An Overview of Meta-Analysis for the Importance of the Overexpression of Detoxification Genes in Insecticide Resistance

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Abstract

Insect-borne diseases, such as malaria, and plant pathogens, like the tobacco mosaic virus, are responsible for human deaths and poor crop yields in communities around the world. The use of insecticides has been one of the major tools in the insect pest control. However, the development of insecticide resistance has been a major problem in the control of insect pest populations that threaten the health of both humans and plants. The overexpression of detoxification genes is thought to be one of the major mechanisms through which pests develop resistance to insecticides. Hundreds of research papers have explored how overexpressed detoxification genes increase the resistance status of insects to an insecticide in recent years; this study is, for the first, a synthesis of these research data aimed at (1) examining the overexpression of metabolic detoxification genes and their involvement in increased levels of resistance to different insecticides in diverse insect species, including agriculturally and medically important insect species. While (2) seeking to determine if the overexpression detoxification genes are most responsible for insecticide resistance in insect pests in general. The strongly correlated with increased levels of insecticide resistance were observed in tested insects with the cytochrome P450 (CYP), glutathione-S-transferase (GST), and esterase gene superfamilies confirming that the overexpression of detoxification genes is indeed involved in insecticide metabolism of insects, including medical (e.g., mosquito and housefly) and agricultural (e.g., planthopper and caterpillar) insect pests.

1. Introduction

There are thought to be approximately five million insect species worldwide with ~ 80% remaining to be discovered (Stork 2018). Among these, agricultural (e.g., whitefly, armyworm, and grasshopper) and medical (e.g., mosquito, housefly, and cockroach) pests can have a significant negative impact on food supplies and human health. Globally, insect pests are responsible for around 35% of potential crop yield losses (Popp et al. 2013; Mesterházy et al. 2020). Besides damage and monetary loss, the transmission of plant viruses by insect vectors can also reduce crop yield and quality (Ferreles and Raccah 2015). Likewise, medically important pests can transfer lethal pathogens capable of compromising human life (e.g., malaria, yellow fever, and dengue, all of which are transferred by mosquitoes) and impose huge socio-economic burdens on the affected populations (Bhatt et al. 2014; Kuehn 2018; Shaw and Catteruccia 2019). For instance, the housefly is capable of transmitting more than 100 human and animal diseases, including cholera, typhoid fever, salmonellosis, and polio (Ostrolenk and Welch 1942; Feng et al. 2018). Common pest management strategies include installing traps to monitor and control the density of pests (Baldacchino et al. 2015), rearing natural enemies to prey on the pests (Lazaro et al. 2015), or spraying insecticide. All these methods are routinely employed to control pest populations in an attempt to avoid production and monetary losses, insect-borne disease outbreaks, and other associated impacts.

Of these various pest management strategies, the use of insecticides is still the most widely-used approach (i.e., roughly two million tons of pesticides being sprayed on insect pests in communities around the world every year (Sharma et al. 2019)) because of its immediate and rapid reduction of pest

populations (Strode et al. 2012). However, this widespread overuse of insecticides has led to the emergence of new problems. The first is that insecticide resistance is rapidly becoming a serious obstacle to the effective pesticide control of many pests. Moreover, and there are only a very limited number of new insecticides being commercialized for vector control (Liu 2015). Because of the frequent and long-term use of insecticides, pests that carry a resistance gene can survive when exposed to an insecticide and inherit this gene on to their next generations, thus leading to the widespread development of insecticide resistance in local populations (Liu 2015, Smith et al. 2016). Crucially, the greater the resistance ability of the pest to a particular insecticide, and hence the survivability of the pest when exposed, the greater the associated crop loss or the transmission of insect-borne diseases. The phenomenon of insecticide resistance was first recognized by scientists in the 1950's when *Aedes* and *Culex* populations began to develop a resistance to DDT larvicide (Giullin and Peters 1952). The resistance of both medical and agricultural pests to a variety of insecticides has now been reported in pest populations worldwide (Naveen et al. 2017; Camara et al. 2018; Okia et al. 2018; Lopes et al. 2019).

