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Editorial overview: Insecticide resistance mechanisms — from behavior and physiology to microbiome science

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Insecticide resistance poses a significant global challenge to managing insect pests, necessitating a deeper understanding of resistance mechanisms to develop effective management strategies and innovative chemical treatments. The majority of data on resistance mechanisms are concentrated on three main detoxification enzymes — cytochrome P450 monooxygenases, esterases, and glutathione-S-transferases — as well as three major target site mutation classes in the nervous system, including *kdr*-type mutations to the sodium channel, *Rdl* mutations to the GABA-gated chloride channel, and altered acetylcholinesterase [1,2]. These mechanisms vary in their activity depending on the specific insecticides, suggesting that not all mechanisms act in concert against any single insecticide.

In this Pest & Resistance issue of *Current Opinion in Insect Science*, an alternative approach was adopted to explore insecticide resistance from various angles, such as behavioral adaptations, the physiological bases of these behaviors, microbiome science, population genetics, mechanisms of transport and sequestration, the impacts of climate change, and the less-understood topic of insecticide resistance in social insects. Contributions to this issue came from a diverse group of early, mid-career, and senior experts from North America, Asia, and Europe, who specialize in insecticide resistance issues across agricultural, public health, veterinary, and urban settings. Additionally, operational perspectives from the Insecticide Resistance Action Committee (IRAC) were incorporated, shedding light on novel resistance mechanisms and offering fresh insights into this complex field.

Despite its significance, behavioral resistance has been less studied compared with physiological/biochemical resistance. Although it has been observed in various insects since the 1940s, it has not been extensively studied due to the complexities of quantifying behavior and the lack of standardized definitions and methodologies. Hubbard and Murillo [3] highlight the critical role of behavioral adaptations in the efficacy of insecticides and the management of resistance. Behavioral resistance is defined as evolved actions that allow insect populations to avoid the lethal effects of insecticides, enhancing survival. It can be innate or develop through exposure, involving changes in behavior such as avoiding treated areas or altering feeding habits. The review categorizes behavioral resistance into stimulus-independent and stimulus-dependent types. The former involves innate behaviors that prevent contact with insecticides,

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while the latter relates to learned behaviors that evolve over time due to direct insecticide exposure. The authors discuss the importance of developing precise bioassays to measure behavioral resistance accurately. They emphasize the need for assays that reflect real-world conditions and allow for the observation of subtle behavioral modifications. Future studies should integrate behavioral components with genetic and molecular approaches to provide a comprehensive mechanistic understanding.

One of the most investigated behavioral resistance mechanisms is glucose aversion in the German cockroach, *Blattella germanica*. This species has become an important model for understanding pest management and adaptive evolution in response to human-driven selection pressures. The review by Wada-Katsumata & Schal [4] highlights the German cockroach's evolution of various resistance mechanisms to toxic baits, particularly focusing on glucose aversion. Glucose aversion emerges from a taste polymorphism that changes glucose's taste from sweet to bitter, leading cockroaches to avoid baits containing it. When insecticide baits containing glucose or related oligosaccharides are not used, this trait is likely preserved at low frequencies in populations. However, because of human-driven selection pressure with these baits, the trait becomes highly adaptive. Recent studies have expanded knowledge by exploring how glucose aversion contributes to learned avoidance of baits, aversion to other sugars, and sexual selection, emphasizing its broad behavioral links to foraging and mating. This adaptation showcases an example of anthropogenic influence altering a species' gustatory traits and, subsequently, its ecological and reproductive behaviors.

Pu and Chung [5] reviewed the new and emerging mechanisms of insecticide resistance, such as cuticular changes mediated by CYP4 family cytochrome P450s, insecticide sequestration by olfactory proteins, and post-transcriptional regulation. Olfactory protein sequestration thus far has been found to be mediated by chemosensory proteins, sensory appendage proteins, and odorant-binding proteins. Because of the hydrophobic nature of odorant molecules, these systems have likely been co-opted to sequester hydrophobic insecticides, in particular, pyrethroids. On the topic of post-transcriptional regulation, the established understanding has been that Cis and Trans-acting genetic elements can control the transcription of resistance genes that encode detoxification enzymes. However, new themes are emerging on resistance-associated translational factors. These include microRNAs and long noncoding RNAs that can affect the expression of more traditionally understood resistance factors related to detoxification and cuticular penetration. Cis element mutations that interact with methylation machinery have also recently emerged in association with resistance.

