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Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids

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ABSTRACT

Pyrethroid insecticides target voltage-gated sodium channels, which are critical for electrical signaling in the nervous system. The intensive use of pyrethroids in controlling arthropod pests and disease vectors has led to many instances of pyrethroid resistance around the globe. In the past two decades, studies have identified a large number of sodium channel mutations that are associated with resistance to pyrethroids. The purpose of this review is to summarize both common and unique sodium channel mutations that have been identified in arthropod pests of importance to agriculture or human health. Identification of these mutations provides valuable molecular markers for resistance monitoring in the field and helped the discovery of the elusive pyrethroid receptor site(s) on the sodium channel.

1. Introduction

Pyrethroids are a major class of synthetic insecticide widely used for controlling arthropod pests and disease vectors because of their fast acting, high insecticidal activities and low mammalian toxicity. However, the intensive use of pyrethroids has led to many instances of pest resistance around the globe. The primary target sites of pyrethroids are voltage-gated sodium channels [1–3]. One of major mechanisms of pyrethroid resistance, which reduces neuronal sensitivity to this class of insecticides, is known as knockdown resistance (*kdr*) [4]. The *kdr* trait was first documented in the house fly and was eventually mapped to a sodium channel locus in house flies where a single nucleotide polymorphism resulted in a substitution of leucine for phenylalanine at position 1014 (L1014F) [5–9]. A second mutation of M918T in conjunction with L1014F (M918T+L1014F) is the genotype that leads to higher levels of resistance to pyrethroids, the so called *super-kdr* phenotype [7,8]. The *kdr* mutation has been documented globally in many major arthropod pests and disease vectors and it is well-established now that mutations in the sodium channel are responsible for *kdr* [10–12]. Identification of *kdr* mutations has already led to successful development of rapid and accurate molecular methods to detect *kdr*-based pyrethroid resistance in field populations [13–16]. Several comprehensive reviews on insect sodium channels and *kdr* have summarized pyrethroid resistance-associated sodium channel mutations identified in pyrethroid-resistant populations of pest species [10–12,17]. However, since these reviews published, more new mutations associated with pyrethroid resistance have been identified in sodium channels from various arthropod pests, particularly in disease vectors, highlighting the complexity of the interactions between pyrethroids and insect sodium channels at the molecular level. The intention of this review is to update the inventory of mutations associated with pyrethroid resistance and discuss both their diversity and convergence among diverse arthropod pest species. In addition, we will examine the relationship of these *kdr* mutations to the pyrethroid receptor(s) on insect sodium channels.

2. Voltage-gated sodium channels

Voltage-gated sodium channels are essential for the initiation and propagation of action potentials in the nervous system and other excitable cells. Our current knowledge of voltage-gated sodium channels originates mainly from molecular and functional analyses of mammalian sodium channels [18]. Mammalian sodium channels are composed of one pore-forming α-subunit of about 260 kDa and up to four much smaller auxiliary β-subunits of about 30–40 kDa. The α-subunit contains four homologous repeats (I–IV), each having six transmembrane segments (S1–S6). The S1–S4 segments in each repeat function as the voltage-sensing domains, whereas the S5 and S6 segment, and the re-entrant loops (called the P-region) connecting the S5 and S6 segments compose the pore-forming domains. The voltage-sensing domain is linked to the pore-forming domain by a small intracellular linker connecting the S4 and S5 segments.

The opening and closing of sodium channels are voltage-gated. In response to membrane depolarization, the S4 segments (the voltage sensors; rich in positively charged residues) move outward, initiating the voltage-dependent activation, which results

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in the opening of the activation gate (presumably formed by the intracellular end of the S6 segments). A few milliseconds after the channel opening, the sodium channel is inactivated (i.e., closed). This inactivation process is mediated by an inactivation particle formed by residues (IFM in mammals, MFM in insects) in the linker between domains III and IV, which blocks the inner pore of the sodium channel. Upon repolarization, the S4 voltage sensors move backward causing the closing of the activation gate, which is known as channel deactivation.