Numerous papers have been published that address the underlying mechanisms that govern the development of insecticide resistance. Some have shown evidence to suggest that gene mutations can decrease the sensitivity of the target site and thus be responsible for increasing insecticide resistance (Dong 2007; Xu et al. 2011; Tmimi et al. 2018; Zeidabadinezhad et al. 2019; Yavaşoglu et al. 2021), while others have argued that the overexpression of the detoxification gene is the most important mechanism driving the development of insecticide resistance (Xu et al. 2016; Feng et al. 2018; Jin et al. 2019). It is generally agreed, however, that target site insensitivity and increased insecticide metabolism are both involved. Consequently, these two mechanisms have been intensively studied (Antonio-Nkondjio et al. 2011; Liu 2015; Dang et al. 2017). Hundreds of papers have explored how overexpressed genes increase the resistance status of insects to insecticides in recent years. This study, for the first time, quantitatively synthesizes these data using meta-analysis to provide an overview of the impact of the overexpression of genes in diverse insect species against different insecticides as well as identify the gene families and clades responsible for the most widely observed examples of this overexpression.

2. Materials And Methods

2.1 Literature search strategy

To fully understand the metabolic detoxification mechanisms conferred by the gene overexpression that are importantly involved in the insecticide resistance development in general, we quantitatively compared the gene expression between resistant and susceptible strains and its correlation by conducting a literature search in Web of Science, PubMed, and AGRICOLA (Ovid) using the following search terms: "gene overexpression" AND "insecticide resistance" OR "pesticide resistance". In addition, "*Culex*" OR "*Aedes*" OR "*Anopheles*" OR "*Aphid*" OR "*Helicoverpa*" OR "**idea*" were used in conjunction with "gene overexpression" AND "insecticide resistance" in Web of Science. The Boolean truncation (*) was used to capture all variations of a search term. A schematic outline of the search strategy is illustrated in Fig. 1. A publication date range of 2000 to 2020 was used and although no restrictions were placed on the country

of publication, only reports in English were included; a study was included only if it was an original journal article (books, review articles, and letters to the editor were excluded). In addition, some of the studies included data for both gene expression and insecticide resistance (Alon et al. 2008; Bass et al. 2011; Arouri et al. 2015) while others only provided gene expression data (Zhu and Liu 2008; Komagata et al. 2010; Cifuentes et al. 2012; Irving et al. 2012; Kaplanoglu et al. 2017). The literature search was performed between January and March 2020 (Fig. 1).

2.2 Study selection, inclusion, and exclusion criteria

To be considered eligible for inclusion, a study had to meet the following criteria:

1. Gene expression must have been measured through relative mRNA abundance by applying qPCR or real-time PCR. Protein-based or enzyme activity-based measurements were excluded because real-time PCR is a more recent and accurate technology for measuring gene expression. Moreover, different gene expression testing methods may generate unexpected variation.
2. A study must have contained a susceptible strain (or susceptible field strain) as the control and a resistant strain (or resistance field strain) as the measurement of comparison. Thus, studies measuring the gene expression level of insects collected from different locations that did not consider significant differences in the resistance levels of insects from different populations were excluded.
3. A study must have reported either the mean value of the gene expression level and some estimate of error (e.g., standard deviation, standard error, or 95% confidence interval) around the mean estimates for the susceptible and resistant strains or the ratio of these gene expression levels.
4. A study must have reported no gene modification of the strains. Thus, studies testing gene overexpression after editing the genomes of the strains using RNAi, CRISPR-Cas9, or other gene-editing methods were excluded.
5. A study must have reported no transformation of the gene to other materials; studies testing gene overexpression after the gene had been transferred to a *sf9* cell, *Drosophila*, or some other material were excluded.

