Chapter

Perspective Chapter: Genomics, Proteomics, and System Biology of Insecticides Resistance in Insects

Rabbiah Manzoor Malik, Sahar Fazal, Narjis Khatoon, Muneeba Ishtiaq, Saima Batool and Syed Tauqeer Abbas

Abstract

Insecticide resistance is an inherited change in pest population exposure to a specific insecticide or group of insecticides. Overuse, misuse, and high interbreeding rates have led to insecticide resistance. Genomic technologies reveal mechanisms of resistance, including decreased target-site sensitivity and increased detoxification. Genomic projects have cloned and identified targeted genes in Drosophila melanogaster and studied resistance-associated mutations in various pest insects. Advancements in genome sequencing and annotation techniques have explored complex multigene enzyme systems, such as glutathione-S-transferases, esterases, and cytochrome P450, which facilitate insecticide resistance. Identifying specific genes involved in resistance and targeted genes is essential for developing new insecticides and strategies to control pests. Insects with resistance metabolize insecticidal compounds faster due to increased catalytic rate and gene amplification. So, system biology plays a very important role in the insect resistance against insecticides and different chemicals such as DDT and permethrin. From system biology, not only the identification of genes was done, but also the protein-protein interactions were found out, which were responsible in the insect resistance.

Keywords: insecticides resistance, system biology of insecticide resistance, p450 and insecticides resistance, genomics of insecticides, proteomics of insecticides

1. Introduction

The most diverse group of animals on Earth is insect, which performs a lot of significant roles. The important functions of the insects are that they act as decomposers of the dead organisms, they are the necessary components of the ecosystems, they are helpful in the pollination of the plants, they also spread the seeds, they are a good source of proteins for the livestock, and they provide us with a variety of products such as dye, silk, wax, and honey [1–4]. Though insects are playing an important role in the lives of humans and livestock, they also prove to be very harmful for the environment and humans [5]. The negative effects of insects on the environment and humans are the major threats for the ecosystem. The most highlighted negative

impact of insects is on agriculture. Some insects such as locusts, caterpillars, and grasshoppers act as pests for crops because they eat the fruits, seed, and leaves of the crops. Some insects affect the development and the growth of the crops and make the plants vulnerable to the diseases; these insects include thrips, weevils, and aphids. Some insects such as locusts damage the crops, which results in famine situations. The pests have negative impacts on the agricultural crops as they spread and carry different diseases of the plants [5, 6]. These harmful impacts of the insects on the crops are resulting in the food shortage in the different parts of the world because the population of the world is increasing rapidly. The damages caused by the insects are also increasing with the increase in climate change [7]. Insects are the main source of causing the infectious diseases among the humans and livestock; for example, mosquitoes are responsible for causing malaria and dengue. Other insects involved in causing infectious diseases are kissing bugs, head lice, body lice, tsetse flies, and so on [8]. Keeping in view these all negative impacts of insects on the humans and environment, there is a need to control the insects.

2. Control of insects and insecticide resistance

Insecticides are used for the control of insects (such as termites, cockroaches, lice, and mosquitoes) in public health, industries, households, and agriculture. Firstly, the DDT was used to control the insects, but the insects got resistant to DDT, and it was reported in the houseflies in 1947. The new and most widely used insecticides are carbamates, organophosphates, formamidines, neonicotinoids, and pyrethroids. These insecticides were very effective against the insects in the beginning, but with the passage of time, insects became resistant to these insecticides [9, 10]. An inherited change in the exposure of a population of a pest to a certain insecticide or a group of insecticides is called insecticide resistance. The insects that cannot be controlled by the repeated use of a particular insecticide are said to be resistant to that insecticide [11]. The insects are getting resistant to the insecticides quickly because the insecticides are overused or misused, the insects' population is greater in size, and they interbreed at a very high rate [12].

3. Genomics and its significance in the field of biological sciences

An interdisciplinary field of biology that focuses on the function, structure, mapping, genome editing, and evolution is called genomics. The complete set of the DNA of an organism is called the genome. The aim of genomics is to study all the collective quantification and characterization of the genes of an organism and their impact on an organism [13]. The field of the biological sciences has become advanced with the help of the genomics as it involves the analysis and the sequencing of the genomes by using the next-generation sequencing and computational tools to analyze and assemble the structure and functions of the genomes [14]. Genomics has brought a revolution in the field of biological sciences such as systems biology, discovery-based research, biotechnology, medical diagnosis, personalized medicines, identifying therapeutic targets, forensics, biology systematics, and finding the evolutionary histories of the organisms. Intra-genomic studies are also involved in genomics such as pleiotropy, epistasis, heterosis, and the interactions between the alleles and loci within the genome [15].

4. Genomics and insecticide resistance

The technologies of genomics are showing different mechanisms of insecticide resistance, which involves decreased target-site sensitivity and increased detoxification [16]. Some possibly important concerns related to the quick insecticide resistance among insects with the evolutionary time are also revealed by the genome projects. Evolutionary biologists are being provided with contemporary and ideal model systems to study the evolution of the resistance among insects for the insecticides [17, 18]. The use of the tools of molecular biology to eliminate the mechanisms of insecticide resistance is of great interest. In 1990s, traditional techniques of molecular biology were used to investigate a few cases of insecticide resistance at a molecular level. The cases that involved the known genes could easily be cloned with the heterologous PCR were manageable. From the early studies, three mechanisms of the insecticides resistance were reported; one mechanism involved target-site insecticide resistance, and the other two mechanisms involved the increased detoxification of the insecticides. In culex mosquitoes [19] and aphids [20], the resistance against carbamate and organophosphate has been reported, and it is an example of the mechanism of detoxification.

Another example of detoxification is in the two species of flies in which the degradation of insecticides takes place. In specific carboxylesterases, the structural mutations had arisen that used to convert them into inefficient but physiologically sufficient organophosphate hydrolases [21]. The third mechanism of detoxification is the mutation of the target molecule in such a way that the target molecule becomes insensitive to the insecticides. The target molecules that become mutant are: for cyclodienes, the γ-aminobutyric acid (GABA) receptors become mutant; for organophosphates, the acetylcholinesterase becomes mutant; and for synthetic pyrethroids and dichlorodiphenyltrichloroethane (DDT), the voltage-gated sodium channels are becoming mutant [22, 23]. These findings were having some remarkable aspects such as the degradation and sequestration mechanisms, the sodium channels becoming insensitive, and repetition of the same amino acid changes in the orthologous proteins among different species, for example, in the acetyl cholinesterases and GABA receptors. The third aspect of these findings was that within a few years of the first use of insecticide, a small amount of the mutant alleles carry the mutations that have spread among the species. These features have shown that insects have very less options to confer the resistance against insecticides [24, 25].

Genomic technologies are able to investigate the previous intractable mechanisms of the resistance. Genomics also discusses the resistance to the proteinaceous biopesticide crystal toxins of *Bacillus thuringiensis* (Bt toxins) and the traditional chemical insecticides [26]. By the help of genomics, some targeted genes of the nervous system of *Drosophila melanogaster* have been cloned and identified and in a wide range of the pest insects, the resistance associated mutations have been studied [27]. Recently, with the advancement in genome sequencing and annotation techniques, genomes of the insects have been sequenced and annotated and the complex multigene enzyme systems such as the glutathione-S-transferases, esterases, and cytochrome P450 that facilitate the insecticides resistance among the insects have been explored [28]. In 2000, the whole genome of *Drosophila melanogaster* was reported, and after that, the partial and complete genomes of different species of insects have started to publish in the biological databases. In the NCBI database, genome sequences of almost 34 species of the orders Hymenoptera, Coleoptra, Diptera, Hemiptera, and Lepidoptera of the insects are available. These species include the most primitive insect human louse and major medical pests such as *Aedes aegyptii* and *Anopheles gambiae* [28].

5. Genes involved in insecticide resistance in insects

Recently, insecticide resistance has become a major concern for the control of many insect pest species. This challenging problem has useful solutions in the genome sequencing, transcriptome analysis, and the global quantization of the gene expression of those genes that are involved in the insecticide resistance. One of the most destructive agricultural pests of the world is *Bactrocera dorsalis* (oriental fruit fly), and it is used as a model to examine the genetic mechanisms of the insecticides resistance. For this species, the molecular data of the genes that were identified by homology was very limited. By using the Ilumina Solexa platform of the next-generation sequencing, the whole transcriptome of *Bactrocera dorsalis* was sequenced and the gene expression in the insecticide resistance was explored [29].

