Chapter 12

Insecticide Resistance and Fitness Cost

Thiago Affonso Belinato and Ademir Jesus Martins

Additional information is available at the end of the chapter

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Abstract

The intensive use of chemicals through decades has been selecting resistant populations of several insect species to distinct classes of insecticides, like neurotoxics, insect growth regulators, and toxins derived from bacteria. Insecticide resistance is nowadays a huge challenge for control programs of pests of rural practices and principally to the management of arthropod vector-borne diseases. Several behavioral, physiological, and molecular mechanisms can be selected for avoiding toxic effects of insecticides in the insect organism. These changes are genetic traits that arise randomly and spread throughout the population along time, under an environment with insecticide selective pressure. However, new rapidly achieved characteristics can present a fitness cost to their harbors, with negative effects in development and reproductive aspects. In this way, in the absence of insecticides, susceptible individuals may present reproductive advantages and then the population resistance levels would tend to decrease. If the selection pressure persists, however, compensatory genes known as modifiers can be selected, ameliorating the negative effects caused by the resistance genes themselves or their pleiotropic effects.

In this chapter, we present a review of research articles that describe some fitness costs associated with insecticide resistance, trying to correlate with the known selected mechanisms whenever possible, under an evolutionary perspective. Examples from natural population, as well as lineages artificially selected for resistance in the laboratory, were considered. Although new tools of vector control are currently being tested under field conditions, the use of insecticides will remain with an important role in the near future at least. In this sense, the knowledge of evolutionary processes of insecticide resistance is crucial to try to revert the resistant status of natural populations and to avoid resistance to new compounds, maintaining this strategy as an effective alternative of insect control.

Keywords: Resistance genes, deleterious effects, modifiers, evolutionary process, adaptation

1. Introduction

1.1. Insecticides and mode of action

Insecticides are traditionally employed in several human activities with the purpose of eliminating or controlling the density of undesired insect populations. At present, albeit the obvious environmental impact, the control of agricultural pests and disease vectors is still largely based on the use of those substances. Moreover, in several cases, chemical compounds represent the principal approach to interrupt the transmission of pathogens. Before the Second World War, most insecticides were constituted of inorganic compounds, and a few organic substances, such as nicotine, pyrethrin, and rotenone [1]. The modern era of organic insecticides began in the 1940s, a period known as the age of the "pesticide revolution", when DDT (dichlorodiphenyltrichloroethane) was used for the first time as an insecticide [2].

Currently, there are 25 groups of insecticides and acaricides based on available evidence about their target sites and mode of action, according to the Insecticide Resistance Action Committee (IRAC) [3]. The World Health Organization Pesticide Scheme (WHOPES) promotes and coordinates the testing and evaluation of pesticides for public health purposes, since 1960. Its recommendations are generally adopted for national campaigns in several countries. The main insecticide classes used for vector control are: organochlorine (OC), organophosphates (OP), carbamates (CA), pyrethroids (PY), insect growth regulators (IGR), spinosyns (SP), and toxins derived from bacteria (*Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus*) [4, 5]. The classes OC, OP, CA, PY, and SP include a broad range of compounds that act on the insect central nervous system and, thus, have an immediate effect.

The target site of OP and CA is the acetylcholinesterase (AChE), a conserved enzyme present in a wide variety of animals, including mammals, birds, reptiles, fish, and insects. This enzyme is responsible for the rapid hydrolytic degradation of the acetylcholine neurotransmitter at synapses, causing momentary interruption of the nerve impulse. The OP and CA insecticides bind in the AChE active site, compromising the acetylcholine hydrolysis and then accumulating the neurotransmitter at the synapses, causing repetitive nerve impulses.

The PY and OC (DDT and analogues) maintain the sodium channels in their opened conformation, generating a continuous influx of ions throughout the axons. Cyclodienes, another group of OC insecticides, act directly on the gamma-aminobutyric acid receptor (GABA), preventing the normal input of chlorine ions in the neurons, just after the nervous impulse. In all cases, regardless of the target site, OP, OC, and PY promote a continuous nerve impulse transmission that culminates in paralysis, convulsions, and death [6].

Unlike neurotoxic insecticides, IGRs do not induce an immediate death of the insects. However, they are toxic mainly against immature stages, affecting the moulting, metamorphosis processes, besides commitments in viability and reproduction of adults [7]. Based on the mode of action, the IGRs are classified into three major categories: (i) juvenile hormone mimics; (ii) ecdysone agonists; and (iii) chitin synthesis inhibitors [8].

