

Insecticides and mosquito-borne disease

Insecticide resistance in mosquitoes can also interfere with developing parasites.

The primary means of controlling mosquito-borne diseases such as malaria and filariasis is still by residual spraying with insecticides. Here we show that insecticide-resistant *Culex quinquefasciatus* mosquitoes are less likely to transmit filariasis than their insecticide-susceptible counterparts. If this surprising finding extends to other combinations of insect species, insecticide-resistance mechanisms and disease, it could have widespread consequences for the control of vector-borne disease.

The development of insecticide resistance in mosquito vectors is common¹. Insecticide resistance is assumed to increase the likelihood of disease transmission by mosquitoes by increasing the population size and allowing mosquitoes to live longer in the presence of insecticide. We have tested the validity of this assumption in *C. quinquefasciatus* from Sri Lanka.

Culex quinquefasciatus uses one predominant resistance mechanism that occurs in more than 80% of insecticide-resistant *Culex* worldwide^{2,3} and which originated in one population before spreading rapidly³. This resistance depends on the stable germline amplification of two esterase enzymes and an aldehyde oxidase encoded on 30 kilobases of DNA⁴; up to 80 copies of this DNA amplicon can be present per cell⁵.

One of the esterases found in resistant insects is expressed at very high levels in the mosquito gut, subcuticular layer, Malpighian tubules and salivary glands, resulting in a change in the redox potential in these cells⁶. As most mosquito-borne parasites must pass through 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-resistance status of the insects.

Lymphatic filariasis, caused by the parasitic worm *Wuchereria bancrofti*, is an endemic disease in Sri Lanka⁷. Since 1974 mosquitoes have been controlled by spraying their breeding sites fortnightly with the organophosphate fenthion⁸. There is a moderate frequency of esterase-mediated resistance in *C. quinquefasciatus* throughout the island, although this mechanism is not particularly effective against fenthion⁸.

We collected blood-fed female *C. quinquefasciatus* from the houses of filariasis patients in seven districts. Mosquitoes were snap-frozen within 48 hours of taking their blood meal and analysed for parasite load by quantitative polymerase chain reaction (PCR) and for insecticide resistance by biochemical assay using *p*-nitrophenyl-

