Published in final edited form as:

Physiol Entomol. 2004 August ; 29(3): 210–213. doi:10.1111/j.0307-6962.2004.00410.x.

Does environmental stress affect insect-vectored parasite transmission?

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Keywords

Anopheles, *Culex*, filariasis, insecticide resistance, malaria

Introduction

In some insects, the cost of parasitic infection can be sufficient to elicit natural selection against competent hosts (Yan *et al.*, 1997). This tendency may be compensated for by increased parasite susceptibility being linked to environmental stress. Obvious examples of variable environmental stress include insecticide selection from disease control operations and seasonal stress through heat and dehydration in some insect species. Vectorial capacity (i.e. the ability of mosquitoes to ingest parasites and to promote their maturation until the infective stage) and the rate of insect survival until parasite maturation (Failloux *et al.*, 1995), can differ geographically according to insect strain (Crans, 1973; McGreevy *et al.*, 1982). The sandfly, *Phlebotomus papatasi*, transmits cutaneous leishmaniasis in desert and savannah regions of the Old World, where seasonal stress and dehydration reduces the quantity of sugar in plant leaves that is available for insect feeding. Selection for hunger tolerance has produced a population of *P. papatasi* that has sufficient longevity from feeding on the sugar depleted leaves to deposit their eggs and transmit *Leishmania* (Schlein & Jacobson, 2000). This inherited trait may alter the insects' physiology and disrupt the interaction between insect and parasite, altering the insects' susceptibility to infection. A similar effect of selection for hunger tolerance on susceptibility to *Leishmania major* infection occurs in Jordanian *P. papatasi* (Schlein & Jacobson, 2001).

Another factor that may explain geographical differences in vectorial capacity is insecticide selection pressure. Many questions remain unresolved on the evolutionary fitness costs and vectorial capacity of insects that are resistant to insecticides (Ferdig *et al.*, 1993; Kraaijeveld & Godfrey, 1997; Yan *et al.*, 1997).

The primary means of controlling mosquito-borne diseases such as malaria and filariasis is by targeting the vector species using residual spraying with insecticides. The development of insecticide resistance in mosquito vectors is common (Hemingway & Ranson, 2000). Insecticides are still commonly used for *Culex* control, although the strategic plan for lymphatic filariasis elimination by 2020 (World Health Organization, 1999) is heavily dependent on drug treatment. *Culex quinquefasciatus*, the major vector of filariasis, uses one predominant esterase-based organophosphate resistance mechanism that occurs in more than 80% of insecticide-resistant populations worldwide (Raymond *et al.*, 1991; Hemingway & Karunaratne, 1998). The most common variant of this mechanism originated once and spread rapidly to all continents (Raymond *et al.*, 1991). This resistance depends on the stable germline amplification of two esterase enzymes and an aldehyde oxidase encoded on a 30-kb DNA amplicon (Hemingway *et al.*, 2000). Up to 80 copies of this amplicon can be present per cell in resistant mosquitoes (Paton *et al.*, 2000). In *Anopheles* mosquitoes, the major malaria vector worldwide, there is a wider variety of insecticide resistance mechanisms, with elevated glutathione S-transferases producing DDT resistance (Prapanthadara *et al.*, 1995; Ranson *et al.*, 2000), elevated P450 monooxygenases combined with an altered sodium channel (kdr) contributing to pyrethroid resistance (Vulule *et al.*, 1999; Chandre *et al.*, 2000) and elevated esterases and altered malathion carboxylesterases producing general organophosphorus and malathion specific resistance, respectively (Brogdon & Barber, 1990; Hemingway, 1982).

Insecticide resistance is assumed to increase the likelihood of mosquito-borne disease transmission by increasing the vector population size and allowing mosquitoes to live longer in the presence of insecticide. The validity of this assumption was tested using Sri Lankan *Culex quinquefasciatus* and lymphatic filariasis as a model system (McCarroll *et al.*, 2000). In insecticide resistant *C. quinquefasciatus*, esterases are highly expressed in the midgut, subcuticular layer, Malpighian tubules and salivary glands, resulting in a change in the redox potential in these cells compared with their susceptible counterparts (Hemingway, 2000). Because most mosquito-borne parasites must pass through some of these tissues to complete their development, it is possible that parasite survival, and hence the vectorial capacity of the insect, may be directly affected by the insecticide status of the insects. The study by McCarroll *et al.* (2000) was the first to attempt to directly examine the relationship between vectorial capacity and insecticide resistance within an insect population. For the first time, this research has now been extended to data from a similar study of malaria, the most prevalent mosquito borne disease, and its *Anopheles* vectors.