Unlike mammals which have at least nine sodium channel genes [19], insects have only one functional sodium channel gene [20]. However, insect sodium channel transcripts undergo extensive alternative splicing and RNA editing to produce functionally and pharmacologically distinct sodium channel variants [11,20]. The first sodium channel gene, *para*, was identified in *Drosophila melanogaster* [21]. Due to the involvement of sodium channels in pyrethroid resistance in various arthropod pest populations, *para*-orthologs have been identified from many insect and arachnid pest species [22]. To date, sodium channels from *D. melanogaster, Musca domestica, and Blattella germanica,* [22], as well as one arachnid sodium channel from *Varroa destructor* [23], have been functionally expressed in *Xenopus* oocytes. Successful expression of these sodium channels *in vitro* allows us to functionally examine pyrethroid resistance-associated mutations.

3. Mode of action of pyrethroids

As discussed above, sodium channels undergo activation (opening) followed by inactivation and deactivation (closing). The gating transitions between the closed and open states are intricately linked to the generation and propagation of electrical impulses (i.e., action potentials). Pyrethroids modify the gating transitions by inhibiting deactivation and inactivation, resulting in prolonged channel opening [1–3]. At the cellular level, pyrethroids disrupt nerve function causing repetitive discharges, membrane depolarization, and synaptic disturbances [1–3]. Studies of insect sodium channels expressed in *Xenopus* oocytes show that pyrethroids, particularly type II pyrethroids, preferably bind to the activated (open) state of insect sodium channels and cause the prolonged opening of sodium channels, evident as a pyrethroid-induced tail current associated with repolarization in voltage-clamp experiments [24–27] provided the initial electrophysiological evidence for pyrethroid trapping sodium channels in the open state in crayfish giant axons. However, very little is known on how pyrethroids trap sodium channels in the open state at the molecular level.

Earlier electrophysiological and pharmacological studies suggest that pyrethroids have a distinct receptor site on the sodium channel [28–32]. Attempts to detect specific pyrethroid binding to the sodium channels in insect nerve tissues failed, mainly because the high lipophilicity of pyrethroids resulted in extremely high levels of nonspecific binding to membranes and filters [33–35]. A high-affinity pyrethroid-binding site has been reported on rat brain sodium channel preparations [36], however, the molecular determinants of the pyrethroid-binding site on the sodium channel have remained elusive until recently with the identification of *kdr* mutations and computer modeling of insect sodium channels (see below).

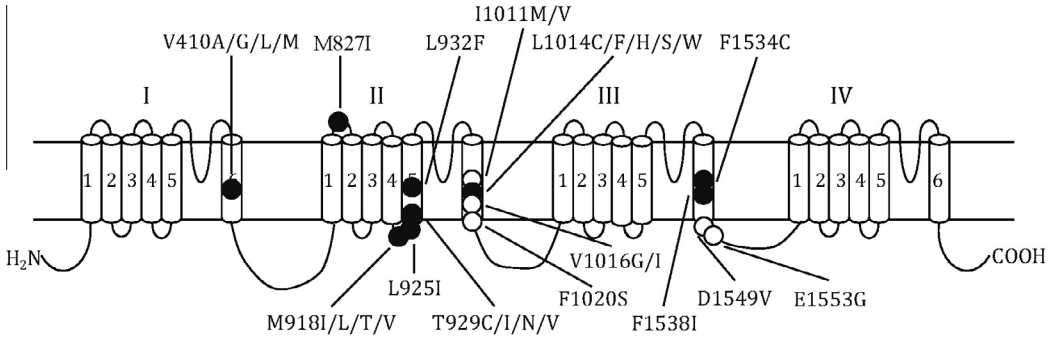

Fig. 1. Position of pyrethroid resistance-associated sodium channel mutations that are detected in more than one species. The mutations with solid circles have been confirmed to reduce sodium channel sensitivity to pyrethroids in *Xenopus* oocytes. The mutations with open circles have not been examined in *Xenopus* oocytes yet. The information on these mutations is presented in Table 1 and Table 3. The sodium channel protein contains four homologous repeats (I–IV), each having six transmembrane segments (S1–S6). Mutations positions are designated based on house fly sodium channel numbering (Genbank accession number: X96668).

