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Current knowledge on the origin of insecticide resistance mechanisms: the tip of the iceberg?

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1 Opinion

Insecticide resistance is characterized by a wide variety of mechanisms including modification of the target-site, overexpression of metabolizing enzymes, structural modification of these same enzymes, and increased excretion or reduced penetration of the insecticide. Furthermore, when the insect has to cope with high selective pressure for insecticide resistance, mutations may accumulate in a single gene as found for acetylcholinesterase (Sindhu 2018) or involve multiple genes and complex genetic interactions (Bass & Nauen 2023; Pym et al. 2023; Roca-Acevedo et al. 2022; Amichot et al. 2004). In the latter case, mutations can interact additively or synergistically to confer high levels of resistance. Clearly, while resistance is advantageous in the presence of insecticide, the cost of such adaptation can be far from negligible in the absence of insecticide (Freeman et al. 2021).

Years of intense research into the factors underlying insecticide resistance has revealed a complex genetic basis for this trait and has helped to identify numerous gene mutations responsible for cases of insecticide resistance. While it is important to continue to monitor the fate of previously described mutations in insect populations and possibly uncover new ones, it is also of paramount importance to understand the driving forces behind these mutations. In this process, we ought to revisit some outstanding questions, such as whether the mutations conferring resistance to insecticides are present before any treatment, or whether the treatment causes them directly or indirectly. We should acknowledge that this type of question has already been addressed from an evolutionary point of view (Hawkins et al. 2019), but our aim in this paper is to shed a different light on the issue of insecticide resistance.

Indeed, a case of pre-existence of a mutation conferring resistance before any insecticidal treatment was described in preserved specimens of *Lucilia cuprina* by Hartley et al. (2006). Individuals of this species collected before the first use of organophosphate (OP) insecticides harbored a mutation (W251L) in their esterase 3 gene that was shown to confer resistance to malathion. Furthermore, the resistance conferred by the W251L mutation was specific to malathion and did not apply to all OP insecticides. It is also interesting to note that this mutation has no fitness cost (ffrench-Constant 2007) and can therefore be present like any other substitution with no associated cost. In addition, to achieve a significant level of resistance against other OP insecticides, another G137D mutation was found, but only in specimens collected after the massive use of OP insecticides. However, this mutation has a high fitness cost. Using the wording of Hawkins et al. (2019), W251L could be an outcome of standing variation and G137D could be a *de novo* mutation. Nevertheless, contrary to the assumption of Hawkins et al. who assigns "significant fitness penalties" to mutations resulting from standing variation, the W251L mutation has no apparent fitness cost. In any case, this situation illustrates very well our present questioning.

We therefore have proof that a mutation conferring resistance can be present in populations before any insecticide treatment. This kind of mutation may be due to chance, but also to diffuse selection resulting from the pressure exerted by natural toxins. Indeed, herbivorous insect species must cope with plant defense molecules and in some cases this ability can also give them resistance to insecticide. This has been observed for example in certain species of Lepidoptera whose enzymes specializing in the metabolization of allelochemicals have been shown to be also able to metabolize

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insecticides (Li et al. 2007; Li et al. 2004; Li et al. 2000). It is clear also that natural molecules such as nicotine, because of their structural proximity to certain synthetic insecticides, could have predisposed insects to resistance (Bass et al. 2013).

These examples illustrate that resistance can pre-exist insecticide treatment, but the question remains: do mutations appear by chance or as a result of genetic events induced by the allelochemical or the xenobiotic/insecticide?

If we accept that mutations conferring resistance occur by chance and before the onset of any stress, we need to think about the putative genetic cost associated with these mutations. This cost must be null or extremely low because in the absence of selective pressure favoring this mutation, it is doomed to disappear from the population. Another possibility is that the mutation rate is very high, so that when stress occurs, whether due to allelochemicals or xenobiotics, an adaptive mutation can be selected. This means that mutations occur at high rates, which should be extremely costly for organisms in terms of fitness, unless we accept that beneficial mutations, i.e. those that confer an ability to adapt to the environment, are preferred. Conversely, deleterious mutations should be counter-selected, but at the population level, this situation is not at all favorable because it implies that a large number of individuals will not be viable or will act as a brake on the development of the population. Furthermore, links may exist between selection pressure, i.e. insecticide treatment, and the appearance of mutations causing resistance. From this point of view, certain references are worth discussed. Let's look at them in chronological order. The first one is from (Muller 1964) who proposes a direct positive link between the rate of recombination and advantageous mutations. The content of this article is theoretical, but its conclusions have been regularly considered to this day (Kaushik 2023). The second one (Flexon & Rodell 1982) demonstrated the link between selection for resistance to DDT and the increase in the rate of chromosomal recombination, supporting the concept of "hitchhicking". This conclusion is in full agreement with Muller's proposals. It should be emphasized that these recombinations must occur in the vicinity or within the genes capable of conferring resistance. It is also interesting to cite another work although not involving insecticides but environmental variations (Turner et al. 2008). Using *genome-wide association* studies on multiple *Drosophila* populations collected in Australia and the United States of America along north-south clines, they demonstrated that the sequence polymorphisms are not randomly distributed and are often found in areas known to be important for the adaptation to the environment. No molecular mechanism has yet been identified or proposed to understand or describe the links between recombination rate and insecticide resistance. Knowledge of recombination mechanisms in *Drosophila melanogaster* (Fellmeth & McKim 2022) and its ability to be selected for insecticide resistance make this model organism an ideal starting point for understanding how recombination and insecticide resistance are actually linked.

Everything that has been written above actually refers to what we might call 'historical' resistance mechanisms. By this we mean mechanisms involving modifications in the targets of the insecticides or enzymes acting directly on insecticides, either through structural mutations modifying their range of substrates, or through overexpression (cis or trans misregulation). In the latter case, epigenetic mechanisms play an important role as shown by the increasing number of descriptions of their involvement in insecticide resistance today (Mogilicherla & Roy 2023) including for example DNA methylation (i.e. Field 2000) or miRNA (Wang et al. 2023). In fact, here we are at the frontier between resistance mechanisms and the mechanisms responsible for the appearance of resistance, even if behind these examples of epigenetics, enzymes are present and can also carry mutations (DNA methylase, RNA polymerase, DICER, etc.). Are we moving the cursor one level upstream?

Biocontrol strategies can also raise entirely new questions about resistance. Indeed, in *Diabrotica virgifera virgifera*, resistance to the bio-insecticide *Bacillus thuringiensis* can be linked to a change of its bacteriome (Paddock et al. 2021). The evolutionary mechanisms leading to such resistance will certainly be challenging to elucidate.

Beyond the exciting scientific aspect, knowledge of the mechanisms by which resistance to insecticides develops can be decisive in managing resistance in the field, especially with new insecticides. While the rate of genetic recombination in a population seems to be important in terms of "historical resistance", cases of resistance involving epigenetic phenomena or the intestinal microbiome cast a certain cloud over our vision of this phenomenon. This makes understanding insecticide resistance and its emergence all the more fascinating...

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