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Functionally characterized arthropod pest and pollinator cytochrome P450s associated with xenobiotic metabolism

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Abstract

The cytochrome P450 family (P450s) of arthropods includes diverse enzymes involved in endogenous essential physiological functions and in the oxidative metabolism of xenobiotics, insecticides and plant allelochemicals. P450s can also establish insecticide selectivity in bees and pollinators. Several arthropod P450s, distributed in different phylogenetic groups, have been associated with xenobiotic metabolism, and some of them have been functionally characterized, using different *in vitro* and *in vivo* systems. The purpose of this review is to summarize scientific publications on arthropod P450s from major insect and mite agricultural pests, pollinators and *Papilio sp*, which have been functionally characterized and shown to metabolize xenobiotics and/or their role (direct or indirect) in pesticide toxicity or resistance been functionally validated. The phylogenetic relationships among these P450s, the functional systems employed for their characterization and their xenobiotic catalytic properties are presented, in a systematic approach, including critical aspects and limitations. The potential of the primary P450-based metabolic pathway of target and non-target organisms for the development of highly selective insecticides and resistance-breaking formulations may help to improve the efficiency and sustainability of pest control.

Key words: cytochrome P450, xenobiotic detoxification, plant allelochemicals, insecticide selectivity, bees, agricultural pests.

1. Introduction

The P450 family of insects and mites includes diverse enzymes involved in endogenous physiological functions and the metabolism of xenobiotics (Nauen et al., 2022). The cytochrome P450 sequences (CYPome) are distributed into four major clans: CYP2, CYP3, CYP4 and mitochondrial. Insect and mite genomes and transcriptomes have facilitated researchers to "extract" their CYPome and study their function. The distribution of P450 genes is not homogenous and they are comprised of few families with many genes and many P450 families with few genes (Dermauw et al., 2020). P450 – based xenobiotic (insecticides and plant toxins) detoxification is typically mediated by hydroxylation, dealkylation and other oxidative reactions, rendering the xenobiotic molecule more excretable and less toxic (Feyereisen, 2012). Several arthropod P450 proteins and genes have been implicated in xenobiotic metabolism in arthropods, identified by a variety of techniques, including *in vitro* heterologous expression systems (Nauen et al., 2021) and *in vivo* (RNAi or transgenic techniques) functional characterization approaches. P450-xenobiotic metabolizers may also play an important role in the specific activation of pro-insecticides, to allow the development and use of low toxicity chemicals for pest control (Vlogiannitis et al., 2021). They also catalyze the detoxification of several active ingredients by pollinators and beneficial insects and determine insecticide selectivity (Jeschke, 2016; Manjon et al., 2018).

The goal of this review is to summarize the current literature on the insect and mite (pests and pollinators) cytochrome P450s, which have been functionally associated with pesticide toxicity, by *in vitro* or *in vivo* means. The phylogenetic relationships, the functional systems employed for the analysis of their role are systematically presented and limitations are discussed. The potential of cytochrome P450s to support the development of highly selective safe insecticides and resistance breaking compounds is discussed.

2. Methods

2.1 Identification / Search of validated P450s from agricultural pests, pollinators and Papilio sp.

Studies including P450s from agricultural pests and bees/pollinators, which have been functionally validated by either *in vitro* or *in vivo* techniques and shown to play a role in xenobiotic metabolism (natural or synthetic compounds) and/or pesticide toxicity/resistance have been included in this analysis. Veterinary pests such as *Musca domestica*, *Lucilia cuprina* and others, or insects regarded as occasional pests, such as Drosophilidae, were not included.

The database used for the literature search was Web of Science® and the latest search date was July 2021. The insect species that are involved in this review, the P450s, the types of methodology used to validate them (*in vitro*: *Escherichiacoli (E. coli)*, yeast, insect cell lines; *in vivo*: RNAi, genome modification: transgenic *Drosophila melanogaster*, CRISPR) were traced and the keywords used were ([“pest” or “insect” or “mite” “P450s” or “CYPs”] and [validated* functional*]and [in vitro* E. coli* yeast* insect cell lines* RNAi* transgenic* Drosophila* CRISPR*]). Then the research articles that included functionally validated P450s either *in vitro* and/or *in vivo*/ genome modification were further categorized based on the species and the order. The data concerning substrate types for the P450s (chemical or natural compounds and the subgroup or exemplifying active ingredient in which they belong), types of methodology for the validation of the role of P450s (*in vitro*: *E.coli*, yeast, insect cell lines; *in vivo*: RNAi, genome modification: transgenic *D. melanogaster*, CRISPR) and the percentage of studies referring to each type of methodology were extracted from the studies with validated P450s, and tables, Venn graphs and pie charts were generated. Another literature search was carried using the Web of Science® database in order to trace the amount of all scientific papers that referred to each P450 either associating it with resistance or validating its contribution in resistance. Hence, a set of additional keywords was used: ([“pest” or “insect” or “mite” “P450s” or “CYPs”] and [resistance*]). The results from this search were used for the generation of a heatmap depicting the number of studies in which each P450 has been studied in the literature.

2.2 Phylogenetic analysis

P450s were manually curated in selected Lepidoptera and Hymenoptera species, either publicly available or private (Table 1). The early-diverged CYP51A1 (Nelson, 1999) from *Homo sapiens* was used as an outgroup. Multiple sequence alignment was performed using the amino acid sequence of these P450s with MAFFT v7.271 (Katoh and Standley, 2013) with the default parameters and then trimming was done with Trimal v1.2rev59 (Capella-Gutierrez et al., 2009), with the parameter “-automated1”. A Maximum Likelihood phylogeny with 100 bootstrap replicates was inferred with RAxML v8.2.11 (Stamatakis, 2014), with parameters “-m PROTGAMMAAUTO”. Branches with <50% bootstrap support were collapsed with TreeGraph2 (Stöver and Müller, 2010) and the resulting Newick tree was loaded to EvolView v2 (He et al., 2016) for post-processing. The vector graphics editor Inkscape v0.92 was used for the final polishing. Two phylogenetic trees were reconstructed using the above approach; one for only the Lepidoptera P450s and another one for only the hymenopteran P450s.