2.3 Data extraction and analyses

To gather data from each study, a standardized form, including author(s), publication year(s), journal(s), insecticide(s), and specie(s), was utilized that facilitated the extraction of the important study characteristics. To perform moderator analysis, the class of insecticides, insect genus, and gene name were collected from each study. The sample size, mean value, and variance components (e.g., standard deviation, standard error, or 95% confidence interval) of gene expression were also collected. Where necessary, standard errors or 95% confidence intervals were converted to standard deviation using sample sizes and t-statistics. Data from the figures were extracted using R with the *metaDigitise* package (1.0.0) (Pick et al. 2019). For figures that presented linear gene expression data on a $\log_{10}$ transformed scale, the data collected from each figure were estimated by raising each data point by 10. Because of

scaling constraints, the error presented in the figures was estimated after recreating the original figures with estimated data and then adjusting the error bars until they were similar to those shown in the original figure. Only 19 observations (4.6% of all data) were collected using this method.

2.4 Effect size calculation and data analyses

For this study, the effect size reflected the strength of the relationship between gene expression and insecticide resistance (Kelley and Preacher 2012; Schäfer and Schwarz 2019). This effect size was calculated for each experiment in a study by comparing the relative gene expression values between the control (susceptible) and the treatment (resistant) strains. The effect size metric used in this study was the natural log response ratio (LnRR; Hedges et al. 1999), which is calculated as the natural log-transformed ratio of mean expression values between the treatment (resistant) and control (susceptible) (Havird et al. 2013). However, to more accurately estimate the magnitude of gene expression involved in insecticide resistance, weighted effect sizes that include the variance of LnRR is needed.

For the studies that only provided the relative expression ratio (i.e., the gene expression ratio between resistant strain and susceptible strain) but did not provide the exact gene expression data for both strains (Cifuentes et al. 2012; Bariami et al. 2012; Feng et al. 2018), LnRR was calculated by directly taking the natural log of the relative expression ratio, and the variance was assigned a value of 0.1. For the studies that provided a mean and error for gene expression for both susceptible and resistance strains (Zhang et al. 2012; Jin et al. 2019; Ma et al. 2019), the LnRR effect size and variance was calculated through R using the “metafor” package (Viechtbauer 2010). An effect size (and lower 95% CI) > 0 indicates that the detoxification gene is overexpressed in the resistant strain compared to the susceptible strain and thus provides evidence that gene overexpression is indeed one of the mechanisms involved in the development of insecticide resistance. In addition, the overexpression of the detoxification gene may not be involved in the mechanism of insecticide resistance. To facilitate the interpretation of the natural log-transformed effect sizes, each was converted back to a linear ratio scale. For example, an LnRR effect size of 0.41 would represent a 1.5 fold overexpression of a detoxification gene in a resistant strain (calculated as $\exp^{0.41} = 1.5$, $1.5/1 = 1.5$) compared to the susceptible strain.

Since our dataset included some studies where error estimates were not provided with mean gene expression, two separate analyses were conducted to evaluate the robustness of our findings. The first analysis was an unweighted analysis that included mean gene expression for all of the studies. The second analysis was a weighted analysis, which only included studies that provided the mean gene expression data and the associated error for both resistant and susceptible strains. Both of the two analyses were conducted using a random effects model for the gene expression of the susceptible and resistant strain in R using both the “metafor” (Viechtbauer 2010) and “meta” (Balduzzi et al. 2019) packages. The statistical heterogeneity, i.e., the magnitude of the changes of the effect sizes in this study, of the treatment effect among studies was assessed using the inconsistency (I^2) test, in which values above 30% and 50% were considered indicative of moderate and high heterogeneity, respectively. To

improve the quality of our results, we conducted sensitivity analyses by omitting each study in turn and re-calculating the effect size to check the resulting difference in the effect size.

