**Editorial overview**: **Pests and Resistance:** **Resistance to pesticides in arthropod crop pests and disease vectors: Mechanisms, Models and Tools**

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Arthropod pests are a profound threat to agricultural production and the health of humans and domestic animals. Worldwide, herbivorous insects and mites cause an estimated 18–20% of crop yield loss per annum representing a value of more than US$470 billion [[1](#_ENREF_1)]. In turn arthropod-vectored diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually [[2](#_ENREF_2)]. The control of these damaging pests has for many years relied heavily on the use of synthetic pesticides, and the impact of chemistry-based interventions has in many cases been spectacularly successful. For example, between 2000 and 2015 the number of deaths due to malaria halved, 80% of which was attributed to the scale-up of insecticide-based vector control interventions [[3](#_ENREF_3)]. Unfortunately, the over-reliance on chemical pesticides has led to the emergence of widespread resistance, posing a serious threat to the sustainable control of a large number of insect pests. To effectively address this growing problem, it is necessary to understand the origin, spread and maintenance of resistance, and the underpinning mechanisms involved. The Pests and Resistance section published last year (2017) explored the ecological and evolutionary drivers of pesticide resistance, and how such knowledge can be used to inform Insect Resistance Management (IRM) and Integrated Pest Management Strategies (IPM) [[4](#_ENREF_4)]. This current section focuses on the *mechanisms* that underpin resistance. An important component of effective IRM/IPM is integrating knowledge on the molecular mechanisms that cause resistance into programmes that prevent, delay, or overcome resistance. For example, once specific resistance-associated genes or mutations are identified and validated, molecular diagnostics can be developed and used to monitor the distribution and frequency of these resistance alleles in the field. Such data can then be used to inform IPM and IRM strategies than aim to slow the further development of resistance mediated by these mechanisms.

Despite the considerable parallels observed in the evolution of resistance in arthropod crop pests and disease vectors, research on each system is often considered (and reviewed) separately. In this section we provide reviews of contemporary work on the evolution of pesticide resistance in both agricultural and medically-important pests with the aim of highlighting commonalities and differences for further exploration.

Despite the applied importance of direct research on pest arthropods we wish to acknowledge the profound impact that model species have contributed to our understanding of resistance, and in particular, the fruit fly, *Drosophila melanogaster*. **Perry** and **Batterham** outline the enormous contribution research on this species has provided on our understanding of insecticide targets, metabolism and transport. In many ways, *D. melanogaster* is a perfect model systemcoming equipped with a sophisticated array of genetic tools and resources. These tools have been of great utility in understanding the interactions between insecticides and the proteins they target. For example, elegant studies using technologies available in *D. melanogaster*, identified the nicotinic acetylcholine receptor subunit, Dα6 as an important target of the insecticide spinosad and characterised mutations that lead to resistance [[5-7](#_ENREF_5)]. Equally significant advances have been made using this model species on the proteins that metabolise and transport pesticides. These include detoxifying enzymes and membrane transport proteins, often providing a causal link between overexpression of metabolic genes and resistance. Finally, as highlighted in this section last year [[8](#_ENREF_8)], while insecticide resistance is often thought to come at a cost, identifying and quantifying these can be difficult if confounded by the different genetic backgrounds of resistant and susceptible pest strains. *D. melanogaster* alleviates this issue by allowing the effect of resistance genes or mutations to be readily examined in a defined genetic background. The combination of this with the wide range of behavioural assays that have been developed for this species has provide unprecedented insights into the range of fitness costs associated with a specific resistance mechanism [[9](#_ENREF_9),[10](#_ENREF_10)].