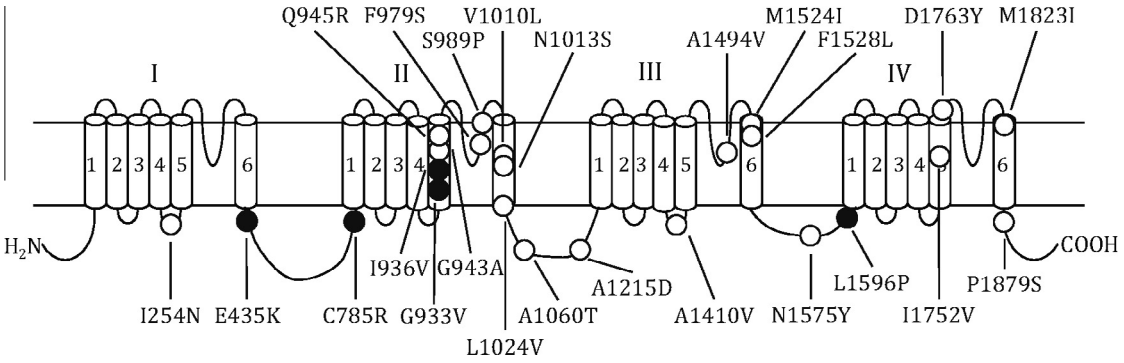

Fig. 2. Position of pyrethroid resistance-associated sodium channel mutations that are detected only in one species. The mutations with solid circles have been confirmed to reduce sodium channel sensitivity to pyrethroids in *Xenopus* oocytes. The mutations with open circles have not been examined in *Xenopus* oocytes yet. The information on these mutations is presented in Table 2 and Table 3. The sodium channel protein contains four homologous repeats (I–IV), each having six transmembrane segments (S1–S6). Mutations positions are designated based on house fly sodium channel numbering (Genbank accession number: X96668).

Table 1
Resistance-associated sodium channel mutations that are found in more than one species.

Mutation	Species	Common name	Original numbering	Reference
V410A	*Helicoverpa zea*	Corn earworm	V421A	[54]
V410G	*Helicoverpa zea*	Corn earworm	V421G	[54]
V410L	*Cimex luctularis*	Common bed bug	V419L	[55]
V410M	*Helicoverpa zea*	Corn earworm	V421M	[54]
	Heliothis virescens	Tobacco budworm	V421M	[56]
M827I+T929I+L932F	*Pediculus humanus capitis*	Human head louse	M815I+T917I+L920F	[57]
	Pediculus humanus corporis	Human body louse	M815I+T917I+L920F	[58]
M918I+L1014F	*Plutella xylostella*	Diamondback moth		[59]
M918L	*Aphis gossypii*	Melon and cotton aphid		[60]
M918L+L925I	*Trialeurodes vaporariorum*	Greenhouse whitefly		[61]
M918T	*Aphis gossypii*	Melon and cotton aphid		[62]
	Tetranychus evansi	Tomato red spider mite		[14]
M918T+L1014F	*Haematobia irritans irritans*	Horn fly		[63]
	Liriomyza huidobrensis	South American leaf miner		[12]
	Musca domestica	House fly		[8]
	Myzus persicae	Green peach aphid		[64]
	Thrips tabaci	Onion thrips		[65]
	Tuta absoluta	Tomato leaf miner		[66]
M918V	*Bemisia tabaci*	Sweet potato whitefly		[67]
L925I	*Bemisia tabaci*	Sweet potato whitefly		[67]
	Cimex luctularis	Common bedbug		[55]
	Trialeurodes vaporariorum	Greenhouse whitefly		[61]
	Rhipicephalus microplus	Southern cattle tick		[68]
T929C	*Frankliniella occidentalis*	Western flower thrips		[69]
T929C+L1014F	*Frankliniella occidentalis*	Western flower thrips		[69]
T929I	*Thrips tabaci*	Onion thrips		[65]
	Thrips palmi	Melon thrips		[70]
	Trialeurodes vaporariorum	Greenhouse whitefly		[61]
	Leptinotarsa decemlineata	Colorado potato beetle		[71]
	Sitophilus zeamais	Maize weevil		[72]
T929I+L932F	*Pediculus humanus capitis*	Human head louse		[73]
T929I+L1014F	*Frankliniella occidentalis*	Western flower thrips		[69]
	Leptinotarsa decemlineata	Colorado potato beetle		[71]
	Plutella xylostella	Diamondback moth		[74]
	Tuta absoluta	Tomato leaf miner		[66]
T929N+L1014F	*Leptinotarsa decemlineata*	Colorado potato beetle		[71]
T929V	*Bemisia tabaci*	Sweet potato whitefly		[75]
	Ctenocephalides felis	Cat flea		[76]
	Frankliniella occidentalis	Western flower thrips		[69]
T929V+L1014F	*Ctenocephalides felis*	Cat flea		[76]
I1011M	*Aedes aegypti*	Yellow fever mosquito	I104M	[77]
I1011V	*Aedes aegypti*	Yellow fever mosquito		[78]
L1014C	*Anopheles sinensis*	Malaria mosquito		[79]
	Culex pipiens pipiens	Southern house mosquito		[80]
L1014F	*Anopheles gambiae*	African malaria mosquito		[81]
	Anopheles stephensi	Malaria mosquito	L31F	[82]
	Anopheles subpictus	Malaria mosquito		[83]
	Aphis gossypii	Melon and cotton aphid		[62]
	Blattella germanica	German cockroach	L993F	[9,46]
	Ctenocephalides felis	Cat flea		[76]
	Culex pipiens pipiens	House mosquito		[84]
	Culex pipiens pallens	Mosquito		[85]
	Culex pipiens quinquefasciatus	Southern house mosquito		[86]
	Cydia pomonella	Codling moth		[87]
	Frankliniella occidentalis	Western flower thrips		[69]
	Haematobia irritans irritans	Horn fly		[63]
	Haematobia irritans exigua	Buffalo fly		[88]
	Leptinotarsa decemlineata	Colorado potato beetle		[89]
	Liriomyza huidobrensis	South American leaf miner		[12]
	Liriomyza sativae	Vegetable leaf miner		[12]
	Meligethes aeneus	Pollen beetle		[90]
	Musca domestica	House fly		[7–9]
	Myzus persicae	Green peach aphid		[91]
	Triatoma infestans	Kissing bug		[92]
L1014H	*Helicoverpa zea*	Corn earworm	L1029H	[54]
	Heliothis virescens	Tobacco budworm	L1029H	[93]
	Liriomyza trifolii	American serpentine leaf miner		[12]
	Musca domestica	House fly		[94,95]
	Stomoxys calcitrans	Stable fly		[96]
L1014S	*Anopheles arabiensis*	Malaria mosquito		[97]
	Anopheles culicifacies	Malaria mosquito		[98]
	Anopheles gambiae	African malaria mosquito		[99]
	Anopheles parilae	Malaria mosquito		[100]
	Anopheles peditaeniatus	Malaria mosquito		[100]