2.5 Moderator analysis

We classified genes into different gene families and clades and insecticides into the different classes according to the modes of action recommended by the Insecticide Resistance Action Committee (IRAC) (Sparks and Nauen 2015). Insect species were grouped according to their genus that is classified as a medical vector or agricultural pest. Medical vectors represent genera that threaten human health; for example, insects in the genus *Aedes* transmit dengue virus to humans. Agricultural pests include genera that threaten plant health such as *Helicoverpa*, which can damage cotton and tomato crops. This made it possible to compare the effect on insecticide resistance of overexpressed genes in different gene families and different P450 gene clades, as well as different insecticide classes, different genera, and different vector types. Finally, moderator analyses were conducted to compare the effect on insecticide resistance of overexpressed genes in different gene families within specific medical or agricultural pests. All moderator analyses were conducted utilizing unweighted meta-analyses for all available effect sizes.

2.6 Publication bias and sensitivity analysis

Publication bias, i.e., the bias of the authors, editors, and/or reviewers to the publication of data, was evaluated by visually analyzing funnel plots (*metaviz* R package), Egger's tests (*dmetar* R package), and regression tests and p-curves (*metafor* R package) (Song et al. 2010). The sensitivity analysis, i.e., a method used to test whether the results will be changed by a certain paper, was performed using the "leave1out" analysis in the *metafor* R package (Saltelli et al. 2008). GOSH (Graphic Display of Study Heterogeneity) plot was performed in the *metafor* R package (Olkin et al. 2012).

3. Results

3.1 Overall dataset

All data used in this study were provided in the online supplemental material for transparency and future analyses (Table S1). Based on our search strategy and inclusion criteria, 51 published papers were selected and analyzed in this meta-analysis contained a total of 12 types of insecticides, the majority of them were three major insecticides, including neonicotinoids (7 papers), organophosphates (4 papers), and pyrethroids (27 papers). Three major gene families, P450 (44 papers), Esterase (10 papers), and GST (9 papers) with over 200 genes and CYP4 (28 papers), CYP6 (35 papers), CYP9 (14 papers), and over 40 gene clades were included in the selected papers. The data distribution includes 19 insect genera that can transmit the disease to either humans (medical vectors) or plants (agricultural pests) (Table 1). The remaining papers ($n > 1950$) were excluded as they failed to meet one or more of the inclusion criteria. Note that all effect sizes below are presented in the form of back-transformed linear scale ratios to facilitate the interpretation of the results (Fig. 1).

Table 1
Number of studies in different categories

		# Papers			# Papers
Total		51	P450 Gene Clade	CYP6	35
Insecticide Classes	Avermectins	2		CYP4	28
	beta-Ketonitrile	1		CYP9	14
	Buprofezin	1		CYP417	4
	Carbamates	1		CYP418	4
	DDT	1		CYP305	4
	Diamides	1		CYP321	1
	Neonicotinoids	7		CYP337	4
	Organophosphate	4		CYP332	2
	Pyrethroid	27		CYP353	5
	Spinosyns	1		CYP306	4
	Sulfoxaflor	1		CYP314	4
	Mixture of insecticides	4		CYP439	4
				CYP340	1
				CYP3085	1
Vector Type	Agricultural Vector*	30		CYP3092	1
	Medical Vector**	21			
Gene Family	P450	44		CYP15	1
	Esterase	10		CYP18	1
	GST	9		CYP301	3
P450 Gene Clade	CYP4	28		CYP302	2
	CYP6	35		CYP303	4
	CYP9	14		CYP304	3
	Others	19		CYP307	1

*: There are 18 paper, 6 paper and 4 paper investigate about the gene expression of P450, GST and Esterase in medical vector respectively.

** : There are 26 paper, 3 paper, and 6 paper investigate about the gene expression of P450, GST and Esterase in agricultural vector respectively.