Concerning the bacterial toxins, *Bacillus thuringiensis* (*Bs*) var. *israelensis* (*Bti*) and *B. sphaericus* are the most employed as insecticides. When ingested by larvae, the *Bt* toxins are activated

by insect proteases and bind to specific receptors in the larvae midgut epithelia. The final effect is an osmotic stress that leads to the disruption of midgut membranes and, consequently, to death [9][1].

2. Insecticide resistance mechanisms

Insecticide resistance is considered the major challenge for control programs involving the use of chemicals. Up to 2014, populations of at least 590 species of insects were diagnosed as resistant to insecticides. Resistance to around 300 compounds, including the neurotoxics (OC, OP, CA, PY, and SP), the IGRs, and *Bt* toxins, has already been registered to one or more insect species [3].

Insecticide resistance has a genetic basis. Randomly arisen mutations can prompt several alterations in aspects of behavior, metabolism, and physiology of the insects, which may gain adaptive advantages in an insecticide-treated environment. Such alterations can be classified as: (i) behavioral changes; (ii) altered penetration (increased production of cuticular components that reduces intake of insecticide); (iii) target site modification; and (iv) metabolic resistance (detoxification enzymes and ABC transporters) [10]. Although evidenced, the two first aspects are less reported, whilst several studies have described and evaluated the target site and metabolic resistance mechanisms. These two, alone or combined, potentially induce a wide range of resistance levels to virtually all available insecticides [11].

Most insecticides target a single protein in the insect organism. The interaction between these molecules disrupts a normal biological process, leading to the toxicant effects. However, some mutations that induce structural alterations in the target protein can change the insecticide levels of toxicity. Moreover, most of these alterations are conserved among distinct insect orders. For instance, cyclodienes inhibit chloride ion transport by keeping the gama-aminobutyric acid (GABA) receptor in a close conformation [12]. The replacement of an alanine to a serine or glycine at the aminoacid position 302 (A302S/G) in the GABA, generally referred to as *rdl* mutations (resistance to dieldrin), confer resistance in several species, such as *Drosophila melanogaster*, *Musca domestica*, *Hametobia irritans, Lucilia cuprina, Tribolium castaneum, Periplaneta americana*, and *Anopheles mosquitos* [13].

The glycine-to-serine substitution (G119S)[2] in the AChE (AChE-1, encoded by the *ace-1* gene) confers resistance to OP and CA in *Anopheles* and *Culex* mosquitoes. Interestingly, this mutation was never found in *Aedes* mosquitoes, regardless of the intense use of OP against their populations. The most accepted hypothesis for this relies on the fact that the AChE-1 119 glycine is encoded by a GGA, differently from the GGC in other species. It means that in other mosquitoes a serine substitution (AGC) requires only one nucleotide change. By contrast, two

1 A complete review about insecticides and their mode of action can be found at Sparks and Nauen (2015).

2 This denomination refers to the aminoacid in the position 119 of the AChE protein (AChE1), based on the Torpedo nomenclature (Toutant, 1989).

concomitantly selected mutations would be necessary in *Aedes* mosquitoes, an unlikely situation referred to as codon constraint [14].

Similarly, several mutations associated with PY and DDT resistance are present in distinct insect orders: the *kdr* mutations, that impair the *knockdown effect* provoked by those insecticides. The most common *kdr* (*knockdown resistance*) mutation is a leucine-to-phenylalanine substitution in the 1014 codon[3], although serine, histidine, cysteine, and tryptophan replacements are also found (reviews presented in Rinkevich et al., 2013 [15]). Several PY-resistant populations of major arthropod pests and disease vectors were found harboring *kdr* mutations. In this sense, for diagnostic purposes, different well-established tools for *kdr* genotyping have been implemented, specific for an increasing number of insect species. This allows a rapid and accurate access of the genetic background for PY resistance in natural populations [16].

The recent commercially introduced SP insecticides, which target the nicotinic acetylcholine receptors (nAChRs) [17], have been used for crop protection, animal health, and against human disease vectors. Three formulations of SP were approved by WHOPES for use in drinking water, increasing the chemical arsenal against mosquitoes [4]. However, resistance to this class of insecticides was already detected in a variety of insect species. A target-site point mutation (glycine-to-glutamate substitution G275E), for example, was identified in the nAChR of a Western flower thrips (*Frankliniella occidentalis*) in association with SP resistance [18]. Besides this single amino acid substitution, alternative splicing in the nAChRα 6 subunit seemed to be the mechanism selected in an SP-resistant population of the diamondback moth *Plutella xylostella* [19].