(*continued on next page*)

Table 1 (*continued*)

Mutation	Species	Common name	Original numbering	Reference
	Anopheles sacharovi	Malaria mosquito		[101]
	Anopheles sinensis	Malaria mosquito		[100]
	Anopheles vagus	Malaria mosquito		[100]
	Culex pipiens pallens	Mosquito		[85]
	Culex pipiens pipiens	House mosquito		[84]
L1014W	*Anopheles sinensis*	Malaria mosquito		[102]
V1016G	*Aedes aegypti*	Yellow fever mosquito	V109G	[77]
V1016I	*Aedes aegypti*	Yellow fever mosquito		[78]
F1020S	*Blattella germanica*	German cockroach	F999S	[103]
	Plutella xylostella	Diamondback moth		[104]
F1534C	*Aedes aegypti*	Yellow fever mosquito	F1269C	[105]
	Aedes albopictus	Asian tiger mosquito		[106]
F1538I	*Rhipicephalus microplus*	Southern cattle tick	F1550I	[107]
	Tetranychus cinnabarinus	Carmine spider mite		[108]
	Tetranychus urticae	Two-spotted spider mite		[109]
D1549V+E1553G	*Helicoverpa armigera*	Cotton bollworm	D1561V+E1565G	[110]
	Heliothis virescens	Tobacco budworm	D1561V+E1565G	[110]

Mutation positions are designated based on house fly sodium channel numbering (Genbank accession number: X96668).
The "original numbering" column refers to the numbering of the mutation in the original paper, if different than house fly numbering.
Reference indicates the first report in the literature.

Table 2
Resistance-associated sodium channel mutations that are found only in one species.