		# Papers		# Papers
Insect Genera	<i>Aedes</i>	3	CYP315	3
	<i>Amsacta</i>	1	CYP380	3
	<i>Anopheles</i>	6	CYP404	2
	<i>Aphis</i>	3	CYP408	2
	<i>Apolygus</i>	1	CYP419	2
	<i>Bemisia</i>	2	CYP425	3
	<i>Blattella</i>	1	CYP426	1
	<i>Ceratitis</i>	1	CYP427	2
	<i>Culex</i>	5	CYP2	1
	<i>Drosophila</i>	1	CYP3323	1
	<i>Frankliniella</i>	1	CYP49	2
	<i>Helicoverpa</i>	4	CYP3	1
	<i>Laodelphax</i>	5	CYP384	1
	<i>Leptinotarsa</i>	1	CYP391	1
	<i>Musca</i>	5	CYP392	1
	<i>Nilaparvata</i>	4	CYP5	1
	<i>Plutella</i>	4	CYP12	1
	<i>Tetranychus</i>	2	CYP319	1
	<i>Triatoma</i>	1	CYP325	1
*: There are 18 paper, 6 paper and 4 paper investigate about the gene expression of P450, GST and Esterase in medical vector respectively.				
**: There are 26 paper, 3 paper, and 6 paper investigate about the gene expression of P450, GST and Esterase in agricultural vector respectively.				

3.2 Effect size summary

The linear scaled effect size (i.e., the number used to reflect the strength of the relationship between the two variable statistically) of the unweighted and weighted analyses (including studies both with and without susceptible strain) were 2.15 (95% confidence interval: 1.89–2.46, P value < 0.01) and 1.96 (95% confidence interval: 1.69–2.27, P value < 0.01), respectively. Overall, the expression level of detoxification genes tested was about double that seen in the resistant strains compared the susceptible strains (effect

size ~ 2), suggesting that the overexpression of detoxifying genes is indeed associated with increased insecticide resistance (Table S2).

To further investigate overexpression of which gene family or clade is responsible for increased insecticide resistance, the overexpression of cytochrome P450, esterase, and GST (glutathione S-transferase) was analyzed. Given the small number of studies on the overexpression of the esterase gene, all esterase related genes, including carboxylesterase (COE) and acetylcholinesterase (AChE), were classified simply as the “esterase” gene. None of the effect sizes for these three gene families overlapped with the null group: the expression level of the esterase gene was 3.5 fold (P value < 0.01) higher in resistant strain compared to the susceptible strain; the expression level of the GST gene was 5 fold (P value < 0.01) higher in resistant strain compared to susceptible strain; and the expression level of the P450 gene was 2 fold (P value < 0.01) higher in resistant strain compared to susceptible strain. All three gene families were clearly involved in insecticide resistance through upregulated gene expression (Fig. 2). Compared to the other two gene families (esterase n = 10, and GST n = 9 papers), P450 had the highest number of studies (n = 44 papers). Interestingly, the effect size for P450 was significantly different from those of both esterase (P value < 0.05) and GST (P value < 0.01). This abundance of studies made it possible to further split the P450 gene group into different clades, in which the expression level of the CYP4, CYP6, and CYP9 genes in the resistant strains were further analyzed showing to be 1.46 fold (P value < 0.05), 2.54 fold (P value < 0.01), and 4.4 fold (P value < 0.01), higher compared to the susceptible strains, respectively. In contrast, the gene expression for the rest of the clade did not significantly contribute to insecticide resistance, with the sole exception being CYP332, which exhibited a significant difference in just two studies (P < 0.05) (Brun-Barale et al. 2010; Xu et al. 2016) (Fig. 3, Table S2). There was, however, a large proportion of heterogeneity in the overall summary effect size ($I^2 > 94\%$) (Figure S1 & S2), suggesting that a single summary effect size may not be as informative. To further explore this heterogeneity, these data were further partitioned into various groups using specific moderators.

3.3 Factors contributing to heterogeneity between studies

First, the papers and effect sizes were classified based on the type of insecticide used when measuring the resistance level of a specific insect strain. If a study involved more than two insecticides, it was classified as “mix-insecticide” (Elzaki et al. 2016; Goindin et al. 2017; Sun et al. 2018; Tchigossou et al. 2018). The detoxification genes in the strains that were resistant to buprofezin, carbamates, organophosphates, and pyrethroid were 1.63 fold (P value < 0.01), 4.11 fold (P value < 0.05), 2.76 fold (P value < 0.01) and 3.52 fold (P value < 0.01) higher compared to the susceptible strain, respectively. In contrast, the overexpression of detoxification genes was not significantly influenced by the insect’s resistance to other classes of insecticides, such as DDT, beta-ketonitrile derivatives, diamides, spinosyns, avermectins, sulfoxaflor, neonicotinoids (Fig. 4, Table S2).