As exemplified above, mutations selected for resistance in the molecular targets of insecticides generally share homologous sites among different insects. These molecules are components of the nervous system, which are highly conserved among animals. Therefore, it is expected that few mutations can be maintained without impairing the essential physiological role of that molecule [20]. Target-site-resistant alleles are increasing in frequency and rapidly spreading, as well-recorded for malaria and dengue vectors. An interactive compilation of these data, organized in time and space scales, can be currently accessed on two distinct online platforms: IR Mapper (http://www.irmapper.com) and Popbio (https://www.vectorbase.org/popbio/).

Detoxifying enzymes are naturally present in living organisms with a protective function against potential damages caused by xenobiotics and endogenous metabolites. In many cases, insecticide resistance occurs due to an increased activity of such enzymes, a mechanism known as metabolic resistance. In general, this mechanism is related with the intense use of insecticides. However, other toxic compounds, such as chemical pollutants and plant toxins can also select for metabolic resistance mechanisms in insect populations. In this sense, different xenobiotics present in the environment are probably related, at least in part, with a preadaptation for insecticide resistance in disease vector and agricultural pests [21, 22]. Basically, xenobiotics pass through a series of enzymatic steps that transform them in polar substances,

3 In the case of the voltage gated sodium channel (NaV), the *M domestica* aminoacid sequence is most commonly taken as reference.

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soluble in water for an easier excretion [23]. The biotransformation is divided into three phases, with the participation of three main groups of enzymes. Phase I includes multiple function oxidases enzymes (MFO or P450) that carry out chemical modifications of a broad variety of xenobiotics. In phase II, glutathione S-transferases (GST) usually conduct conjugation reactions in the products resulting from the previous phase. The esterases (EST) can participate in both phase I and II, hydrolyzing ester bonds present in the xenobiotics. Finally, during phase III, the metabolites produced in the two first phases are actively exported out of the cells via ATP-binding cassette (ABC) transporters [24-27].

The metabolic resistance mechanisms are characterized by a gain in the ability for detoxifying molecules of insecticides, preventing them from reaching their targets. This acquisition can be selected by either an increase in the enzymatic activity over the insecticide (mutations that improve the detoxifying power) or an augment in the amount of copies of a specific enzyme (due to an increase in the transcription rate, for instance). Glutathione S-transferases, EST and MFO P450 enzymes are each comprised of tens of genes, composing supergene families, possibly resulting from duplication events along the evolutionary process, as well as independent gene duplications inside distinct species [28, 29]. Differently from target site mutations that can arise in homologous sites among different insect groups, several detoxifying genes are unique for some species and may be selected for insecticide resistance in a particular way.

The main questions that lie upon the molecular basis of insecticide resistance mechanisms are how many (and which) genes control the phenotype of resistance, how many mutations were selected within that gene(s), and if they are just spreading from one origin or appearing multiple times [30]. The advent of high-throughput screening molecular tools expands the searches for selected resistance mechanisms and their overall effects, toward beyond the target site mechanisms. Recent advances have revealed the complexity of metabolic systems enrolled in insecticide resistance at transcriptomic and genomic levels. Comparisons of the whole transcriptional profile between susceptible and resistant individuals generally indicate the participation of several genes in the physiological process of resistance [31-33]. In addition, genetic loci influencing the resistance can be physically mapped in the chromosomes through quantitative trait loci (QTL) approaches [34-36]. Likewise, a recent study identified several single nucleotide polymorphisms (SNPs), as well as an important and previously neglected copy number variation (CNV) related to insecticide resistance in *Aedes aegypti*, by combining genomic target enrichment with next-generation sequencing technologies [37].