110 The nomenclature for genes encoding P450s includes the root symbol CYP for the superfamily,
111 a number indicating the gene family, a capital letter indicating the subfamily and a numeral for
112 the individual gene name.

113 **3. Identity and phylogeny of arthropod P450s implicated in xenobiotic metabolism and**
114 **resistance**

115 *3.1 Identity*

116 Several P450s have been functionally implicated in xenobiotic metabolism and insecticide
117 resistance across different arthropod taxa, in agricultural pests, pollinators and mite species
118 (Table 2A-D). More specifically, 56 Lepidoptera P450s; 73 non-Lepidoptera P450s from
119 several orders (Homoptera, Diptera, Coleoptera and Orthoptera), 13 from mite species
120 (Trombidiformes); and 23 from pollinator and *Papilio* species (Hymenoptera and Lepidoptera)
121 have been functionally validated in the literature (Table 2A-D). These P450s (Table 2A-D)
122 have been reported in individual, few or multiple studies (Figure 1A-D).

123 The Lepidoptera *Helicoverpa armigera* is one of the most well studied species in terms of the
124 number of P450s (21 P450s) (Figure 1A). *H. armigera* CYP6B6 has been reported in more
125 than 25 publications (Figure 1A), and CYP6B7 in 20. Several publications (6 to 19) focused
126 on *H. armigera* CYP337B3, CYP9A12, CYP9A14 and CYP6AE14 and fewer (less than five)
127 involve the CYP6AE cluster, while CYP6B2 has been mentioned in 15 publications and
128 CYP337B1 in 8 publications (Figure 1A). Some *Spodoptera* species demonstrate an abundance
129 of studied P450s: *S. exigua* has ten P450s (CYP6AE10, CYP6AB14, CYP9A98, CYP9A10,
130 CYP9A21v3, CYP9A105, CYP321A16, CYP332A1, CYP321A8, CYP9A168) while *S. litura*
131 has six functionally verified P450s (CYP321B1, CYP6B50, CYP6AB14, CYP9A40,
132 CYP6AB60, CYP6AB12), all of them studied in less than five publications. *Helicoverpa zea*
133 and *Chilo suppressalis* have three and four identified P450s respectively: CYP6B8 from *H. zea*
134 has been studied in ten publications and CYP321A1 in nine scientific publications, while all
135 *C. suppressalis* P450s have been mentioned in less than five (Figure 1A). *Plutella xylostella*
136 and *S. frugiperda* have three functionally validated P450s each (CYP6BG1, CYP321E1,
137 CYP340W1 and CYP321A8, CYP321A9, CYP321B1, respectively) (Figure 1A). They are all
138 mentioned less than five times in the literature with one exception: *P. xylostella* CYP6BG1
139 appeared in the literature six times (Figure 1A). The rest of the species depicted in Figure 1A,
140 *Amyelois transitella*, *Cydia pomonella* and *Depressaria pastinacella* include either one or two
141 characterized P450s, appearing in less than five publications.

142 Among non-Lepidoptera pest species, 16 P450s belong to the Orthoptera species *Locusta*
143 *migratoria*, nine P450s of *Laodelphax striatellus* (Hemiptera), with only CYP353D1 having
144 been studied more than five times, eight P450s of *Leptinotarsa decemlineata* (Coleoptera) and
145 nine P450s of *Aphis gossypii* (Hemiptera) have been implicated in xenobiotic detoxification
146 (all of them mentioned in less than five publications each) (Figure 1B). Following up, five
147 P450s from *Diaphorina citri* (Hemiptera) and *Bradysia odoriphaga* (Diptera) have been
148 implicated to xenobiotic detoxification, while nine P450s from *Nilaparvata lugens* (Hemiptera)
149 have been studied for their role in detoxification. All the above have been identified in the
150 literature less than five times, with the exceptions of *N. lugens* CYP6ER1, CYP6AY1,
151 CYP6CW1 which are mentioned in 19, 16 and 6 publications, respectively. *Sogatella furcifera*
152 (Homoptera) and *Tribolium castaneum* (Coleoptera) have respectively three and four P450s
153 contributing to xenobiotic metabolism, while *Myzus persicae*, the Diptera *Ceratitis capitata*
154 and the Coleoptera species *Brassicogethes (Meligethes) aeneus* and *Sitophilus zeamais* have
155 one P450 each (studied less than five times. The *M. persicae* CYP6CY3 appeared in
156 approximately 13 publications (Figure 1B). Two P450s of the Hemiptera *Bemisia tabaci* have
157 a validated role in metabolism/resistance respectively: CYP6CM1 and CYP6CX4. The *B.*
158 *tabaci* CYP6CX4 is referred to less than five publications, while it is notable that CYP6CM1
159 from the same species is one of the most extensively studied P450s, with 40 publications
160 (Figure 1B), referring also to its different biotype origins (CYP6CM1vB, CYP6CM1vQ).

161 From the agriculturally important mite species, seven P450s of *Tetranychus cinnabarinus* and
162 six of *T. urticae* have been functionally validated for their contribution in xenobiotic
163 detoxification and resistance (identified in less than five publications each) (Figure 1C). The
164 impact of insecticides on the health of bee and bumblebee pollinators is a topic of intensive
165 research and considerable current debate, as they are exposed to a wide variety of natural and
166 synthetic xenobiotics (including pesticides) (Johnson, 2015). In the category of pollinators and
167 *Papilio* sp., *Apis mellifera* (Hymenoptera) presents the largest number of studied P450s for
168 their role in xenobiotic detoxification (n =8), which are studied less than five times each, except
169 for CYP9Q1 (mentioned in seven publications) (Figure 1D). The hymenopteran species *A.*
170 *cerana cerana*, *Bombus terrestris* and the Lepidoptera *Papilio glaucus* have each three P450s
171 studied for their detoxifying role, reported less than five times (except for *P. glaucus* CYP6B4
172 which is reported in nine publications), while the hymenopteran *Osmia bicornis* and the
173 Lepidoptera *Papilio polyxenes* have two xenobiotic metabolizing P450s each (*P. polyxenes*
174 CYP6B1 has been mentioned in the literature 30 times) (Figure 1D).