Next, the studies and their effect sizes were classified based on their target species to investigate whether the positive relationship between gene expression and insecticide resistance exists across species. The analysis revealed that *Aedes* (5.56 fold, P value < 0.01), *Anopheles* (3.21 fold, P value < 0.01), *Apolygus*

(15.85 fold, P value < 0.01), *Ceratitis* (18.60 fold, P value < 0.01), *Culex* (1.96 fold, P value < 0.01), *Drosophila* (11.14 fold, P value < 0.01), *Helicoverpa* (6.77 fold P value < 0.01), *Laodelphax* (2.13 fold, P value < 0.01), *Leptinotarsa* (12.60 fold, P value < 0.01), *Musca* (5.15 fold, P value < 0.01), and *Plutella* (1.57 fold, P value < 0.05) all showed significant differences between gene expression in susceptible and resistant strains, while other genera, such as *Triatoma*, *Blattella*, *Frankliniella*, *Bemisia*, *Tetranychus*, *Aphis*, *Nilaparvata*, *Amsacta* did not (Fig. 5, Table S2).

Finally, the insect genera were grouped according to their role as medical or agricultural vectors. The overexpression of detoxification genes in resistant strains relative to susceptible strains was noticeably significant in the medical vectors group (3.40 fold, P value < 0.01) than in the agricultural pests group (1.82 fold, P value < 0.01); (P value of medical vector compared with agricultural vector < 0.01). To investigate which gene families are involved in insecticide resistance in both agricultural pests group and medical vectors group, each group were further classified into three groups based on their gene families. In the medical vector group, the expression of detoxification genes in all three gene families was higher in the resistant strains than in the susceptible strains by 7.31 fold in the GST genes (P value < 0.01), 5.35 fold in the esterase genes (P value < 0.01), and 2.86 fold in the P450 genes (all P value < 0.01), respectively (Fig. 6). In contrast, across the agricultural vectors, the expression levels of the GST genes and the P450 gene were 3.17 fold (P value < 0.05) and 1.78 fold (P value < 0.01) higher, respectively, in the resistant strains than in the susceptible strains with the esterase genes (P value > 0.05) being the sole exception (Fig. 7, Table S2).

3.4 Publication bias and sensitivity analysis

Publication bias (i.e. the bias about the direction or significance of the manuscripts from authors, editors and reviewers when they are submitting or accepting manuscripts) was assessed using multiple approaches, including funnel plot evaluations, regression and Egger's tests (Egger et al. 1997). The regression test of the funnel plot of the weighted meta-analysis showed a slight asymmetry ($P = 0.043$), while an Egger's test revealed a marginally significant symmetry ($P = 0.054$; Fig. 8), indicating there is no clear publication bias in this study. To further address the potential for publication bias, a P-curve analysis was used to check whether any studies showed false positives (i.e., findings that statistics suggest are meaningful when they are not (Simonsohn et al. 2014)). The resulting curve was right-skewed with > 95% of the studies showing $p < 0.01$, suggesting truly significant p -values dominated the dataset (Figure S3). A leave-one-out sensitivity analysis (i.e., the method used to examine whether the result will change significantly due to one certain paper) was also conducted for both weighted and unweighted meta-analyses to determine whether individual studies influenced the overall results. The sensitivity analysis showed that no single effect size significantly biased the overall effect size (Table S3 & S4). Consequently, a Graphic Display of Study Heterogeneity (GOSH) plot was created to visually depict the heterogeneity, showing it to be equally distributed across the dataset (Figure S4) (Olkin et al. 2012).

