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Using fruit flies to delve into mosquito insecticide resistance

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With more than 3,000 species and an almost ubiquitous presence, the economic importance of mosquitoes cannot be overemphasized. *Anopheles* mosquitoes are vectors for infectious diseases such as malaria – an endemic disease in tropical and sub-tropical regions of the world that infects more than 200 million people worldwide and causes over 400,000 deaths annually, with most casualties being infants or inhabitants of sub-Saharan Africa. The *Aedes aegytpi* and *Culex quinquefasciatus* species of mosquitoes are also vectors for arboviruses such as chikungunya virus, dengue virus, western equine encephalitis virus, and Zika virus. Consequently, insecticides are frequently used to stem the population of mosquitoes. Nevertheless, mosquito insecticide resistance has emerged as a major problem that has contributed to numerous failed eradication campaigns for the aforementioned diseases. In this mini-review, we expound on how fruit flies (*Drosophila melanogaster*) could be a complementary model system for studying mosquito insecticide resistance, with the ultimate goal of confirming any promising leads in mosquitoes.

KEYWORDS

malaria, insecticide resistance, insect vectors, mosquitoes, fruit flies, *Drosophila melanogaster*

Introduction

Malaria is a blood-borne disease caused by various plasmodium species that live within erythrocytes. However, while malaria can, on rare occasions, be transmitted *via* blood transfusions or congenital transmission, it is predominantly transmitted through the bite of a female mosquito of the *Anopheles* genus. Consequently, vector control strategies have long been a major component of attempts to limit the spread of infectious diseases transmitted by mosquitoes, such as malaria. This proved to be somewhat successful between 2000 and 2015, as the number of people infected with malaria steadily dropped, due at least in part to the use of insecticide-treated bed nets. However, over the past few years, mosquito insecticide resistance has become increasingly evident. Here, we briefly explore the major insecticide resistance mechanisms found in mosquitoes and discuss how we think studies in fruit flies can improve our understanding of mosquito insecticide resistance and contribute to the identification of new targets for insecticides. Thus, the rationale for this mini-review is not to provide a thorough understanding of the subject of

mosquito insecticide resistance, but rather to provide an overview of the research themes in the field to *Drosophila melanogaster (Dm)* biologists, and briefly draw attention to how *Dm* genetics and cell biology can be co-opted to address questions relevant to mosquito insecticide resistance. For more thorough discussions of the subject of mosquito insecticide resistance, we refer readers to a number of reviews that have been published in recent years on this subject (Dusfour et al., 2019; Susanna and Pratiwi, 2021; Suh et al., 2023).

Types of insecticide resistance

Insecticide resistance usually develops gradually, as a result of several mechanisms that culminate in reducing the effectiveness of the insecticide. Nevertheless, there are four main insecticide resistance mechanisms referred to as target-site resistance, metabolic resistance, cuticular resistance and behavioral resistance; and many mosquito species can develop resistance through multiple mechanisms. We begin by briefly reviewing each type of insecticide resistance mechanism.

Target-site resistance

Target-site resistance develops when a mutation (usually a point mutation) develops in a specific insecticide target in a few flies that renders them refractory to the insecticide. This may arise when the mutation renders the molecular target incapable of binding to the insecticide to inhibit its activity, or the molecular target's ability to engage and activate or inhibit downstream signaling cascades is impaired. This leads to a selective advantage for this initial small population of mosquitoes, eventually culminating in the whole population becoming largely resistant to the insecticide. Target-site resistance has been observed for insecticides that target genes encoding for the voltage-gated sodium channel (VGSC), acetylcholinesterase (AChE) and the γ-aminobutyric acid (GABA) receptor, Rdl (Resistance to dieldrin) (Hemingway et al., 2004).

Two classes of insecticides – pyrethroids and dichlorodiphenyltrichloroethane (DDT) – inhibit VGSCs. Pyrethroids modify the gating kinetics of VGSCs by inhibiting both activation and inactivation of the channel. Several single or multiple amino acid substitutions in the VGSC, such as the I1011M/V, L1014C/F/S/W and V1016G/I substitutions, block or at least reduce the affinity of the insecticide for the channel (Martinez-Torres et al., 1998; Brengues et al., 2003; Enayati et al., 2003; Chang et al., 2009).

