

Minireview

The genomics of insecticide resistance

John G Oakeshott, Irene Horne, Tara D Sutherland and Robyn J Russell

Address: Commonwealth Scientific and Industrial Research Organisation (CSIRO) Entomology, Clunies Ross Street, Black Mountain, ACT 2601, Australia.

Correspondence: John Oakeshott. E-mail: John.Oakeshott@csiro.au

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Abstract

Genomic technologies are revealing several mechanisms of insecticide resistance involving enhanced detoxification or reduced target-site sensitivity that had previously defied molecular analyses. Genome projects are also revealing some potentially far-reaching consequences for pest-insect genomes of the rapid accumulation of multiple resistance mutations in very short periods of evolutionary time.

The evolution of insecticide-resistant insects provides evolutionary biologists with ideal, contemporary model systems for studying how new adaptations can be very rapidly acquired. There is therefore great interest in the use of the tools of molecular biology to elucidate the mechanisms of insecticide resistance. Four recent reports [1-4] have shown how genomic techniques can access mechanisms that had previously proven intractable to molecular analysis.

A few cases of insecticide resistance were investigated at the molecular level during the 1990s using 'traditional' molecular techniques. The number was limited because essentially only those cases involving known genes that could readily be cloned by heterologous PCR or reverse genetics were tractable. Three types of mechanism were revealed by these early studies, two involving enhanced detoxification of the insecticide and one rendering the target site for the insecticide insensitive to its effects. One detoxification mechanism involving sequestration of the insecticide was seen in two cases of resistance to organophosphate and carbamate insecticides in aphids [5] and culicine mosquitoes [6]. In these examples, carboxylesterases with high affinity for the insecticides but very low degradative activity were massively overexpressed as a result of 100-400 fold amplifications of the genes encoding them. The second detoxification mechanism,

involving active degradation of the insecticide, was seen in two species of flies in which structural mutations had arisen in specific carboxylesterases that converted them to kinetically inefficient - but apparently physiologically sufficient - organophosphate hydrolases [7-9]. The third mechanism, in which the target molecule mutates in such a way that it becomes insensitive to the insecticides, has now been found in several cases covering a range of species and types of chemical [10-14]. The mutant target molecules include acetylcholinesterase for organophosphates, γ -aminobutyric acid (GABA) receptors for cyclodienes and voltage-gated sodium channels for the synthetic pyrethroids and dichlorodiphenyltrichloroethane (DDT).

There were several remarkable aspects to these findings. One was the biochemical inelegance of the sequestration and degradation mechanisms: sequestration incurred a metabolic cost and degradation was kinetically inefficient. Another was the recurrence of exactly the same amino-acid changes in orthologous proteins across different species, which was true for some of the esterases that could degrade insecticides and in some of the acetylcholinesterases, GABA receptors and sodium channels that became insensitive to them. A third remarkable aspect was that, in all cases, one or a small number of mutant alleles carrying the respective mutations have

spread through the species within a few years of first use of the insecticide. All these features suggested that there was a very limited set of options available to insects to confer resistance to insecticides. Genomic technologies are now allowing investigation of some previously intractable resistance mechanisms, however. These cover resistance to both the traditional chemical insecticides mentioned above and the proteinaceous 'biopesticide' crystal toxins from *Bacillus thuringiensis* ('Bt toxins'). Here, we reassess the prevailing dogmas on resistance genetics in the light of the new results.