Mutation	Species	Common name	Original numbering	Reference
I254N	*Drosophila melanogaster*	Common fruit fly	I286N	[111]
E435K+C785R+L1014F[c]	*Blattella germanica*	German cockroach	E434K+C764R+L993F	[112]
M827I[c]	*Pediculus humanus capitis*	Human head louse	M815I	[13]
M827I+T929I[c]	*Pediculus humanus capitis*	Human head louse	M815I+T917I	[13]
M827I+L932F[c]	*Pediculus humanus capitis*	Human head louse	M815I+L920F	[13]
T929I+L1014F+A1060T[a]+P1879S	*Plutella xylostella*	Diamondback moth	A1101T	[113]
G933V[c,d]	*Rhipicephalus microplus*	Southern cattle tick	G72V	[114]
I936V[c]	*Helicoverpa zea*	Corn earworm	I951V	[54]
Q945R	*Lepeophtheirus salmonis*	Sea flea		[115]
F979S+L1014F	*Myzus persicae*	Green peach aphid		[116]
S989P+V1016G	*Aedes aegypti*	Yellow fever mosquito		[117]
V1010L+L1014S	*Anopheles culicifacies*	Malaria mosquito		[98]
N1013S	*Anopheles sinensis*	Malaria mosquito		[102]
L1014F+A1060T[a]+P1879S	*Plutella xylostella*	Diamondback moth	A1101T	[113]
L1014F+N1575Y	*Anopheles gambiae*	African malaria mosquito		[118]
V1016G+D1763Y	*Aedes aegypti*	Yellow fever mosquito	D1794Y	[119]
L1024V	*Tetranychus urticae*	Two-spotted spider mite	L1022V	[120]
A1060T[a]+P1879S	*Plutella xylostella*	Diamondback moth	A1101T	[59]
A1215D[a]	*Tetranychus urticae*	Two-spotted spider mite		[109]
A1410V	*Drosophila melanogaster*	Common fruit fly	A1549V	[111]
A1494V	*Drosophila melanogaster*	Common fruit fly	A1648V	[111]
M1524I	*Drosophila melanogaster*	Fruit fly	Not numbered	[111]
F1528L+L1596P[e]+I1752V+M1823I[b]	*Varroa destructor*	Varroa mite	F758L, L826P , I982V, M1055I	[121]

Mutation positions are designated based on house fly sodium channel numbering (Genbank accession number: X96668).
The "original numbering" column refers to the numbering of the mutation in the original paper, if different than house fly numbering.
Reference indicates the first report in the literature.

[a] This residue aligns poorly to $MdNa_v1$. Numbering relative to original numbering is retained.
[b] M in $VdNa_v1$ is V in $MdNa_v1$.
[c] Reduced sensitivity to pyrethroids has been confirmed in *Xenopus* oocytes (Table 3).
[d] C933A is resistant to deltamethrin [39].
[e] P1596L increases sensitivity to fluvalinate [122].

4. Common and unique resistance-associated mutations

Pyrethroid resistance-associated mutations were identified by comparing partial or complete coding sequences of the *para*-orthologous sodium channel genes from pyrethroid-sensitive and pyrethroid-resistant strains of various arthropod species. With the increased use of pyrethroids in controlling arthropod pests and disease vectors, more pyrethroid-resistant populations have been documented [37]. This trend, coupled with the recent increase in the accessibility and affordability of molecular tools has had a tremendous influence on the identification of a wide array of mutations associated with pyrethroid resistance in various species. These unique amino acid substitutions are found throughout the sodium channel protein (Figs. 1 and 2).

More than 30 unique resistance-associated mutations or combinations of mutations have been detected in more than one species (Table 1 and Fig. 1). In contrast, Table 2 and Fig. 2 summarize resistance-associated mutations that have been detected in only a single species. Most of these mutations in Table 1 have been shown to reduce the pyrethroid sensitivity of house fly, cockroach and/or *Drosophila* sodium channels expressed in *Xenopus* oocytes confirming their role in *kdr* (Table 3). However, the mutations in Table 2 and Fig. 2 are mostly functionally uncharacterized.

Intriguingly, the majority of functionally confirmed mutations are found in IIS5, IIS6, and IIIS6 segments (Fig. 1 and Table 3). Computer modeling (see below) predicts that IIS5, IIIS6 and the linker connecting S4 and S5 in domain II compose a pyrethroid binding site and most of the *kdr* mutations in these regions (such as M918T and L925I) likely confer resistance by reducing binding of pyrethroids to sodium channels [38]. Subsequent studies from systematic site-directed mutagenesis of residues in the linker connecting S4 and S5 in domain II, IIS5 and IIIS6 uncovered more pyrethroid-sensing residues in these regions, supporting this model [39,40]. However, according to this model, many other *kdr* mutations including the L1014F mutation in IIS6 that has been detected in many species is not close to this receptor site (further discussion below).