AChE, the enzyme that catalyzes the hydrolysis of acetylcholine (ACh) in the nervous system, is the target site for organophosphate and carbamate-based insecticides [reviewed in (Fournier and Mutero, 1994)]. Organophosphate and carbamates have similar structures to ACh, as a result, they act as competitive inhibitors at the AChE active site. Biochemical assays in many mosquito species have revealed that the gene encoding AChE is a mutational hotspot. Relatively common single amino acid substitutions in many mosquito species that render the enzyme resistant to insecticides are the G119S, F290V and F331W substitutions (Weill et al., 2002; Weill et al., 2003; Alout et al., 2007a; Alout et al., 2007b; Djogbénou et al., 2008).

Cyclodiene insecticides, such as dieldrin, and phenyl pyrazones, such as fipronil, target the GABA receptor, Rdl, a gated chloride ion channel composed of five subunits [reviewed in (Kim and Hibbs, 2021)]. Dieldrin resistance has been linked to an A296S/G substitution found in many *Anopheles* species (Du et al., 2005; Wondji et al., 2011). Resistance to fipronil has also been reported in mosquitoes; however, the molecular lesion(s) responsible for it remain unclear (Liu et al., 2004).

Metabolic resistance

Metabolic resistance is the most common form of insecticide resistance and arises as a result of increased biodegradation of insecticides due to the activation of stress response pathways. It typically involves upregulation of glutathione-S-transferases (GSTs), cytochrome P450 monooxygenases (P450 enzymes), and esterases – a heterogeneous group of enzymes that typically includes carboxylesterase and cholinesterases (Salinas and Wong, 1999; Enayati et al., 2005; Liu, 2015). GSTs catalyze the conjugation of electrophilic compounds with reduced glutathione (GSH), which renders the resulting product less toxic and more hydrophilic, and thus more easily excreted than the hitherto unconjugated product (Habig et al., 1974). Some GSTs are also capable of catalyzing a dehydrochlorination reaction using GSH as a cofactor; and this appears to be the predominant mechanism by which resistance to DDT occurs (Clark and Shamaan, 1984). Nevertheless, in many other instances where elevated GST activity has been linked to insecticide resistance, the actual GST enzyme that confers resistance to the insecticide is unknown. This is partly due to the fact that the repertoire of GSTs in insects has rapidly expanded (many mosquito species have about 30 GST genes) as a result of gene duplications in the insect-specific delta and epsilon GSTs; and multiple delta- or epsilon-type GSTs can be overexpressed in a DDT-resistant strain such as *Anopheles gambiae* (Ortelli et al., 2003).

Similarly, genes encoding for esterases and P450 enzymes in insects can sometimes exceed 50 and 100, respectively, as amplification and duplication events have greatly increased their number. This has led to challenges in elucidating which P450 genes are required for insecticide resistance, as multiple P450 genes are typically induced as a result of exposing mosquitoes to insecticides. Further, the three major classes of enzymes linked to metabolic resistance are likely to act in concert, as the co-elevation of P450 enzymes and esterases in response to insecticide treatment has been reported (Vulule et al., 1999). In line with this, the mosquito P450 enzyme, CYP6Z8, metabolizes common pyrethroid metabolites produced by carboxylesterases such as 3-phenoxybenzoic alcohol and 3-phenoxybenzaldehyde, to 3-phenoxybenzoic acid and other more soluble derivatives (Chandor-Proust et al., 2013).

Cuticular resistance

Cuticular resistance arises from a thickening or alteration of the cuticle that reduces its permeability to the insecticide. Many cuticle proteins belonging to different protein families are expressed in

various mosquito species (Zhou D. et al., 2017; Zhou Y. et al., 2017). In this regard, it has been shown that transcripts of the cuticle proteins, CPR63 and CPR47, are induced in pyrethroid-resistant strains of *Culex pipiens pallens* species than in non-resistant strains (Sun et al., 2017). Further studies showed that CPR63 might contribute to pyrethroid resistance by thickening the cuticle and thus, possibly, increasing the tolerance of mosquitoes to deltamethrin (Xu et al., 2022). Other cuticle proteins expressed at higher levels in pyrethroid-resistant relative to susceptible mosquito strains are CPR124, CPR127, CPR129 and CPR131 (Nkya et al., 2014; Vannini et al., 2014; Yahouédo et al., 2017; Huang et al., 2018). Lastly, CPLCG3, CPLCG4 and CPLCG5 are essential for cuticular resistance as they are involved in a putative cuticle thickening process (Vannini et al., 2014; Huang et al., 2018; Yahouédo et al., 2017).

