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The Pyrethroid Knockdown Resistance

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1. Introduction

New promising insect control efforts are now being evaluated such as biological alternatives or even transgenic insects and *Wolbachia* based strategies. Although it is increasingly clear that successful approaches must involve integrated actions, chemical insecticides unfortunately still play a central role in pest and vector control (Raghavendra et al., 2011). Development of new safe and effective compounds in conjunction with preservation of those currently being utilized are important measures to insure insecticide availability and efficiency for arthropod control. In this sense, understanding the interaction of insecticides with the insect organism (at physiological and molecular levels), the selected resistance mechanisms and their dynamics in and among natural populations is obligatory.

Pyrethroids are synthetic compounds derived from pyrethrum, present in *Chrysanthemum* flowers. Currently, pyrethroids are the most used insecticides against arthropod plagues in agriculture and livestock as well as in the control of vectors of veterinary and human health importance. They are chemically distinguished as type I (such as permethrin, compounds that lack an alpha-ciano group) and type II (with an alpha-ciano group, like deltamethrin) (T. G. Davies et al., 2007b). Pyrethroid insecticides have been largely adopted against vector mosquitoes through indoor, perifocal or ultra-low volume (ULV) applications. As of yet pyrethroids are the only class of insecticides approved for insecticide treated nets (ITNs), an important tool under expansion against malaria, mainly in the African continent (Ranson et al., 2011). The consequence of intense and uncontrolled pyrethroid use is the extremely rapid selection of resistant populations throughout the world.

Just like DDT, pyrethroids act very fast in the central nervous system of the insects, leading to convulsions, paralysis and eventually death, an effect known as *knockdown*. However, unlike DDT, pyrethroids are not claimed to cause severe risks to the environment or to animal or human health, hence its widespread use. The main pyrethroid resistance mechanism (the knockdown resistance phenotype, *kdr*) occurs due to a point mutation in the voltage gated sodium channel in the central nervous system, the target of pyrethroids and DDT.

Herein we aim to discuss the main mechanism of pyrethroid resistance, the knockdown resistance (*kdr*) mutation, its effect and its particularities among arthropods. The most common methods presently employed to detect the *kdr* mutation are also discussed. Some aspects regarding the other main pyrethroid resistance mechanisms, like alterations in behaviour, cuticle and detoxifying enzymes will be only briefly addressed. The proposal of this chapter is to review knockdown resistance to pyrethroids, nowadays the preferred insecticide class worldwide. This topic discusses aspects of general biology, physiology,

biochemistry, genetics and evolution, with focus on disease vector mosquitoes. It is expected that the amount and diversity of material available on this subject may well illustrate insecticide resistance in a broader context.

2. Insecticide resistance mechanisms

Besides the resistance to chemical insecticides caused by modifications in the target site (also called phenotypic resistance), other mechanisms commonly associated are: metabolic resistance, behavioral modification and alterations in the integument. In the first case, endogenous detoxifying enzymes become more efficient in metabolizing the insecticide, preventing it from reaching its target in the nervous system. This occurs due to 1) increase in the number of available molecules (by gene amplification or expression activation) or 2) mutation in the enzyme coding portion of the gene, so that its product metabolizes the insecticide more efficiently. These processes can be very complex and involve three major enzyme superfamilies: Esterases, Multi function Oxidases P450 and Glutathion-S-Transferases (Hemingway & Ranson, 2000; Montella et al., 2007). In contrast, there are few examples in literature regarding insect behavioral changes and tegument alterations.

Resistance to insecticides may be functionally defined as the ability of an insect population to survive exposure to dosages of a given compound that are lethal to the majority of individuals of a susceptible lineage of the same species (Beaty & Marquardt, 1996). Resistance is based on the genetic variability of natural populations. Under insecticide selection pressure, specific phenotypes are selected and consequently increase in frequency. Resistance can result from the selection of one or more mechanisms. In order to elucidate the molecular nature of resistance, many studies report laboratory controlled selection of different species (Chang et al., 2009; Kumar et al., 2002; Paeoporn et al., 2003; Rodriguez et al., 2003; Saavedra-Rodriguez et al., 2007). With selected lineages, it becomes easier to separate the role of each distinct mechanism. In a more direct approach, the current availability of a series of molecular tools enables detection of expression of altered molecules in model organisms so that the effect of the insecticide can be evaluated under specific and controlled circumstances (Smith et al., 1997).

Regardless of the mono or multi-factorial character of resistance, this phenomenon may be didactically divided into four categories: behavioral, cuticular, metabolic and phenotypic resistance. In the first case the insect simply avoids contact with the insecticide through behavioral adaptations, which are presumably related to genetic inheritance (Sparks et al., 1989). Among arthropods, mosquitoes are by far the group most intensely investigated in relation to behavioral resistance (Lockwood et al., 1984). For instance, *Anopheles* malaria vector mosquitoes from the Amazon Region had the habit of resting in the walls after a blood meal. There are registers that some populations changed their behavior after a period of indoor residual application of DDT to the dwelling walls (Roberts & Alecrim, 1991). Behavioral changes that minimize contact between insect and insecticide may cause a severe impact in the insecticide application efficacy, especially if resistance is selected by physiological features (Ranson et al., 2011).

Certain alterations in the insect cuticle may reduce insecticide penetration. However, these effects are unspecific, leading to resistance to a series of xenobiotic compounds. This mechanism is known as reduced penetration or cuticle resistance. It is probably not related to high levels of resistance by itself, but it can interact synergistically with other mechanisms. The physiological processes or molecular pathways which describe this type of

resistance remain to be elucidated. With respect to pyrethroid resistance, recent evidences point to an increase in the levels of expression of two cuticle genes in populations of two *Anopheles* species (Awolola et al., 2009; Vontas et al., 2007).

The increased ability to detoxify insecticides is one of the main types of resistance, commonly referred to as metabolic resistance. It takes place when the activity of naturally detoxifying enzymes is enhanced, impeding the insecticide to reach its target. Among these enzymes, Multi function Oxidases (or Monoxigenases P450), Esterases and Glutathion-S-Transferases (GST) (ffrench-Constant et al., 2004; Hemingway & Ranson, 2000) are the major representative families. Although the molecular basis of metabolic resistance has been extensively studied, only few reports have investigated the specific metabolic pathways involved or their location in the insect organism. Many different mutations may be attributed to metabolic resistance, such as those leading to production of more enzymes, via gene duplication events or either increases in gene transcription rates, alterations in the normal tissue/time specificity of expression, point mutations leading to a gain of function or changes in the substrate specificity (ffrench-Constant et al., 2004; Hemingway et al., 2004; Perry et al., 2011). Detoxifying enzymes belong to superfamilies composed of numerous genes (Ranson et al., 2002), and it is not unusual for different enzymes to produce the same metabolites. Additionally, an alteration in one type of enzyme may lead to cross-resistance among different classes of insecticides (Ranson et al., 2011). However, population genetic markers that make feasible a complete diagnostic of the resistance mechanisms or their distribution are not yet available. Current studies are generally based on biochemical assays (Valle et al., 2006) and, to a lesser extent, on *microarray detox chips* (David et al., 2005; Vontas et al., 2007). Due to technical limitations, the most common reports are hence oriented to single gene responses, such as punctual mutations that increase the ability of a specific enzyme in detoxifying an insecticide (Lumjuan et al., 2011; Morin et al., 2008).

Multi function P450 Oxidases are the enzymes most commonly associated to metabolic resistance to pyrethroids. However, despite much indirect evidence of P450 total activity increase or even detection of higher expression of some related genes (*cyp*), little is known about their metabolic activity. For instance, 111 genes code for P450 in *Anopheles gambiae*, but only two (*cyp6p3* and *cyp6m2*) were described to be involved in pyrethroid metabolism (Muller et al., 2008). Surprisingly, metabolic resistance can still vary during the course of the day. This is the case of an *Ae. aegypti* population whose resistance to the pyrethroid permethrin is mediated by the *cyp9M9* gene. Expression of this gene is regulated by transcriptional factors enrolled in the circadian rhythm of the insect, varying along the day (Y. Y. Yang et al., 2010).

Finally, phenotypic or target site resistance is designated by modification of the insect molecule where the insecticide binds, inhibiting its effects. Neurotoxic insecticides have as their ultimate target different molecules from the insect central nervous system: the enzyme Acetylcholinesterase (for organophosphates and carbamates), the gama-aminobutiric acid receptor (for ciclodienes), the nicotinic acetylcholine receptors (for spinosyns and neonicotinoids) and the voltage gated sodium channel (for DDT and pyrethroids). Although the mutated target molecule decreases or even abolishes its affinity for the insecticide, it is essential that this alteration does not result in loss of function regarding the insect physiological processes. Since the classical target molecules are much conserved among animals, few mutations are permissive to guarantee the viability of their carriers (ffrench-Constant et al., 1998; Raymond et al., 2001).

The voltage gated sodium channel (Na_V) is the effective target for a number of neurotoxins produced by plants and animals, as components of their predation or defense strategies. Knowledge that mutations in the Na_V gene can endow resistance to both the most popular insecticides of the past (DDT) and nowadays (pyrethroids) is leading to significant progress in the understanding of the physiology, pharmacology and evolution of this channel (ffrench-Constant et al., 1998; O'Reilly et al., 2006).

3. The role of the voltage gated sodium channel (Na_V) in the nerve impulse propagation in insects

The membrane of all excitable cells (neurons, myocites, endocrinous and egg cells) have voltage gated ion channels responsible for the generation of action potential. These cells react to changes in the electric potential of the membrane, modifying their permeability status (Alberts et al., 2002; Randall et al., 2001). Voltage gated sodium channels (Na_V) are transmembrane proteins responsible for the initial action potential in excitable cells (Catterall, 2000). They are members of the protein superfamily which also includes voltage gated calcium (Ca_V) and potassium (K_V) channels (Jan & Jan, 1992). Both Na_V and Ca_V channels are constituted of four homologous domains whilst K_V is a tetramer with only one domain. A proposed evolution pathway assumes that Ca_V have evolved from K_v by gene duplication during the evolution of multicelular eukaryotes. Na_V channels are supposed to have evolved from an ancestral Ca_V family (family Ca_V3) (Spafford et al., 1999). Accordingly, the four Na_V domains are more similar to their Ca_V counterparts than among themselves (Strong et al., 1993). The sodium channel is completely functional by itself, unless the kinetics of opening and closure of the voltage gated channel can be modified by other proteins, sometimes referred to as complementary subunits (beta subunit in mammals and TipE in *Drosophila*) (Catterall et al., 2003).