3. Evolution of insecticide resistance

Insecticide resistance is an adaptive trait in which a set of genes are favorably selected to maintain the insect alive and able to reproduce under an environment exposed to pesticides. After being introduced, insecticides gradually eliminate the susceptible specimens, usually found at higher frequencies within populations. By contrast, harbors of resistant alleles, supposedly rare in the population, increase their frequencies along the time of continuous pesticide application. The importance of resistance alleles occurring prior to insecticide

employment has been discussed since the 1950s with the works of Crown [38] and more recently incremented on ffrench-Constant's reviews [39, 40]. If resistance mechanisms hold elevated fitness cost in absence of insecticide (as discussed subsequently), the rareness of these alleles in nonexposed populations is then a direct assumption. In this case, the selection of resistance genes is a post adaptive response. On the other hand, pre adaptive selection of resistant alleles might have happened before the insecticide pressure, presumably if those alleles had another physiological role. Consequently, this type of resistance alleles would be less likely to carry a fitness cost [39].

The presence of insecticides in the environment is the basis for resistance selection. Operational factors, like formulation, dosage, frequency, and intensity of application, will determine the strength of that selection pressure. Likewise, environmental and intrinsic biological elements will determine the extension and velocity for the dispersion course of resistance alleles. The amount of resistance alleles and their initial frequency, as well as their dominance, penetrance, expressiveness, and interaction within the whole genetic background are the genetic components. In parallel, biological and ecological pieces in this scenario include the offspring size, generation turnover, mono or polygamy behaviors, together with degrees of mobility, isolation, and migration, mono or polyphagia, use of refuges, etc. [41]. Naturally, the knowledge of most of these aspects will optimize the design for more effective insect control strategies. Even considering all those parameters, insecticide application can play a strong selection pressure, able to change the profile of a population very quickly [42].

One parameter that probably has a large impact on the evolution of insecticide resistance is the side effects, usually negative, related to the resistance mechanisms. This is likely the main reason that explains the low frequency of resistance alleles in populations not exposed to chemicals. Therefore, the most common assumption is that when the use of insecticides is interrupted, the frequency of nonresistant specimens would tend to increase toward the establishment of the previous susceptibility levels of the population. This is especially what managers of campaigns against vector of pathogens anxiously look for, once the arsenal of insecticide compounds to this end is very restricted [4, 5].

The mode of insecticide application is crucial to the velocity of resistance evolution. Since the main goal of these control strategies is a prompt reduction of the targeted insect population, they often apply high dosages of insecticides, which combined with the indiscriminate use of the household or agriculture products, result in a strong selective pressure. Hence, even with a high impact on the fitness, some resistance alleles can spread among populations [43]. Besides physiological and reproductive hitched-hiked costs for resistance, a continuous pressure may favor the spread of mechanisms with lower side effects. An important factor resulting from the refining aspect of Natural Selection over the adaptation for resistance is the selection of "modifier genes", which neutralize or compensate deleterious effects [44]. The modifier genes can reduce drastic effects on the overall fitness previously induced by some resistance alleles, enhancing the adaptation to the environment with insecticides.

An emblematic example occurred in the in the Australian sheep blowfly *L. cuprina*, where a mutant allele for the carboxylesterase E3 is responsible for resistance to the OP diazinon, presenting, however, high disadvantage in environments free of insecticide. One of the effects

on the overall fitness was a bilateral asymmetry in the resistant flies. With continuous use of insecticide over the resistant population, a modifier gene was subsequently selected, increasing the fitness and also neutralizing the negative effects over the asymmetry [45]. Later, it was verified that the candidate for that modifier was a gene with an important role in oogenesis, spermatogenesis, embryonic mesoderm formation, and eyes development. The authors hypothesized that the resistance allele had a broad pleiotropic effect causing developmental perturbations that affected bristles and wing development, presumably impelled by a role of the carboxylesterase E3 in cell adhesion. The selection of the modifier gene compensated these effects [46].

In *Culex* mosquitoes the *ace-1*^{R} allele codes for the G119S mutant AChE resistant to OP, however, with 60% lower activity than the wild-type enzyme. Consequently, resistant individuals present a severe fitness cost, reflected with the decrease of the *ace-1*^{R} allele frequency in the absence of insecticide, as observed in some *Culex pipiens* populations [47, 48]. The G119S mutation in *Anopheles gambiae* followed the same tendency [49]. The emergence of gene duplication in the *ace-1* locus containing both resistant *ace-1*^{R} and susceptible *ace-1*^{S} alleles not only guaranteed resistance to OP but also diminished the resistance deleterious effects, once the physiological role of the enzyme was no longer compromised [50].