Behavioral resistance

Perhaps the most intractable of all the insecticide resistance mechanisms is behavioral resistance, which, as the name suggests, refers to behavioral changes evoked by insects as a means to avoid further exposure to the insecticide. Forms of behavioral resistance include a reduction in the extent of mosquito entry into homes, an elevated rate of exit from the area sprayed with the insecticide, and a change in feeding times. In Tanzania, an increased proportion of *Anopheles gambiae* and *Anopheles fenestus* mosquitoes have been reported to feed outdoors as a result of the use of insecticide-resistant bed nets (Russell et al., 2011). Additionally, the introduction of indoor vector control in Bioko Island, Equatorial Guinea, led to an increase in the proportion of *Anopheles gambiae* mosquitoes that sought human hosts outdoors (Reddy et al., 2011).

Using fruit flies to gain a better understanding of target-site resistance

While the ideal model system for studying mosquito insecticide resistance and vector control are mosquitoes themselves, several reports lend credence to the proposition that *Dm* are also useful in studying the effects of mutations linked to insecticide resistance in mosquitoes. The well-developed genetic toolkit in *Dm*, make them an ideal insect model to first identify a broad class of genes that regulate a process with implications for developing insecticides to mosquitoes. Subsequently, more focused studies in mosquitoes examining multiple genes in the same class of genes, may identify the specific genes that regulate the process in mosquitoes. With regard to target-site resistance, both synonymous and nonsynonymous mutations in VGSCs have been associated with target-site resistance; but, in general, the significance of synonymous mutations are unclear. Previously assumed to be benign, growing evidence indicates that synonymous mutations can influence phenotypes by regulating gene expression and/or protein stability *via* an alteration in mRNA stability, a disruption of splicing activity, a change in the efficiency of miRNA binding, a perturbation of translational activity, and an interference with the function of long non-coding RNAs. Studies in *Dm* can test the ability of synonymous mutations in VGSCs to impact these cellular processes.

A gene that encodes for a VGSC in *Dm* is *paralytic* (*para*). This gene can encode for about 60 isoforms as a result of alternative splicing (Lin et al., 2009; Lin et al., 2012). As there are varied periods and patterns of expression of some of these isoforms, it would be interesting to determine how knock-in mutations in fruit flies that mimic the relevant synonymous mutations in mosquitoes regulate expression of these isoforms. Further, a *Dm* strain in which Para is tagged with GFP endogenously exists (Ravenscroft et al., 2020). The relevant knock-in mutations can be created in this strain to ascertain how Para localization and/or stability is affected. Lastly, the GAL4/UAS system can be exploited to overexpress constructs carrying the appropriate mutations (Fischer et al., 1988; Brand and Perrimon, 1993). Similar analyses can be performed for GABAR and AChE.

Fruit flies can also be used to gain a better understanding of how allelic drive can be used to reverse insecticide resistance. In a particularly noteworthy study, CRISPR/Cas9 gene editing of *para* was used to generate a series of common VGSC mutations found in mosquitoes and tested for susceptibility to various insecticides (Kaduskar et al., 2022). The study revealed that it was possible to replace the resistant allele with a native (susceptible) allele in population cages. Thus, this proof-of-principle study highlights how fruit flies can be employed to elucidate the potential of performing a targeted reversion of an insecticide-resistant strain to a wild-type susceptible state.