Cell action potential starts with the depolarization of the membrane, with the internal side attaining a more positive state (compare Figure 1, pannels A and B). A stimulus that causes the depolarization in a given region of the cell membrane promotes activation (opening) of the Na_V in the vicinity. This process results in the influx of Na^+ to the cell, enhancing depolarization of the membrane. The action potential works in a positive feedback, that is, once started there is no need of additional stimuli to progress. However, one millisecond after the channel has been activated, the surrounding membrane reaches the Na^+ equilibrium potential, and the channel is deactivated. In this state, the pore is still open, but it assumes a conformation that halts the ion influx into the cell (Figure 1, C). After some further milliseconds, the membrane is repolarized and the channel closes, finally returning to its resting configuration (Figure 1, D). This whole process occurs in consonance with other channels and pumps, such as K_V and sodium/ potassium pumps that restore the original electric potential of the cell (Catterall et al., 2003; Randall et al., 2001). The correct operation of sodium channels is essential for nerve impulse propagation. Hence, if the regular propagation of an impulse is altered, as due to the interaction with an insecticide, the organism suffers paralysis and can eventually die.

The structure of Na_V is organized in four homologous domains (I-IV), each containing six hydrophobic segments (S1-S6) and a *P-loop* between S5 and S6 (Figure 2). The segments S1-S4 work as a voltage sensitive module. Since S4 segments are positively charged and sensitive to voltage changes, they move across the membrane in order to initiate the channel activation in response to membrane depolarization (schematically represented in Figure 1,

compare relative position of the Na_v blue domains in the different pannels). The pore forming module is composed of the S5-S6 segments and the loop between them, the latter acting as an ion selective filter in the extracellular entrance of the pore (Catterall et al., 2003; Goldin, 2003; Narahashi, 1992). Additionally, the *P-loop* residues D, E, K and A, respectively from domains I, II, III and IV, are critical for the Na^+ sensitivity (Zhou et al., 2004).

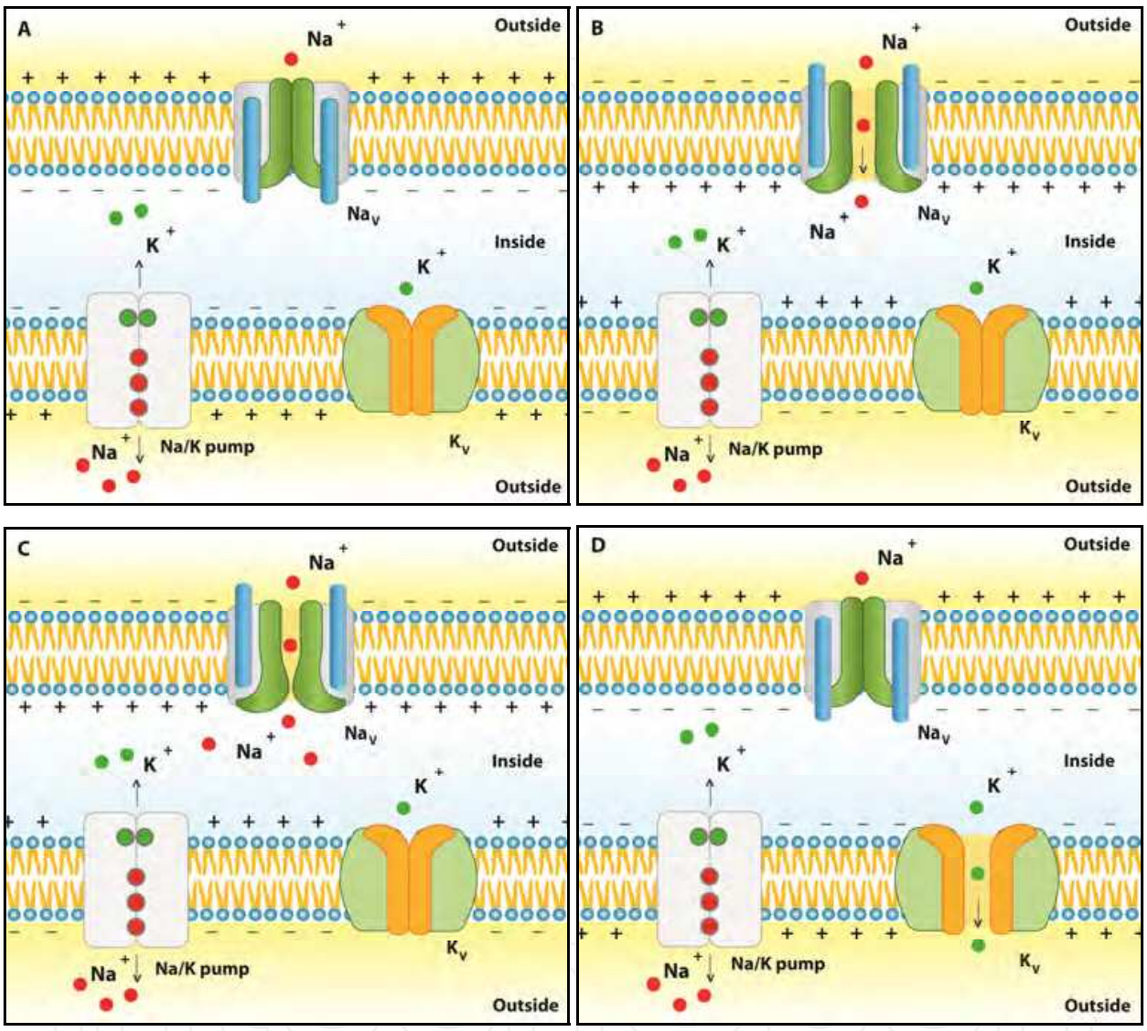

Fig. 1. Propagation of the action potential through a neuronal axon - In the resting potential stage (A) the axon cytoplasm has Na^+ and K^+ respectively in low and high concentrations compared to the surrounding extracellular fluid. The Na/K pump is constantly expelling three Na+ from the cell for every two K+ it transfers in, which confers a positive charge to the outer part of the membrane. When there is a nervous stimulus, the Na_V opens and the membrane becomes permeable affording the influx of Na^+, depolarizing the membrane charge (B). This is the rising phase of the action potential. Soon (~1 millisecond), the Na_V is deactivated, precluding further Na+ entrance to the cell (C), whilst K^+ exits the cell through K_V which is now opened, characterizing the falling phase of the action potential (D). The Na/K pump helps to reestablish the initial membrane potential. The action potential generates a wave of sequential depolarization along the axon. Figure based on T. G. Davies et al. (2007b).

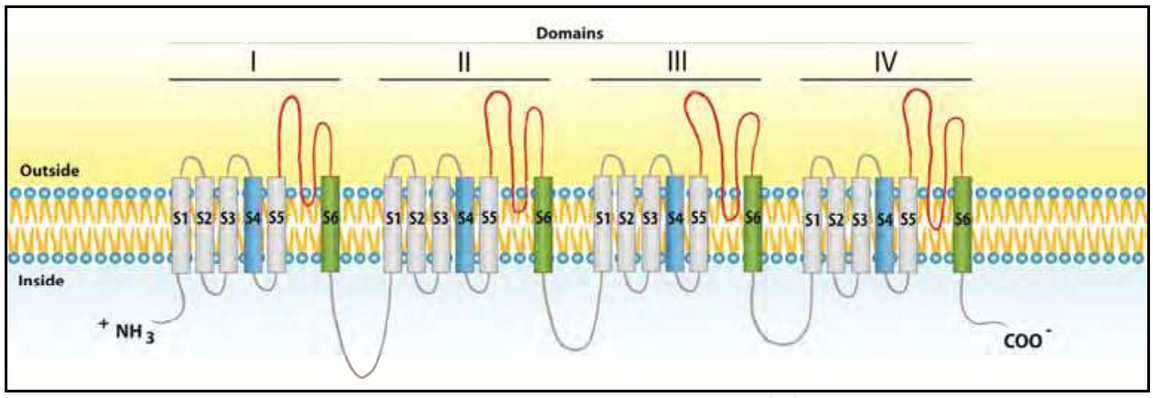

Fig. 2. The voltage gated sodium channel - Scheme representative of the Na_V inserted in a cell membrane, showing its four homologous domains (I-IV), each with six hydrophobic segments (S1-S6). In blue, the voltage sensor segments (S4); in green, the S6 segments, which form the channel pore together with the S5 segments and the link (*P-loop*, in red) between them. Figure adapted from Nelson & Cox (2000).

In the closed state, the putative insecticide contact sites are blocked, corroborating the assumption that pyrethroids and DDT have more affinity to the Na_v channel in its open state when the insecticide stabilizes the open conformation (O'Reilly et al., 2006). These insecticides, therefore, inhibit the channel transition to the non-conducting and deactivated states (T. E. Davies et al., 2008). By interacting with the channel, they form a sort of wedge between segments IIS5 and IIIS6 that restricts displacement of the pore forming helices S5 and S6, preventing closure of the channel. Consequently, the influx of Na^+ is prolonged, and the cell is led to work at an abnormal state of hyper-excitability. The amplitude of the Na^+ current will not decrease unless the cell's level of hyper-excitability is overcome by its ability to keep the sodium-potassium pump under operation. This process is responsible for the pyrethroid sublethal effect in insects, known as *knockdown* effect, which may lead to paralysis and death if prolonged (T. E. Davies et al., 2008; T. G. Davies et al., 2007b).

Predictive models suggest that DDT and pyrethroids interact with a long and narrow cavity delimited by the IIS4-S5 linker and the IIS5 and IIIS6 helices, accessible to lipophilic insecticides. Moreover, some of the aminoacids belonging to the helices engaged in contact with these insecticides are not conserved among arthropods and other animals, and this could be responsible for the selectivity of pyrethroid effects against insects (O'Reilly et al., 2006). The crystal structure of a Na_V has been recently published (Payandeh et al., 2011), pointing to a better understanding of the channel function and to its interaction with targeted compounds in a near future.