4. Discussion

We further confirmed that the overexpression of detoxified gene is indeed responsible to insecticide resistance through the rigorous analysis of data from 51 studies, including three major detoxification gene families (P450s, GSTs, and Esterases), various species (e.g. *Aedes*, *Anopheles*, *Plutella*, *Helicoverpa*, etc.), and different type of insecticides (e.g. permethrin, neonicotinoids, organophosphates, etc.). Plenty of studies has also proven the importance of the overexpression of detoxified gene in insecticide resistance (Feyereisen 1999; Enayati et al. 2005; Liu 2015) and confirmed their large responsibility for enhancing the metabolic detoxification of insecticides, such as affecting the thickness of the insect cuticle (Plapp 1976; Yahouédo et al. 2017; Balabanidou et al. 2018).

However, not all of the insecticides showed significant differences in gene expression of detoxification in resistant and susceptible strains. For example, traditional classes of insecticide (e.g., pyrethroid, organophosphate, carbamates, and etc.) exhibited significant differences, while some insecticides (e.g., diamides, avermectins, sulfoxaflor, and etc.) showed little difference between resistant and susceptible strains. As we have known, the long-term and overuse of an insecticide can lead to resistance due to diverse mechanisms (Liu 2015). Thus, the insecticides that were not shown to have large differences in detoxification gene expression in resistant and susceptible strains may be because they are not as widely used as traditional insecticides. So, even if resistance develops, resistance may be due to other mechanism than the high expression of detoxification genes. Most species were found to be able to upregulate the expression of detoxification genes against insecticides, as most insecticides are broad-spectrum and are used to control multiple insect species (Ahmad et al. 2002; Sparks et al. 2020).

Interestingly, although both medical and agriculture pests can upregulate their gene expression when facing pressure from insecticides, medical pests showed much higher expression levels compared to agricultural pests. This may be because medical pests live closer to humans, so face more insecticide selection pressure compared to agricultural pests. However, further investigations are required to validate this hypothesis. When comparing each type of pest in a specific gene family, the expression of esterase in agricultural pests tended not to be significantly different between susceptible and resistant strains, a marked contrast to what was observed in medical pests. However, there was still a slight difference between the resistant and susceptible strains. More studies are needed that investigate the relationship between insecticide resistance and the gene expression of esterase if we are to understand the underlying causes of these differences.

Declarations

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Availability of data and material: Data available within the article and its supplementary

Materials.

Code availability: R code for meta-analyses was available as Supplementary Data.

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Supplementary Tables

Supplementary Tables are not available with this version

Figures

Figure 1

Flow diagram based on PRISMA.

Figure 2

Meta-analytical, sub-group estimate of gene expression related to insecticide resistance, with 95% CI in the P450, GST, and esterase gene families. The overall effect represents the collective effect of all the studies in terms of their unweighted analyses.

Figure 3

Meta-analytical, sub-group estimate of gene expression related to insecticide resistance, with 95% CI in the P450 gene families classified by gene clade. The overall effect represents the collective effect of all P450 studies.

Figure 4

Meta-analytical, sub-group estimate of the gene expression related to insecticide resistance, with 95% CI across different insecticide classes. The overall effect represents the collective effect of all the relevant studies.

Figure 5

Meta-analytical, sub-group estimate of the gene expression related to insecticide resistance, with 95% CI in different insect genus and vector types. The overall effect represents the collective effect of all the relevant studies.

Figure 6

Meta-analytical, sub-group estimate of the gene expression related to insecticide resistance, with 95% CI in the medical vector with different gene families and the P450 gene clade. The overall effect represents the collective effect of all the studies investigating the medical vector.

Figure 7

Meta-analytical, sub-group estimate of the gene expression related to insecticide resistance, with 95% CI in the agricultural vector with different gene families and the P450 gene clade. The overall effect represents the collective effect of each insecticide class in all the relevant studies.

Figure 8

Funnel plot of the weighted meta-analysis of the studies, including both susceptible and resistant expression data. The dark blue area means represent the 95% confidence interval.

Supplementary Files

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