Using fruit flies to gain a better understanding of metabolic resistance

Forced expression of the mosquito P450 enzymes, CYP6P9a and CYP6P9b, in *Dm* conferred tolerance to both permethrin and deltamethrin (Riveron et al., 2013). Nevertheless, some discrepancies have been observed between data obtained from overexpressing detoxification genes in *Dm* and *Anopheles gambiae* mosquitoes. In such instances, the natural tendency is to dismiss the observations in *Dm*. However, the interpretation of such results is complicated by the fact that the outcome of a GAL4/UAS overexpression experiment is largely dependent on the extent and domain of overexpression. Even in fruit flies, where the GAL4/UAS toolkit is well-established, contradictory results can be obtained when the same gene is overexpressed in a specific *Dm* tissue using different tissue-specific GAL4 lines. Therefore, it appears the yardstick for success when overexpressing detoxification genes should be whether a protective effect against insecticides can be observed at all, as a result of using many GAL4 lines – both tissue-specific and ubiquitous. In this regard, the Gene-Switch system in *Dm* may be particularly valuable as it allows a dose-dependent expression of a transgene in response to varying concentrations of the RU486 (mifepristone) drug (Roman et al., 2001; McGuire et al., 2004; Nicholson et al., 2008; Ke and Hsu, 2019). While some studies in which a mosquito cDNA was overexpressed in *Dm* to test the effect of an insecticide have yielded promising results (Pavlidi et al.,

2012; Riveron et al., 2013), it is possible that mosquito cDNAs may code for proteins that are incapable of interacting optimally with other endogenous regulatory factors. Hence, when overexpression of a mosquito cDNA fails to rescue *Dm* from insecticide-induced toxicity, it may be necessary to also test the effect of overexpressing the closest fruit fly ortholog of the mosquito gene. Ultimately, however, any promising results in fruit flies will have to be confirmed in mosquitoes.

Several peptidases, regulators of lipid and carbohydrate metabolism, sodium-calcium exchangers, and signaling molecules are induced alongside GSTs, P450 genes, and esterases in insecticide-resistant strains (Vontas et al., 2005; Liu et al., 2007). A relatively less explored aspect of metabolic insecticide resistance are the signaling pathways that regulate expression of what we refer to as insecticide resistance effector molecules (i.e., GSTs, P450 genes, and esterases). Identifying upstream regulators of these effector molecules is crucial, as it will furnish knowledge about the possibility of dismantling metabolic resistance mechanisms. A breakdown of the metabolic resistance mechanism should, in principle, enable an erstwhile resistant insecticide to be effective again. In one informative study, it was shown that GPCR signaling upregulates the expression of some P450 genes, raising the possibility that inhibitors of this specific GPCR signaling cascade may suppress insecticide resistance (Li et al., 2014). The readily available transgenic RNAi strains and sophisticated genetic tools in *Dm* make it particularly suited for exploring the regulatory relationships between the multiple genes induced in insecticide-resistant strains.

When mitochondrial function is impaired, it results in the activation of a mitochondrial stress signaling cascade that culminates in the induction of genes required for conferring tolerance to mitochondrial distress. As an example, we recently showed that RNAi-mediated knockdown of isocitrate dehydrogenase 2 (IDH2) leads to an upregulation of genes involved in Fe-S cluster biogenesis, redox and protein homeostasis, GSTs, P450 enzymes, esterases, regulators of lipid and carbohydrate metabolism, transporters, and signaling molecules, among other genes (Murari et al., 2022). This highlights the remarkable similarity in gene expression profile resulting from activating mitochondrial stress signaling or insecticide resistance. Further, it is widely accepted that a combination of piperonyl butoxide (PBO) and pyrethroids is more effective at reducing mosquito populations than using pyrethroids alone (Gleave et al., 2021). As PBO inhibits P450 enzymes, this observation raises the possibility that knocking down genes induced as a result of insecticide resistance may enhance the insecticidal effect of an ineffective insecticide. This is a phenomenon referred to as synergism. Given the similarity between the gene expression profile associated with insecticide resistance and mitochondrial stress signaling, we hypothesize that disrupting some components of the mitochondrial stress signaling cascade identified as a result of IDH2 disruption could synergize with low doses of an insecticide to cause lethality.

Furthermore, we have found that disruption of IDH2 in *Dm* flight muscles culminates in the induction of a ferroptosis-like form of cell death that may provide opportunities for addressing the issue of insecticide resistance. Ferroptosis is a non-apoptotic form of cell death that is triggered by iron-dependent lipid peroxidation (Stockwell et al., 2017); however, it has not been explored extensively in *Dm* or mosquitoes. As emerging evidence indicates that there are likely to be *Dm*-specific aspects of ferroptosis, if similar observations are found in mosquitoes, it may provide an additional mechanism for combating insecticide-resistant mosquitoes.