Mosquitoes are the major carriers of pathogens, and they are the source of causing infectious diseases among humans such as dengue and malaria, and the control of mosquitoes is the biggest threat as they are resistant to insecticides. In natural populations, the alternative tools for the control of mosquitoes have been implemented and the mechanism of the resistance was studied. A common mechanism of the resistance is the biodegradation of the insecticides by detoxification enzymes; during this mechanism, the changes in the genome of the mosquitoes have been identified except the individual genotyping of the resistance. Particularly, polymorphisms of the detoxification enzymes and the function of the copy number variations (CNVs) have not been examined at the genomic level though they represent strong markers for metabolic resistance. With the use of next-generation sequencing, the genes and polymorphisms associated with insecticide resistance in mosquitoes have been explored. According to a research, 760 candidate genes were sequenced and identified to be the cause of resistance against deltamethrin in the dengue mosquito (*Aedes aegypti*) [30]. The analysis of the CNVs showed the amplification of 41 genes to be associated with the resistance and in the resistant populations, the cytochrome P450 was over transcribed. More than 30,000 variants were detected in the analysis of the polymorphism. By combing the filtering of allele frequency and the Bayesian 55 nonsynonymous variants that were strongly associated in causing the resistance were identified. Both the polymorphisms and the CNVs within the regions were conserved but differed across the continents, which confirm that the changes in the genome causes the metabolic resistance against insecticides are not universal. The novel DNA markers for insecticide resistance were identified, which open the way for tracing the metabolic changes established by the mosquitoes for resisting the insecticides within and among the populations [31].

Anopheles gambiae is resistant to the four classes of insecticides, that is, the carbamates, pyrethroids, organophosphates, and organochlorines; that is why the control of the malaria is difficult in Africa. The functional validation of the detoxifying enzymes is lacking in *Anopheles gambiae*, but the expression of the detoxifying enzymes increases in resisting the insecticides. In the resistant *Anopheles gambiae*, the three genes Cyp6p3, Cyp6m2, and Gste2 are upregulated; for these findings and to explore the phenotype of the insecticide resistance, the transgenic analysis was performed using the UAS/GAL4 system. The evidence was reported that the resistance against organochlorine and organophosphate in *Anopheles gambiae* explains the overexpression of GSTE2 in a wide tissue profile. Carbamate and pyrethroid resistance is given by the overexpression of Cyp6p3; in the same tissues, pyrethroid resistance is explained by Cyp6m2. According to a research conducted on 757

samples of *Anopheles gambiae*, the mutations in the rdl, ace-1, and kdr gene were detected using sequencing and SNaPshot. In the insecticide resistance in *Anopheles gambiae* populations, the multiple mutations were also detected in the kdrW, ace-1, and A296G rdl alleles [32].

6. Mechanisms of insecticide resistance

Insecticide resistance is primarily caused by changes in the genes of insects. The genes that are involved in the insecticide resistance include those that encode for detoxification enzymes such as cytochrome P450 (CYP) and glutathione S-transferase (GST), which metabolize and detoxify the insecticides. These enzymes can also have mutations that increase their activity, making the insecticides less toxic. Target-site resistance mechanisms are also driven by mutations in the genes encoding the target proteins of the insecticides. On the other hand, insecticides target specific genes in insects to kill them. These genes are responsible for vital processes such as nerve impulse transmission, muscle contraction, and metabolism. For example, many insecticides target the voltage-gated sodium channels in the insects' nervous system, which are necessary for nerve impulse transmission [11, 16].

Other insecticides target enzymes that are involved in the production of energy in the insects, such as the mitochondrial electron transport chain, making it impossible for the insects to survive. Insecticides also target genes that are responsible for the synthesis of chitin, which is an important component of the insects' exoskeleton and necessary for their survival. It is important to note that the mechanisms of resistance and the target of the insecticides are constantly evolving due to the insects' adaptation to the environment and the insecticides. Therefore, the identification of the specific genes involved in resistance and the genes targeted by insecticides is essential for the development of new insecticides and strategies to control the pests [33]. There are different mechanisms of insecticide resistance, which include target-site insecticide resistance, metabolic insecticide resistance, penetration resistance, and behavioral resistance.

7. Penetration resistance

The susceptible insects absorb the toxin more quickly than the resistant insects. When the insects' outer cuticle develops the barriers of the slow absorption of the insecticides in their bodies, the penetration resistance occurs. Due to the penetration resistance, insects are protected from a wide range of the insecticides. Along with the other mechanisms of the insecticides, the penetration resistance takes place, and due to the reduced intensity of the penetration, these mechanisms of resistance dominate among insects [34].

8. Behavioral resistance

The insects that are resistant to insecticides are able to recognize and detect a danger and to avoid the toxin. For various classes of insecticides such as organophosphates, carbamates, organochlorines, and pyrethroids, the behavioral mechanism has been reported [35].

9. Target-site insecticide resistance

The specific binding site of an insecticide is mutated or modified during the resistance of the target site, due to which the target site becomes incompatible for the activation. In most common pests (such as *Myzus persicae*, *Musca domestica*, and *Drosophila melanogaster*), the mutations occur in the target regions, that is, knockdown resistance to pyrethroids, reduced sensitivity of the sodium channels against DDT, and the resistance against spinosad and subunits like nicotinic acetylcholine receptors for the neonicotinoids [24, 25]. Because of these mutations, the binding of the target region with the insecticides becomes impossible, and this leads to a loss of binding affinity. Moreover, the overproduction of the enzymes occurs in the metabolic resistance, which detoxify or break down the insecticides, leading to the resistance of the pests. Some metabolic enzymes such as hydrolases, cytochrome p450 monooxygenase, and glutathione S-transferase play a major role in the evolution of metabolic resistance. In the wild-type AChE gene (ace), the point mutations were found in the resistant *B. dorsalis*. In some species of the insects, the resistance also arises from the novel variants that represent the genetic changes such as the RNA edited product or alternatively spliced RNA [36].

10. Metabolic insecticide resistance

Metabolic insecticide resistance, also known as detoxification-based resistance, is a mechanism by which insects are able to detoxify the toxic compounds present in insecticides through the action of enzymes. This type of resistance is becoming increasingly common and is a significant threat to the control of insect pests. According to recent research, metabolic insecticide resistance has been primarily mediated by the activity of enzymes such as cytochrome P450 monooxygenases (P450s), esterases, and glutathione S-transferases (GSTs). These enzymes are able to detoxify the toxic compounds present in insecticides, rendering them harmless to the insect [10].

11. Proteomics- proteins and compounds involved in developing resistance

One example of metabolic insecticide resistance is found in the cotton bollworm, *Helicoverpa armigera*. Research has shown that this pest is able to detoxify the insecticide deltamethrin through the action of P450 enzymes. Specifically, the study found that the insect had an increased expression of the P450 gene CYP6B8, which was responsible for detoxifying the insecticide. Another example can be found in the red flour beetle, *Tribolium castaneum*. Research has shown that this pest is able to detoxify the insecticide chlorpyrifos through the action of esterases. Specifically, the study found that the insect had an increased activity of the esterase enzyme, which was responsible for detoxifying the insecticide [36–38].

Metabolic insecticide resistance can also be found in the mosquito, *Aedes aegypti*. Research has shown that this pest is able to detoxify the insecticide temephos through the action of GSTs. Specifically, the study found that the insect had an increased activity of the GST enzyme, which was responsible for detoxifying the insecticide. It is important to note that the evolution of resistance in insects is a complex process, influenced by a combination of genetic, biochemical, and environmental factors. To ensure effective control of insect pests, it is crucial to adopt integrated pest management strategies that include the use of insecticides in combination with other control

measures such as source reduction, biological control, and the use of alternative treatments such as essential oils. Research shows that metabolic insecticide resistance is a significant problem that is becoming increasingly common. The resistance is primarily mediated by the activity of enzymes such as P450s, esterases, and GSTs, which are able to detoxify the toxic compounds present in insecticides. To effectively control insect pests, it is crucial to adopt integrated pest management strategies that include the use of insecticides in combination with other control measures [30, 31].

The mechanism of the insecticide resistance of some insects is explained here:

11.1 Cockroaches

In more than half a dozen insect pest species, point mutations in the para sodium channel gene have been linked to knockdown resistance (kdr) to pyrethroids insecticides. In this investigation, we found two novel para variants in five strains of German cockroaches with high levels of resistance to kdr. The first intracellular linker, which joins domains I and II, contains the two alterations, which change glutamic acid (E434) to lysine (K434) and cysteine (C764) to arginine (R764), respectively. Closest to domain I is E434K, which is found near the beginning of the linker. C764R is found near the end of the linker (closest to domain II). One of the resistant strains has two further mutations, one from proline (P1880) to leucine (L1888) and another from aspartic acid (D58) to glycine (G58). The four mutations are exclusively seen in the most resistant individuals of a particular strain, and they coexist with the previously discovered leucine to phenylalanine (L993F) kdr mutation in IIS6. These findings imply that these mutations may be in charge of the German cockroach's high levels of knockdown resistance to pyrethroids pesticides [39, 40].