The functional impact of mutations identified in insect species other than the house fly, cockroach or *Drosophila* has been assessed by introducing those mutations into one of the three sodium channels by site-directed mutagenesis and then characterizing the mutated channels in *Xenopus* oocytes. For example, the *Heliothis virescens* V410M mutation in IS6 reduced the pyrethroid sensitivity by 10-fold when introduced into *Drosophila*, house fly and cockroach sodium channels [27,41,42], the T929I mutation in IIS5, identified in diamondback moth and other pest species, drastically reduced the Para channel sensitivity to deltamethrin [43], and the F1538I mutation in IIIS6 completely abolishes the pyrethroid sensitivity of the cockroach sodium channel to structurally diverse pyrethroids [44].

The L1014F mutation in IIS6 was the first resistance-associated mutation that was detected and confirmed to be the cause of *k*dr [8,25,26,45]. Since the first reports from the house fly and the German cockroach [7–9,46], pyrethroid resistance has been attributed to substitution of F, H, S, C, or W at this position in other insect species across evolutionarily divergent insect groups (Table 1). Variability (e.g. L1014F/H/S/C/W) in substitution at a single site resulting in pyrethroid resistance is not unique for L1014. Divergent substitutions have been shown at other sites, including V410 (M/A/G/L) in IS6, M918 (T/L/V) in the linker connecting S4 and S5 in domain II, and T929 (I/C/N/V) in IIS5 (in each of these three cases only the first amino acid substitution has been functionally confirmed to cause a reduction in pyrethroid sensitivity). This massively parallel and divergent evolution of resistance demonstrates not only are these sites critical for pyrethroid action, but also raises the possibility that the substituting amino acid at these sites can potentially vary based on the type of pyrethroid used to select the resistant population. For example, sodium channels with the L1014F, L1014H, and L1014S mutations provide variable levels of protection to Type I or Type II pyrethroids or DDT [47]. Similarly, M918T in the linker connecting S4 and S5 in domain II provides extremely high levels of protection against permethrin and deltamethrin [26], but does not provide protection from DDT [48], and F1534C confers channel resistance to type I, but not type II pyrethroids [49]. It will be of great interest to determine if compound-specificity is evident with other sites of divergent substitution that have not yet been functionally characterized.

Co-occurrence of more than one resistance-associated mutation often more drastically reduces the channel sensitivity to pyrethroids than individual mutations alone. For example, the L1014F mutation or the M918T mutation alone caused about 5–10 fold reduction in the sensitivity of the Para channel to deltamethrin, but the double mutations almost abolished the sensitivity of the Para channel to deltamethrin [26,50]. Similarly, the T929I mutation either alone or in combination with M827I and L932F completely eliminated permethrin sensitivity in Vssc1 channels [51]. Two mutations, E435K and C785R, in the linker connecting domains I and II were found to co-exist with the L1014F only in the German cockroach. Each mutation alone did not reduce the sensitivity of the cockroach sodium channel to deltamethrin [25]. However, when either the E435K or C785R mutation was combined with the L1014F mutation, the channel sensitivity was reduced by 100-fold [25]. Concomitant presence of all three mutations reduced channel sensitivity to deltamethrin by 500-fold [25]. Similarly, E435K and C785R mutations also further reduced the sensitivity of the V410M channel to pyrethroids [41]. These two mutations are considered as enhancers of the V410M and L1014F mutations [25,41].

5. Do *kdr* mutations define the pyrethroid receptor site(s)?

Relying on information of key *kdr* mutations, O'Reilly et al. [38] used the X-ray structure of the $K_v1.2$ potassium channel as a template to predict the open conformation of the house fly sodium channel. This interesting model predicts that the pyrethroid receptor site is located in a hydrophobic cavity delimited by the IIS4–S5 linker and IIS5 and IIIS6 helices. Subsequent systematic site-directed mutagenesis of these regions uncovered more pyrethroid-sensing residues in these segments, providing further experimental support for this model [39,40]. Electrophysiological studies also showed that *kdr* mutations in the IIS4–S5 linker and IIS5 and IIIS6 helices likely reduce pyrethroid binding to the receptor site on the sodium channel [26,43,44,52]. How this mutation reduces sodium channel sensitivity to pyrethroids remains elusive. The L1014F mutation has been shown to increase close-state inactivation which could reduce the availability of a *Drosophila* sodium channel in the open state for pyrethroid action [52,53]. In the same study, Hill plot analysis showed that the house fly M918T mutation in the linker connecting IIS4 and IIS5 (part of the first receptor site) reduces the number of pyrethroid-binding sites per channel from two to one in the *Drosophila* sodium channel [52]. This led to another possibility that L1014 in IIS6 could be at a second pyrethroid receptor site; and the L1014F mutation confers resistance by reducing pyrethroid binding. This hypothesis is supported by results from specialized analysis of pyrethroid binding utilizing the competitive binding of active and inactive isomers of permethrin, called Schild analysis. Schild analysis shows that the L1014F mutation (i.e., L993F in the cockroach sodium channel) reduces pyrethroid binding to the cockroach sodium channel [44]. Future mutational analysis coupled with computer modeling is necessary