Besides pyrethroids and DDT, other insecticides act on the voltage gated sodium channel, like the sodium channel blocker insecticides (SCBIs) and N-alkylamide inseticides (like BTG 502). There are few reports about these compounds. However, it is known that SCBIs, such as indoxicarb, act by blocking the impulse conduction, an effect opposite to that of DDT and pyrethroids (Du et al., 2011).

4. The knockdown effect and the *kdr* phenotype

In the early 1950s, no sooner had DDT been introduced as an insecticide than resistant strains of houseflies were described. When exposed to DDT, these insects in general did not

suffer paralysis followed by death (*knockdown*) but, at most, presented a momentary paralysis followed by complete locomotion recovery, this phenotype being named *kdr* (*knockdown* resistance) (Busvine, 1951; Harrison, 1951; Milani, 1954). Since the introduction of pyrethroids, plenty of insect species exhibiting the *kdr* phenotype have been observed, attributed to previous DDT selection pressure, characterizing cross-resistance between both insecticides (Hemingway & Ranson, 2000). *Kdr* resistance results in 10-20 fold decrease in the sensitivity to the insecticide. However, *kdr* lineages of some species can exhibit up to 100 X increased pyrethroid resistance, an effect denominated *super-kdr*. *Kdr* and *super-kdr* alleles act as recessive traits and hence may persist at low levels in the population in heterozygous individuals (T. G. Davies et al., 2007a).

Over three decades after the description of the *kdr* effect, electrophysiological studies based on neuronal cells and tissues suggested that Na_V had to be the target site for pyrethroids. These reports also indicated that cross-resistance between pyrethroids and DDT must be related to that channel (Pauron et al., 1989). Concomitantly, the gene *paralytic* (*para)* from *Drosophila melanogaster* was cloned and sequenced. This gene is placed in the *locus* related to behavioral changes and paralysis after exposure to high temperatures, similar to the *knockdown* effect produced by DDT and pyrethroids (Loughney et al., 1989). Comparisons within vertebrate nucleotide sequences revealed that *para* is homologous to the voltage gated sodium channel gene (Na_V) (Loughney & Ganetzky, 1989). It was then shown, with a DDT resistant housefly lineage, that the *locus* homologous to *para* was in strong linkage with the *kdr* phenotype (Williamson et al., 1993). This evidence was soon extended to other insect species plagues or vectors, such as the tobacco budworm *Heliothis virescens* (Taylor et al., 1993), the cockroach *Blatella germanica* (Dong & Scott, 1994) and the mosquito *Aedes aegypti* (Severson et al., 1997).

Hitherto, Na_V is the only molecule incriminated as the target site for DDT and pyrethroids. Although it has been implied that type II pyrethroids can interact with the GABA receptor (which is the target, for instance, of the insecticide dieldrin), this interaction has not been considered toxically important (Soderlund & Bloomquist, 1989). Research on the molecular interaction between pyrethroids and their target site presently guides a series of approaches towards the development of a great variety of natural and synthetic neurotoxicants acting on the Na_V (Soderlund, 2010).

5. Molecular biology of the insect Na_V and the *kdr* mutation

A great variety of sodium channels have been identified by electrophysiological assays, purification and cloning (Goldin, 2001). In mammals, nine Na_V genes are known, with distinct electrophysiological properties as well as different expression profiles in the tissues and throughout development (Goldin, 2002; Yu & Catterall, 2003), phylogenetic analyses revealing that all are members of only one unique family, deriving from relatively recent gene duplications and chromosome rearrangements. On the other hand, Ca_V and Ka_V have little protein sequence identity and present diverse functions, indicative of more ancient segregation of their coding genes (Catterall et al., 2003).

The Na_V orthologous genes and cDNAs from *D. melanogaster* and *An. gambiae* share, respectively, 56-62% and 82% of nucleotide identity, evidencing a high degree of conservation between these species. The Na_V C-terminal is the most variable region, but as in all dipterans, it is mainly composed of aminoacids of short (Gly, Ala, Ser, Pro) or negative (Asp, Glu) side chains, suggesting a conserved function in this domain (T. G. Davies et al.,

2007a). Concerning size, the voltage gated sodium channel of *Ae. aegypti* (*AaNa$_V$*), for instance, presents 293 Kb of genomic DNA, with 33 exons. Its longer observed transcript has an ORF of 6.4 Kb, coding for 2,147 aminoacids for a protein estimated in 241 KDa (Chang et al., 2009).

The existence of two Na$_V$ evolutionary lines in invertebrates, represented by the genes *para* and *DSC1* in *D. melanogaster*, has been suggested (Spafford et al., 1999). These lines do not correspond to the different genes observed among vertebrates, and they are supposed to have arisen after vertebrate and invertebrate splitting (Goldin, 2002). DSC1 plays a role in the olfactory system (Kulkarni et al., 2002) as it has been found in the peripheral nervous system and also at high density in the synaptic regions. DSC1 is sensitive to tetradotoxin, a specific Na$_V$ blocker (Zhang et al., 2011), while BSC1, its homologous in *B. germanica*, has also been identified as a putative sodium channel, being expressed in the cockroach nerve cord, muscle, gut, fat body and ovary (Liu et al., 2001). Neither *DSC1* nor *BSC1*, however, mapped with any *locus* related to insecticide resistance (Loughney et al., 1989; Salkoff et al., 1987). Actually, these channels probably represent prototypes of a new Ca$_V$ family, highly related to the known Na$_V$ and Ca$_V$ (Zhang et al., 2011; Zhou et al., 2004). On the other hand, in invertebrates, the *D. melanogaster para* gene (or *DmNa$_V$*) and its equivalent in other species actually code for sodium channels and are related to pyrethroid/DDT resistance and to behavioral changes, as aforementioned.

In his review, Goldin (2002) suggested that two to four genes coding for sodium channels should exist in insects and that differences among them would not result from distinct genes but from pos-transcriptional regulation. Accordingly, even after publication of many insect genome sequences, there has been no mention whatsoever of Na$_V$ gene duplication. Furthermore, recent reports attribute the diversity in Na$_V$ sequences to alternative splicing and RNA editing. These modifications seem to be tissue and stage specific and might also have some influence on pyrethroid resistance (Liu et al., 2004; Song et al., 2004; Sonoda et al., 2008).

5.1 Alternative mRNA splicing in the Na$_V$

Briefly, alternative splicing is a post-transcriptional regulated event characterized when certain exons are removed together with introns. This is a common mechanism of gene expression regulation and increment of protein diversity in eukaryotes. The process may occur in different ways: complete exons can be included or excluded (optional exons), splicing sites can be altered and introns can be retained in the mature mRNA. There are also mutually exclusive pairs of exons, when two exons never unite in the same transcript. Alternative mRNA splicing introduces variability in both sequence and size of the Na$_V$ intracellular region, which by itself should have an impact on its operation (T. G. Davies et al., 2007a).

The regulation for excision of an exon, in detriment of others, may be tissue and development specific. In the context of pyrethroid resistance, it is important to know to what extent alternative splicing events compromise the interaction between the insecticide and the channel. It is also necessary to investigate the amount of alternative transcripts in the course of development and their distribution in the different tissues of the insect. The sodium channel genes have alternative exons that potentially synthesize a great number of different mRNAs (Figure 3). There are also mutually exclusive exons that occur in the transmembrane regions of domains II and III (T. G. Davies et al., 2007a). In *D. melanogaster*, many alternative splicing sites have been identified, with seven optional regions and two

pairs of mutually exclusive exons (Figure 3) (Olson et al., 2008). These sites are conserved in *M. domestica* (Lee et al., 2002) generating, in both species, 512 potential Na_V transcripts by alternative splicing. However, they are not all necessarily expressed as less than 10 were actually observed in mRNA pools (Soderlund, 2010).

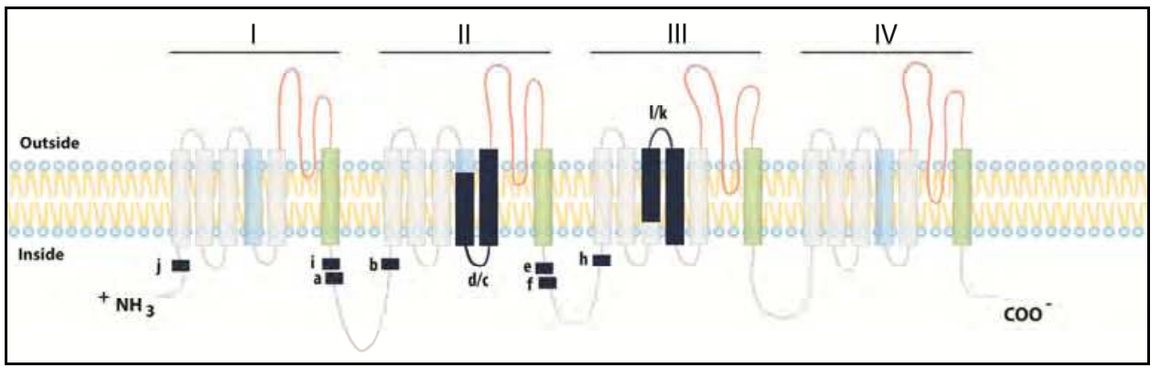

Fig. 3. Alternative splice in the insect voltage gated sodium channel gene. Scheme of Na_V with the sites of alternative exons of $DmNa_V$ indicated in dark color. Exons *a*, *b*, *i*, *j*, *e* and *f* are optional, while *d*/*c* and *l*/*k* are mutually exclusive. Figure adapted from Oslon et al. (2008).

The aminoacid sequences translated from optional exons are conserved and generally consist of intracellular domains of the channel, suggesting functional relevance to these events. Na_V transcript diversity derived from alternative splicing has been investigated in insects of many orders, revealing a high level of conservation, as shown in the cockroach *B. germanica* (Liu et al., 2001; Song et al., 2004), the silk worm *Bombyx mori* (Shao et al., 2009), the moth *Plutella xylostella* (Sonoda et al., 2008) and the mosquitoes *An. gambiae* (T. G. Davies et al., 2007a) and *Ae. aegypti* (Chang et al., 2009). However, in some species not all exons were observed nor their expression detected (see Davies et al., 2007a).