11.2 Head lice

Pediculus humanus capitis, often known as the human head louse, is a blood-sucking ectoparasite that primarily affects kids in both industrialized and developing nations. Permethrin is the primary active component of chemical pediculicides, which are the first line of defense. Despite the prolonged usage of these products, no studies have been conducted to determine if head lice in Honduras are resistant to insecticides. Knockdown resistance (kdr), the most prevalent mechanism in head lice, is caused by two point mutations and the corresponding amino acid substitutions, T917I and L920F, in the voltage-sensitive sodium channel (VSSC) [41]. The most significant contributing factor to the rise in head lice infestations worldwide may be pyrethroids resistance [42, 43]. Knockdown resistance (kdr), which reduces an insect's nerve sensitivity, is a property of lice resistant to pyrethroids and is brought on by single nucleotide point mutations (SNPs) in the para-orthologous voltage-sensitive sodium channel (VSSC) gene. It is well recognized that resistance is caused by the key amino acid substitutions T917I and L920F, which are found in domain II [44]. The locations of the housefly VSSC's amino acid sequence revealed that the mutations T929I and L932F, which have been linked to permethrin resistance, were expressed (rather than in the head louse amino acid sequence). Additionally, it has been shown that this group of mutations coexists as a resistant haplotype; when T197I was produced in *Xenopus oocytes*, either alone or in combination, it effectively inhibited permethrin sensitivity. The T917I amino acid change is relevant to pyrethroid resistance via the kdr-type nerve insensitivity mechanism and can be employed as a molecular marker for resistance detection [45].

11.3 Fruit fly

Cyclodiene and phenylpyrazole insecticides affect the GABA-gated chloride channel component that the resistance to dieldrin gene, or Rdl, encodes. By genetically mapping cyclodiene dieldrin resistance in *Drosophila melanogaster*, the gene was first identified. The change from Ala301 to Ser, one amino acid, caused the 4000-fold resistance. A wide variety of resistant insect species' Rdl orthologs were later found to contain the same alteration. In a research, a duplication at the Rdl gene in *D. melanogaster* was discovered. Rdl is present in two copies, one of which is WT and the other of which has two point mutations: An Ala301 to Ser resistance mutation and a Met360 to Ile substitution. Individuals with this duplication had lower temperature sensitivity, altered RNA editing linked to the resistant allele, and intermediate dieldrin resistance compared to single copy Ser301 homozygotes. This genomic rearrangement is caused by ectopic recombination between Roo transposable elements. By building a transgenic, artificial duplication integrating the 55.7-kb Rdl locus with a Ser301 mutation into an Ala301 background, the duplication phenotypes were confirmed. In most cases, gene duplications increase the amount of gene product generated, which has a considerable impact on the evolution of pesticide resistance. However, in this instance, duplication of the Rdl target site results in permanent heterozygosity, offering a rare opportunity for adaptive mutations to accumulate in a single copy without removing the essential gene's innate function [46].

11.4 Mosquito

The environmental changes in nature and the adaptive genes are easily identifiable; *Culex pipiens* mosquito's resistance to organophosphorus pesticides provides a useful model for analyzing the fitness cost of resistance genes and their origin. This resistance is caused by two loci, the super-locus Ester and the locus Ace.1, each of which contains a number of resistance alleles. According to population surveys, the fitness costs of various resistance genes and even resistance alleles at the same locus vary. The consequences of these resistance genes on various fitness-related variables are being investigated in order to better understand this fitness cost and its unpredictability. The impact of three resistance alleles such as Ester4, Ester1, and Ace.1R on paternity success relative to susceptible males and relative to one another in the research using competition trials between two males for accessing a single female were examined. The impact of susceptible and resistant female genotypes on male mating success was eventually examined. The strains utilized in this investigation have a common genetic history. Males who competed against any of the resistant males had a mating advantage, indicating a high cost of resistance genes for this feature. Regardless of the genotype of the female, resistant male had the same paternity success rate when competing against susceptible males [31, 32, 38, 47].

12. Pathways involved in metabolic resistance

Xenobiotics are detoxified by enzymes into a less or nontoxic compound, resulting in the formation of a more suitable form of metabolite for rapid removal from the body. Insects having resistance metabolize these insecticidal compounds faster due to presence of enzyme with increased catalytic rate and in higher quantities because

of increased amplification and transcription of their genes. There are two phases of detoxification: phase I (primary), consisting of oxidation or hydrolysis, and phase II (secondary), consisting of conjugation reactions of products of phase I with different endogenous compounds, like glucuronic acid or glutathione, facilitating their subsequent dissolution and excretion from all over the body [48–51]. Sequestration is also an important mechanism of defense that has been adopted by insects to tolerate these xenobiotics, in addition to such processes of detoxification that are based on cleavage and excretion of insecticides by using enzymes. This strategy involves selective and specific uptake, transportation, and storing of secondary metabolites from the plants on which they are feeding. These metabolites provide them resistance against the insecticides, interfering with their physiological mechanisms [52, 53]. One of the examples of such mechanisms is hematophagy, found in mosquitoes. It could be probably a way of secondary adaptation in which they obtain food of high quality in order to maintain egg production [54].

The enzymes that are majorly involved in xenobiotics detoxification in living organisms are synthesized by transcription of members of large families multigene complexes of enzymes like oxidases, esterases, and glutathione transferases (GSTs).

12.1 Esterases

Esterases belong to a large group of enzymes that catalyze phase 1 reactions, which can metabolize a large variety of endogenous and exogenous substrates. Their role in detoxification of insecticide metabolites is well reported, and they have been shown to act against a wide range of chemical compounds, including organophosphates, pyrethroids, and carbamates [48]. Studies have shown their probable involvement in resistance against Bt toxin [55] and even against neonicotinoid [56]. Insecticide compounds can be detoxified through enzymatic cleavage or sequestration. Insecticides esters are hydrolyzed into their corresponding alcohols and acids by the Esterases and are excreted from the insects' body more easily due to their increased solubility. Insecticides can also be sequestered by Esterases so that the availability of toxic molecules is no longer possible for interacting with the target proteins [57–59]. Esterases are linked to insecticide resistance, due to some qualitative or quantitative or both types of changes in many species of insects, causing the enzymes' overproduction or their structures modifications [48]. Esterases are overexpressed due to upregulation of their genes or amplification or both. One of the most studied examples of detoxification of insecticide through gene amplification is seen in the green peach aphid *Myzus persicae*, which involves the overproduction of a specific enzyme carboxylesterase (Hemiptera: Aphididae) [60–63]. Such amplified esterases have also been seen in mosquitoes of the genus Culex, associated with insecticide resistance (Diptera: Culicidae) [19, 64, 65] and some other species, like the brown planthopper *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae) [66]. In some species, like *Aphis gossypii* Glover (Hemiptera: Aphididae) or B-biotype *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), the expression of enzymes esterases is increased due to increased levels of transcription, due to corresponding gene upregulation [67, 68]. Esterases are also involved in changing the structure of enzymes that are involved in enhanced ability of insects to metabolize the compounds of insecticides. "Mutant ali-esterase theory" was presented by scientists based on this type of mechanism described in the housefly *Musca domestica* (Diptera: Muscidae) for the first time [69]. The insects with resistance exhibited a decreased activity of esterase apparently, as compared to susceptible compounds, resulting due to structural modifications in the

enzyme facilitating the process of hydrolysis of the metabolite of insecticide, but it reduced or prevented the hydrolysis of the molecule used for determination of the esterase activity. This mechanism of resistance was considered to be based on substitution of two amino-acid (Gly137Asp and Trp251Leu) in houseflies as well as in many other insect species belonging to the order of Diptera [22, 23, 70].

Other mechanisms like chromosomal rearrangements or demethylation also effect the overproduction of esterase. Mechanisms of demethylation can lead to gene silencing and subsequent reduction of levels of esterase among E4 populations [71], whereas no correlations have been seen between esterase activity and methylation levels in the esterase variant of FE4 [61]. In some Italian populations of aphid species, autosomal rearrangements of same type for the FE4 isoforms of esterase have been reported but only in those with low activity of esterase, showing that resistance due to esterase and translocation are not correlated always [62, 72].