Another scenario of amelioration of resistance was richly described by Labbé et al. (2009) for a gradual replacement of resistant genes in a decade's time among populations of *C. pipiens* from Montpelier, Southern France. In that study, the authors found that the *Ester*^{1} allele (from *Ester* locus, enrolled with over production of EST) was selected for resistance to OP; however, it was later replaced by the *Ester*^{4} allele. This newer one conferred the same advantages over insecticides, nonetheless with lower pleiotropic effects and fitness cost. Interestingly, a third allele *Ester*^{2} with both higher advantage and fitness cost seemed to be replacing the previously selected *Ester*^{4}. The hypothesis raised was that the first replacement (*Ester*^{1} to *Ester*^{4}) occurred as a compensatory amelioration, since *Ester*^{4} is less costly and more "generalist". On the other hand, the *Ester*^{2} allele would be more "specialist" to insecticide-treated areas, conferring high resistance but with strong pleiotropic effects. The practices of insecticide use in different areas of Montpelier during that time certainly influenced the evolution of this *Ester* locus. If the intensity of treatment had decreased, *Ester*^{4} would have possibly been favored over the stronger resistant *Ester*^{2} allele, given the former's lower fitness cost [44].

Although a common class of insecticide can select the same mutation for resistance in different insects, its effects on fitness vary through the species or even among different populations of the same species. For instance, the A302S *rdl* mutation remained under high frequencies in natural populations and the resistance persisted despite the withdrawal of cyclodienes in the field for years, as reported to natural populations of *Drosophila* [51], the German cockroach [52], and to the mosquito *A. gambiae* [53]. On the other hand, a reduction in the *rdl* resistant allele without insecticide selection pressure was observed in natural populations of the horn fly *H. irritans* [13] and the Australian sheep blowfly *L. cuprina* from both field and laboratory caged strain [54]. In the same way, *rdl* mutant *A. gambiae* and *Anopheles stephensi* mosquitoes presented reduced fertility and fecundity [55].

One has to consider that the evaluation of the overall fitness effects of a given mutation is very challenging, once it is difficult to separate their own effects from those caused by other mechanisms possibly coselected for resistance. In these aforementioned *rdl* examples, the reduced fitness might be related to the A302S mutation itself, and/or to metabolic resistance mechanisms. Similarly, the persistence of the resistance allele in an environment free of dyeldrin might be explained by the rdl cross-resistance with other insecticide that had been continually applied, as well as by the selection of modifiers genes, as previously discussed.

4. Evaluation of fitness cost of insecticide resistance

The main approach to investigate the fitness cost of resistance in field populations is to monitor the levels of resistance along the time in environments distinctly exposed to insecticides. Moreover, if the principal mechanism selected for resistance is known, the genotyping of resistance genes in place and time scales render important assumptions about their fitness cost. It is very difficult to access this kind of data from the field, however, since there are many variables occurring simultaneously.

For example, one population of *A. gambiae* from M'Bé, Côte D'Ivoire, used to be considered susceptible to most insecticides up to 2002, when a civil crisis broke and the monitoring was discontinued. Ten years later, a new study revealed important changes of the resistance mechanisms among *A. gambiae* populations from that locality. The main mechanisms that led them to become highly resistant to OC, PY, and CA were the L1014F *kdr* mutation and elevated activity of MFO and EST. The only well-known contexts that might explain this severe shift from susceptible to highly resistant were the pressure with deltamethrin-based products from rice paddles and the distribution of long-lasting PY impregnated nets (LLINs) since 2006 [56]. The alteration in the resistance profile over the time would suggest a low cost of the resistance alleles. However, little was known regarding the actual levels of insecticide pressure, migration from vicinity areas, and about the extent of the influence of surrounding environment. In this case, controlled laboratory assays could help to estimate the fitness costs of the selected resistance mechanisms.

For fitness studies in the laboratory, population cage experiments can evaluate the fluctuation of resistance itself and the selected mechanisms over successive generations, under an environment clearly free of insecticide and without interference of migration. In this matter, the cost of resistance can be measured according to the velocity that the resistance alleles decrease in confined lineages along the time. A laboratory lineage of *A. aegypti* resistant to PY due to the Na_VR2 *kdr* mutation[4] presented deleterious effects in a series of life-trait parameters. Population cage assays corroborated these negative costs, showing that the *kdr* allele severely decreased from 75% to almost zero along 15 generations [57]. Most of the studies have been making use of an opposite direction: populations from the field are confined and submitted to a selection pressure in the laboratory. In another example, also with *A. aegypti*, populations

4 NaVR2 is the kdr allele mutant in both 1016 (Val to Ile) and 1534 (Phe to Cys) of the voltage gated sodium channel (NaV), found in American populations of *A. aegypti*.