There are two mutually exclusive exons (called c/d) that code for a region between IIS4 and IIS5 segments (Figure 3). The absence of one of these exons might be important for pyrethroid resistance, since the *super-kdr* mutation (Met918Thr) is located in this region, as will be discussed further. In the cockroach *B. germanica*, the mutually exclusive exon pair *k*/*l* codes for the voltage sensitive region at domain III. The two varieties $BgNa_V1.1a$ and $BgNa_V1.1b$[1], which contain the exons *l* and *k* respectively, exhibit distinct electrophysiological properties. Furthermore, $BgNa_V1.1b$ is 100X more resistant to the pyrethroid deltamethrin than $BgNa_V1.1a$ (Du et al., 2006).

5.2 Sodium channel RNA editing

RNA editing has an important role in the regulation of gene expression and protein diversity. Recent studies implicate RNA editing in the removal of exons in alternative splicing sites, in the antagonism of interference RNA process (iRNA), in the modulation of mRNA processing and in the generation of new exons (for a review see Y. Yang et al., 2008). The basic mechanism of diversity generated by RNA editing includes nucleoside modifications such as C to U or A to I deaminations. Besides, it is possible that non-

[1] The genes annotation is in accordance with the nomenclature suggested by Goldin (2000).

templated nucleotides can be inserted in the edited mRNA. This process alters the protein aminoacid constitution so that it differs from the predicted genomic DNA sequence (Brennicke et al., 1999).

Liu et al. (2004) claimed that RNA editing should be the main regulatory mechanism to modulate the insect Na_V function. For instance, no correlation was found between a variety of *DmNa*$_V$ originated by alternative splicing and the observed changes in gating properties. Therefore it was implied that RNA editing might play a primary role in determining the voltage dependence of activation and deactivation of *DmNa*$_V$ variants (Olson et al., 2008). At least 10 A/I RNA editing substitutions were observed in the *DmNa*$_V$ in different points of the Drosophila life cycle indicating developmental regulation (Palladino et al., 2000). These sites are highly conserved in various organisms. Type U/C editing, which is more usual in mitochondria and plastids from higher plants, was also observed in *DmNa*$_V$ and *BgNa*$_V$, with electrophysiological alterations in both cases (Liu et al., 2004). Hence, RNA editing should play an important role in the generation of channels with distinct affinities to insecticides. Thus, it seems reasonable to infer that insecticide pressure selects for an adaptive mechanism which might spatially and temporally modulate Na_V mRNA editing. Still, in *Cx. quinquefasciatus* mosquitoes, diversity based on U/A editing in the sodium channel mRNA was shown to be related to pyrethroid resistance (Xu et al., 2006). In *Ae. aegypti*, however, recent analysis of *AaNa*$_V$ transcripts from a pyrethroid resistant lineage did not identify any sign of RNA editing (Chang et al., 2009).

5.3 The *kdr* mutation

The very first mutation identified as responsible for the *kdr* trait was a leucine to phenylalanine substitution (Leu1014Phe)[2] in the Na_V IIS6 segment of *M. domestica* (Ingles et al., 1996). Since then, the genomic sequence spanning the region coding for the IIS6 segment has been explored in a vast number of insects, in most of which, the same substitution being found at homologous sites (1014). Besides Phe, Ser is also encountered replacing Leu at the 1014 site in *An. gambiae*. They were initially observed respectively in western and eastern African regions, being commonly referred to as *w-kdr* and *e-kdr* mutations (Pinto et al., 2006). However, nowadays it is known that none of these alleles is restricted to either part of the continent (Ranson et al., 2011). A different substitution in the same 1014 site, Leu1014His, was also associated to pyrethroid resistance in the tobacco budworm *Heliothis virescens* (Park et al., 1999). Many studies identified at least 20 additional substitutions in the Na_V sequence, the majority being placed between segments S4 and S5, or internally to segments S5 or S6 of domain II. However, for most of them, the relationship with pyrethroid resistance is only speculative. Good compilations have recently been presented (T. G. Davies et al., 2007a; Dong, 2007; Du et al., 2009).

It is noteworthy that many of these mutations are not in the precise domain of interaction between insecticide and Na_V (O'Reilly et al., 2006). On the other hand, it is likely that substitutions in these points of interaction could result in the *super-kdr* trait, which has a more pronounced resistance effect (T. G. Davies et al., 2007b). This phenotype was also first described in *M. domestica* (Williamson et al., 1996) and *Haematobia irritans* (Guerrero et al., 1997). In both species, beyond the Leu1014Phe substitution, a Met918Thr mutation (in the IIS4-S5 linker) was disclosed in flies with very high resistant ratios to pyrethroids, referred

[2] Number refers conventionally to the position in the voltage gated sodium channel primary sequence of *M. domestica Vssc1*, according to Soderlund & Knipple 2003.

to as the *super-kdr* mutation (Jamroz et al., 1998). However, since it occurs only in association with the Leu1014Phe mutation, its isolated effects are as yet unknown. Although no *super-kdr* mutation has so far been identified in mosquitoes, it was suggested that Leu932Phe, in association with Ile936Val (both also in the IIS4-S5 linker), in *Culex* might play this role, being the first example of *super-kdr* in this group (T. G. Davies et al., 2007a). Accordingly, these sites have proved to be important for the interaction between Na_V, in the *D. melanogaster* sodium channel and pyrethroids or DDT (Usherwood et al., 2007).

Substitutions in site 929 are also involved in enhanced pyrethroid resistance, as is the case with the Lepidoptera *Plutella xylostella* mutation Thr929Ile, detected in association with Leu1014Phe (Schuler et al., 1998). However, in the maize weevil *Sitophilus zeamais*, the Thr929Ile was found alone (Araujo et al., 2011). In the louse *Pediculus capitis*, in turn, the Thr929Ile mutation was together with Leu932Phe (Lee et al., 2000). There were other substitutions in the same site: Thr/Cys and/or Thr/Val in the diamondblack moth *Frankliniella occidentalis* (Forcioli et al., 2002) and in the cat flea *Ctenocephalides felis* (Bass et al., 2004).

Ae. aegypti mosquitoes do not present any substitution in the classic 1014 *kdr* site, unlike many other insects or even mosquitoes from other genera, such as *Anopheles* and *Culex*, very likely because the 1014 site of *Ae. aegypti* Na_V is coded by a CTA, in place of the TTA codon present in the majority of other insects. For this reason, two simultaneous nucleotide substitutions would be necessary in order to change from Leu (CTA) to Phe (TTT) or Ser (TCA) (Martins et al., 2009a; Saavedra-Rodriguez et al., 2007). Instead, mutations in different positions have been observed in *Ae. aegypti* populations from Latin America and Southeast Asia, but at least two sites seem to be indeed related to pyrethroid resistance: 1016 (Val to Ile or Gly) and 1534 (Phe to Cys), respectively in the IIS6 and IIIS6 segments (Brengues et al., 2003; Harris et al., 2010; Martins et al., 2009a, b; Saavedra-Rodriguez et al., 2007). Mutations in the vicinity of this site in the IIIS6 segment were also encountered in the southern cattle tick *Rhipicephalus microplus* (He et al., 1999) and in the two-spotted spider mite *Tetranychus urticae* (Tsagkarakou et al., 2009).

Although different Na_V site mutations are known to confer resistance to pyrethroids, their number is quite restricted; additionally, far related taxa present alterations in the same homologous sites. For instance, the Leu1014Phe *kdr* mutation must have arisen at least on four independent occasions in *An. gambiae* (Pinto et al., 2007). Alterations that do not interfere with the endogenous physiological functions of the Na_v must be rare as it is much conserved among animals (ffrench-Constant et al., 1998). As a matter of fact, most of the species studied so far have the *kdr* mutation in the 1014 site, few species proving otherwise due to codon constraints, like *Ae. aegypti* and some anopheline species.

6. Molecular assays for monitoring frequency of *kdr* mutation in insect natural populations

Currently, there are many PCR based diagnostic methods for *kdr* mutation available with elevated sensitivity and specificity. For technique choice, one must consider mainly the laboratory resources, facilities and training of technical personnel, which is as important as establishing an defining localities and frequency of sampling. There is neither consensus nor strict rules suitable for all insect species or even for different populations of the same species. Resistance is a very dynamic process depending upon a series of external factors. Therefore, resistance level as well as the selected mechanisms may fluctuate in a short

period of time and space (Kelly-Hope et al., 2008). Moreover, one must be aware about the patterns of distribution and structure of the evaluated populations in order to determine an adequate frequency and sampling size (Ranson et al., 2011).

Allele-specific PCR assays (AS-PCR), as the name suggests, consists of amplification and detection of a specific allele from the DNA of an individual, who is further classified as hetero or homozygous for that allele. Many methodologies based on this strategy have been well succeeded in high-throughput individual diagnostic of *kdr* mutations. Herein, we highlight some PCR based amplifications by allele-specific primers and TaqMan genotyping.

There is ample variation for PCR methods based on allele specific primers. As a first example, one can use two primers (forward and reverse) common for both alleles that amplify a region spanning the mutation site. In this case, additional specific primers, bearing the SNP (single nucleotide polymorphism) at the 3'-end, have opposite orientations in relation to each other (Figure 2-A). The common primers will pair themselves giving rise to a bigger product (that can also be assumed as the positive control reaction) and shorter ones, the consequence of pairing with each allele-specific primer of contrary orientation. The common primers must anneal at sites that result in differently sized products when paring with the specific ones. If both alleles are present (cases when the individual is heterozygous) three products with distinct sizes will be produced (Chen et al., 2010; Harris et al., 2010).

Instead of amplifying a common region for both alleles, it is possible to directly obtain only the specific products (Figure 2-B). This can be accomplished by using only one common primer in one orientation and the two allele specific primers in the opposite sense. However, since the specific primers are at the same orientation and their specificity continues lying upon the 3'-end, something should be incremented in order to obtain distinguishable products. Germer & Higuchi, (1999), later improved by Wang et al. (2005), proposed attaching a GC-tail of different sizes to the 5'-end of the specific primers in a way that the products could be distinguishable by their Tm in a melting curve analysis. In this case the mix reaction contains a fluorescent dye, which lights up when bounded to double strand DNA, carried out in a fluorescence-detecting thermocycler ("Real time PCR"). Additionally, a different mismatch (pirimidine for purine or vice–versa) is added to the third site before the 3'-end of each allele specific primer, in order to strengthen their specificity (Okimoto & Dodgson, 1996). Alternatively, the products can also be distinguishable in a gel electrophoresis.