12.2 Monooxygenases

Another class of enzymes involved in metabolism of Xenobiotics is microsomal oxidases or mixed function oxidases (MFOs). These are enzymes of phase 1 reactions and are also involved in metabolism of endogenous metabolites like fatty acids, pheromones, or hormones. These enzymes can convert hydrophobic molecules to hydrophilic substances so that they can easily be eradicated from the body. Their major localization is in digestive tract [37, 73, 74]. Microsomal oxidases are Cytochrome P450 monooxygenases (P450s) that are from the group of enzymes composed of heme thiolate proteins. They exhibit a characteristic peak of absorbance at 450 nm when they are in a reduced form and complexed with molecule of carbon monoxide. The reactions catalyzed by these enzymes involve the transfer of one atom of molecular oxygen to a substrate and the reduction of the second atom of oxygen to form water. This process needs the transfer of at least two electrons, which are provided by NADPH cytochrome P450 reductase [73, 75]. P450s possess a large variety of enzymes that are highly specific for substrate and can catalyze various reactions like hydroxylation, epoxidation, desulfurization, O-dealkylation, or N-dealkylation. They play a major role in interactions between plants and insects and metabolizing many insecticides like organophosphates, carbamates, neonicotinoids, and pyrethoids [50, 76–79]. The enzymes of P450 family are named by abbreviation CYP with an Arabic number of the respective family, a capital letter designating the subfamily with an Arabic numeral designating the individual protein. Every each has its own gene to be coded. More than 600 P450 genes have been characterized from insects, and it has been found that genes of families CYP6, CYP4, CYP12, and CYP9 are associated with resistance in insects against insecticides (**Figures 1** and **2**) [48, 73, 74]. The MPOs are majorly found in the midgut, Malpighian tubules, and fat bodies of insects. The housefly is the candidate whose MPOs system has been extensively studied [80]. Studies have reported that higher concentrations of P450s and an increased activity of monooxygenases are found in resistant insects. Overexpression of such activities occurs as a result of upregulation of their genes, that is mediated by the modifications of regulatory elements [73]. They have also shown amplification of genes or qualitative modifications in other studies [78, 81–83]. Some type of insecticides can also be activated by enzymes of insect P450 system. One of the example is the formation of phosphate (P=O) from phosphorothioates (P=S). This causes an increased 330 potency for inhibiting acetylcholinesterase by a magnitude of 3 or 4 orders. The synthesis of juvenile hormone, pheromone components, and ecdysone also needs involvement of P450s [84].

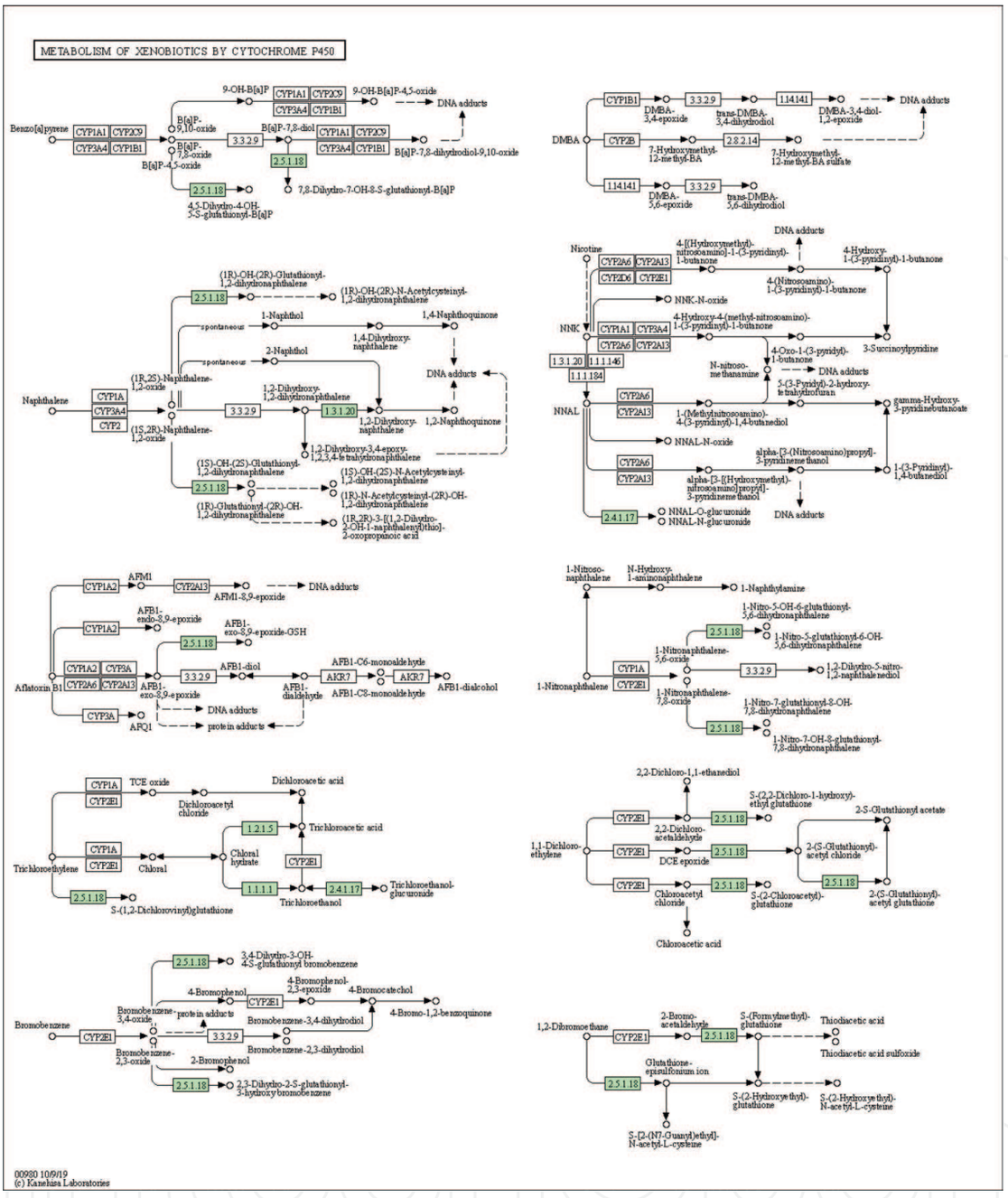

Figure 1.
Metabolism of xenobiotics by cytochrome P450 - Anopheles gambiae (malaria mosquito).

Aerobic organisms are characterized by the presence of a diverse group or family of enzymatic proteins named Glutathione transferases (GSTs), which are found ubiquitously. They play a major function in the detoxification of xenobiotic as well as endogenous compounds. They are also found to be associated with synthesis of hormones, intracellular transport, and protection in contradiction of oxidative stress [85]. GST enzymes are involved in catalyzing the conjugation reactions of reduced glutathione with electrophilic molecules or substrates as well as in sequestrating substrate. This results in increased hydrophilicity or water solubility and decreased toxicity of reactive molecules and in turn facilitates their removal or excretion from the body. Specifically, they catalyze conjugations by facilitating nucleophilic attack of the sulfhydryl group of endogenous reduced glutathione (GSH) on electrophilic centers of a range of xenobiotic