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from distinct Mexican localities were pressured with the PY permethrin for at least five generations in the laboratory. All the lineages had an increase in the resistance levels, correlated with an augment in the frequency of the Val1016Ile *kdr* mutation and with a number of detoxifying genes differentially transcribed, generally distinct at each lineage. Interestingly, the lineages that reached the highest frequencies of the *kdr* mutation presented a lower number of altered detoxifying genes [58]. These results strongly suggested that this *kdr* mutation had a lower fitness cost compared to the metabolic resistance genes occurring at each genetic background.

The knowledge of alterations in physiological and reproductive aspects is generally achieved by comparing life-trait parameters between susceptible and resistant individuals. As the result of pleiotropic effects of an altered gene will depend on the whole genomic structure, it is important that susceptible and resistant groups have the most similar genetic background as possible. The parameters usually evaluated are larval developmental time, adult longevity, ability to avoid predators, fecundity, fertility, mating competitiveness, and reproductive potential. When treating of blood-sucking insects, probing, acceptance of blood meal, and amount of ingested blood can also be tested. Such studies demand well-controlled conditions and are generally highly laborious, so that most of them follow few parameters at a time. In addition, the knowledge of the biology of the species under investigation is a prerequisite for the definition of which aspects would be more informative.

Fitness studies in the laboratory necessarily have to consider a well-representative collection from the field, in order to contemplate most part of the whole amplitude of variable traits from the original population. An F1 offspring of this sampling may then be raised in the laboratory to sufficiently amplify the number of individuals to be tested, as well as to normalize the physiological condition among the different populations. A laboratory lineage control of susceptibility and vigor should also be raised in parallel, as an endogenous control of experimental conditions, whenever possible.

Selection pressure for insecticide resistance in the laboratory has the advantage of controlled strength of selection and environmental conditions, population size, and absence of migration. On the other hand, if a monogenic key-mechanism for resistance was under lower frequency in the field, it is likely that this gene is not present in the sampling that established the first generation in the laboratory. For this reason laboratory pressures tend to result in polygenic resistance, where several resistance traits of minor effects are selected, but with a larger response when emerged together in the same genetic background [30]. This could also explain the different patterns of selected mechanisms to the same class of insecticide, especially metabolic resistance, in a same species.

Another important issue to be aware of when evaluating fitness costs in the laboratory environment is that most of the studies have investigated the possible life-trait alterations under optimal conditions. The amount and quality of food, the composition of substrate (or water in case of aquatic insects), density of individuals along life cycle phases, and mainly temperature and humidity are usually controlled. By contrast, insects are continually exposed to a wide range of abiotic or biotic stresses in the field. Therefore, the physiological costs of resistance alleles are probably underestimated in laboratory optimal conditions [41, 59]. The

evaluation of the fitness costs in resistant insects under stress conditions (in terms of nutrition, temperature, and larval density, for instance) can bring forth relevant data related to the evolution of resistance in the field. However, such investigations are still scarce [60-62].

5. Possible changes on development and reproduction of insecticide-resistant insects

As previously discussed, resistance genes may cause changes or even dysfunctions upon direct physiological process and indirect life history traits. The knowledge of the insecticide resistance costs and which parameters are altered are important to better design strategies of insect control, especially considering vectors of pathogens, once general developmental and reproductive life-traits are strongly associated to their vectorial capacity. In the following, we present some examples of resistance side effects in vector mosquitoes.

The longevity of insects is generally evaluated in fitness investigations as a key parameter of vector/parasite relationship. Decreased longevity has been detected in species resistant to different classes of insecticides. Both *Culex pipiens pallens* and *A. aegypti* selected for PY resistance in laboratory presented decreased longevity [63-65]. Pyrethroid resistance also induced similar effects on the longevity of *A. gambiae* females, in this case presumably due to affected energy metabolism and oxidative stress [66]. Defenses to non neurotoxic compounds can also affect longevity, as observed in one *A. aegypti* lineage selected in the laboratory for diflubenzuron (a chitin synthesis inhibitor) resistance [67]. As resistance mechanisms vary among species and populations, especially when metabolic, the life span of the resistant insects is not always affected, even when high resistance ratios are observed. This was the case of two Brazilian field populations of *A. aegypti* resistant to both OP and PY insecticides [68].