The second group of techniques is based on the amplification of a region spanning the *kdr* mutation site followed by the detection of the different alleles by specific hybridization with minor groove binding (MGB) DNA fluorescent probes, also known as TaqMan assay (Figure 2-C). Different alleles can be detected in the same reaction, since each probe is attached to a distinct fluorophore. The probe is constituted of an oligonucleotide specific for the SNP with a reporter fluorescent dye in the 5'-end and a non fluorescent quencher in the 3'-end (Araujo et al., 2011; Morgan et al., 2009; Yanola et al., 2011). Bass et al., (2007) concluded that TaqMan probes were the most accurate for *kdr* genotyping among six different evaluated methods.

Other techniques have also been applied. The *Hola* (Heated Oligonucleotide Ligation Assay, see details in Black et al., 2006) revealed high specificity in detecting different Na_V alleles in the 1011 (Ile, Met and Val) and 1016 (Val, Ile and Gly) sites from Thai *Ae. aegypti* populations (Rajatileka et al., 2008) and in the 1014 site of *Cx. quinquefasciatus* from Sri Lanka (Wondji et

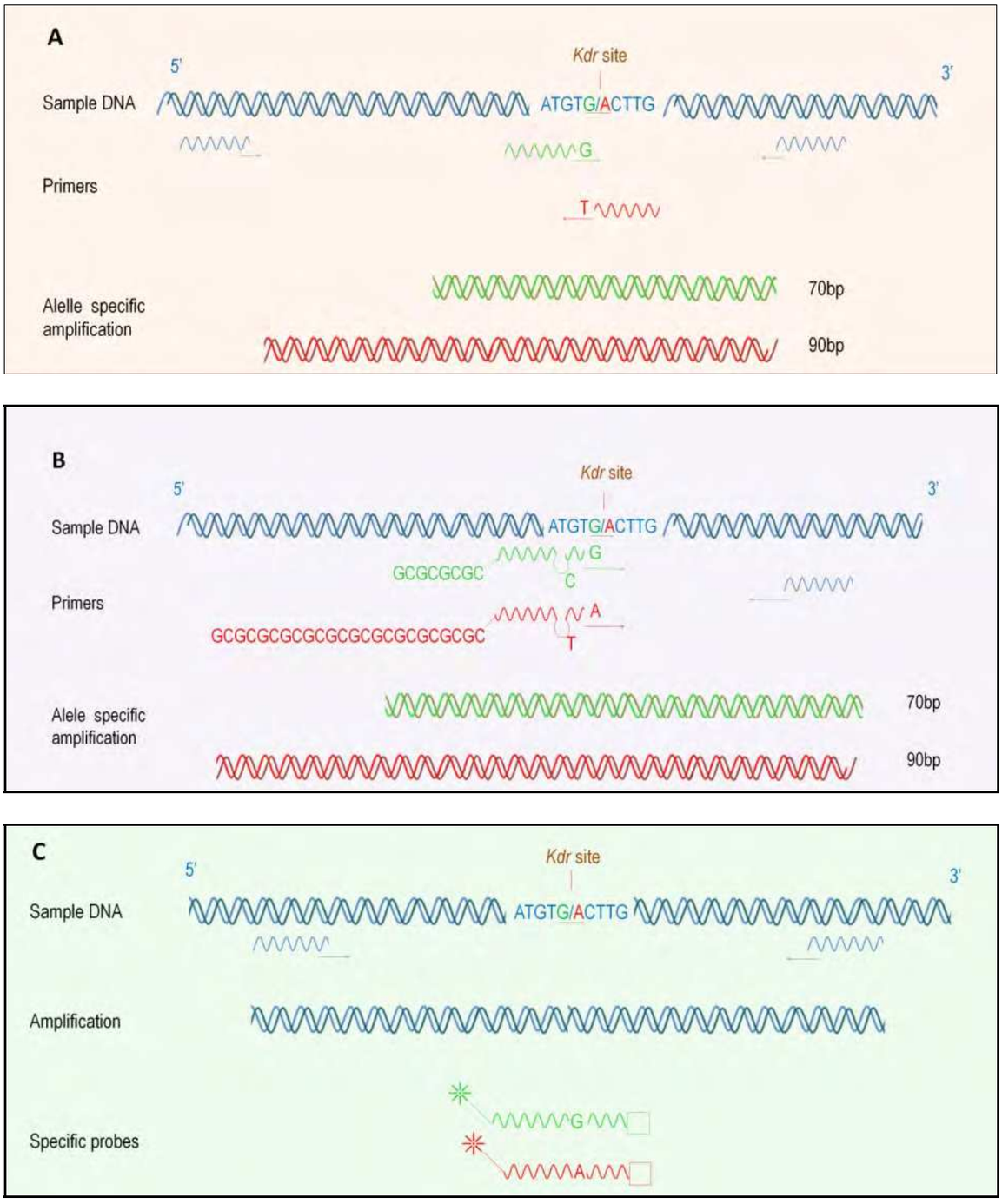

Fig. 4. Examples for kdr genotyping based on PCR methods. A – Allelic specific PCR with specific primers in different orientations; B – Allelic specific PCR with specific primers in the same orientation but with additional and differently sized $[GC]_n$ tails, in addition to a mismatch in the 3rd base before the 3'-end; C – TaqMan assay based on specific probes with a different luminescence for each allele. Figure adapted from Yanola et al. (2011).

al., 2008). However, comparison between *HOLA* and pyrosequencing revealed more specificity for this latter method in the diagnostic of the *kdr* mutation Leu1014Phe in *Cx. quinquefasciatus* (Wondji et al., 2008). Sequencing of regions that encompass the SNP allows a direct visualization of the nucleotide allele sequences, eliminating the problem of unspecific amplification or hybridization of PCR based protocols. Moreover, it enables visualizing potential novel variations that would never be identified by PCR diagnostic SNP techniques. However, sequencing in large scale is much more expensive than the aforementioned genotyping tools. It is also mandatory that the eletropherograms generated have a clean profile, so that the heterozygous individuals can be undoubtedly discriminated.

7. Conclusions

New strategies for arthropod control based on the release of laboratory manipulated insects that would suppress or substitute natural populations are being tested in the field with great prospect. The release of transgenic insects carrying a dominant lethal gene (RIDL) (Black et al., 2011) or of mosquitoes with the intracellular *Wolbachia*, that lead to refractoriness to other parasites (Werren et al., 2008) are currently the most discussed strategies. However, the laboratory handling process has to consider specific and sometimes complex aspects for each insect species, and it may take many years until field control based on this kind of approach can be effectively accomplished. Moreover, field studies that guarantee the environmental safety of releasing manipulated insects may take even longer. Hence, even if these strategies prove to be efficient to reduce, extinguish, or substitute a target insect population, the use of insecticides may still indeed play an essential role for many years to come, especially during periods of high insect or disease incidence.

Pyrethroids are largely the most adopted insecticide class in agriculture and for public health purposes. Their use tends to increase, since pyrethroids are the only safe compound to impregnate insecticide treated nets (ITNs), a strategy under expansion against mosquitoes. Advances regarding knowledge of its target, the voltage gated sodium channel, can contribute to the design of new compounds as well as the rapid identification of resistance related mutations. The continuous monitoring of insecticide resistance status, and its mechanisms, in natural populations has proven to be an important tool in the preservation of these compounds.

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9. References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). *Molecular Biology of the Cell* (4th), Garland Science, New York.

Araujo, R.A., Williamson, M.S., Bass, C., Field, L.M., & Duce, I.R. (2011). Pyrethroid resistance in *Sitophilus zeamais* is associated with a mutation (T929I) in the voltage-gated sodium channel. *Insect Mol Biol*, Vol.20, No. 4, (Aug), pp. 437-445, ISSN 1365-2583.

Awolola, T.S., Oduola, O.A., Strode, C., Koekemoer, L.L., Brooke, B., & Ranson, H. (2009). Evidence of multiple pyrethroid resistance mechanisms in the malaria vector *Anopheles gambiae* sensu stricto from Nigeria. *Trans R Soc Trop Med Hyg*, Vol.103, No. 11, (Nov), pp. 1139-1145, ISSN 1878-3503.

Bass, C., Nikou, D., Donnelly, M.J., Williamson, M.S., Ranson, H., Ball, A., Vontas, J., & Field, L.M. (2007). Detection of knockdown resistance (kdr) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods. *Malar J*, Vol.6, No. pp. 111, ISSN 1475-2875.

Bass, C., Schroeder, I., Turberg, A., Field, L.M., & Williamson, M.S. (2004). Identification of mutations associated with pyrethroid resistance in the para-type sodium channel of the cat flea, *Ctenocephalides felis*. *Insect Biochem Molec*, Vol.34, No. 12, (Dec), pp. 1305-1313, ISSN 0965-1748.

Beaty, B.J., & Marquardt, W.C. (1996). *The biology of disease vectors*, University Press of Colorado, Colorado.

Black, W.C.t., Alphey, L., & James, A.A. (2011). Why RIDL is not SIT. *Trends Parasitol*, Vol.No. (Jun 7), ISSN 1471-5007.

Black, W.C.t., Gorrochotegui-Escalante, N., & Duteau, N.M. (2006). Heated oligonucleotide ligation assay (HOLA): an affordable single nucleotide polymorphism assay. *J Med Entomol*, Vol.43, No. 2, (Mar), pp. 238-247, ISSN 0022-2585.

Brengues, C., Hawkes, N.J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P., Manguin, S., Morgan, J.C., & Hemingway, J. (2003). Pyrethroid and DDT cross-resistance in *Aedes aegypti* is correlated with novel mutations in the voltage-gated sodium channel gene. *Med Vet Entomol*, Vol.17, No. 1, (Mar), pp. 87-94, ISSN 0269-283X.

Brennicke, A., Marchfelder, A., & Binder, S. (1999). RNA editing. *FEMS Microbiol Rev*, Vol.23, No. 3, (Jun), pp. 297-316, ISSN 0168-6445.

Busvine, J.R. (1951). Mechanism of resistance to insecticides in housefly. *Nature*, Vol.168, No. pp. 193-195,

Catterall, W.A. (2000). From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron*, Vol.26, No. 1, (Apr), pp. 13-25, ISSN 0896-6273.

