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Does resistance really carry a fitness cost?

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Insecticide resistance mutations are widely assumed to carry fitness costs. However studies to measure such costs are rarely performed on genetically related strains and are often only done in the laboratory. Theory also suggests that once evolved the cost of resistance can be offset by the evolution of fitness modifiers. But for insecticide resistance only one such example is well documented. Here we critically examine the literature on fitness costs in the absence of pesticide and ask if our knowledge of molecular biology has helped us predict the costs associated with different resistance mechanisms. We find that resistance alleles can arise from pre-existing polymorphisms and resistance associated variation can also be maintained by sexual antagonism. We describe novel mechanisms whereby both resistant and susceptible alleles can be maintained in permanent heterozygosis and discuss the likely consequences for fitness both in the presence and absence of pesticide. Taken together these findings suggest that we cannot assume that resistance always appears *de novo* and that our assumptions about the associated fitness costs need to be informed by a deeper understanding of the underlying molecular biology.

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Our ability to manage xenobiotic resistance (both to drugs and pesticides), relies on the 'alternation' (or 'mixture') of classes of compound with differing modes of action. Management strategies using such alternation of differing chemical classes assume that resistance to compound A will decline during the subsequent use of compound B. This assumption is based on the prediction that *de novo* resistance to compound A will carry a fitness cost and that the frequency of resistance to A will therefore decline while compound B (or no compound) is used instead. This assumption, that resistance carries a cost in the absence of the xenobiotic, is therefore central to current resistant management strategies in both agriculture (pesticide resistance) and medicine (antibiotic resistance and cancer tumour drug resistance). Despite the widespread reliance on such predicted fitness costs to decrease the frequency of xenobiotic resistance, and an ample literature on the subject, the documentation of such costs is in fact fraught with technical difficulty. Here we will focus our discussion on fitness costs associated with insecticide resistance but it is important to remember that such principles also apply to the management of resistance to all pesticides and drugs.

In the year 2000, Coustau *et al.* suggested that 'fitness costs can only be fully interpreted in the light of the molecular mutations that might underlie them' [1]. Here, some 17 years later, and following an explosion in the molecular analysis of insecticide resistance, we therefore now examine the extent to which this is true. Classical theory predicts that *de novo* mutations that confer resistance to pesticides should carry a fitness cost in the absence of pesticide. This theory is based on a model developed by Fisher [2] which suggests that independent selection pressures shape the present (almost) optimal phenotypes via complex gene coevolution. In view of this gene interdependence any new resistance associated mutation of major effect would therefore be predicted to be highly deleterious. Similarly, theory also suggests that once a new mutation has arisen then other loci within the genome can act as 'modifiers' to ameliorate the negative fitness costs associated with resistance in the absence of pesticide. However, as discussed below, well documented examples of such fitness modifiers are in fact very rare [3,4]. Here we will therefore critically examine if the current body of literature supports the assumption that resistance always carries a cost. We will do this by addressing several fundamental questions. First, under what conditions can we realistically measure any potential fitness costs for different resistant strains? Second, what evidence is there that fitness costs are offset by the evolution of modifiers or are many resistance mutations in fact pre-existing polymorphisms with pleiotropic effects? Third, has the explosion of resistance associated molecular biology really helped us to understand when and where resistance might carry a cost?

Counting the cost

Numerous case studies of fitness costs attributed to insecticide resistance have been recently and comprehensively reviewed elsewhere [5]. A review of this review

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suggests to us several basic rules for experiments designed to study the fitness costs of resistance. First and foremost, if resistance is defined as a genetic change leading to control failure in the *field*, then resistant strains should be both *field* derived and the costs of resistance should be studied in the *field*. Experiments on chronically selected resistant laboratory strains or on field collected strains tested in the laboratory, cannot really tell us much about likely fitness costs in the field. Second, the field collected strains that are compared should be both of known resistance genotype (homozygous susceptible *SS*, homozygous resistant *RR* or heterozygous *RS*) and should be compared in a similar genetic background (usually achieved by back-crossing resistance into a known susceptible background). Finally, if an experiment is conducted in the field, then ideally the resistant and susceptible strains should be competed directly against one another. If we apply these simple genetic criteria to the plethora of studies on fitness costs in the literature then very few studies pass all three of these tests. Therefore laboratory cage based competition studies showing, for example, a lack fitness cost associated with CYP6D1 mediated pyrethroid resistance in the house fly [6], need to be repeated under field conditions. In short the literature has therefore become a confusing array of studies conducted on a range of unrelated strains that may or may not have anything to do fitness costs in the field. Bearing all this in mind, it is now worth examining the few studies in which related strains or populations have been examined in the field.