175 *3.2 Phylogeny*

176 An overlay of literature reports on a phylogeny of complete Lepidoptera CYPomes of *H.*
177 *armigera, S. littoralis* and *P. xylostella* (Figure 2A) shows that virtually all P450s that are
178 known to be involved in detoxification of xenobiotics belong to Clan 3 (Figure 2A, genes with
179 black circles), except for CYP340W1, a P450 belonging to Clan 4 which is involved in the
180 detoxification of macrocyclic lactones. Within Clan 3, however, there is no particular clade
181 that is associated with resistance to insecticides and many clades have genes involved in
182 detoxification. The most studied P450 families with regards to pesticide resistance are CYP6B
183 (pyrethroids, OPs (organophosphorus pesticides)), CYP6BG (pyrethroids, ryanoids), CYP6AE
184 (pyrethroids and many classes of compounds), CYP9A (ryanoids, capsaicin), CYP332A (OPs),
185 and CYP337B (pyrethroids; the CYP337B3 gene, specifically). In contrast, P450s that are
186 involved either in known physiological functions (Figure 2A, genes decorated with black
187 triangles), or are suspected to be involved in some physiological function (Figure 2A, black/red
188 stars) are found in all four clans. It should be noted, however, that there are P450s in other clans
189 that are involved in detoxification in non-Lepidoptera species as in case of the mitochondrial
190 CYP12A1 of *Musca domestica* (Guzov et al., 1998).

191 Similarly, in the hymenopterans, the detoxification-related P450s are found in Clan 3 (Figure
192 2B, genes with black circles) and more specifically in one of two families; CYP9Q/CYP9BU
193 (neonicotinoids, pyrethroids, quercetin) (Beadle et al., 2019; Mao et al., 2011; Troczka et al.,
194 2019), or CYP6AS (quercetin) (Mao et al., 2009). Genes in the CYP9Q/9BU branch are present
195 in the Apidae and some Megachilidae species, conferring selective protection from
196 thiaclorprid, but not imidacloprid, even though both insecticides belong to the neonicotinoid
197 class (Manjon et al., 2018; Troczka et al., 2019). Of particular importance is the expansion in
198 the CYP6AS family, that appears to be Hymenoptera specific (Oakeshott et al., 2010).
199 Nevertheless, not all CYP6AS genes are necessarily involved in detoxification, since in
200 addition to the detoxifiers (CYP6AS1, 3, 4, and 10), others are involved in physiology
201 (CYP6AS8, and 11) (Wu et al., 2017). More specifically, CYP6AS8 and CYP6AS11 are
202 candidates for omega and omega-1 hydroxylations of short chain fatty acids to pheromones
203 (Plettner et al., 1996), while recent RNAi experiments indicated that knock-down of CYP6AS8
204 affects the short chain fatty acid biosynthesis (Wu et al., 2020). Even though such omega
205 hydroxylations are carried out by CYP4 enzymes in vertebrates, it has been proven for other
206 CYP6 genes that this P450 family is also capable of this biochemical function (Helvig et al.,

207 2004). To date, however, neither CYP6AS8, nor CYP6AS11 have been biochemically
208 characterized.

209 The key difference, however, when compared to Lepidoptera, is that there are far fewer cases
210 of functionally characterized P450s in Hymenoptera. This is true even for Hymenoptera-
211 specific P450s such as CYP6BC and CYP4AV that are present in all Hymenoptera and should
212 therefore have an important and specific role in these insects. Similarly, Lepidoptera specific
213 P450s are equally interesting, such as the CYP428A, which appears to be fast evolving
214 according to our phylogenetic analysis (Figure 2A).

215 Concerted research efforts should be undertaken in order to develop selective insecticides,
216 targeting major Lepidoptera pests on the one hand, which will be harmless to the ecologically
217 and agriculturally important Hymenoptera, on the other hand. In this quest for selective
218 insecticides, it is important to take into consideration two facts related to P450s. The first is
219 that it is not possible to predict the catalytic competence of a P450, simply through similarity
220 to another P450 that has been functionally characterized (Dermauw et al., 2020). The second
221 fact is that there is no clear dichotomy between P450s involved in detoxification of xenobiotics,
222 and P450s involved in physiological functions (Dermauw et al., 2020). Both facts stress that
223 finding P450s that confer selective protection to an insecticide will not be a trivial task, and
224 will require extensive expertise and most likely wet lab screening.

225 **4. Systems used for functional validation.**

226 Several approaches have been exploited to characterize the role of P450s from different species
227 in xenobiotic metabolism, including *in vitro* expression in heterologous systems, RNAi-based
228 reverse genetics, or transgenic approaches (*in vivo* overexpression in *D. melanogaster* or non-
229 model organisms, or CRISPR/CAS9 knock out) which provided different levels of validation
230 for the involvement of P450s in detoxification and implication in resistance (Nauen et al.,
231 2022).

232 Eighty four P450s originating from all four categories of pests and pollinators/*Papilio* sp.
233 reported in this review (Lepidoptera, non-Lepidoptera, mite pest species and
234 pollinators/*Papilio sp.*) have been functionally validated to be associated to pesticide
235 toxicity/resistance using only the RNAi approach (Figure 3A); thirty eight using only *in vitro*
236 methodologies and one using only genome modification approaches; twelve P450s have been
237 characterized utilizing both *in vitro* systems and RNAi, twenty three using both genome
238 modification approaches (reverse genetics in non-model organisms or *D. melanogaster*

239 heterologous expression) and *in vitro*; two using genome modification and RNAi and five using
240 all approaches (RNAi, genome modification and *in vitro*) (Figure 3A). The validation systems
241 used for each P450 are mentioned in Table 2 (last two columns). It is notable that CRISPR in
242 non-model organism has been so far utilized only for Lepidoptera P450 validation (*H. armigera*
243 6AE cluster, (Wang et al., 2018) and *S. exigua* 9A186 (Zuo et al., 2021)).