Comparative genomics and divergent evolution of detoxification genes

One of the major advances in the study of insecticide resistance enabled by genomics has simply been the cataloguing of relevant gene families. The lack of this information has not been so problematic for genes that mutate to give an insensitive target site, which for most chemical insecticides involves just one or occasionally two candidate genes, but it has been a major constraint on the study of resistance by sequestration and degradation. Now, Ranson *et al.* [1] have compared the three major gene families that have so far been implicated in insecticide detoxification between the two insect genomes that have been fully sequenced. The three gene families are the cytochrome P450s, carboxylesterases and glutathione-S-transferases (GSTs), and the two species are the dipterans *Drosophila melanogaster* and the malarial mosquito *Anopheles gambiae*. In total, nearly 150 members of the three families were found in each species, with the P450 family roughly twice as large as either of the others. Most of the P450s and GSTs are thought to have detoxification or related digestive and/or metabolic roles [15,16], although many of the esterases are also expected to have specialist non-detoxification functions [17]. There would therefore seem to be substantial scope for the secondment of various members of the families to resistance-related functions. Furthermore, as biochemists have long suggested [18], the P450s seem to be the main resource for the evolution of resistance by enhanced detoxification.

Significantly, although *D. melanogaster* and *A. gambiae* are in the same insect Order, only a small minority of the members of the three gene families could clearly be identified as orthologs between the two species [1]. Most major clades of genes within each of the three families were clearly represented in both species, but the origins of most genes within each clade were best explained by independent duplication events within each species after the two species had diverged from one another. It follows from this that the finding of the same resistance mutations in orthologous genes should prove to be the exception rather than the rule, at least in the case of resistance by enhanced detoxification.

Microarrays and regulatory mutations in cytochrome P450s

Daborn *et al.* [2] have now provided us with the first example of a mutation in a P450 gene that leads to insecticide resistance elucidated at a molecular level. They used expression profiling with microarrays to show that the high level of DDT resistance found in many strains of *D. melanogaster* is due to an approximately 100-fold upregulation of a specific P450 enzyme (Cyp6g1), owing to the insertion of a transposable element into its promoter. Although this mechanism is completely different from those elucidated in earlier studies [5-14], one finding that is reminiscent of the earlier work is the rapid proliferation of a very small number of resistant alleles (in this case just one) throughout the species range.

Perhaps the critical feature of the expression-profiling approach taken by Daborn *et al.* [2] was that they were able to identify a specific causal change in a specific member of a large gene family without any *a priori* knowledge or assumption as to the identity of that gene within the family. In fact, although Daborn *et al.* [2] knew they were dealing with a P450 gene and therefore analyzed an array of only that family, they could in theory have proceeded without that knowledge and simply analyzed a more comprehensive array. The significance of this is that there are many cases of resistance whose biochemical mechanisms differ from those in the cases resolved at a molecular level so far. Moreover, several enzymes that metabolize pesticides but that do not belong to the three major detoxification gene families (P450s, carboxylesterases and GSTs) have been found in soil bacteria, and several of these have homologs of as-yet unknown function in insects [17]. All this suggests that additional resistance mechanisms will be found as the power of genomic technologies is applied to further examples of resistance.

Microarrays will not, of course, be the appropriate tool to resolve all of these cases. Proteomic technologies will also have their place, along with positional cloning using quantitative trait loci (QTLs). And, significantly, there will remain some mechanisms that are difficult to resolve even with genomic technologies, for example upregulation mediated by changes to *trans*-acting factors, a mechanism that appears to underlie some cases of resistance involving P450s, carboxylesterases and GSTs [18,19].

QTLs, positional cloning and multiple types of resistance to Bt toxins

The Bt toxins differ from most other insecticides in that they are proteins and are not neurotoxins. In fact, their modes of action are complex and poorly understood, involving binding to sites on at least four different protein and carbohydrate targets in the insect midgut [20-23]. Bt-toxin resistance has become a critical concern in the last five years as expression of Bt toxins in transgenic broad-acre crops has become

widespread [21]. Resistance has already been reported in natural populations of the diamondback moth *Plutella xylostella*, and it has proven to be relatively straightforward to select for resistance in laboratory populations of several species [20-23]. Several Bt-toxin resistance genes have been reported from *P. xylostella*, most of which probably encode the proteins that act as toxin-binding sites [20,22]. Similarly, most of the laboratory-selected examples of resistance to Bt toxins involve multiple genes [20-22]. Some of the individual genes underlying Bt-toxin resistance have now been mapped onto high-density linkage maps using QTL mapping [22-24].