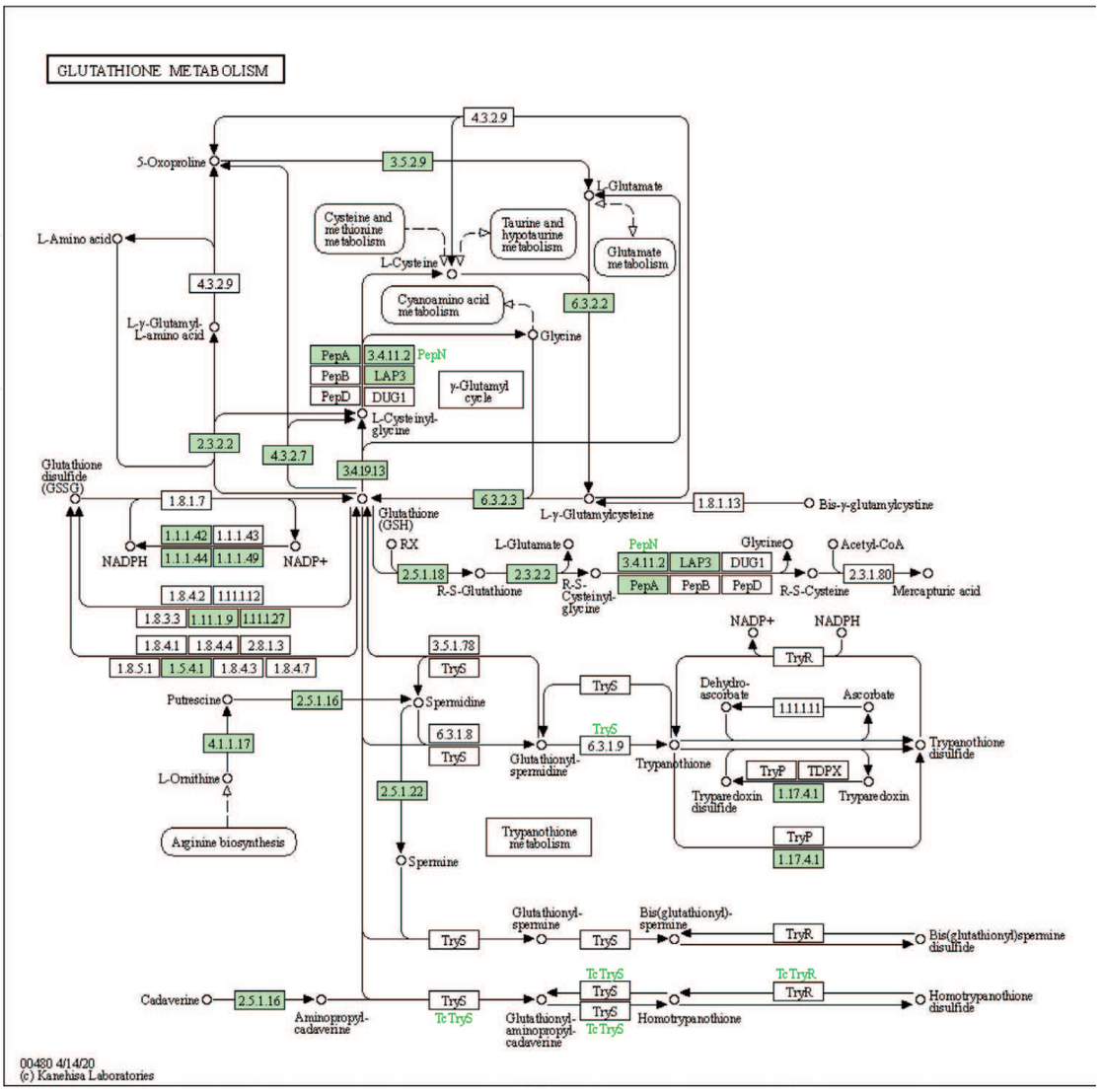

Figure 2.
Glutathione metabolism - Drosophila melanogaster (fruit fly).

compounds, including insecticides or acaricides [86] and various plant toxins [76]. This results in the conversion of xenobiotics into derivatives of mercapturic acid, which are more soluble and easily excreted out from the insect body [87, 88]. Species of free oxygen radicals are formed in insects by the action of pesticides that are highly toxic and can be removed with the help of these GSTs. They also help in metabolizing insecticides through their reductive dehydrochlorination [89]. In insects, there are two groups of GSTs: cytosolic and microsomal. They are classified on the basis of their occurrence in the cell. Only cytosolic GSTs have been reported to be involved in insecticides metabolism. They play an important role in developing resistance against some insecticides like pyrethroids and organophosphates. In mosquitoes and houseflies, resistance against DDT has been eveloved due to a DDT dehydrochlorinase GST [88]. The full extent of family of this enzyme has been revealed in genomes of the *Drosophila melanogaster* Meigen and *Anopheles gambiae* Giles [88]. Generally, the quantity of enzyme is increased due to either overexpression of gene or its amplification, which results in enhanced resistance on the basis of GSTs [90, 91]. Insecticides can also be sequestered by GSTs that provide the insects protection against the toxicity of these insectcides, for example, pyrethroid [83].

12.3 Pgp pumps

Pgp pumps are transporters composed of P-glycoprotein (Pgp) that are integral membrane proteins and belong to the ATP-binding cassette (ABC) superfamily, which utilizes the energy produced from ATP breakdown and translocates different metabolites as well as xenobiotics across the cell membranes [92]. This type of mechanism has been majorly observed in fungi and bacteria for developing resistance against antibiotics [93], but very little work has been reported on it regarding insects. Only recently, these ABC transporters have been found in insects as a supposed mechanism that can contribute in resistance by facilitating the efflux transport mechanism of insecticides as well as their compounds or metabolites that are derived from phase I and II reactions [94–98]. ABC transporters can produce resistance in insects through different modes like quantification of protein or transcript and by synergistic mechanisms of ABC inhibitors [94, 99]. Furthermore, in different lepidopteran species, a mutant allele has been discovered that confers resistance to the pore-forming Cry1Ac toxin from *Bacillus thuringiensis* (Bt) by a mechanism that is not related to toxin extrusion, but because it causes the loss of Cry1Ac binding to membrane vesicles [100, 101].

13. What is systems biology?

The study of the relationships and behavior of biological entity components such as molecules, cells, organs, and organisms is known as systems biology. Individual roles are played by microbes, plants, animals, and entire ecosystems in the natural world, which is a complex system of interconnected pieces. The investigation of living creatures is approached comprehensively in systems biology. It studies how diverse biological creatures interact at different sizes. Every person, for example, is a system. The system includes our organs, tissues, cells, and the components they are formed of, as well as bacteria and other creatures that dwell on our epidermis and in our digestive system [102].

Computational and mathematical analysis and modeling are important to systems biology. It gathers data from a wide variety of biological sciences and technologies known as "-omics" by researchers. Among these "omics" are genomics (the study of whole gene sets in an organism) and proteomics (the study of all the proteins in a cell, tissue, or organism). The emphasis in these fields is on describing and measuring the biological molecules that underpin how organisms are produced, operate, and live [103].

14. What is a significant role of systems biology in causing insect resistant?

Insecticide resistance is regarded as a typical similar pattern of microevolution, in which a powerful selection agent is given to a large natural community, resulting in a shift in the frequency of alleles conferring resistance. While numerous pesticide resistance variations have been identified at the gene level that was in term of systems biology, they are usually single genes with a big influence seen in highly resistant insect pest. With *Drosophila melanogaster,* many polymorphisms have been involved in DDT resistance; however, only Cyp6g1 locus has already been proven to be meaningful to field populations. They uncover DDT-associated polygenes using genome-wide association studies (GWAS) and assess their

adaptive importance using selective sweep analysis. As a result, they validate two DDT resistance loci. This was considered as the significant role of system biology in causing insect resistant [104].

15. What are the main pathways involved in insects resistance?

The two major pathways involved in insecticide resistance were metabolic resistance and target-site resistance. Metabolic resistance is a typical defense strategy that relies on enzymatic mechanisms to protect the insect by detoxifying/sequestering pesticide compounds. In order to overcome the potential toxicity of the plants they feed on, the enzymes involved are those that insects have evolved as support against naturally occurring plant poisons (study will focus) such as alkaloids, terpenes, and phenols. This might explain the modernization of metabolic resistance to a wide range of insecticides, many of which have direct or indirect botanical origins. Enzymes may detoxify xenobiotics into a nontoxic chemical and/or a form that is more suited for fast removal from the body [105].

Resistant insects metabolize the pesticide quicker because they have enzymes with a better catalytic rate, or because they have more enzymes as a result of enhanced transcription or gene duplication. Detoxification can be separated into two phases: phase I (primary) activities involving hydrolysis or oxidation, and phase II (secondary) processes involving coupling of phase I results with endogenous molecules such as glutathione and eventual elimination from the body. In addition to such enzymatic cleavage and excretion-based detoxification methods, sequestration is a significant defense mechanism that certain insects have evolved to withstand xenobiotics [67].

This is a typical phenomenon in insect herbivores that involves the precise and selective absorption, transport, and storing of secondary metabolites from plants in order to avoid interference with the insects' physiological processes. Such behavior has been seen in mosquitos, where hematophagy is most likely a subsequent adaption to get high-quality food for egg formation. Members of vast multigene families of isoenzymes, oxidoreductases, and GSTs transcribe the enzymes involved in xenobiotic detoxification in living organisms [106].

On the other hand, target-site resistance was explained as the pesticide's target site of action in the insect that can be genetically engineered to inhibit the insecticide from bonding or interacting at the site of action, lowering or eliminating the insecticide's pesticidal impact. During target-site resistance, an insecticide's particular binding site is transformed (mutated) and/or removed, rendering the target site unsuitable with activation. Most frequent insect (*Myzus persicae*, *Musca domestica*, and *Drosophila melanogaster*) target areas are mutated, including subunits such as cholinergic acetyl cholinergic receptor (nAChRs), knockdown resistance (KDR), and others. Insecticides are not able to bind inside the target area as a result of these changes, resulting in a reduction of binding affinity [107].