Using fruit flies to gain a better understanding of cuticular resistance

For the most part, the molecular basis of cuticular resistance is still being unraveled; but some candidate genes have emerged that may regulate expression of cuticle proteins. As a case in point, the laccase gene, which encodes for a diphenol oxidase, is induced in fenvalerate-resistant strains of *Culex pipiens pallens* (Pan et al., 2009). This gene can be expressed in fruit flies exposed to fenvalerate to examine whether it regulates the expression of cuticle proteins or confers tolerance to the insecticide. Further, fruit flies can be exposed to various insecticides and a time series RNA-Seq experiment performed to assess the order of induction of genes involved in cuticle biogenesis. Subsequently, RNAi and overexpression analyses can be used to decipher the regulatory relationships between the genes and the degree to which they impact insecticide resistance.

Using fruit flies to gain a better understanding of behavioral resistance

The challenge of using fruit flies to study behavioral insecticide resistance lies in the fact that a paradigm for studying it has to be established first, as there is some debate about whether behavioral insecticide resistance mechanisms should be classified as such, or simply regarded as avoidance mechanisms (Zalucki and Furlong, 2017). Nevertheless, one area in which research in fruit flies could prove informative for dissecting the mechanism of behavioral resistance to insecticides, is by examining whether the insecticide in question can trigger an aversive response in the olfactory system. It has been shown that pyrethrum extracts from flower heads of *Tanacetum cinerariifolium*, which have long been used as insect repellants, evoke olfactory responses to cause aversion in both *Dm* and *Drosophila suzukii* (Liu et al., 2021). Further studies revealed that pyrethrin, the major component of pyrethrum, activates at least three distinct odorant receptors – Or7a, Or42b and Or59b – to elicit the insecticide repellent response. A similar set of experiments in *Aedes aegypti* mosquitoes revealed the importance of AaOr31 in conferring pyrethrum repellence in mosquitoes (Liu et al., 2021). While the result of this olfactory system-induced aversive response is beneficial, conceivably, a comparable phenomenon could occur as a result of insecticide spraying to trigger an aversive response which culminates in behavioral resistance.

The extensive arsenal of genetic tools for probing the *Dm* olfactory system make it an ideal system to uncover a possible role of the olfactory system in behavioral resistance. The *Dm*

olfactory system senses airborne molecules *via* the activation of receptors located on olfactory receptor neurons (ORNs). Each ORN expresses G-protein-coupled receptors which elicit a unique odorant response profile for that neuron. Each olfactory receptor functions together with an obligate co-receptor dubbed Orco (olfactory receptor co-receptor). As a start, fruit flies can be exposed to an insecticide and the phenomenon of behavioral resistance examined in *orco* mutants. If the behavioral insecticide resistance response is abrogated in *orco* mutants, it will mean further studies dissecting the effect of eliminating individual olfactory receptors may uncover which ones are required for behavioral insecticide resistance.

Using fruit flies to identify new targets of insecticides

Established in 2005 through a $50 million grant from the Bill and Melinda Gates Foundation to the Liverpool School of Tropical Medicine, the Innovative Vector Control Consortium (IVCC) has been spearheading efforts to identify new and improved insecticides. In this respect, more than 4.5 million compounds have been evaluated for potential use as insecticides, which resulted in the identification of six classes of new active ingredients that have implications for malaria vector control (see https://www.ivcc.com/research-development/insecticide-discovery-and-development/). Other screening efforts have involved the use of malaria vector species (Lees et al., 2019; Lees et al., 2020). Nevertheless, fruit flies (*Dm*) can be a complementary model system for identifying new molecular targets for insecticides. As previously alluded to with respect to metabolic resistance, studies in fruit flies can identify how the multiple genes induced in response to insecticides are regulated; and how this can be exploited to suppress insecticide resistance. Moreover, there are many other pharmaceutical products that may be useful insecticides but have not yet been studied extensively in fruit flies. These include pymetrozine and flonicamid, which are modulators of chordotonal organs; semicarbazones, some classes of diamides and several other modulators of nerve and muscle function; and growth and development targets such as ecdysone receptor agonists and inhibitors of respiration. Studies in fruit flies are likely to provide useful information about their roles, but with the ultimate aim of confirming any promising results in mosquitoes, and working with the IVCC to further exploit any promising findings to prevent disease transmission.