244 More specifically, looking at the different systems utilized for the functional validation of
245 individual P450s in different arthropods ((a) Lepidoptera; (b) non-Lepidoptera insect species;
246 (c) mites and; (d) pollinators/ *Papilio sp.*) the information is depicted in Figure 3 B-E:

247 (a) Twenty two P450s (Figure 3B) have been validated to resist/tolerate xenobiotics using only
248 the system of RNAi in Lepidoptera, despite the debate in the literature concerning the
249 effectiveness of RNAi in this order (Terenius et al., 2011). For example, all the P450s from
250 *Spodoptera litura* (CYP321B1, CYP6B50, CYP6AB14, CYP9A40, CYP6AB60, CYP6AB12)
251 have been validated for their contribution to resistance using RNAi (Lu et al., 2020, 2019b,
252 2019a; Sun et al., 2019; Wang et al., 2017; Wang et al., 2015b, 2015a). Eighteen Lepidoptera
253 P450s have been validated using *in vitro* systems only, like *H. zea* CYP6B27 (Wen et al., 2009)
254 and *H. armigera* CYP9A12 (Chen et al., 2019; Shi et al., 2021; Tian et al., 2019; Yang et al.,
255 2008), while only one P450 (*H. armigera* CYP6AE20) has been validated using only genome
256 modification approaches (Wang et al., 2018) (Figure 3B and Table 2A). Additionally, two
257 P450s have been verified using both RNAi and *in vitro* systems: *H. armigera* CYP6B6 and
258 CYP9A14 (Shi et al., 2021; Tao et al., 2012; Tian et al., 2017; Yang et al., 2008; Zhao et al.,
259 2016) while 11 P450s have been verified using both *in vitro* and genome modification systems:
260 the *H. armigera* 6AE cluster CYP6AE11, 6AE12, 6AE15, 6AE16, 6AE17, 6AE18, 6AE19
261 (both heterologous expression and CRISPR) (Wang et al., 2018) and *S. exigua* CYP321A16,
262 CYP332A1, CYP321A8 (both heterologous expression and *D. melanogaster* transgenic
263 expression) (Bo et al., 2020) and CYP9A168 both heterologous expression and *D.*
264 *melanogaster* system (Zuo et al., 2021) (Figure 3Band Table 2A). *H. armigera* CYP6AE14 has
265 been validated for xenobiotic tolerance and metabolism using all three categories: *in vitro*
266 systems, genome modification and RNAi (Mao et al., 2007; Shi et al., 2018; Tao et al.,
267 2012)(Figure 3B and Table 2A);

268 (b) Non-Lepidoptera pest P450s are distributed differently, with the great majority being
269 characterized only with the use of RNAi (50/74 validated P450s- Figure 3C and Table 2B). For
270 example, the *L. migratoria* P450s (CYP6FF1, CYP6FD2, CYP6FE1, CYP4G102, CYP4G62,
271 CYP9AQ2, CYP409A1, CYP408B1, CYP9AQ1, CYP9A3, CYP6HC1, CYP6HL1,

272 CYP6HN1, CYP6HQ1 and CYP303A1 (except for CYP6FD1)) have been associated with
273 insecticide toxicity/resistance with the use of RNAi. Exclusively *in vitro* systems have been
274 exploited for only six P450s from this category- and one of them is *L. striatellus* CYP6FU1
275 (Elzaki et al., 2018) (Figure 3C and Table 2B). Notably, CRISPR is not detected in the literature
276 so far for non-Lepidoptera pest P450 validation. It appears more common to use both RNAi
277 and *in vitro* systems, as it is depicted in Figure 3C: nine P450s have been validated using both
278 sets of techniques. For example, *A. gossypii* CYP6CY22 and CYP6CY13 assessed for its
279 metabolic ability and involvement in resistance with *in vitro* expression system and RNAi
280 (Chen et al., 2020; Hirata et al., 2017; Ma et al., 2019) (Figure 3C and Table 2B). Four P450s
281 were studied using both *in vitro* and *D. melanogaster* transgenic expression (genome
282 modification) approaches: *L. striatellus* CYP6AY3v2 (Wang et al., 2017), *M. persicae*
283 CYP6CY3 (Bass et al., 2013; Nakao et al., 2019), *C. capitata* CYP6A51 (Tsakireli et al., 2019)
284 and *B. (M.) aeneus* CYP6BQ23 (Samantsidis et al., 2020; Zimmer et al., 2014). One P450 has
285 been validated for its role in resistance using both RNAi and genome modification systems: *N.*
286 *lugens* CYP6CS1 using *D.melanogaster* heterologous expression system and the RNAi
287 approach (Wang et al., 2021) (Figure 3C and Table 2B). Three P450s have been validated using
288 *in vitro* and *in vivo* (RNAi and genome modification) methodologies: *B. tabaci* CYP6CM1
289 (Daborn et al., 2012; Nauen et al., 2013), *N. lugens* CYP6ER1 (Bao et al., 2016; Pang et al.,
290 2016) and *T. castaneum* CYP6BQ9 (Zhu et al., 2010)(Figure 3C and Table 2B);