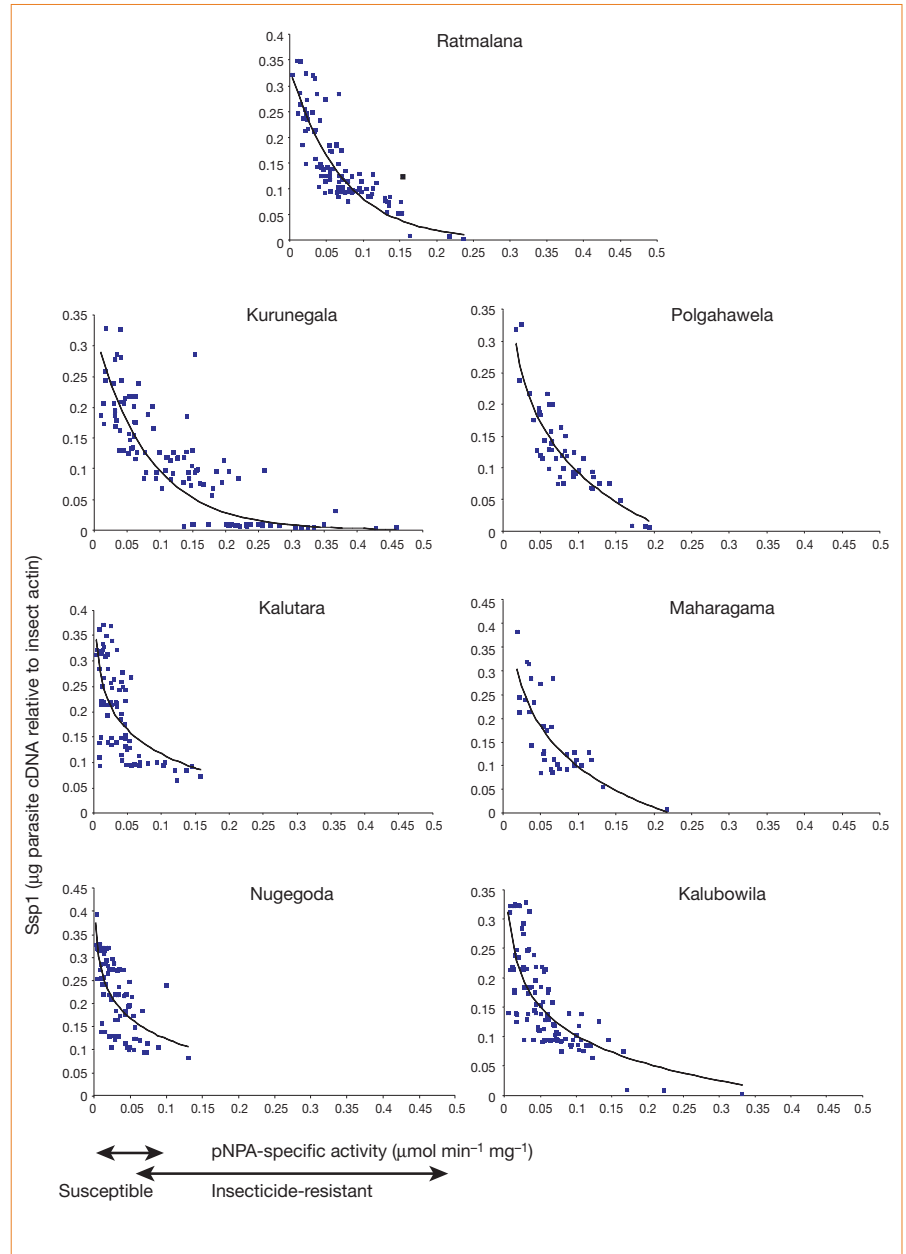


Figure 1 Relation between insecticide-resistance status, as determined by esterase activity using *p*-nitrophenylacetate (pNPA), and quantity of *Wuchereria bancrofti* RNA (as determined by the quantity of parasite Ssp1-repeat complementary DNA sequence relative to the insect actin cDNA in the same extraction) carried by individual field-collected mosquitoes less than 48 h after an infected blood meal; insects were collected from seven locations in Sri Lanka (for further details, see Supplementary Information). Arrows indicate ranges of esterase activity in insecticide-susceptible and in insecticide-resistant insects.

acetate. For quantitative PCR, we used primers specific to *W. bancrofti*⁹, standardized against a mosquito actin control¹⁰ from a complementary DNA template produced from RNA extracted from whole infected mosquitoes.

Almost 80% of the insecticide-susceptible and resistant mosquitoes that we collected were infected with *W. bancrofti*. However, at all seven localities there was a

strong negative correlation between esterase activity, as determined with *p*-nitrophenylacetate, and parasite RNA levels (Fig. 1). The reduction in parasite RNA in insecticide-resistant mosquitoes was not due to differential mortality of parasite-infected resistant insects, as insecticide-resistance gene frequencies were similar in infected mosquitoes, in field-caught mosquito larvae, and in uninfected mosquito adults;

blood-meal sizes were also comparable for the resistant phenotype.

Artificially feeding insecticide-resistant and insecticide-susceptible mosquito colonies (PelRR and PelSS, respectively¹¹) with blood infected by *W. bancrofti* to an intermediate level of parasitaemia — which should result in the infection of mosquitoes without substantial insect mortality — produced stage-L3 infective parasite larvae after 12 days in 76% of PelSS females ($n=250$), but no larvae in any of the PelRR females ($n=200$). Our results indicate that an increase in esterase activity could affect the development of stage-L1 *W. bancrofti* larvae, which may be arrested in the gut cells of insecticide-resistant but not insecticide-susceptible mosquitoes.