Many respiratory inhibitors proposed as insecticides exert their effect largely by altering reactive oxygen species (ROS) production or other mitochondrial processes that do not involve a disintegration of the oxidative phosphorylation (OXPHOS) complexes. Examples include several inhibitors of complex I (CI), such as fenazaquin, pyridaben, fenpyroximate, pyrimidifen, tolfenpyrad and tebufenpyrad. Nonetheless, the broad specificity of these CI inhibitors has limited their use. As a case in point, tebufenpyrad and pyridaben alter mitochondrial dynamics in rat dopaminergic neuronal cultures (Charli et al., 2016). Their broad specificity has also meant that concentrations used in the field, during clinical trials, had to be chosen judiciously. As sub-lethal levels of mitochondrial ROS can activate compensatory stress responses (Owusu-Ansah et al., 2008; Owusu-Ansah and Banerjee, 2009; Owusu-Ansah et al., 2013; Murari et al., 2021; Murari et al., 2022), this may explain, at least in part, why a clinical trial in Tanzania exploring the effectiveness of fenpyroximate and abamectin-treated durable wall liners as a control mechanism for malaria was not successful (Mpangala et al., 2021). We postulate that a more favorable outcome could be achieved with respiratory chain inhibitors that block aspects of the assembly of the OXPHOS system. We have been studying the mechanism of mitochondrial CI assembly in *Dm* and found that severe RNAi-mediated disruption of multiple CI subunits is lethal (Garcia et al., 2017). We hypothesize that a similar phenomenon may occur in other dipterans such as mosquitoes that could be exploited to develop new insecticides.

Mitochondrial CI is the most elaborate component of the OXPHOS system (Fiedorczuk et al., 2016; Agip et al., 2018; Rhooms et al., 2019). Mammalian CI has 45 subunits organized into two domains of the complex, oriented almost perpendicularly to each other, and referred to as the matrix and membrane domains (Figure 1) (Agip et al., 2019). There are three distinct functional modules of CI dubbed the N, Q, and P modules. The N module contains the flavin mononucleotide (FMN) cofactor and accepts electrons from NADH in the mitochondrial matrix. The Q module is situated between the N module and the membrane domain and transfers electrons to ubiquinone. The proton-pumping P module is essentially the membrane domain; and can be further sub-divided into a proximal P_P and distal P_D module [reviewed in (Vartak et al., 2014; Formosa et al., 2018; Rhooms et al., 2019)]. A total of 14 core subunits contain all the catalytic centers of CI and are conserved from the ancestral enzyme in bacteria to the eukaryotic enzyme. Seven core subunits (NDUFS1, NDUFS2, NDUFS3, NDUFS7, NDUFS8, NDUFV1 and NDUFV2) are encoded by the nucleus, translated in the cytoplasm, and imported into the mitochondrion; while the other core subunits are encoded and translated within the mitochondrion (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6). The 31 remaining subunits are referred to as accessory or supernumerary subunits, as they are not directly involved in performing the bioenergetics functions of CI. During CI assembly, specific subcomplexes consisting of a few CI subunits form largely independently of each other and merge in a stereotypic fashion en route to forming the mature complex (Stroud et al., 2016; Garcia et al., 2017; Formosa et al., 2018). CI assembly factors (CIAFs) are proteins that are usually found in association with specific subcomplexes and assist with the assembly process; but they are subsequently released when assembly is complete. The N, Q and P modules are synthesized from specific subcomplexes or assembly intermediates that can be tracked by immunoblotting or complexome profiling techniques (Guerrero-Castillo et al., 2017; Formosa et al., 2020; Murari et al., 2020; Szczepanowska et al., 2020).