Catterall, W.A., Chandy, K.G., Clapham, D.E., Gutman, G.A., Hofmann, F., Harmar, A.J., Abernethy, D.R., & Spedding, M. (2003). International Union of Pharmacology: Approaches to the nomenclature of voltage-gated ion channels. *Pharmacol Rev*, Vol.55, No. 4, (Dec), pp. 573-574, ISSN 0031-6997.

Chang, C., Shen, W.K., Wang, T.T., Lin, Y.H., Hsu, E.L., & Dai, S.M. (2009). A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in *Aedes aegypti*. *Insect Biochem Mol Biol*, Vol.39, No. 4, (Apr), pp. 272-278, ISSN 1879-0240.

Chen, L., Zhong, D., Zhang, D., Shi, L., Zhou, G., Gong, M., Zhou, H., Sun, Y., Ma, L., He, J., *et al.* (2010). Molecular ecology of pyrethroid knockdown resistance in *Culex pipiens pallens* mosquitoes. *PLoS One*, Vol.5, No. 7, pp. e11681, ISSN 1932-6203.

David, J.P., Strode, C., Vontas, J., Nikou, D., Vaughan, A., Pignatelli, P.M., Louis, C., Hemingway, J., & Ranson, H. (2005). The *Anopheles gambiae* detoxification chip: a highly specific microarray to study metabolic-based insecticide resistance in malaria vectors. *Proc Natl Acad Sci U S A*, Vol.102, No. 11, (Mar 15), pp. 4080-4084, ISSN 0027-8424.

Davies, T.E., O'Reilly, A.O., Field, L.M., Wallace, B., & Williamson, M.S. (2008). Knockdown resistance to DDT and pyrethroids: from target-site mutations to molecular modelling. *Pest Manag Sci*, Vol.64, No. 11, (Nov), pp. 1126-1130, ISSN 1526-498X.

Davies, T.G., Field, L.M., Usherwood, P.N., & Williamson, M.S. (2007a). A comparative study of voltage-gated sodium channels in the Insecta: implications for pyrethroid resistance in Anopheline and other Neopteran species. *Insect Mol Biol*, Vol.16, No. 3, (Jun), pp. 361-375, ISSN 0962-1075.

Davies, T.G., Field, L.M., Usherwood, P.N., & Williamson, M.S. (2007b). DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life*, Vol.59, No. 3, (Mar), pp. 151-162, ISSN 1521-6543.

Dong, K. (2007). Insect sodium channels and insecticide resistance. *Invert Neurosci*, Vol.7, No. 1, (Mar), pp. 17-30, ISSN 1354-2516.

Dong, K., & Scott, J.G. (1994). Linkage of kdr-type resistance and the para-homologous sodium channel gene in German cockroaches (*Blattella germanica*). *Insect Biochem Mol Biol*, Vol.24, No. 7, (Jul), pp. 647-654, ISSN 0965-1748.

Du, Y., Khambay, B., & Dong, K. (2011). An important role of a pyrethroid-sensing residue F1519 in the action of the N-alkylamide insecticide BTG 502 on the cockroach sodium channel. *Insect Biochem Mol Biol*, Vol.41, No. 7, (Jul), pp. 446-450, ISSN 1879-0240.

Du, Y., Liu, Z., Nomura, Y., Khambay, B., & Dong, K. (2006). An alanine in segment 3 of domain III (IIIS3) of the cockroach sodium channel contributes to the low pyrethroid sensitivity of an alternative splice variant. *Insect Biochem Mol Biol*, Vol.36, No. 2, (Feb), pp. 161-168, ISSN 0965-1748.

Du, Y., Nomura, Y., Luo, N., Liu, Z., Lee, J.E., Khambay, B., & Dong, K. (2009). Molecular determinants on the insect sodium channel for the specific action of type II pyrethroid insecticides. *Toxicol Appl Pharmacol*, Vol.234, No. 2, (Jan 15), pp. 266-272, ISSN 1096-0333.

ffrench-Constant, R.H., Daborn, P.J., & Le Goff, G. (2004). The genetics and genomics of insecticide resistance. *Trends Genet*, Vol.20, No. 3, (Mar), pp. 163-170, ISSN 0168-9525.

ffrench-Constant, R.H., Pittendrigh, B., Vaughan, A., & Anthony, N. (1998). Why are there so few resistance-associated mutations in insecticide target genes? *Philos Trans R Soc Lond B Biol Sci*, Vol.353, No. 1376, (Oct 29), pp. 1685-1693, ISSN 0962-8436.

Forcioli, D., Frey, B., & Frey, J.F. (2002). High nucleotide diversity in the para-like voltage-sensitive sodium channel gene sequence in the western flower thrips (Thysanoptera : Thripidae). *Journal of Economic Entomology*, Vol.95, No. 4, (Aug), pp. 838-848, ISSN 0022-0493.

Germer, S., & Higuchi, R. (1999). Single-tube genotyping without oligonucleotide probes. *Genome Res*, Vol.9, No. 1, (Jan), pp. 72-78, ISSN 1088-9051.

Goldin, A.L. (2001). Resurgence of sodium channel research. *Annu Rev Physiol*, Vol.63, No. pp. 871-894, ISSN 0066-4278.

Goldin, A.L. (2002). Evolution of voltage-gated Na(+) channels. *J Exp Biol*, Vol.205, No. Pt 5, (Mar), pp. 575-584, ISSN 0022-0949.

Goldin, A.L. (2003). Mechanisms of sodium channel inactivation. *Curr Opin Neurobiol*, Vol.13, No. 3, (Jun), pp. 284-290, ISSN.

Goldin, A.L., Barchi, R.L., Caldwell, J.H., Hofmann, F., Howe, J.R., Hunter, J.C., Kallen, R.G., Mandel, G., Meisler, M.H., Netter, Y.B., *et al.* (2000). Nomenclature of voltage-gated sodium channels. *Neuron*, Vol.28, No. 2, (Nov), pp. 365-368, ISSN 0896-6273.

Guerrero, F.D., Jamroz, R.C., Kammlah, D., & Kunz, S.E. (1997). Toxicological and molecular characterization of pyrethroid-resistant horn flies, *Haematobia irritans*: identification of kdr and super-kdr point mutations. *Insect Biochem Mol Biol*, Vol.27, No. 8-9, (Aug-Sep), pp. 745-755, ISSN 0965-1748.

Harris, A.F., Rajatileka, S., & Ranson, H. (2010). Pyrethroid resistance in *Aedes aegypti* from Grand Cayman. *Am J Trop Med Hyg*, Vol.83, No. 2, (Aug), pp. 277-284, ISSN 1476-1645.

Harrison, C.M. (1951). Inheritance of resistance of DDT in the housefly, *Musca domestica* L. *Nature*, Vol.167, No. 4256, (May 26), pp. 855-856, ISSN 0028-0836.

He, H., Chen, A.C., Davey, R.B., Ivie, G.W., Wagner, G.G., & George, J.E. (1999). Sequence analysis of the knockdown resistance-homologous region of the para-type sodium channel gene from pyrethroid-resistant *Boophilus microplus* (Acari: Ixodidae). *J Med Entomol*, Vol.36, No. 5, (Sep), pp. 539-543, ISSN 0022-2585.

Hemingway, J., Hawkes, N.J., McCarroll, L., & Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Insect Biochem Mol Biol*, Vol.34, No. 7, (Jul), pp. 653-665, ISSN 0965-1748.

Hemingway, J., & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annu Rev Entomol*, Vol.45, No. pp. 371-391, ISSN 0066-4170.

Ingles, P.J., Adams, P.M., Knipple, D.C., & Soderlund, D.M. (1996). Characterization of voltage-sensitive sodium channel gene coding sequences from insecticide-susceptible and knockdown-resistant house fly strains. *Insect Biochem Mol Biol*, Vol.26, No. 4, (Apr), pp. 319-326, ISSN 0965-1748.

Jamroz, R.C., Guerrero, F.D., Kammlah, D.M., & Kunz, S.E. (1998). Role of the kdr and super-kdr sodium channel mutations in pyrethroid resistance: correlation of allelic frequency to resistance level in wild and laboratory populations of horn flies (*Haematobia irritans*). *Insect Biochem Mol Biol*, Vol.28, No. 12, (Dec), pp. 1031-1037, ISSN 0965-1748.

Jan, L.Y., & Jan, Y.N. (1992). Tracing the roots of ion channels. *Cell*, Vol.69, No. 5, (May 29), pp. 715-718, ISSN 0092-8674.

Kelly-Hope, L., Ranson, H., & Hemingway, J. (2008). Lessons from the past: managing insecticide resistance in malaria control and eradication programmes. *Lancet Infect Dis*, Vol.8, No. 6, (Jun), pp. 387-389, ISSN 1473-3099.

Kulkarni, N.H., Yamamoto, A.H., Robinson, K.O., Mackay, T.F., & Anholt, R.R. (2002). The DSC1 channel, encoded by the smi60E locus, contributes to odor-guided behavior in *Drosophila melanogaster*. *Genetics*, Vol.161, No. 4, (Aug), pp. 1507-1516, ISSN 0016-6731.

Kumar, S., Thomas, A., Sahgal, A., Verma, A., Samuel, T., & Pillai, M.K. (2002). Effect of the synergist, piperonyl butoxide, on the development of deltamethrin resistance in yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Arch Insect Biochem Physiol*, Vol.50, No. 1, (May), pp. 1-8, ISSN 0739-4462.

Lee, S.H., Ingles, P.J., Knipple, D.C., & Soderlund, D.M. (2002). Developmental regulation of alternative exon usage in the house fly Vssc1 sodium channel gene. *Invert Neurosci*, Vol.4, No. 3, (Apr), pp. 125-133, ISSN 1354-2516.

Lee, S.H., Yoon, K.S., Williamson, M.S., Goodson, S.J., Takano-Lee, M., Edman, J.D., Devonshire, A.L., & Clark, J.M. (2000). Molecular analysis of kdr-like resistance in permethrin-resistant strains of head lice *Pediculus capitis*. *Pestic Biochem Physiol*, Vol.66, No. pp. 103-143.