16. Which mechanism of resistance affects the behavior of the insects?

Metabolic resistance serves as the most common mode and frequently poses the most difficult barrier. Insects break down pesticides using their internal enzyme systems. These enzymes may be present in larger concentrations or in more effective forms in resistant strains. It was also explained by the case study of P450 gene in *House Flies* [108].

Insects may employ a variety of metabolic processes to avoid the fatal effects of pesticides. Increased cytochrome P450 detoxification, for example, is known to play a key role in many insect species. P450s' constitutively elevated overexpression and induction are hypothesized to somehow be responsible for enhanced levels of pesticide detoxification. However, unlike continuously upregulation P450 genes, whose regulation connection with pesticide resistance has been well explored; P450 induction in insecticide resistance is less well understood. The current work focuses on the identification of particular P450 genes that are activated in permethrin-resistant house flies in response to permethrin treatment. As a result, Permethrin administration co-upregulated the expression of three P450 genes, CYP4D4v2, CYP4G2, and CYP6A38, in permethrin conferring resistance ALHF house flies in a period and dose-dependent way. The protein sequences among these 3 P450s from resistant ALHF as well as vulnerable aabys and CS house flies were found to be similar. CYP4D4v2 and CYP6A38 were found on autosome 5, correlating to the association of P450-mediated resistance in ALHF, while CYP4G2 was found on autosome 3, where the key insecticide susceptibility factors for ALHF had been mapped, but no P450 genes had been reported previously to this investigation.

This study provided the first direct proof that numerous P450 genes are co-upregulated in permethrin-resistant house flies via the induction process, which boosts total P450 gene expression levels in resistant house flies. This research provides new information on the functional importance of P450 genes as they react to insecticide therapies, detoxification of insecticides, insect adaptation to their atmosphere, and the evolution of insects [108].

17. What is the role of protein-protein interaction pathway in insect resistance according to system biology?

At the moment, the problem of resistance is not fundamentally solved since the development speed of new insecticides cannot keep up with the progression speed of resistance, and there is a lack of knowledge of the molecular mechanism of resistance.

Researchers used literature mining and the String database to identify seed genes and their interacting proteins involved in the biological mechanism of pesticide resistance in *Drosophila melanogaster*. They discovered 528 proteins molecules and 13,514 protein-protein interactions. String and Pajek built the protein interaction network, and we looked at topological features like degree centrality and eigenvector centrality. KEGG pathway enrichment analyses revealed an enrichment for proteasome complexes and drug metabolism of cytochrome P450. This is the first time that the pesticide resistance in molecular level mechanism of *D. melanogaster* has been investigated using network biology methodologies and tools, and it can provide a bioinformatic basis for further understanding of insecticide resistance mechanisms [108].

So, systems biology plays a very important role in the insect resistance against insecticides and different chemicals such as DDT and permethrin. From systems biology, not only the identification of genes was done, but also the protein-protein interactions were found out, which were responsible for insect resistance.

Author details

Rabbiah Manzoor Malik[1,2,3*], Sahar Fazal[3], Narjis Khatoon[3], Muneeba Ishtiaq[3], Saima Batool[3] and Syed Tauqeer Abbas[1,2]

1 Wah Medical College, Wah Cantt, Pakistan

2 National University of Medical Sciences, Rawalpindi, Pakistan

3 Capital University of Sciences and Technology, Islamabad, Pakistan

*Address all correspondence to: rabbiahmanzoor@gmail.com

IntechOpen

References

[1] Van Huis A. Potential of insects as food and feed in assuring food security. Annual Review of Entomology. 2013;**58**:563-583

[2] Pietro Campobasso C, Di Vella G, Introna F. Factors affecting decomposition and Diptera colonization. Forensic Science International. 2001;**120**(1-2):18-27

[3] Mueller UG, Gerardo NM, Aanen DK, Six DL, Schultz TR. The evolution of agriculture in insects. Annual Review of Ecology, Evolution, and Systematics. 2005;**36**(1):563-595

[4] Wang Y-S, Shelomi M. Review of black soldier fly (Hermetia illucens) as animal feed and human food. Food. 2017;**6**(10):91

[5] Nghiem LTP et al. Economic and environmental impacts of harmful non-indigenous species in Southeast Asia. PLoS One. 2013;**8**(8):e71255

[6] Gregory PJ, Johnson SN, Newton AC, Ingram JSI. Integrating pests and pathogens into the climate change/food security debate. Journal of Experimental Botany. 2009;**60**(10):2827-2838

[7] Carvalho FP. Agriculture, pesticides, food security and food safety. Environmental Science and Policy. 2006;**9**(7-8):685-692

[8] Delaunay P et al. Bedbugs and infectious diseases. Clinical Infectious Diseases. 2011;**52**(2):200-210

[9] Ware GW, Whitacre DM. An introduction to insecticides. Pesticide Biochemistry. 2004;**6**

[10] Perry T, Batterham P, Daborn PJ. The biology of insecticidal activity and resistance. Insect Biochemistry and Molecular Biology. 2011;**41**(7):411-422

[11] Liu N. Insecticide resistance in mosquitoes: Impact, mechanisms, and research directions. Annual Review of Entomology. 2015;**60**:537-559

[12] Ranson H, Burhani J, Lumjuan N, Black WC IV. Insecticide resistance in dengue vectors. Trop. net [online]. 2010;**1**(1)

[13] Ellegren H. Genome sequencing and population genomics in non-model organisms. Trends in Ecology & Evolution. 2014;**29**(1):51-63

[14] Allendorf FW, Hohenlohe PA, Luikart G. Genomics and the future of conservation genetics. Nature Reviews. Genetics. 2010;**11**(10):697-709

[15] Brown TA. Genomes 4. Garland Science. 2018. pp. 1-24

[16] Ffrench-Constant RH. The molecular genetics of insecticide resistance. Genetics. 2013;**194**(4):807-815

[17] Ranson H et al. Evolution of supergene families associated with insecticide resistance. Science (80-.). 2002;**298**(5591):179-181

[18] Daborn PJ et al. A single P450 allele associated with insecticide resistance in drosophila. Science (80-.). 2002;**297**(5590):2253-2256

[19] Raymond M, Chevillon C, Guillemaud T, Lenormand T, Pasteur N. An overview of the evolution of overproduced esterases in the mosquito Culex pipiens. Philosophical Transactions of the Royal Society London Series B Biological Sciences. 1998;**353**(1376):1707-1711

[20] Field LM, Devonshire AL, Forde BG. Molecular evidence that insecticide resistance in peach-potato aphids (Myzus persicae Sulz.) results from amplification of an esterase gene. The Biochemical Journal. 1988;**251**(1):309-312

[21] Newcomb RD, Campbell PM, Ollis DL, Cheah E, Russell RJ, Oakeshott JG. A single amino acid substitution converts a carboxylesterase to an organophosphorus hydrolase and confers insecticide resistance on a blowfly. Proceedings of the National Academy of Sciences. 1997;**94**(14):7464-7468

[22] Claudianos C, Russell RJ, Oakeshott JG. The same amino acid substitution in orthologous esterases confers organophosphate resistance on the house fly and a blowfly. Insect Biochemistry and Molecular Biology. 1999;**29**(8):675-686

[23] Campbell PM, Newcomb RD, Russell RJ, Oakeshott JG. Two different amino acid substitutions in the ali-esterase, E3, confer alternative types of organophosphorus insecticide resistance in the sheep blowfly, Lucilia cuprina. Insect Biochemistry and Molecular Biology. 1998;**28**(3):139-150

[24] French-Constant RH, Anthony N, Aronstein K, Rocheleau T, Stilwell G. Cyclodiene insecticide resistance: From molecular to population genetics. Annual Review of Entomology. 2000;**45**(1):449-466

[25] Williamson MS, Martinez-Torres D, Hick CA, Devonshire AL. Identification of mutations in the houseflypara-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Molecular and General Genetics MGG. 1996;**252**(1):51-60

[26] Ibrahim MA, Griko N, Junker M, Bulla LA. Bacillus thuringiensis: A genomics and proteomics perspective. Bioengineering Bugs. 2010;**1**(1):31-50

[27] Olazcuaga L et al. A whole-genome scan for association with invasion success in the fruit fly Drosophila suzukii using contrasts of allele frequencies corrected for population structure. Molecular Biology and Evolution. 2020;**37**(8):2369-2385

[28] Homem RA, Davies TGE. An overview of functional genomic tools in deciphering insecticide resistance. Current Opinion in Insect Science. 2018;**27**:103-110