Most of the mammalian accessory subunits are conserved in *Dm* (Garcia et al., 2017), but the extent to which each accessory subunit contributes to CI assembly differs between organisms. For instance, while RNAi-mediated disruption of NDUFA12 potently impairs CI assembly in *Dm* flight muscles and can cause lethality, a CRISPR-mediated knockout of NDUFA12 in human cells has

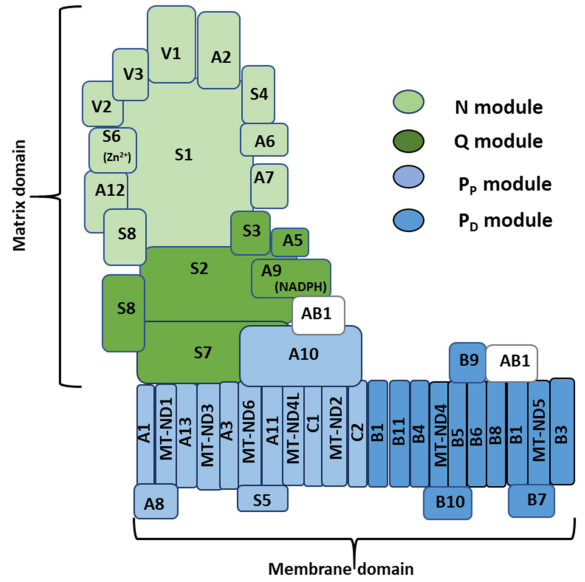

FIGURE 1
Architecture of mitochondrial complex I. A diagram of mitochondrial CI – based on mammalian Cryo-EM studies – depicting the hydrophilic matrix and hydrophobic membrane domains oriented almost perpendicularly to each other; and the relative positions of subunits localized to the N, Q, P_P and P_D modules. There are 45 subunits in mammalian CI, 44 of which are unique subunits, and NDUFAB1 (AB1), which appears twice in the complex. Fourteen of the 45 subunits are referred to as core subunits, as they are conserved in all organisms in which CI is present (see text for further details). NDUFS6 (S6) and NDUFA9 (A9) contain Zn^{2+} and NADPH as co-factors, respectively. Additional co-factors omitted for clarity include a flavin mononucleotide molecule and several Fe-S clusters found in association with some subunits in the matrix domain, and a number of phospholipid molecules intertwined with several subunits in the membrane domain.

minimal effects on CI assembly (Stroud et al., 2016; Garcia et al., 2017). We anticipate that such differences in roles of CI subunits in CI assembly can be exploited to make new arthropod- or diptera-specific CI assembly inhibitors, which can ultimately result in the development of novel insecticides.

Conclusion and future perspectives

There is an ever-present need to identify novel mosquito insecticides that are more effective and selective, and entail new mechanisms of action. This may require homology modeling for identifying novel chemistries and insect-specific modes of regulation of already established targets, as was performed for the housefly VGSC (O'Reilly et al., 2006). Alternatively, it may be necessary to screen for novel sites in genes that are well established insecticide targets, as was performed for the isooxazoline insecticide, A1443, a ligand-gated chloride channel antagonist (García-Reynaga et al., 2013). It is also crucial to uncover entirely novel biochemical targets, as was the case with specific classes of diamides that were found to modulate insect ryanodine receptor function (Sattelle et al., 2008). Molecular targets that regulate developmental pathways in insects, such as cell death, autophagy, molting, and basic organelle function could also be exploited. In fact, the importance of autophagy in the fat body, and ecdysone signaling in regulating molting and other aspects of mosquito physiology, has been described extensively, highlighting their potential utilization as targets of insecticides (Bryant and Raikhel, 2011; Childs et al., 2016; Shaw and Catteruccia, 2019; Werling et al., 2019; Brown et al., 2020; Ekoka et al., 2021; Maharaj et al., 2022). While, undoubtedly, studies in fruit flies can contribute to our understanding of all these putative aspects of mosquito insecticide development, we propose that developing insecticides that disrupt organelle function to cause cell death, especially those that inhibit mitochondrial CI assembly, *via* a mechanism distinct from how CI assembly is regulated in humans, holds great promise for identifying new and insect-specific targets of insecticides. However, we note that at best, *Dm* should only be regarded as a complementary model system for studying mosquito insecticide resistance as any promising leads would have to be confirmed in mosquitoes.

Author contributions

EO-A wrote the review and got feedback from KFBH and DV. All authors contributed to the generation of the figure. All authors approve the submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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