291 (c) The majority of mite pest P450s and their role in xenobiotic metabolism and/or
292 toxicity/resistance has been investigated with the use of RNAi only, which is in fact nine out
293 of the 13 validated P450s in the literature (Figure 3D and Table 2C): *T. cinnabarinus*
294 CYP389B1, CYP392A26, CYP391A1, CYP384A1, CYP392D11, CYP392A28 were
295 validated by Shi et al., 2016 using RNAi (Shi et al., 2016). Also, *T. urticae* CYP392D8,
296 CYP392A12, CYP389C10 were evaluated for their contribution to resistance using RNA
297 interference (Xu et al., 2021). *T. cinnabarinus* CYP389C16 has been validated for its role in
298 metabolism and resistance using both *in vitro* and RNAi approaches (Feng et al., 2020). *T.*
299 *urticae* CYP392E10 has been validated for xenobiotic detoxification using *in vitro* systems
300 (Demaeght et al., 2013). Additionally, *T. urticae* CYP392A11 is involved in insecticide
301 metabolism, demonstrated by the use of *in vitro* systems, *D. melanogaster* heterologous
302 expression (genome modification), and also RNAi (Riga et al., 2015; Xu et al., 2021), while
303 CYP392A16 was expressed *in vitro*, to prove its catalytic activity against insecticides (Riga et
304 al., 2014) and genome modification techniques were also explored (Riga et al., 2020);

305 (d) Pollinator and *Papilio sp*. P450 functional validation seem to rely more on the *in vitro*
306 systems, and also *in vivo*, mostly using genome modification set of techniques. Three P450s
307 have been validated only using RNAi (*A. cerana cerana* CYP301A1, CYP303A1, CYP306A1
308 (Zhang et al., 2019))(Figure 3E and Table 2D). Thirteen P450s were validated using only *in*
309 *vitro* systems. More specifically, *A. mellifera* CYP6AS1, CYP6AS3, CYP6AS4, CYP6AS10,
310 *B. terrestris* CYP9Q5, *O. bicornis* CYP9BU2 and all the *Papillio* P450s (CYP6B1, 6B3, 6B4,
311 6B17, 6B21, 6B25, 6B33) were shown to contribute to xenobiotic metabolism using *in vitro*
312 systems (Beadle et al., 2019; Hung, 1997; Li et al., 2003, 2004; Manjon et al., 2018; Mao et
313 al., 2011, 2009; Wen et al., 2006, 2003) (Figure 3E and Table 2D). There have also been seven
314 P450s identified in the literature for which the researchers exploited both *in vitro* and genome
315 modification strategies: *A. melifera* CYP9Q1, CYP9Q2, CYP9Q3, CYP6AQ1, *B. terrestris*
316 CYP9Q4, CYP9Q6 and *O. bicornis* CYP9BU1 (Beadle et al., 2019; Haas et al., 2021; Manjon
317 et al., 2018; Mao et al., 2009; Troczka et al., 2019).

318 *4.1 Heterologous expression systems used for in vitro validation*

319 Different *in vitro* expression systems (bacterial, insect cell/baculovirus, yeast) and strategies
320 (including modifications at the DNA or protein sequence or electron delivery, fusion enzyme
321 systems) have been recruited in order to functionally characterize P450s associated with
322 insecticide resistance and toxicity *in vitro* and confirmed their role of metabolizing several
323 insecticides at different rates (Nauen et al., 2021).

324 The systems that have been exploited include different heterologous expression hosts: the
325 prokaryote *Escherichia coli* and the eukaryotic systems *Saccharomyces cerevisiae, Pichia*
326 *pastoris*, insect cells (baculovirus mediated) and stable insect cells (Figure 4). The *E. coli*
327 system offers important benefits as an expression system, like the highly produced protein
328 yields, the inexpensive culture media and most importantly, the lack of endogenously produced
329 P450s (Nauen et al., 2021). The yeast systems that are recruited for P450 expression, especially
330 *S. cerevisiae* and *P. pastoris*, offer the significant advantage of performing post-translational
331 modifications combined with an environment of organelles similar to other eukaryotic
332 organisms, enabling proper protein membrane anchoring (Hausjell et al., 2018). Insect cells
333 also constitute a frequently utilized expression system, as they resemble the natural insect
334 protein production system (Feyereisen, 2012), using either the baculovirus transient expression
335 approach, or stable insect cell lines. The total distribution of the *in vitro* systems that are
336 exploited in the literature for P450 expression leads to almost equal use of the baculovirus
337 mediated system (47%) and the *E.coli* expression system (44%), while stable cell line comprise

338 only 6% and yeast systems 3% according to published research (Figure 4A). The set of
339 methodologies used for the validation of each P450 are mentioned in the last two columns of
340 Table 2.

341 There is a wide distribution of the *in vitro* systems used for the Lepidoptera P450 metabolism
342 exploration (Figure 4B and Table 2A). The majority of the studies (72%) concerning
343 Lepidoptera insect pest P450s took advantage of the baculovirus mediated transient expression
344 system, as shown in Figure 4B. Fourteen percent of the studies exploited *E. coli* and 10% yeast
345 systems, while only 4% of the studies used stable insect cell lines. For example, researchers
346 using Sf9- baculovirus mediated expression, confirmed the detoxifying role of the *A. transitella*
347 CYP6AB11 (Niu et al., 2011), *D. pastinacella* CYP6AB3v1 and CYP6AB3v2 (Mao et al.,
348 2008, 2006), *H. zea* CYP6B27 (Wen et al., 2009)(*H. armigera* CYP6B2, CYP6B6, CYP6B7,
349 CYP9A3, CYP9A12, CYP9A14, CYP9A16, CYP9A17 and CYP9A23 (Shi et al., 2021) and
350 CYP321A16, CYP332A1, CYP321A8 from *S. exigua* (Bo et al., 2020; Hu et al., 2021).
351 Additionally, P450s belonging to the *H. armigera* CYP6AE subfamily were proven to
352 participate in the detoxification of several insecticides and phytochemicals using baculovirus
353 mediated expression in Hi5 insect cells (Shi et al., 2018; Wang et al., 2018) The widely used
354 *E.coli* system is also used for Lepidoptera P450 expression (14% of the studies) (Figure 4B).
355 After heterologously expressing the proteins in *E.coli*, Calla et al., 2019 (Calla et al., 2020)
356 described the ability of CYP6AE89 from *D. pastinacella* for metabolism. Similarly, Tian et al.,
357 2019 expressed the *H. armigera* proteins CYP9A14, 9A12, 9A17 and CYP6B6 in the *E.coli*
358 system, showing their contribution to detoxification. Close to the percentage of studies that
359 used the *E.coli* expression system is the use of yeast (10%) (Figure 4B). For example, *H.*
360 *armigera* CYP6B7 was proven to degrade chemical substances, using the *P. pastoris*
361 expression system (Zhao et al., 2018). On the contrary, stable insect cells have been used only
362 in 4% of the publications concerning Lepidoptera P450s (Figure 4B). Ha2302 stable cells were
363 used to express CYP337B3 (Joußen et al., 2012; Rasool et al., 2014) and CYP337B1v1 (Joußen
364 and Heckel, 2021) from *H. armigera*.

