with the immune system of their host: an overview. *Archives of Insect Biochemistry and Physiology,* 13, 3-27.

Wang, Y. and Gaugler, R., 1999. *Steinernema glaseri* surface coat protein suppresses the immune response of *Popillia japonica* (Coleoptera: Scarabaeidae) larvae. *Biological Control,* 14 (1), 45-50.

Zheng, L.B., Cornel, A.J., Wang, R., et al., 1997. Quantitative trait loci for refractoriness of *Anopheles gambiae* to *Plasmodium cynomolgi* B. *Science,* 276 (5311), 425-428.