Does insecticide resistance alter malaria parasite development in *Anopheles* vectors?

The study by McCarroll *et al.* (2000) demonstrated a negative correlation between filarial infection and insecticide resistance of *Culex* mosquitoes. *Plasmodium*, like filarial parasites in *Culex*, encounters a different cellular environment in insecticide resistant mosquitoes compared with insecticide susceptible mosquitoes, caused by physiological changes accompanying resistance that may affect parasite survival. Malaria parasites undergo a complex developmental cycle when passing through several different tissues within the mosquito vector.

The extensive use of pyrethroid impregnated bednets and other insecticides for malaria control in endemic regions has resulted in the emergence of insecticide resistance in many *Anopheles* vectors, which makes malaria control problematic. *Anopheles gambiae* is the major malaria vector in Africa, but pyrethroid resistance in laboratory strains of this species (such as the RSP isolated from East Africa) is relatively low (less than 10-fold). Target site insensitivity (kdr) is an important element in resistance, in combination with a metabolic-based resistance mechanism in East Africa (Nikou *et al.*, 2003; Ortelli *et al.*, 2003). By contrast, there are highly pyrethroid resistant strains of the major Asian malaria vector

Anopheles stephensi, such as the pyrethroid-selected strain DUB-R with 182-fold resistance compared with a pyrethroid susceptible strain 'BEECH' (Enayati *et al.*, 2003). Target site insensitivity only partially explains resistance in *An. stephensi* because the majority is contributed by up-regulation of metabolic enzymes, such as esterases, monooxygenases and glutathione S-transferases. These insecticide resistant and susceptible *An. stephensi* strains and the malaria parasite *Plasmodium yoelii nigeriensis*, which develops very successfully in this *Anopheles* vector, were used to investigate any effect of resistance on malaria parasite survival.

Plasmodium yoelii nigeriensis Killick-Kendrick (N67) was maintained in CD1 male mice (Ahmed *et al.*, 1999) and parasitemias of infected mice were determined from Giemsa-stained blood films prepared from tail blood. The presence of exflagellating microgametocytes was confirmed by examination of fresh thick blood films before mosquito feeding. Larvae of the resistant (DUB-R) and susceptible (BEECH) *An. stephensi* strains (Enayati *et al.*, 2003) were reared under standard conditions to produce adults of uniform size. Access to glucose solution was denied to the adult females 12 h before blood feeding. Discrete batches of 35 6-day-old mosquitoes from each strain were fed on the same mouse to ensure that any observed variation was not attributable to differences in the blood/infection quality or the body temperature of the mice. Female mosquitoes that were not fully engorged after feeding were removed from the cage. Four independent pair-wise experiments were performed. Mosquitoes were maintained under standard conditions for 8 days postinfection to determine the prevalence and intensity of infection.

Mosquito midguts were dissected and suspended in a 2% aqueous solution of mercurochrome to allow contrasting vital staining of the oocysts. The number of oocysts and melanization spots per gut was counted under a light microscope and the mean number of developing oocysts/midgut was determined.

Table 1 gives a comparison of the number of ookinetes that successfully traversed the midgut epithelium to form oocysts in pyrethroid resistant (DUBS-R) vs. susceptible (BEECH) *An. stephensi*. There was no significant difference between the two strains, and a similar number of melanized ookinetes were produced in both mosquito strains (data not shown).

These data are in contrast to recent indications that insecticide resistance and the capacity of the *Anopheles* vector to host malaria parasite are correlated. For example, a recent morphological, biochemical and genomic approach demonstrated broad physiological differences between parasite refractory and susceptible *Anopheles* mosquitoes, implicating a detected elevated level of reactive oxygen species as a major factor contributing to parasite melanotic encapsulation (Kumar *et al.*, 2003; Christophides *et al.*, 2004). An enhanced ability of the insecticide resistant insects to tolerate oxidative stress was also implied by the protective role of glutathione S-transferases (Vontas *et al.*, 2001), whereas comparison of superoxide dismutase activity (a major antioxidant enzyme) between the *An. stephensi* strains used in this study confirmed the presence of enhanced antioxidant defence in the resistant insects (Vontas, unpublished). The lower oxygen free radical damage in insecticide resistant insect tissues containing malaria parasites should improve pathogen survival, although the effect of elevated monooxygenases in the same tissues is not known. Finally, preliminary microarray data indicate that gene expression and associated physiological changes during insecticide metabolism strongly overlap with the oxidoreductive response of *Anopheles gambiae* to *Plasmodium berghei* infection (Vontas, Christophides and Ranson, unpublished data). Nevertheless, the redox-oxidoreductive link hypothesized by the above indications between vectorial capacity and insecticide resistance was not found in this study.