365 Non-Lepidoptera insect pest P450s are also mostly expressed in insect cells with the use of
366 baculovirus (41% of the studies) (Figure 4C and Table 2B), while 34% of the studies used
367 *E.coli* and 25% the stable insect cell line approach. Interestingly, expression in the yeast system
368 was not identified in the literature (Figure 4C). *L. striatellus* CYP417A2v2, CYP439A1v3,
369 CYP4C71 have all been successfully expressed and validated in Sf9 (baculovirus mediated)
370 cell lines (Miah et al., 2019, 2017; Xiao et al., 2020). *N. lugens* CYP6AY1 on the contrary has

371 been expressed in *E.coli* cells by Ding et al., 2013, in an effort to assess xenobiotic metabolism.
372 The smallest amount of publications (25%- Figure 4C) has exploited stable insect cell lines:
373 Nakao et al., 2019 (Nakao et al., 2019) used S2 cells from *D. melanogaster* in order to express
374 *M. persicae* CYP6CY3 and Kalsi et al., 2017 (Kalsi and Palli, 2017) handled SL1 stable cell
375 lines to express *L. decemlineata* CYP6BJ variants, CYP9Z25 and CYP9Z29.

376 The great majority of mite P450s have been expressed in *E.coli* cells (83% of the studies),
377 while 17% used baculovirus mediated expression in insect cells (Figure 4D and Table 2C). The
378 yeast expression system as well as the stable cell lines are absent for the specific category. In
379 fact, from all mite P450s that were evaluated for their metabolic capacity, only CYP392E10
380 from *T. urticae* was transiently expressed in Hi5 cell lines, showing its detoxifying ability
381 (Demaeght et al., 2013). Concerning pollinator and *Papilio sp.* P450s, the distribution of the
382 systems is completely different from the previous categories, as all the studies (100%)
383 employed the baculovirus mediated expression system (Figure 4E).

384 Mosquito P450s (*Anopheles* and *Aedes* species), included as a separate category for comparison
385 reasons only, have been elucidated for their metabolic activity utilizing mostly the *E.coli*
386 system for their expression (90% of the studies), while the yeast and baculovirus mediated
387 expression have been equally used (5% each) (Adolfi et al., 2019; Chandor-Proust et al., 2013;
388 Edi et al., 2014; Ibrahim et al., 2018, 2016; Kasai et al., 2014; Mclaughlin et al., 2008; Mitchell
389 et al., 2012; Müller et al., 2008; Riveron et al., 2017, 2014; Stevenson et al., 2012, 2011; Vontas
390 et al., 2018; Yunta et al., 2019, 2016) (Figure 4F).

391 *4.2 Reverse/functional genetic systems used for in vivo validation*

392 *In vivo* functional validation of the role of P450s to xenobiotic tolerance and resistance has
393 been facilitated by the RNA interference (RNAi) approach (Bai-Zhong et al., 2020; Ding et al.,
394 2013; Gao et al., 2016; Kalsi and Palli, 2017; Mao et al., 2007; Pang et al., 2016; Wang et al.,
395 2018; Wang et al., 2018) as well as *Drosophila* transformation tools (Daborn et al., 2012;
396 Manjon et al., 2018; Riga et al., 2020; Samantsidis et al., 2020; Troczka et al., 2019; Tsakireli
397 et al., 2019; Zhu et al., 2010; Zimmer et al., 2018)and the recent advances in genome editing
398 technology in non-model organisms (Wang et al., 2018; Zuo et al., 2021).

399 RNAi has been used in various agricultural insect and mite species in order to validate the role
400 of P450s in xenobiotic tolerance/resistance. For example, targeting of CYP6BJ[a/b], CYP6BJ1v1,
401 CYP9Z25, CYP9Z29 from *L. decemlineata* indicated their involvement in defense against
402 natural and synthetic compounds (Kalsi and Palli, 2017), while RNAi mediated knockdown of

403 CYP6BQ9 from *T. castaneum* revealed increased susceptibility of the QTC279 resistant strain
404 to deltamethrin (Zhu et al., 2010). This tool has been also used outside of the coleopteran
405 species, such as the hemipteran species *N. lugens* where independent targeting of CYP6AY1
406 or CYP6ER1 indicated their involvement in imidacloprid tolerance (Ding et al., 2013; Pang et
407 al., 2016). Moreover, P450s that are associated to xenobiotic resistance from major Lepidoptera
408 pest species have been subjected to RNAi mediated knockdown, either through plant (Mao et
409 al., 2007), diet or droplet feeding (Bai-Zhong et al., 2020; Wang et al., 2018) or injections (Gao
410 et al., 2016; Hu et al., 2014) indicating their involvement in tolerance to natural and synthetic
411 compounds. RNAi is the main P450 *in vivo* validation system in agricultural insect pests, while
412 this methodology is equally used with *in vitro* systems in Lepidoptera species (Figure 3B) ,
413 despite the concerns about the effectiveness of this tool in Lepidoptera order (Terenius et al.,
414 2011). Although, RNAi is a fast and easy approach linking P450 functional validation and
415 resistance *in vivo*, dsRNA stability and/or cellular uptake may impair the methodology in
416 certain insect species (Cooper et al., 2020).