Table 3
Resistance-associated sodium channel mutations that have been confirmed to reduce sodium channel sensitivity to pyrethroids in *Xenopus* oocytes.

Mutation	Location	Reference
V410M	IS6	[27,41,42,123]
V410M+E435 K+C785R	IS6+Li-II[a]+Li-II	[41]
E485K+C785R + L1014F	Li-II+Li-II+IIS6	[25]
E485K+L1014F	Li-II+IIS6	[25]
C785R+L1014F	Li-II+IIS6	[25]
M827I	Linker IIS1–S2	[51]
M827I+T929I	Linker IIS1–S2+IIS5	[51]
M827I+T929I+L932F	Linker IIS1–S2+IIS5+IIS5	[51]
M827I+L932F	Linker IIS1–S2+IIS5	[51]
M918T	Linker IIS4-S5	[43,48,50]
M918T+L1014F	Linker IIS4–S5+IIS6	[26,48,50]
L925I	Linker IIS4–S5	[39]
T929I	IIS5	[39,43,51]
T929I+L1014F	IIS5+IIS6	[43]
T929I+L932F	IIS5+IIS5	[51]
L932F	IIS5	[39,51]
C933A[b]	IIS5	[39]
I936V	IIS5	[39]
L1014F	IIS6	[25,26,44,45,47,48]
L1014H	IIS6	[27,47]
L1014S	IIS6	[47]
F1534C	IIIS6	[49]
F1538I	IIIS6	[124]
L1596P[c]	LIII-IV	[122]

[a] The linker connecting repeats I and II of the sodium channel.
[b] G933V is associated with resistance in *Rhipicephalus microplus* [114].
[c] P1596L increases sensitivity to fluvalinate [122].

to identify any possible new pyrethroid receptor site on insect sodium channels.

6. Conclusions

Investigations into the molecular mechanism of pyrethroid resistance due to mutations in the voltage-gated sodium channel has yielded a tremendous amount of information that has far-reaching implications in both basic and applied aspects of research. Identification of mutations associated with pyrethroid resistance provides precise molecular markers for rapidly assessing the frequency of resistance alleles in field populations. The diversity in amino acid positions that harbor mutations and the divergent mutations found in those positions likely reflect the evolutionary plasticity of pyrethroid resistance manifested in the field. This plasticity is reinforced by the observation from functional analyses that these mutations provide varying levels of protection against different pyrethroids, implying that the diversity of resistance mutations may also be driven in part by the specific pyrethroid used to select for resistance. In addition to deepening our understanding of the pyrethroid resistance mechanism, information accumulated from the functional characterization of pyrethroid resistance mutations in *Xenopus* oocytes and the elucidation of the binding and action of pyrethroids at the molecular and atomic levels has also contributed significantly to the general knowledge of the gating mechanisms and toxin pharmacology of the sodium channel.

While research on *kdr* mutations has yielded many answers, many important questions still remain. Will new mutations emerge as pyrethroids continue to be a major component of many pest control programs? What are the physiological and evolutionary reasons that restrict the number and frequency of resistance alleles in the field? While it is easy to understand why *kdr* mutations that are located in the predicted pyrethroid receptor site would confer pyrethroid resistance, how do the mutations that are not located at the receptor site confer pyrethroid resistance? Are there additional pyrethroid receptor sites on the sodium channel? Can altered channel gating (e.g., activation, deactivation and/or inactivation) by *kdr* mutations confer pyrethroid resistance by counteracting the action of pyrethroids? One can only hope that the next decade of research in this field will be as productive as it has been for the past two decades and provide answers to these remaining questions. Note: Since the preparation of this review, several new sodium channel mutations have been identified to be associated with pyrethroid resistance in *Culex quinquefasciatus* [125,126].

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