The time to complete the larval development is also of particular interest, since the longer it takes the higher is the exposure to adverse conditions of the breeding site and to natural predators and pathogens. Likewise longevity, resistance to several insecticides can affect this parameter. Increased developmental time was observed in *Culex quinquefasciatus* and *A. aegypti* selected in the laboratory for PY resistance [64, 65], and also to an *A. aegypti* field population with high resistance level to OP [65]. Natural populations of *C. pipiens* harboring the resistance alleles *ace-1*^{R} (modified AChE), *Ester*^{1} and *Ester*^{4} (overproduction of EST) also presented a longer larval developmental time [69]. The *kdr* mutation was also the prime cause for a delay in the larval development of *A. aegypti*, especially when mutant and PY susceptible larvae were reared together and under more stringent conditions [57]. Again, impacts on this parameter were not restricted to neurotoxic insecticides, as demonstrated for an *A. aegypti* laboratory strain resistant to *Bti* toxins, which presented impairment on the larval development time [70].

Some behavioral aspects can also be affected by resistance, as the ability to detect a potential host. Under laboratory conditions, for example, fewer OP resistant *A. aegypti* females responded to the blood meal stimuli, compared to their susceptible counterparts [68]. Similar results were observed in lineages of the same vector selected for resistance to a chitin synthesis

inhibitor. Additionally, these blood-fed females ingested 18-26% less blood than the susceptible lineage [67]. The blood meal acceptance and the amount engorged can directly influence the pathogen loads ingested, potentially influencing the vector competence. These parameters are also directly connected with fecundity, since blood feeding is related to the production of eggs. Indeed, the reduction in the amount of ingested blood in resistant *A. aegypti* mosquitoes was directly proportional to a lower number of eggs [67, 68]. Several studies evidenced the impact of insecticide resistance in blood-feeding aspects [64, 71, 72].

Besides longer developmental time, lower longevity, and problems with blood feeding, reproductive traits are potentially stronger parameters against dispersion and maintenance of resistance in the field. Some studies have addressed these aspects with laboratory-resistant lineages. *Aedes aegypti* populations resistant to OP and an IGR showed lower reproductive capacity, where resistant males were able to fecundate a lower number of females [67, 68]. In the same way, susceptible *C. pipiens* males had a mating advantage when competing with *Ester-4*, *Ester-1*, and *Ace-1*^{R} resistant individuals [47].

Some advantageous resistance side effects also occur. A *D. melanogaster* with increased expression of GST enzymes lived longer. The authors suggested that this alteration also promoted a tissue protection against reactive oxygen species [73]. In the same context, the resistance allele *Cyp6g1*, also in *D. melanogaster*, conferred resistance to DDT and was associated with a higher adult fecundity and increased viability of eggs and larvae in absence of insecticide [74]. Females of the mosquito *C. quinquefasciatus* resistant to PY by MFO overexpression survived longer when maintained with sugar solution [75].

6. Conclusions

The idea of "evolution-proof insecticides" is a challenge for the introduction of new compounds. A possible strategy proposed to slow the evolution of insecticide resistance would be to apply compounds with action over older mosquitos, i.e., when females have already laid most of their eggs. In this direction, there would be a very weak selection pressure over resistance genes, once practically all the offspring of susceptible and resistant individuals have emerged at each generation [76]. This is particularly interesting to the control of vector-borne diseases, because several pathogens have an intrinsic incubation time of their life cycle inside the insect organism. where the insects are able to feed on blood and lay their eggs several times before become infective. Nonetheless, they cannot live long enough to have the opportunity of a infective blood feeding. Mathematical models have shown that this kind of approach against old insects would dramatically affect the course of insecticide resistance [77].

New strategies are currently being tested in the field, like the release of genetically modified mosquitoes that suppress the natural population [78, 79] and of a strain carrying endosymbiont bacteria that diminishes the mosquito vectorial capacity [80, 81]. However, until these tools are not available for a high-scale application and considering distinct vectors, the use of insecticides must continue to play a central role, especially during epidemic outbreaks. In this sense, physiological, molecular, and evolutionary aspects of insecticide resistance need to be

further studied and discussed with the aim to better improve the control of undesired insect populations.

Author details

Thiago Affonso Belinato and Ademir Jesus Martins

*Address all correspondence to: ademirjr@ioc.fiocruz.br

Laboratório de Fisiologia e Controle de Artrópodes Vetores, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro-RJ, Brazil

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