Two laboratory-selected Bt-toxin resistance genes have been identified using genomic technologies. The first, by Gahan *et al.* [3], involved positional cloning in the caterpillar *Heliothis virescens*. Gahan *et al.* [3] analyzed the sequence of the region to which the resistance gene had been mapped and found that it included an open reading frame for a protein, cadherin, which biochemical work had implicated as part of the target site for some Bt toxins. The coding region of this gene was apparently disrupted in resistant lines by insertion of a transposable element. This result shows some interesting similarities to and differences from the examples of mutations causing target-site resistance to chemical insecticides summarized earlier [10-14]. Once again, the mutant target site appears to be effectively insensitive to the insecticide, although in this case the native function of the target molecule has probably also been lost, leading, it is expected, to a significant fitness penalty in the absence of insecticide (a phenomenon that is also characteristic of the resistant *P. xylostella* found in the field [20-22]). Interestingly, it is also another example of a resistance mutation that is due to insertion of a transposon, although in this case it is the coding region, not the gene promoter, that has been disrupted.

Griffitts *et al.* [4] identified the second Bt-toxin resistance gene, this time in the nematode *Caenorhabditis elegans*, by testing various cosmids cloned from the region containing the gene from a resistant strain for their ability to confer resistance when transformed into a susceptible strain. The cosmid found to do this encodes a β -1,3 glycosyltransferase, which Griffitts *et al.* [4] suggest may be involved in the assembly of a carbohydrate component of binding site(s) for Bt toxins. Both the resistance alleles of this gene that were sequenced had point mutations in their coding region with radical effects: one introduced a premature stop codon and the other replaced an otherwise highly conserved residue. Again, one might expect that these presumptive loss-of-function mutations would incur a fitness penalty in the absence of the pesticide. It will be interesting to see whether the putative fitness costs in the absence of the pesticide are borne out by further work on the mutant Bt-toxin target sites. With a couple of notable exceptions, such costs are not generally observed for chemical insecticide resistance mechanisms [25,26].

Selective sweeps and their genomic consequences

One feature of the evolution of insecticide resistance in the field that recurs through all the pre-genomic and genomic studies is the rapid spread of resistance alleles after the initial outbreak. Because it happens so quickly, there is relatively little time for recombination to separate the favored resistance gene from the particular combination (haplotype) of closely linked genes in which it arose. For example, it appears likely that the sweep of the mutant esterase/organophosphate hydrolase gene in the organophosphate-resistant blowflies [7,8] replaced most of the variation throughout the cluster of ten esterase genes in which it lies with just a couple of whole-cluster haplotypes [27]. This cluster is likely to make up a large proportion of the detoxifying esterase genes in the blowfly's genome [17]. If, as seems quite probable, similar sweeps have occurred in clusters containing resistant mutant P450s and GSTs [28], then a major reduction may have occurred in the genetic variation in this species' chemical defence system in a very short interval of evolutionary time. It must be said that the blowfly and some other major pests have proven remarkably adept in evolving resistance to many insecticides. As noted above, there may also be additional detoxification systems beyond the three major gene families so far studied. Nevertheless, it is tempting to suggest that, on top of any direct fitness costs in the absence of insecticide that may occur for some resistance mutants [25], there may also be a substantial 'opportunity cost' in terms of lost variation with which the species can respond to changes in its chemical environment in the future.

In conclusion, the application of genomic technologies to previously intractable cases of insecticide resistance has greatly expanded our views on the range of options available to insects to evolve insecticide resistance. On the other hand, however, we can also now see that the speed with which multiple resistance mutations are sweeping through some insect species will be substantially reducing the variation in linked genes. In so far as many detoxification genes occur in tightly linked clusters, these selective sweeps will impinge on the genetic variation available to these species to respond to future insecticide or other xenobiotic challenges.

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