Liu, Z., Chung, I., & Dong, K. (2001). Alternative splicing of the BSC1 gene generates tissue-specific isoforms in the German cockroach. *Insect Biochem Mol Biol*, Vol.31, No. 6-7, (Apr 27), pp. 703-713, ISSN 0965-1748.

Liu, Z., Song, W., & Dong, K. (2004). Persistent tetrodotoxin-sensitive sodium current resulting from U-to-C RNA editing of an insect sodium channel. *Proc Natl Acad Sci U S A*, Vol.101, No. 32, (Aug 10), pp. 11862-11867, ISSN 0027-8424.

Lockwood, J.A., Sparks, T.C., & Story, R.N. (1984). Evolution of insect resistance to insecticides: a reevaluation of the roles of physiology and and behavior. *Bull Entomol Soc Am*, Vol.30, No. pp. 41-51.

Loughney, K., & Ganetzky, B. (1989). The *Para* Locus Encodes a Protein Homologous to the Vertebrate Sodium-Channel. *J Neurogenet*, Vol.5, No. 4, pp. 262-262, ISSN 0167-7063.

Loughney, K., Kreber, R., & Ganetzky, B. (1989). Molecular Analysis of the Para Locus, a Sodium-Channel Gene in *Drosophila*. *Cell*, Vol.58, No. 6, (Sep 22), pp. 1143-1154, ISSN 0092-8674.

Lumjuan, N., Rajatileka, S., Changsom, D., Wicheer, J., Leelapat, P., Prapanthadara, L.A., Somboon, P., Lycett, G., & Ranson, H. (2011). The role of the *Aedes aegypti* Epsilon glutathione transferases in conferring resistance to DDT and pyrethroid insecticides. *Insect Biochem Mol Biol*, Vol.41, No. 3, (Mar), pp. 203-209, ISSN 1879-0240.

Martins, A.J., Lima, J.B., Peixoto, A.A., & Valle, D. (2009a). Frequency of Val1016Ile mutation in the voltage-gated sodium channel gene of *Aedes aegypti* Brazilian populations. *Trop Med Int Health*, Vol.14, No. 11, (Nov), pp. 1351-1355, ISSN 1365-3156.

Martins, A.J., Lins, R.M., Linss, J.G., Peixoto, A.A., & Valle, D. (2009b). Voltage-gated sodium channel polymorphism and metabolic resistance in pyrethroid-resistant *Aedes aegypti* from Brazil. *Am J Trop Med Hyg*, Vol.81, No. 1, (Jul), pp. 108-115, ISSN 1476-1645.

Milani, R. (1954). Comportamento medeliano della resistenza alla azione del DDT e correlazione tra abbattimento e e mortalitá in *Musca domestica* L. *Revista di Parassitologia*, Vol.15, No. pp. 513-542.

Montella, I.R., Martins, A.J., Viana-Medeiros, P.F., Lima, J.B., Braga, I.A., & Valle, D. (2007). Insecticide resistance mechanisms of Brazilian *Aedes aegypti* populations from 2001 to 2004. *Am J Trop Med Hyg*, Vol.77, No. 3, (Sep), pp. 467-477, ISSN 0002-9637.

Morgan, J.A., Corley, S.W., Jackson, L.A., Lew-Tabor, A.E., Moolhuijzen, P.M., & Jonsson, N.N. (2009). Identification of a mutation in the para sodium channel gene of the cattle tick *Rhipicephalus (Boophilus) microplus* associated with resistance to synthetic pyrethroid acaricides. *Int J Parasitol*, Vol.No. (Jan 17), ISSN 1879-0135.

Morin, S., Alon, M., Alon, F., & Nauen, R. (2008). Organophosphates' resistance in the B-biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae) is associated with a point mutation in an ace1-type acetylcholinesterase and overexpression of carboxylesterase. *Insect Biochem Molec*, Vol.38, No. 10, (Oct), pp. 940-949, ISSN 0965-1748.

Muller, P., Warr, E., Stevenson, B.J., Pignatelli, P.M., Morgan, J.C., Steven, A., Yawson, A.E., Mitchell, S.N., Ranson, H., Hemingway, J., *et al.* (2008). Field-caught permethrin-resistant *Anopheles gambiae* overexpress *CYP6P3*, a P450 that metabolises pyrethroids. *PLoS Genet*, Vol.4, No. 11, (Nov), pp. e1000286, ISSN 1553-7404.

Narahashi, T. (1992). Nerve membrane Na+ channels as targets of insecticides. *Trends Pharmacol Sci*, Vol.13, No. 6, (Jun), pp. 236-241, ISSN 0165-6147.

Nelson, D.L., & Cox, M.M. (2000). *Lehninger - Principles of Biochemistry* (3rd), Worth Publishers, New York.

O'Reilly, A.O., Khambay, B.P., Williamson, M.S., Field, L.M., Wallace, B.A., & Davies, T.G. (2006). Modelling insecticide-binding sites in the voltage-gated sodium channel. *Biochem J*, Vol.396, No. 2, (Jun 1), pp. 255-263, ISSN 1470-8728.

Okimoto, R., & Dodgson, J.B. (1996). Improved PCR amplification of multiple specific alleles (PAMSA) using internally mismatched primers. *Biotechniques*, Vol.21, No. 1, (Jul), pp. 20- 26, ISSN 0736-6205.

Olson, R.O., Liu, Z., Nomura, Y., Song, W., & Dong, K. (2008). Molecular and functional characterization of voltage-gated sodium channel variants from *Drosophila melanogaster*. *Insect Biochem Mol Biol*, Vol.38, No. 5, (May), pp. 604-610, ISSN 0965-1748.

Paeoporn, P., Komalamisra, N., Deesin, V., Rongsriyam, Y., Eshita, Y., & Thongrungkiat, S. (2003). Temephos resistance in two forms of *Aedes aegypti* and its significance for the resistance mechanism. *Southeast Asian J Trop Med Public Health*, Vol.34, No. pp. 786-792.

Palladino, M.J., Keegan, L.P., O'Connell, M.A., & Reenan, R.A. (2000). A-to-I pre-mRNA editing in Drosophila is primarily involved in adult nervous system function and integrity. *Cell*, Vol.102, No. 4, (Aug 18), pp. 437-449, ISSN 0092-8674.

Park, Y., Taylor, M.F.J., & Feyereisen, R. (1999). Voltage-gated sodium channel genes hscp and hDSC1 of *Heliothis virescens* F-genomic organization. *Insect Molecular Biology*, Vol.8, No. 2, (May), pp. 161-170, ISSN 0962-1075.

Pauron, D., Barhanin, J., Amichot, M., Pralavorio, M., Berge, J.B., & Lazdunski, M. (1989). Pyrethroid Receptor in the Insect Na+ Channel - Alteration of Its Properties in Pyrethroid-Resistant Flies. *Biochemistry*, Vol.28, No. 4, (Feb 21), pp. 1673-1677, ISSN 0006-2960.

Payandeh, J., Scheuer, T., Zheng, N., & Catterall, W.A. (2011). The crystal structure of a voltage-gated sodium channel. *Nature*, Vol.475, No. 7356, (Jul 21), pp. 353-358, ISSN 1476-4687.

Perry, T., Batterham, P., & Daborn, P.J. (2011). The biology of insecticidal activity and resistance. *Insect Biochem Mol Biol*, Vol.41, No. 7, (Jul), pp. 411-422, ISSN 1879-0240.

Pinto, J., Lynd, A., Elissa, N., Donnelly, M.J., Costa, C., Gentile, G., Caccone, A., & do Rosario, V.E. (2006). Co-occurrence of East and West African kdr mutations suggests high levels of resistance to pyrethroid insecticides in *Anopheles gambiae* from Libreville, Gabon. *Med Vet Entomol*, Vol.20, No. 1, (Mar), pp. 27-32, ISSN 0269-283X.

Pinto, J., Lynd, A., Vicente, J.L., Santolamazza, F., Randle, N.P., Gentile, G., Moreno, M., Simard, F., Charlwood, J.D., do Rosario, V.E., *et al.* (2007). Multiple origins of knockdown resistance mutations in the Afrotropical mosquito vector *Anopheles gambiae*. *PLoS One*, Vol.2, No. 11, pp. e1243, ISSN 1932-6203.

Raghavendra, K., Barik, T.K., Reddy, B.P., Sharma, P., & Dash, A.P. (2011). Malaria vector control: from past to future. *Parasitol Res*, Vol.108, No. 4, (Apr), pp. 757-779, ISSN 1432-1955.

Rajatileka, S., Black, W.C.t., Saavedra-Rodriguez, K., Trongtokit, Y., Apiwathnasorn, C., McCall, P.J., & Ranson, H. (2008). Development and application of a simple colorimetric assay reveals widespread distribution of sodium channel mutations in Thai populations of *Aedes aegypti*. *Acta Trop*, Vol.108, No. 1, (Oct), pp. 54-57, ISSN 1873-6254.

Randall, D., Burggren, W., & French, K. (2001). *Eckert Animal Physiology: Mechanisms and Adaptations* (5th), W.H. Freeman and Company.

Ranson, H., Claudianos, C., Ortelli, F., Abgrall, C., Hemingway, J., Sharakhova, M.V., Unger, M.F., Collins, F.H., & Feyereisen, R. (2002). Evolution of supergene families associated with insecticide resistance. *Science*, Vol.298, No. 5591, (Oct 4), pp. 179-181, ISSN 1095-9203.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z., & Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends Parasitol*, Vol.27, No. 2, (Feb), pp. 91-98, ISSN 1471-5007.

Raymond, M., Berticat, C., Weill, M., Pasteur, N., & Chevillon, C. (2001). Insecticide resistance in the mosquito *Culex pipiens*: what have we learned about adaptation ? *Genetica*, Vol.112, No. (Nov), pp. 287-296, ISSN 0016-6707.

Roberts, D.R., & Alecrim, W.D. (1991). [Response of *Anopheles darlingi* to spraying with DDTin amazonas, Brazil]. *Bol Oficina Sanit Panam*, Vol.110, No. pp. 480-488.

Rodriguez, M.M., Bisset, J.A., Diaz, C., & Soca, L.A. (2003). [Cross resistance to pyrethroids in *Aedes aegypti* from Cuba induced by the selection with organophosphate malathion]. *Rev Cubana Med Trop*, Vol.55, No. 2, (May-Aug), pp. 105-111, ISSN 0375-0760.