[29] Jin T, Zeng L, Lin Y, Lu Y, Liang G. Insecticide resistance of the oriental fruit fly, Bactrocera dorsalis (Hendel) (Diptera: Tephritidae), in mainland China. Pest Management Science. 2011;**67**(3):370-376

[30] Ocampo CB, Salazar-Terreros MJ, Mina NJ, McAllister J, Brogdon W. Insecticide resistance status of Aedes aegypti in 10 localities in Colombia. Acta Tropica. 2011;**118**(1):37-44

[31] Faucon F et al. Identifying genomic changes associated with insecticide resistance in the dengue mosquito Aedes aegypti by deep targeted sequencing. Genome Research. 2015;**25**(9):1347-1359

[32] Yin J et al. Molecular detection of insecticide resistance mutations in Anopheles gambiae from Sierra Leone using multiplex SNaPshot and sequencing. Frontiers in Cellular and Infection Microbiology. 2021;**11**:778

[33] Karaa SU. Insecticide resistance. London, UK: IntechOpen; 2012

[34] Balabanidou V, Grigoraki L, Vontas J. Insect cuticle: A critical determinant of insecticide resistance. Current Opinion in Insect Science. 2018;**27**:68-74

[35] Zalucki MP, Furlong MJ. Behavior as a mechanism of insecticide resistance: Evaluation of the evidence. Current Opinion in Insect Science. 2017;**21**:19-25

[36] Ilias A, Vontas J, Tsagkarakou A. Global distribution and origin of target site insecticide resistance mutations in Tetranychus urticae. Insect Biochemistry and Molecular Biology. 2014;**48**:17-28

[37] Liu N, Li M, Gong Y, Liu F, Li T. Cytochrome P450s--their expression, regulation, and role in insecticide resistance. Pesticide Biochemistry and Physiology. 2015;**120**:77-81

[38] Stevenson BJ, Pignatelli P, Nikou D, Paine MJI. Pinpointing P450s associated with pyrethroid metabolism in the dengue vector, Aedes aegypti: Developing new tools to combat insecticide resistance. PLoS Neglected Tropical Diseases. 2012;**6**(3):e1595

[39] Yanola J, Somboon P, Walton C, Nachaiwieng W, Prapanthadara L. A novel F1552/C1552 point mutation in the Aedes aegypti voltage-gated sodium channel gene associated with permethrin resistance. Pesticide Biochemistry and Physiology. 2010;**96**(3):127-131

[40] Dong KE, Scott JG. Linkage of kdr-type resistance and the Para-homologous sodium channel gene in German cockroaches (Blattella germanica). Insect Biochemistry and Molecular Biology. 1994;**24**(7):647-654

[41] Larkin K et al. First evidence of the mutations associated with pyrethroid resistance in head lice (Phthiraptera: Pediculidae) from Honduras. Parasites & Vectors. 2020;**13**(1):1-7

[42] Falagas ME, Matthaiou DK, Rafailidis PI, Panos G, Pappas G. Worldwide prevalence of head lice. Emerging Infectious Diseases. 2008;**14**(9):1493-1494

[43] Ichard R, Oberts JR. Clinical Practice Head Lice. 2002 [Online]. Available: www.nejm.org [Accessed 16 February 2019]

[44] Karakuş M, Atıcı T, Karabela ŞN, Baylan O, Limoncu ME, Balcıoğlu İC. Detection of permethrin resistance and phylogenetic clustering of turkish head lice (*Pediculus humanus capitis*; De Geer, 1767 populations). Acta Tropica. 2020;**204**:105362

[45] Eldefrawi ME, Eldefrawi AT. Nervous-system-based insecticides. In: Safer Insecticides. London, UK: CRC Press; 2020. pp. 155-207

[46] Dong K et al. Molecular biology of insect sodium channels and pyrethroid resistance. Insect Biochemistry and Molecular Biology. 2014;**50**:1-17

[47] Paul A, Harrington LC, Zhang L, Scott JG. Insecticide resistance in Culex pipiens from New York. Journal of the American Mosquito Control Association. 2005;**21**(3):305-309

[48] Li X, Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology. 2007;**52**:231-253

[49] Hollingworth RM, Dong K. The biochemical and molecular genetic basis of resistance to pesticides in arthropods. In: Whalon ME, MotaSanchez D, Hollingworth RM, editors. Global Pesticide Resistance in Arthropods. Wallingford, UK: CABI; 2008. pp. 5-31

[50] Yu SJ. The Toxicology and Biochemistry of Insecticide. Boca Raton, FL, USA: CRC Press; 2008. p. XVI+276

[51] Berenbaum MR, Johnson RM. Xenobiotic detoxification pathways in honey bees. Current Opinion in Insect Science. 2015;**10**:51-58

[52] Erb M, Robert CAM. Sequestration of plant secondary metabolites by insect herbivores: Molecular mechanisms and ecological consequences. Current Opinion in Insect Science. 2016;**14**:8-11

[53] Petschenka G, Agrawal AA. How herbivores coopt plant defenses: Natural selection, specialization, and sequestration. Current Opinion in Insect Science. 2016;**14**:17-24

[54] Moore SJ. Plant-based insect repellents. In: Debboun M, Frances SP, Strickman DA, editors. Insect Repellents Handbook. Boca Raton, FL, USA: CRC Press; 2015. pp. 179-212

[55] Gunning RV, Dang HT, Kemp FC, Nicholson IC, Moores GD. New resistance mechanism in Helicoverpa armigera threatens transgenic crops expressing bacillus thuringiensis Cry1Ac toxin. Applied and Environmental Microbiology. 2005;**71**:2558-2563

[56] Zhu YC, Luttrell R. Altered gene regulation and potential association with metabolic resistance development to imidacloprid in the tarnished plant bug, Lygus lineolaris. Pest Management Science. 2015;**71**:40-57

[57] Devonshire AL, Moores GD. A carboxylesterase with broad substrate specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (Myzus 333 persicae). Pesticide Biochemistry and Physiology. 1982;**18**:235-246

[58] Oakeshott JG, Claudianos C, Campbell PM, Newcomb RD, Russell RJ. Biochemical genetics and genomics of insect esterases. In: Gilbert LI, Iatrou K, Gill SS, editors. Comprehensive Insect Molecular Science. Vol. 5. Oxford, UK: Elsevier; 2005. pp. 309-381

[59] Wheelock CE, Shan G, Ottea J. Overview of carboxylesterases and their role in the metabolism of insecticide. Journal of Pest Science. 2005;**30**:75-83

[60] Field LM, Devonshire AL, Forde BG. Molecular evidence that insecticide resistance in peachpotato aphids (Myzus persicae Sulz.) results from amplification of an esterase gene. The Biochemical Journal. 1988;**251**:309-312

[61] Bizzaro D, Mazzoni E, Barbolini E, Giannini S, Cassanelli S, Pavesi F, et al. Relationship among expression, amplification and methylation of FE4 esterase genes in Italian populations of Myzus persicae (Sulzer) (Homoptera: Aphididae). Pesticide Biochemistry and Physiology. 2005;**81**:51-58

[62] Rivi M, Monti V, Mazzoni E, Cassanelli S, Panini M, Anaclerio M, et al. A1-3 chromosomal translocations in Italian populations of the peach potato aphid Myzus persicae (Sulzer) not linked to esterase-based insecticide resistance. Bulletin of Entomological Research. 2013;**103**:278-285

[63] Bass C, Puinean M, Zimmer TC, Denholm I, Field LM, Foster SP, et al. The evolution of insecticide resistance in the peach potato aphid, *Myzus persicae*. Insect Biochemistry and Molecular Biology. 2014;**51**:41-51

[64] Severini C, Marinucci M, Raymond M. Insecticide resistance genes in Culex pipiens (Diptera: Culicidae) from Italy: Esterase B locus at the DNA level. Journal of Medical Entomology. 1994;**3**:496-499

[65] Hemingway J, Hawkes NJ, McCarroll L, Ranson H. The molecular basis of insecticide resistance in mosquitoes. Insect Biochemistry and Molecular Biology. 2004;**34**:653-665

[66] Small GJ, Hemingway J. Molecular characterization of the amplified

carboxylesterase gene associated with organophosphorus insecticide resistance the brown planthopper, *Nilaparvata lugens*. Insect Molecular Biology. 2000;**9**:647-653

[67] Alon M, Alon F, Nauen R, Morin S. 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 carboxylesterases. Insect Biochemistry and Molecular Biology. 2008;**38**:940-949