Turning to pest insects, four reviews in this section highlight recent work on the mechanisms and mutations underpinning resistance evolution. The first of these concerns metabolic resistance – the enhanced metabolism/sequestration of insecticides by detoxification enzymes such as glutathione S-transferases (GSTs), cytochrome P450s (P450s) and carboxylcholinesterases (CCEs). **Pavlidi**, **Vontas** and **Van Leeuwen** review recent work on the role of GSTs in the pesticide resistance of crop pests and disease vectors. They find that GSTs in resistant insects and mites confer resistance in two principle ways. Firstly, as for enzymes belonging to other well characterised detoxification enzyme superfamilies, such as P450s and CCEs, GSTs can confer resistance via direct metabolism or sequestration of chemicals. However, in contrast to these other enzyme groups, GSTs may also indirectly mediate resistance by providing protection against oxidative stress induced by insecticide exposure [[11](#_ENREF_11)]. GST resistance by both mechanisms is primarily mediated by overexpression of the specific enzyme involved, however, recent work on insecticide resistance in the African malaria vector *Anopheles funestus*, has demonstrated that qualitative as well as quantitative alterations to GSTs can lead to resistance [[12](#_ENREF_12)]. The GST AfGSTe2 is overexpressed in DDT resistant populations of *An. funestus*, however, a single amino acid substitution (L119F) in this enzyme, only found in resistant populations, enlarges the DDT-binding cavity, leading to increased DDT metabolism [[12](#_ENREF_12)].

While reports of resistance mediated by metabolic or target-site mechanisms are now commonplace a third mechanism - reduced penetration of insecticide through the insect cuticle - has been much less frequently reported, and consequently is comparatively poorly understood. **Balabanidou**, **Grigoraki** and **Vontas** highlight contemporary work on this topic and discuss outstanding knowledge gaps. To date, two primary mechanisms of penetration resistance have been described, physical changes in cuticular thickness and alterations in the chemical composition of the cuticle. The challenge for recent studies has been understanding how these alterations arise. New insights on these topics have been provided by recent work on the malaria vector *Anopheles gambiae* [[13](#_ENREF_13)]. A multi-insecticide resistant strain of *An. gambiae* was found to have a significantly thicker cuticle than susceptible mosquitoes due to enriched deposition of hydrocarbons to the epicuticle (the thin, cuticular hydrocarbon (CHC) rich, protective outer layer of the cuticle). Significantly, this was further linked to the over-expression of two cytochrome P450s (CYP4G16 and CYP4G17) which catalyse the final step of CHC synthesis. This work illustrates another way that the overexpression of metabolic enzymes may confer resistance beyond direct metabolism or sequestration of a pesticide.

The increased expression of genes encoding detoxification enzymes is now well established as one of the most common mechanisms by which arthropod pests evolve resistance. Given this, it is somewhat surprising that the regulatory factors that mediate the (over)expression of resistance genes are so poorly understood. Two reviews in this section cover current knowledge on two different aspects of this topic. The first of these by **Wilding** explores the genomic basis of transcriptional regulation of detoxification loci in insect pests and vectors. Several of the studies detailed have focused on Cap ‘n’ Collar isoform-C (CncC); an insect transcription factor that, as a heterodimer with a second transcription factor, muscle aponeurosis fibromatosis (Maf), regulates the transcription of a range of detoxification genes. Resistance involving these transcription factors does not appear to result from mutations in the coding sequence of the genes encoding these highly conserved proteins. Rather, resistance has been linked to constitutive overexpression of CncC or alterations in the binding site, antioxidant response element (ARE) sequences, in the promoters of detoxification genes [[14](#_ENREF_14)]. Despite the progress made in understanding the role of this important transcription factor in resistance, as cautioned by Wilding (this issue), a focus on CncC and ARE alone is short-sighted, and growing evidence is emerging of the role of other transcription factors and their associated binding sites. This topic of research continues to be somewhat challenging, however, recent genomic and functional advances provide optimism that a deeper understanding of this important subject can be gained.