Filarial infection severely damages the mosquito host, often killing it. The spread of esterase-based insecticide resistance in field populations of *C. quinquefasciatus* may therefore be influenced by selection pressures for both insecticide detoxification and reduction of the microfilarial burden. Similar esterase-based insecticide-resistance mechanisms have been selected in field populations of the malaria vectors *Anopheles albimanus*¹² and *A. culicifacies*¹³, which could directly affect the transmission of malaria.

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Second, does Doncaster *et al.*'s model aid our understanding of the patterns of sexuality observed in nature? Several theoretical problems have been pointed out with the tangled bank model, and empirical studies have tested its (identical) assumptions and predictions.

For example, the advantage of sex is reduced if asexual populations are made up of multiple clones that fill multiple portions of the niche space^{4,5}; coexistence of sexuals and asexuals is rarely observed⁴; plant and algae populations consisting of mixtures of genotypes have carrying capacities not much greater than that of the average of their component genotypes, and rarely greater than that of the best genotype⁸; and results from natural populations indicate that the ecological and demographic predictions of the tangled bank model are not met^{6,9}.

Third, does the model of Doncaster *et al.* provide new insights into the cost of sex (males)? The authors argue that the cost of males is ecology dependent, so there is not necessarily a twofold advantage to be recouped in adaptive payoffs. However, one of the model's implicit assumptions is that different genotypes use different niches (genotype-by-environment interactions), so the model is not purely ecological.

The way in which this type of model allows coexistence between sexuals and asexuals has already been discussed^{4,5}. Moreover, this mechanism is more appropriately viewed as an adaptive payoff in its own right, as in previous formulations^{1,3–5}. In this case, it may reduce the cost to be paid by other mechanisms, but such interactions between models have been discussed extensively¹⁰.

Nonetheless, Doncaster *et al.* remind us of the role that the tangled bank mechanism could play in a pluralist explanation of sex, possibly by interacting with deleterious mutations in a fashion analogous to the Red Queen¹⁰.

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Evolution

Paying for sex is not easy

Explaining the maintenance of sexual reproduction remains one of the greatest challenges for biology, with more than 20 hypotheses having been advanced so far¹. Doncaster *et al.*² have proposed another possible explanation, but we question the novelty and importance of their suggested mechanism.

First, does this model² provide a new mechanism to help explain sex? The model assumes that different genotypes exploit different parts of the environment, and that an asexual clone is not able to occupy all the environmental niches that are open to a sexual population. This provides an advantage to sex, and allows coexistence between sexuals and asexuals.

However, the assumptions are the same as those of the 'tangled bank' mechanism for the maintenance of sex^{1,3–5}. The message that has emerged from previous considerations of this mechanism is therefore the same as that proposed by Doncaster *et al.*² — namely, that competition within a fixed set of niches can provide an advantage to sex, with "the success of the clone [being] restrained by the narrowness of its ecological range" and leading to "a stable equi-

librium at which both sexual and clonal individuals persist"⁴.

Although the mechanisms favouring sex appear to be identical, the elegantly simple form of Doncaster *et al.*'s Lotka–Volterra model means that it requires implicit assumptions and is therefore hard to compare with previous tangled bank models, which were based on numerical simulations and which made more explicit assumptions^{4,5}. For example, does Doncaster *et al.*'s model require competition between siblings (as with some formulations of the tangled bank), or is it the special case in which sib competition is excluded³?

It is hard to test the implicit assumptions in the new model², but it may render some aspects of the tangled bank mechanism more testable by emphasizing two parameters: the maximum population growth rate, R_0 , and the degree of overlap between sexual and asexual niches, α . However, the importance of these parameters has been discussed previously — for example, it has been pointed out⁶ that, analogous to variation in R_0 , higher fecundity leads to greater sib competition and so increases the advantage of sex. It has also been shown⁴ how the advantage of sex varies with a 'competition coefficient', which is a version of α . Furthermore, such competition coefficients are notoriously difficult to measure⁷.