Saavedra-Rodriguez, K., Urdaneta-Marquez, L., Rajatileka, S., Moulton, M., Flores, A.E., Fernandez-Salas, I., Bisset, J., Rodriguez, M., McCall, P.J., Donnelly, M.J., *et al.* (2007). A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American *Aedes aegypti*. *Insect Mol Biol*, Vol.16, No. 6, (Dec), pp. 785-798, ISSN 0962-1075.

Salkoff, L., Butler, A., Wei, A., Scavarda, N., Giffen, K., Ifune, C., Goodman, R., & Mandel, G. (1987). Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila*. *Science*, Vol.237, No. 4816, (Aug 14), pp. 744-749, ISSN 0036-8075.

Schuler, T.H., Martinez-Torres, D., Thompson, A.J., Denholm, I., Devonshire, A.L., Duce, I.R., & Williamson, M.S. (1998). Toxicological, electrophysiological, and molecular characterisation of knockdown resistance to pyrethroid insecticides in the diamondback moth, *Plutella xylostella* (L.). *Pestic Biochem Phys*, Vol.59, No. 3, (May), pp. 169-182, ISSN 0048-3575.

Severson, D.W., Anthony, N.M., Andreev, O., & ffrenchConstant, R.H. (1997). Molecular mapping of insecticide resistance genes in the yellow fever mosquito (*Aedes aegypti*). *J Hered*, Vol.88, No. 6, (Nov-Dec), pp. 520-524, ISSN 0022-1503.

Shao, Y.M., Dong, K., Tang, Z.H., & Zhang, C.X. (2009). Molecular characterization of a sodium channel gene from the *Silkworm Bombyx* mori. *Insect Biochem Mol Biol*, Vol.39, No. 2, (Feb), pp. 145-151, ISSN 1879-0240.

Smith, T.J., Lee, S.H., Ingles, P.J., Knipple, D.C., & Soderlund, D.M. (1997). The L1014F point mutation in the house fly Vssc1 sodium channel confers knockdown resistance to pyrethroids. *Insect Biochem Mol Biol*, Vol.27, No. 10, (Oct), pp. 807-812, 0965-1748 (Print) 0965-1748 (Linking).

Soderlund, D.M. (2010). State-Dependent Modification of Voltage-Gated Sodium Channels by Pyrethroids. *Pestic Biochem Physiol*, Vol.97, No. 2, (Jun 1), pp. 78-86, ISSN 0048-3575.

Soderlund, D.M., & Bloomquist, J.R. (1989). Neurotoxic actions of pyrethroid insecticides. *Annu Rev Entomol*, Vol.34, No. pp. 77-96, ISSN 0066-4170.

Song, W., Liu, Z., Tan, J., Nomura, Y., & Dong, K. (2004). RNA editing generates tissue-specific sodium channels with distinct gating properties. *J Biol Chem*, Vol.279, No. 31, (Jul 30), pp. 32554-32561, ISSN 0021-9258.

Sonoda, S., Igaki, C., & Tsumuki, H. (2008). Alternatively spliced sodium channel transcripts expressed in field strains of the diamondback moth. *Insect Biochem Mol Biol*, Vol.38, No. 9, (Sep), pp. 883-890, ISSN 0965-1748.

Spafford, J.D., Spencer, A.N., & Gallin, W.J. (1999). Genomic organization of a voltage-gated Na+ channel in a hydrozoan jellyfish: insights into the evolution of voltage-gated Na+ channel genes. *Receptors Channels*, Vol.6, No. 6, pp. 493-506, ISSN 1060-6823.

Sparks, T.C., Lockwood, J.A., Byford, R.L., Graves, J.B., & Leonard, B.R. (1989). The Role of Behavior in Insecticide Resistance. *Pestic Sci*, Vol.26, No. 4, pp. 383-399, ISSN 0031-613X.

Strong, M., Chandy, K.G., & Gutman, G.A. (1993). Molecular evolution of voltage-sensitive ion channel genes: on the origins of electrical excitability. *Mol Biol Evol*, Vol.10, No. 1, (Jan), pp. 221-242, ISSN 0737-4038.

Taylor, M.F.J., Heckel, D.G., Brown, T.M., Kreitman, M.E., & Black, B. (1993). Linkage of Pyrethroid Insecticide Resistance to a Sodium-Channel Locus in the Tobacco Budworm. *Insect Biochem Molec*, Vol.23, No. 7, (Oct), pp. 763-775, ISSN 0965-1748.

Tsagkarakou, A., Van Leeuwen, T., Khajehali, J., Ilias, A., Grispou, M., Williamson, M.S., Tirry, L., & Vontas, J. (2009). Identification of pyrethroid resistance associated mutations in the para sodium channel of the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). *Insect Mol Biol*, Vol.18, No. 5, (Oct), pp. 583-593, ISSN 1365-2583.

Usherwood, P.N., Davies, T.G., Mellor, I.R., O'Reilly, A.O., Peng, F., Vais, H., Khambay, B.P., Field, L.M., & Williamson, M.S. (2007). Mutations in DIIS5 and the DIIS4-S5 linker of *Drosophila melanogaster* sodium channel define binding domains for pyrethroids and DDT. *FEBS Lett*, Vol.581, No. 28, (Nov 27), pp. 5485-5492, ISSN 0014-5793.

Valle, D., Montella, I.R., Medeiros, P.F.V., Ribeiro, R.A., Martins, A.J., & Lima, J.B.P. (2006). *Metodologia para quantificação de atividade de enzimas relacionadas com a resistência a inseticidas em Aedes aegypti/ Quantification metodology for enzyme activity related to insecticide resistance in Aedes aegypti*, Editora MS, ISBN: 853341291-6 1., DF: Brasília.

Vontas, J., David, J.P., Nikou, D., Hemingway, J., Christophides, G.K., Louis, C., & Ranson, H. (2007). Transcriptional analysis of insecticide resistance in *Anopheles stephensi* using cross-species microarray hybridization. *Insect Mol Biol*, Vol.16, No. 3, (Jun), pp. 315-324, ISSN 0962-1075.

Wang, J., Chuang, K., Ahluwalia, M., Patel, S., Umblas, N., Mirel, D., Higuchi, R., & Germer, S. (2005). High-throughput SNP genotyping by single-tube PCR with Tm-shift primers. *Biotechniques*, Vol.39, No. 6, (Dec), pp. 885-893, ISSN 0736-6205.

Werren, J.H., Baldo, L., & Clark, M.E. (2008). *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol*, Vol.6, No. 10, (Oct), pp. 741-751, ISSN 1740-1534.

Williamson, M.S., Denholm, I., Bell, C.A., & Devonshire, A.L. (1993). Knockdown resistance (*kdr*) to DDT and pyrethroid insecticides maps to a sodium channel gene locus in the housefly (*Musca domestica*). *Mol Gen Genet*, Vol.240, No. 1, (Jul), pp. 17-22, ISSN 0026-8925.

Williamson, M.S., Martinez-Torres, D., Hick, C.A., & Devonshire, A.L. (1996). Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. *Mol Gen Genet*, Vol.252, No. 1-2, (Aug 27), pp. 51-60, ISSN 0026-8925.

Wondji, C.S., Priyanka De Silva, W.A., Hemingway, J., Ranson, H., & Parakrama Karunaratne, S.H. (2008). Characterization of knockdown resistance in DDT- and pyrethroid-resistant *Culex quinquefasciatus* populations from Sri Lanka. *Trop Med Int Health*, Vol.13, No. 4, (Apr), pp. 548-555, ISSN 1365-3156.

Xu, Q., Wang, H., Zhang, L., & Liu, N. (2006). *Kdr* allelic variation in pyrethroid resistant mosquitoes, *Culex quinquefasciatus* (S.). *Biochem Biophys Res Commun*, Vol.345, No. 2, (Jun 30), pp. 774-780, ISSN 0006-291X.

Yang, Y., Lv, J., Gui, B., Yin, H., Wu, X., Zhang, Y., & Jin, Y. (2008). A-to-I RNA editing alters less-conserved residues of highly conserved coding regions: implications for dual functions in evolution. *RNA*, Vol.14, No. 8, (Aug), pp. 1516-1525, ISSN 1469-9001.

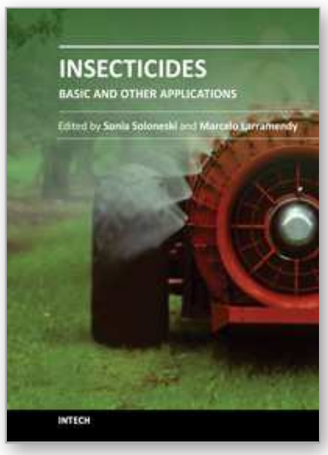
Yang, Y.Y., Liu, Y., Teng, H.J., Sauman, I., Sehnal, F., & Lee, H.J. (2010). Circadian control of permethrin-resistance in the mosquito *Aedes aegypti*. *J Insect Physiol*, Vol.56, No. 9, (Sep), pp. 1219-1223, ISSN 1879-1611.

Yanola, J., Somboon, P., Walton, C., Nachaiwieng, W., Somwang, P., & Prapanthadara, L.A. (2011). High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Trop Med Int Health*, Vol.16, No. 4, (Apr), pp. 501-509, ISSN 1365-3156.

Yu, F.H., & Catterall, W.A. (2003). Overview of the voltage-gated sodium channel family. *Genome Biol*, Vol.4, No. 3, ISSN 1465-6914.

Zhang, T., Liu, Z., Song, W., Du, Y., & Dong, K. (2011). Molecular characterization and functional expression of the DSC1 channel. *Insect Biochem Mol Biol*, Vol.41, No. 7, (Jul), pp. 451-458, ISSN 1879-0240.

Zhou, W., Chung, I., Liu, Z., Goldin, A.L., & Dong, K. (2004). A voltage-gated calcium-selective channel encoded by a sodium channel-like gene. *Neuron*, Vol.42, No. 1, (Apr 8), pp. 101-112, ISSN 0896-6273.



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It is our hope that this book will be of interest and use not only to scientists, but also to the food-producing industry, governments, politicians and consumers as well. If we are able to stimulate this interest, albeit in a small way, we have achieved our goal.

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