417 The model organism *D. melanogaster* and the expansion of the genetic tools offer an alternative
418 method for functional validation of P450s in resistance to xenobiotics. The employment of
419 GAL4/UAS system has enabled the conditional expression of P450s in different tissues and
420 investigation of their role in xenobiotic resistance and tolerance (McLeman et al., 2020).
421 Examples from the Drosophila system include the heterologous expression of P450s from
422 agricultural pests and the category of pollinators/ *Papilio sp.* such as, *C. capitata* CYP6A51
423 (Tsakireli et al., 2019), *P. xylostella* CYP6BG1 (Li et al., 2018), CYP6CM1vQ from *B. tabaci*
424 (Daborn et al., 2012), *B. (M). aeneus* CYP6BQ23 (Samantsidis et al., 2020), CYP321A16 and
425 CYP332A1 from *S. exigua* (Bo et al., 2020), *M. persicae* CYP6CY3 (Bass et al., 2013), *N.*
426 *lugens* CYP6ER1 (Pang et al., 2016; Zimmer et al., 2018), *T. urticae* CYP392A11 and
427 CYP392A16 (Riga et al., 2020, 2015), *A. mellifera* CYP9Q2 and CYP9Q3 (Manjon et al.,
428 2018), *B. terrestris* CYP9Q4 and CYP9Q6 (Manjon et al., 2018; Troczka et al., 2019).

429 Although this system provides evidence for the functional link between P450s and their role in
430 xenobiotic resistance, the levels achieved are usually less than 5-fold compared to the striking
431 phenotypes observed in the original populations (often >100-fold). However, it is not clear if
432 this is the actual contribution of the P450 or concerns limitations of the system. It has been
433 suggested that additional factors may contribute to resistance. Recently, Samantsidis et al.,
434 2020 combined transgenic expression and CRISPR/Cas9 modification in *D. melanogaster*.
435 Transgenic flies expressing the *B.*(*M). aeneus* CYP6BQ23 while also bearing the L1014F

436 mutation in voltage gated sodium channel contributed greater resistance levels to the pyrethroid
437 deltamethrin, than each mechanism separately, an indication of their synergistic role
438 (Samantsidis et al., 2020).

439 CRISPR editing tool has been successfully employed not only in *D. melanogaster* and other
440 model organisms, but also in the Lepidoptera species *H. armigera* and *S. exigua.* The reverse
441 genetic approach followed by knocking out a cluster of CYP6AEs provided *in vivo* evidence
442 for the involvement of this cluster in detoxification and tolerance to esfenvalerate, indoxacarb
443 and phytochemicals (Wang et al., 2018), while knock out of *CYP9A186* from *S. exigua* restores
444 susceptibility to emamectin benzoate (Zuo et al., 2021).

445 **5. Xenobiotic specificity of functionally validated P450s**

446 P450s from all arthropodal categories that are mentioned in this review (Lepidoptera pests,
447 non- Lepidoptera pests, mite pests and pollinators/ *Papilio sp*.) have been associated with the
448 metabolism of active ingredients and/or functionally linked *in vivo* with xenobiotic
449 resistance/tolerance from two or more insecticide classes (pyrethroids, neonicotinoids, OPs,
450 organochlorines, etc.), as well as diverse natural allelochemicals (furanocoumarins,
451 phenylpropenes, ketones etc.). The substances are categorized and summarized in Table 2 for
452 each P450.

453 *5.1 Lepidoptera*

454 Several Lepidoptera P450s have been identified to metabolize substances from only one
455 chemical group. For example, *A. transitella* CYP6AB11 contributes to imperatorin (natural
456 compound-furanocoumarin class) detoxification (Niu et al., 2011). The codling moth *C.*
457 *pomonella* CYP9A61 contributes to the metabolism of cypermethrin, permethrin and λ-
458 cyhalothrin, all belonging to the class of pyrethroid insecticides (Yang et al., 2017), while
459 CYP6B2 was found to contribute to deltamethrin (pyrethroid) and azinphos methyl
460 (organophosphate) resistance (Wan et al., 2019) (Table 2A). *C. suppressalis* CYP6CV5,
461 CYP9A68, CYP321F3, CYP324A12 have been associated with chlorantraniliprole (diamide)
462 resistance (Xu et al., 2019) (Table 2A). CYP6B8 originating from the generalist *H. zea,* has a
463 broad insecticide- detoxifying role, being able to metabolize a great number of synthetic
464 compounds from distant groups, such as cypermethrin (pyrethroids), aldrin (organochlorines),
465 carbaryl (carbamates), diazinon (organophosphates), as well as natural substances belonging
466 to the groups of furanocoumarins, flavonoids, indolyl alcohols, cinnamate esters (Li et al.,
467 2004; Rupasinghe et al., 2007; Wen et al., 2009) (Table 2A). Similarly, *H. zea* CYP321A1 has

468 the ability to metabolize the above mentioned compounds (except for carbaryl (carbamates)
469 and indole carbinol (indolyl alcohols) with various efficiencies (Rupasinghe et al., 2007;
470 Sasabe et al., 2004). Additionally, P450s belonging to the *H. armigera* CYP6AE subfamily
471 were proven to participate in the detoxification of a broad spectrum of insecticides and
472 phytochemicals. For example, CYP6AE17 and CYP6AE18 contribute to the metabolism of
473 pyrethroids, neonicotinoids, organochlorines, oxadiazines and carbamates, but also
474 furanocoumarin compounds (Shi et al., 2021, 2018; Wang et al., 2018)(Table 2A). The *H.*
475 *armigera* CYP6B6 is capable of detoxifying both natural (capsaicin) and chemical compounds
476 (the pyrethroid esfenvalerate) while also contributing to resistance to chlorpyriphos
477 (organophosphate) and bifenthrin, cyfluthrin (pyrethroids) (Shi et al., 2021; Tian et al., 2019,
478 2017; Zhao et al., 2016). The *H. armigera* CYP6B7 has been shown to metabolize two different
479 insecticide classes: pyrethroids (esfenvalerate, fenvalerate and bifenthrin) and OPs
480 (chloryrifos) (Shi et al., 2021; Zhao et al., 2018, 2017). CYP6BG1 from *P. xylostella* has been
481 functionally implicated in chlorantraniliprole (diamide) as well as b-cypermethrin, permethrin
482 (pyrethroid) resistance *in vivo* (Bautista et al., 2009; Li et al., 2018) (Table 2A). *S. exigua*
483 CYP6AB14, CYP9A98, CYP9A10 contribute to pyrethroid resistance (deltamethrin for
484 CYP6AB14, CYP9A98 and a- cypermethrin for CYP9A10) (Hafeez et al., 2020a, 2019), while
485 CYP6AE10 is implicated in resistance to lamda- cyhalothrin (pyrethroid) and metabolic
486 adaptation to the its plant host defense allelochemicals (quercetin (flavonoid)) (Hafeez et al.,
487 2020b). *S. litura* CYP9A40 is implicated in resistance/tolerance of both insecticides
488 (deltamethrin (pyrethroids), methoxyfenozide (diacylhydrazines)) and natural compounds
489 (cinnamic acid, quercetin (flavonoid)) (Wang et al., 2015b).