The second review on the theme of resistance gene regulation is provided by **Weetman**, **Djogbenou** and **Lucas** who describe recent work on the role of copy number variation (CNV) in the resistance phenotype of mosquitoes. The authors illustrate that resistance mediated by this mechanism can be caused by duplication/amplification of both genes encoding detoxification enzymes and insecticide target sites. In the case of the former, amplified gene copies, such as those encoding esterases, are identical and confer resistance because of the enhanced capacity to break-down insecticides resulting from increased gene dosage [[15](#_ENREF_15)]. In contrast, CNV affecting insecticide target-proteins, such as the acetylcholinesterase (*Ace-1*) gene is considerably more complex. Duplication of *Ace-1* was first described in the house mosquito, *Culex pipiens* [[16](#_ENREF_16)], but has more recently been identified in *An. gambiae* and *Anopheles coluzzi*, both major vectors of malaria [[17](#_ENREF_17)]. In these species duplication of *Ace-1* is associated with a target-site mutation, G119S, which confers strong resistance to carbamate and organophosphate (OP) insecticides, but also results in a very inefficient enzyme. As first reported for *C. pipiens*, resistant mosquito vectors were initially found to carry a copy of the gene with the G119S mutation and a wild type copy, creating a state of permanent heterozygosis that provides the benefit of resistance to carbamates and OPs but with a reduction of fitness costs [[17](#_ENREF_17)]. Since then a range of genotypes that combine different numbers of resistant or susceptible copies of *Ace-1* have been described [[18](#_ENREF_18)], with the type of duplication selected presumably reflecting the intensity and distribution of insecticide selection pressure.

Recent technical advances have greatly facilitated the speed and ease with which resistance mechanisms can be identified and functionally validated. As outlined in this section, two of the most significant include the application of genomics to study resistance, and the development of post-genomic methods which can be used to validate candidate resistance alleles. **Clarkson**, **Temple** and **Miles** review recent research on the former and the insights provided by genomic studies of resistance in malaria vectors. The falling cost of DNA sequencing, over the last decade has made whole genome sequencing (WGS) of hundreds to thousands of resistant and susceptible insects collected from wild populations feasible for the first time. An exceptional example of this is provided by the efforts of the *Anopheles gambiae* 1000 Genomes Consortium who in the first phase of this project sequenced 765 mosquitoes from 8 African countries [[19](#_ENREF_19)]. This mammoth effort revealed strong signals of recent positive selection at several genes that are known to have a role in resistance. These included the gene encoding the voltage-gated sodium channel, *Vgsc*, the target site for pyrethroid insecticides, where 47 non-synonymous mutations were observed of which 17 appeared to be under selection. Significantly, while at least some of the observed variants had been previously described, many were completely novel. Furthermore, strong signals of selection were also observed at multiple loci with no known resistance genes, and these clearly merit further detailed investigation. The rich data these new approaches provide may facilitate the development of more dynamic and predictive forms of IRM, and Clarkson *et al.* outline how WGS might be used in this context. As illustrated by the studies highlighted in this review the increasing use of global genomic and transcriptomic approaches to study resistance are generating a huge number of resistance gene candidates that require functional validation. **Homem** and **Davies** highlight three functional genomic technologies which can meet this need and are being used to demonstrate the causality of resistance genes and mutations. These are RNA interference (RNAi), which can be used to knock-down candidate gene expression, the GAL4/UAS system, which can be used to express genes of interest in a spatiotemporal controlled manner, and CRISPR/Cas9, which can be used to introduce or delete sequences of interest. While *D. melanogaster* has been used to pioneer these approaches in insects (Batterham & Perry, this issue), they are increasingly being used in non-model insects. For example, introduction of the G4946E substitution into the ryanodine receptor gene of beet armyworm, *Spodoptera exigua*, (which was lethal when introduced into *D. melanogaster*) confirmed that this mutation confers high levels of resistance to diamides [[20](#_ENREF_20)].

To conclude, this is an exciting time to explore the molecular mechanisms that mediate pesticide resistance. New technological advances now allow previously unimaginable data sets to be produced, with the challenges now frequently relating to the handling and interpretation of the sheer volume of data obtained. We hope the reviews in this section provide a glimpse of some of the recent studies on the mechanisms of resistance evolution in crop pests and disease vectors and that this work can influence and inform future work on this important topic.

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