The vectorial capacity of various *Anopheles* taxa to different *Plasmodia* is highly influenced by the diverse ecoethological characteristics of the mosquitoes and the complex molecular interactions between the two organisms. The success of the ookinete to oocyst differentiation, which is a critical stage in parasite development, appears to differ significantly between different parasite–mosquito combinations (Christophides *et al.*, 2004). The refractory phenotypes in a genetically selected strain of *Anopheles* vectors are highly *Plasmodium* species-specific with refractory strains remaining totally susceptible (Somboon *et al.*, 1999), emphasizing the specificity of the interaction between mosquito and parasite. It is possible that the physiological differences, related to the production and detoxification of reactive oxygen species, identified in *An. gambiae* refractory and susceptible mosquitoes (Kumar *et al.*, 2003) are specific to the *An. gambiae*–rodent *P. berghei* model system and this might explain our negative result in the *An. stephensi*–*P. y. nigeriensis* system. However, there is sufficient justification for further investigation of the possible association between insecticide resistance and vectorial capacity phenotypes, in other mosquito–*Plasmodium* systems, such as the primary vector *An. gambiae* (using a recently isolated highly insecticide resistant strains from West Africa) and the rodent parasite *P. berghei* or human parasites. This work is ongoing. In addition, parasite survival at the sporozoite level is being determined in insecticide resistant vs. susceptible strains. The research should ultimately lead to novel measures for malaria control, via insect transgenesis or the development of ‘smart’ insecticides capable of disrupting interactions that are protective to the parasite and/or enhancing antagonistic ones.

Alternative forms of stress selection can produce a different response

By contrast to the decreased susceptibility resulting from insecticide pressure in *Culex*, selection for hunger tolerance in *P. papatasi* produced increased susceptibility to parasite infection (Schlein & Jacobson, 2001). Selection of a colony of *P. papatasi* of Jordanian origin for hunger tolerance increased susceptibility to *L. major* infection from 25% to between 80 and 64%. This increase in susceptibility was an inherited characteristic and not a direct result of preinfection hunger. It is not clear what physiological changes are being selected in the sandflies to produce the hunger tolerance, although simple gross differences in sandfly size have been ruled out. In this case, the selective effect seen in the laboratory colony also appeared to hold in the field, with larger proportions of the F_1 sandflies from an arid (sugar deprived) area becoming infected compared with F_1 insects from an oasis collection site. The results from both studies show the interdependence of different selectable insect characteristics, where phenotypes influenced by different environmental conditions can affect the balance between parasite and vector.

Acknowledgments

We thank Pam Taylor, Ann Underhill and Victoria Carter at Keele University for assistance. J.V. is supported by the General Secretariat of Research and Technology, Greece. L.McC. is supported by a special research fellowship from the Leverhulme Trust and the Medical Research Council. S.H.P.P.K. is supported by a Wellcome Trust Fellowship.

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Table 1

Comparison of number of oocysts developed in insecticide resistant and susceptible *Anopheles stephensi* mosquitoes.

<i>An. stephensi</i> species	Mouse	Prevalence of infection	Arithmetic mean of oocysts	Arithmetic mean of oocysts per group	Probability ($P=0.05$)
BEECH	1	5.3%	16±11 (n=23)	14	$P=0.41$
	2	8%	15±12 (n=25)		
	3	9.2%	13±10 (n=30)		
	4	7.8%	14±11 (n=29)		
DUBS-R	1	5.3%	20±18 (n=27)	16	
	2	8%	16±18 (n=28)		
	3	9.2%	14±9 (n=25)		
	4	7.8%	13±9 (n=35)		

Each replicate experiment used different batches of 25 mosquitoes from each strain, which were fed on the same infected mouse (1–4). The probability indicating whether the number of oocysts in the midgut of each strain are similar was determined by Student's *t*-test.