One species where considerable efforts have been made to study resistance costs in well defined strains in the field is the Australian sheep blowfly, *Lucilia cuprina*. In this insect 70% mortality is observed in the overwintering (diapausing or developmentally arrested) larvae and diazinon resistant flies overwinter less successfully than their susceptible counterparts [7]. Critically, a 'modifier' locus of diazinon resistance has also been documented (see following discussion). When this modifier is restored to the resistant flies the overwintering success of resistant and susceptible flies is similar [7]. Similarly, dieldrin resistant (*Resistant to dieldrin* or *Rdl*) blowflies are also more strongly selected against during the Australian winter than at other times of the year [8]. These careful studies in the blowfly, which use genetically related susceptible and resistant strains with and without a fitness modifier, show us that the time of year in which field based fitness studies are performed is critical. Two further studies support the conclusion that overwintering can exacerbate the cost of resistance and that careful work studying resistance frequencies at all times of year are required. The first study examined the changes in resistance allele frequency of *Culex pipiens* mosquitoes overwintering in caves in the South of France. These mosquitoes carried two different resistance mechanisms either amplified esterases (termed as a single super locus, *Ester*) or altered acetylcholinesterase (encoded by *ace-1*). Whilst the changes in resistance frequencies observed can be altered by immigration of susceptible insects into the cave, changes in the frequency of *Ester* over the winter suggest that this super locus may be associated with a fitness cost as large as 42%. Similarly, a cost of 7% could be inferred for individuals that are homozygous resistant for *ace-1* or *ace-1*^{RR} [9]. Finally, highly resistant clones of *Myzus persicae* aphids (clones R_2 and R_3) that over-express esterase-4 (E4), which can sequester and hydrolyse a range of insecticides. They show a reduced capacity to overwinter in the United Kingdom when compared to their susceptible (S) and moderately resistant (R_1) counterparts [10].

Mechanisms and modifiers

Even the most simplistic consideration of resistance mechanisms can give us a set of predictions about when and where mutation of a gene product might lead to a fitness cost. For target site resistance involving point mutations in so called 'lethal' genes encoding essential ion channel subunits, we would predict severe functional constraints on the nature and location of resistance associated mutations. The classic example of such constraints is shown by amino acid replacements in the GABA receptor subunit encoded by the *Rdl* gene. Here replacements of alanine301 both affect drug binding and also destabilise the drug preferred desensitised state of the receptor. Given this unique 'dual' resistance mechanism, nearly all insects showing cyclodiene resistance carry replacements of alanine301. In *Drosophila* at least, and in common with many other ion channel mutants, *Rdl-RR* flies show temperature sensitivity (paralysis at high temperatures) in comparison to their *SS* counterparts and like resistance this phenotype is also semi-dominant. However to our knowledge the effects of such temperature sensitive paralysis have not been investigated in the field for *Rdl* or indeed other target sites such as the *para* encoded sodium channel (*para*^{ts} mutants were indeed originally isolated on this basis). Surprisingly however this narrow range of constraints does not apply to all ion channel subunits targeted by insecticides, despite the fact that these native ligand-gated ion channels are all composed of complex hetero-multimers of different ion channel subunits encoded by several different genes. Thus even native (rather than recombinant) GABA gated chloride ion channels containing *Rdl* encoded subunits are known to contain other subunits (despite the Rdl subunit alone conferring much of the insecticide relevant pharmacology). Thus a wide range of different mutations (including both point mutations [11,12], exon-skipping [13••] or the production of truncated proteins [14–16]) can give rise to spinosad resistance in the α6 subunit of the nicotinic acetylcholine receptor. This is explained by the surprising finding that α6 knock-out strains of *Drosophila* are in fact not 'lethal' and also confer high levels of resistance to spinosad, leading the authors to speculate

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