Uemura et al. [6] reviewed the recent advances in the study of knockdown resistance (*kdr*) mutations in *Aedes* mosquitoes, focusing on several key mutations. *kdr* mutations specifically impact insecticides that target neuronal sodium channels like Dichlorodiphenyltrichloroethane, pyrethrins, and pyrethroids. Several mutations that are summarized occur in a number of positions along the gene and translate to the mature protein. Different complements of mutations have arisen in different areas of the world, and some likely have been exported/imported to other areas. These mutations occur singly or in combination, and the presence of multiple mutations generally increases resistance levels. However, importantly, some mutations have no impact on resistance but may play a 'balancing' role to offset fitness

costs, that is, help to assure normal channel gating kinetics in the absence of insecticide or assure normal function in the face of variable ambient temperatures. Understanding the complements of *Aedes kdr* mutations will be essential for comprehending their ecological implications, fitness tradeoffs, management implications (for rotations), and potential for enhancing selective toxicity.

The article by Amezian et al. [7] provides new insights into the emerging role of ABC (ATP-dependent binding cassette) transporters in pesticide toxicity and resistance. ABC transporters function by moving toxicants and other biochemicals across membranes and other biological barriers. Transporter expression localizes to tissues relevant to xenobiotic action, for example, midgut, Malpighian tubules, brain–blood barrier, and even legs. ABC transporters are relevant to the mode of action and resistance of Bt (*Bacillus thuringiensis* protein toxin), as well as resistance to chemical insecticides. Transporter downregulation is more relevant to Bt, with upregulation being more relevant for chemical resistance. Transacting transcription factors appear to be involved in upregulation, whereas frameshift mutations appear responsible for truncated proteins and downregulation in cases of Bt resistance. Regarding xenobiotic transport and resistance, the resistance-associated transporters also function in lipid/hydrocarbon transport. ABC transporter upregulation has further been correlated with penetration resistance via cuticle thickening, with apparent cooperation with CYP4 P450s involved in hydrocarbon synthesis. The authors recommend a battery of approaches for developing a better understanding of ABC transporters in arthropod toxicity and resistance, including synergism with inhibitors, reverse genetics, substrate specificity, subcellular localization, quantification of transport, docking studies, and more.

Besides behavioral and physiological mechanisms, the microbiome has also been incriminated to play roles in pesticide toxicology and resistance. The article by Peterson [8] overviews the emerging field of arthropod microbiome toxicology, which is now connected with both pesticide detoxification and the activation of propesticides. Symbiont-mediated pesticide degradation is widespread across insects. As shown through recent studies, bacteria use pesticide molecules as a carbon, elemental, and/or nutritional source, which can result in neutralization (detoxification) or activation (enhanced toxicity). Moving forward, the field of pest management will likely need to consider the roles of insect microbiomes in both pest control failures and successes, as well as the roles of insect microbiomes in a broader ecological IPM context. As with other examples of host–symbiont interaction, gaining a full understanding of bacteria-mediated insecticide metabolism will require a 'holobiont' approach that considers host and bacteria together as a single phenotype.

Booth [9] highlights the critical role of genetic studies in understanding the dynamics of pest invasions and the evolution of resistance. This contribution explores the global distribution, insecticide resistance, and genetic dynamics of indoor urban pests such as bed bugs and German cockroaches, noting their widespread presence facilitated by human movement and their cryptic nature, which aids inadvertent transport. Also highlighted is the evolution of multiple insecticide-resistance mechanisms, emphasizing the importance of understanding these mechanisms for effective management using their underlying genetic mutations, like *kdr*, as markers. The use of population genetics with high-throughput genomic technologies (to provide precise tracing capabilities) is discussed as a means to gain detailed insights into pest population structure, patterns of invasion, spread, and resistance development. Despite the typical association of successful invasions with high propagule pressure and genetic diversity, many urban pest populations can be founded by a small number of individuals that manage to establish and thrive due to their reproductive strategies and adaptability. Booth [9] further underscores the importance of understanding the genetic basis of pest behavior and resistance to develop targeted and sustainable management strategies, highlighting the potential of genetic tools in predicting and mitigating resistance spread.

Lastly, Scharf and Lee [10] examine insecticide resistance within eusocial insects such as bees, ants, and termites, noting their unique social structures that contribute to varied susceptibility to insecticides. The review discusses how the cooperative behaviors, overlapping generations, and specialized roles of castes within these colonies might limit or obscure resistance development, contrasting with more commonly studied nonsocial insects such as German cockroaches, bed bugs, flies, and mosquitoes. There has been minimal documented resistance in eusocial species, likely because of the challenges in studying resistance due to the complexity of rearing full colonies. However, there have been observed adaptations in bees exposed to agricultural pesticides. The review also proposes future research directions emphasizing multidisciplinary approaches to better understand and document resistance dynamics in socially complex insects. It also highlights the urgent need for improved experimental models and comprehensive studies to uncover the resistance capabilities of eusocial insects, extending beyond the well-documented cases in bees.

We invite readers to explore this special issue for a more comprehensive understanding of the current breadth of knowledge available on pest resistance, as well as for synthesizing paths forward to reach more sustainable management outcomes.

Declaration of Competing Interest

The authors declare that the content of this paper was not affected by any financial, commercial, legal, or professional interest.

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