[68] Cao CW, Zhang J, Cao XW, Liang P, Cuo HL. Overexpression of carboxylesterase gene associated with organophosphorous insecticide resistance in cotton aphids, Aphis gossypii (glover). Pesticide Biochemistry and Physiology. 2008;**90**:175-180

[69] Oppenoorth FJ, van Asperen K. Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. Science. 1960;**132**:298-299

[70] Carvalho RA, Torres T, Azeredo-Espin AML. A survey of mutations in the Cochliomyia hominivorax (Diptera: Calliphoridae) esterase E3 gene associated with organophosphate resistance and the molecular identification of mutant alleles. Veterinary Parasitology. 2006;**140**:344-351

[71] Field LM. Methylation and expression of amplified esterase genes in the aphid Myzus persicae (Sulzer). The Biochemical Journal. 2000;**349**:863-868

[72] Rivi M, Monti V, Mazzoni E, Cassanelli S, Panini M, Bizzaro D, et al. Karyotype variations in Italian populations of the peach-potato aphid Myzus persicae (Hemiptera: Aphididae). Bulletin of Entomological Research. 2012;**102**:663-671

[73] Feyereisen R. Insect cytochrome P450. In: Gilbert LI, Iatrou K, Gill SS, editors. Comprehensive Molecular Insect Science. Vol. 4. Oxford: Elsevier BV; 2005. pp. 1-77

[74] Feyereisen R. Insect P450 inhibitors and insecticides: Challenges and opportunities. Pest Management Science. 2015;**71**:793-800

[75] Guengerich FP. Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. Chemical Research in Toxicology. 2008;**14**:611

[76] Després L, David JP, Gallet C. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology & Evolution. 2007;**22**:298-307

[77] Philippou D, Field L, Moores G. Metabolic enzyme(s) confer imidacloprid resistance in a clone of Myzus persicae (Sulzer) (Hemiptera: Aphididae) from Greece. Pest Management Science. 2010;**66**:390-395

[78] Puinean AM, Foster SP, Oliphant L, Denholm I, Field LM, Millar NS, et al. Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid Myzus persicae. PLoS Genetics. 2010;**6**:e1000999

[79] Alptekin S, Bass C, Nicholls C, Paine MJ, Clark SJ, Field L, et al. Induced thiacloprid insensitivity in honeybees (Apis mellifera L.) is associated with up-regulation of detoxification genes. Insect Molecular Biology. 2016;**25**:171-180

[80] Markussen MDK, Kristensen M. Cytochrome P450 monooxygenase-mediated neonicotinoid resistance in the house fly Musca domestica L. Pesticide Biochemistry and Physiology. 2010;**98**:50-58

[81] Amichot M, Tarés S, Brun-Barale A, Arthaud L, Bride JM, Berge JB. Point mutations associated with insecticide resistance in the drosophila cytochrome P450 CYP6A2 enable DDT metabolism. European Journal of Biochemistry. 2004;**271**:1250-1257

[82] Wondji SC, Irving H, Morgan J, Lobo NF, Collins FH, Hunt RH, et al. Two duplicated P450 genes are associated with pyrethroid resistance in Anopheles funestus, a major malaria vector. Genome Research. 2009;**19**:452-459

[83] Kostaropoulos I, Papadopoulos AI, Metaxakis A, Boukouvala E, Papadopoulou-Mourkidou E. Glutathione S-transferase in the defence against pyrethroids in insects. Insect Biochemistry and Molecular Biology. 2001;**31**:313-319

[84] Feyereisen R. Molecular biology of insecticide resistance. Toxicology Letters. 1995;**82**:83-90

[85] Ketterman AJ, Saisawang C, Wongsantichon J. Insect glutathione transferases. Drug Metabolism Reviews. 2011;**43**:253-265

[86] Konanz S, Nauen R. Purification and partial characterization of a glutathione S-transferase from the two-spotted spider mite, Tetranychus 334 urticae. Pesticide Biochemistry and Physiology. 2004;**79**:49-57

[87] Habig WH, Pabst MJ, Jakoby WB. Glutathione Stransferases. The first enzymatic step in mercapturic acid formation. The Journal of Biological Chemistry. 1974;**249**:7130-7139

[88] Enayati AA, Ranson H, Hemingway J. Insect glutathione transferases and insecticide resistance. Insect Molecular Biology. 2005;**14**:3-8

[89] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annual Review of Pharmacology and Toxicology. 2005;**45**:51-88

[90] Ranson H, Hemingway J. Glutathione transferases. In: Gilbert LI, Iatrou K, Gill SS, editors. 335 Comprehensive Molecular Insect Science. Oxford: Elsevier; 2005. pp. 383-402

[91] Vontas JG, Small GJ, Nikou DC, Ranson H, Hemingway J. Purification, molecular cloning and heterologous expression of a glutathione S-transferase involved in insecticide resistance from the rice brown planthopper, Nilaparvata lugens. The Biochemical Journal. 2002;**362**:329-337

[92] Hollenstein K, Dawson RJP, Locher KP. Structure and mechanism of ABC transporter proteins. Current Opinion in Structural Biology. 2007;**17**:412-418

[93] Lage H. ABC transporters: Implications on drug resistance from microorganisms to human cancers. International Journal of Antimicrobial Agents. 2003;**22**:188-199

[94] Dermauw W, Van Leeuwen T. The ABC gene family in arthropods: Comparative genomics and role in insecticide transport and resistance. Insect Biochemistry and Molecular Biology. 2014;**45**:89-110

[95] Bariami V, Jones CM, Poupardin R, Vontas J, Ranson H. Gene amplification, ABC transporters and cytochrome P450s: Unravelling the molecular basis of pyrethroid resistance in the dengue vector, *Aedes aegypti*. PLOS Neglected Tropical Diseases. 2012;**6**:e1692

[96] Aurade RM, Jayalakshmi SK, Sreeramulu K. Pglycoprotein ATPase from the resistant pest, Helicoverpa

armigera: Purification, characterization and effect of various insecticides on its transport function. Biochimica et Biophysica Acta. 2010;**1798**:1135-1143

[97] Porretta D, Gargani M, Bellini R, Medici A, Punelli F. Defence mechanisms against insecticides temephos and diflurobenzuron in the mosquitos Aedes caspius: The P-glycoprotein efflux pumps. Medical and Veterinary Entomology. 2008;**22**:48-54

[98] O'Donnell MJ. Insect excretory mechanisms. Advances in Insect Physiology. 2008;**35**:1-122

[99] Buss DS, Callaghan A. Interaction of pesticides with p-glycoprotein and other ABC proteins: A survey of the possible importance to insecticide, herbicide and fungicide resistance. Pesticide Biochemistry and Physiology. 2008;**90**:141-153

[100] Heckel DG. Learning the ABCs of Bt: ABC transporters and insect resistance to bacillus thuringiensis provide clues to a crucial step in toxin mode of action. Pesticide Biochemistry and Physiology. 2012;**104**:103-110

[101] Gahan LJ, Pauchet Y, Vogel H, Heckel DG. An ABC transporter mutation is correlated with insect resistance to bacillus thuringiensis Cry1Ac toxin. PLoS Genetics. 2010;**6**:e1001248

[102] Babtie AC, Kirk P, Stumpf MP. Topological sensitivity analysis for systems biology. In: Proceedings of the National Academy of Sciences. 2014;**111**(52):18507-18512

[103] Karr JR, Sanghvi JC, Macklin DN, Gutschow MV, Jacobs JM, Bolival B Jr, et al. A whole-cell computational model predicts phenotype from genotype by system biology. Cell. 2012;**150**(2):389-401

[104] Duneau D, Sun H, Revah J, San Miguel K, Kunerth HD, Caldas IV, et al. Signatures of insecticide selection in the genome of *Drosophila melanogaster*. G3: Genes, Genomes, Genetics. 2018;**8**(11):3469-3480

[105] Panini M, Manicardi GC, Moores GD, Mazzoni E. An overview of the main pathways of metabolic resistance in insects. Invertebrate Survival Journal. 2016;**13**(1):326-335

[106] Dang K, Doggett SL, Veera Singham G, Lee CY. Insecticide resistance and resistance mechanisms in *Myzus persicae, Musca domestica, and Drosophila melanogaster*. Parasites & Vectors. 2017;**10**(1):1-31

[107] Zhu F, Li T, Zhang L, Liu N. Co-up-regulation of three P450 genes in response to permethrin exposure in permethrin resistant *house flies, Musca domestica*. BMC Physiology. 2008;**8**(1):1-13

[108] Zhang G, Zhang W. Protein–protein interaction network analysis of insecticide resistance molecular mechanism in *Drosophila melanogaster*. Archives of Insect Biochemistry and Physiology. 2019;**100(1)**:e21523