490 *5.2 Non- Lepidoptera pests (Homoptera, Hemiptera, Diptera, Coleoptera, Orthoptera)*

491 *L. striatellus* CYP353D1v2 is able to degrade the chemically unrelated insecticides buprofezin
492 and imidacloprid (neonicotinoid) (Elzaki et al., 2017), while CYP417A2v2, CYP439A1v3,
493 CYP4C71 have been found to metabolize chemical substrates: imidacloprid (neonicotinoid),
494 deltamethrin (pyrethroid) and imidacloprid (neonicotinoid), respectively (Miah et al., 2019,
495 2017; Xiao et al., 2020). CYP6CW1 has been associated with buprofezin and pymetrozine
496 (pyridine azomethine derivative) resistance, CYP4DE1 and CYP6W3v2 with ethiprole (phenyl
497 pyrazole) resistance (Elzaki et al., 2015; Zhang et al., 2015). Three *L. decemlineata* P450s have
498 been associated with imidacloprid resistance (neonicotinoid): CYP4Q3, CYP9e2- like and
499 CYP9Z26 (Clements et al., 2017; Kaplanoglu et al., 2017; Naqqash et al., 2020) while
500 CYP350D1 has been associated with chlorantraniliprole (diamide) resistance (Dumas et al.,

501 2020). *A. gossypii* CYP6CY14, CYP6CY22 and CYP6UN1 have been found to be involved in
502 resistance to dinotefuran (CYP6CY14 also in acetamiprid resistance- (Ullah et al., 2020)) and
503 potentially involved in its detoxification (Chen et al., 2020), while CYP380C6 is involved in
504 spirotetramat (tetronic and tetramic acid derivative) resistance (Pan et al., 2018) and CYP6A2
505 in a- cypermethrin (pyrethroid) and spirotetramat (tetronic and tetramic acid derivative) (Peng
506 et al., 2016)(Table 2B). The *N. lugens* CYP4DE1, CYP353D1, CYP439A1, CYP6AY1v2 have
507 been associated with chlorpyriphos and imidacloprid resistance (organophosphate and
508 neonicotinoid, respectively)(Xu et al., 2020). Additionally, CYP4CE1 and CYP6CW1
509 metabolize and are also involved in resistance of imidacloprid (neonicotinoid) (Zhang et al.,
510 2016). CYP6ER1 detoxifies several members of the neonicotinoid insecticide family
511 (imidacloprid, thiamethoxam, dinotefuran and nitenpyram), as well as sulfoxaflor (group of
512 sulfoximines) (Hamada et al., 2020; Liao et al., 2019; Mao et al., 2019; Pang et al., 2016; Sun
513 et al., 2018). Several CYP6ER1 variants have been investigated for their capacity to detoxify
514 imidacloprid, with positive results (Zimmer et al., 2018). A follow up study on the
515 aforementioned CYP6ER1 variants that bear deletions was published by Hamada and
516 colleagues (Hamada et al., 2020) and assayed for their ability to metabolize/detoxify several
517 neonicotinoid insecticide (acetamiprid, thiacloprid, clothianidrin, thiamethoxam, nitenpyram)
518 and one butenolide (flupyradifurone) (Table 2B). *S. zeamais* CYP6MS1 knockdown
519 experiments revealed its role in terpinen-4- ol (terpineol) susceptibility (Huang et al., 2020).
520 The *S. avenae* CYP6A14-1 and CYP307A1 contribute to imidacloprid (neonicotinoid)
521 resistance (Zhang et al., 2020). *S. furcifera* CYP6FD1 and CYP4FD2 contribute to sulfoxaflor
522 (sulfoximine) resistance (Wang et al., 2019) and CYP6ER4 to chlorpyriphos
523 (organophosphate) resistance (Ruan et al., 2021). *T. castaneum* CYP346 has been
524 experimentally associated with phosphine resistance (Wang et al., 2020). Five *D. citri* P450s
525 (CYP4C67, CYP4DA1, CYP4C68, CYP4G70, CYP4DB1) are involved in imidacloprid
526 (neonicotinoid) resistance (Killiny et al., 2014). Chen and colleagues depicted in several studies
527 the relationship of *B. odoriphaga* CYP9b2, CYP49a1, CYP12b1 and CYP6FV12 and
528 imidacloprid resistance (neonicotinoid), while CYP3356A1 has been related to imidacloprid,
529 thiamethoxam (neonicotinoids) and b- cypermethrin (pyrethroid) resistance (Chen et al., 2018;
530 Chen et al., 2019a, 2019b) (Table 2B).

531 The whitefly CYP6CM1 is capable of metabolizing a broad range of compounds that belong
532 to neonicotinoids (imidacloprid, thiacloprid, nitenpyram, clothianidin), pyriproxyfen and
533 pyridine azomethine- derivative (pymetrozine) groups (Daborn et al., 2012; Hamada et al.,

534 2019; Karunker et al., 2009; Nauen et al., 2015, 2013; Roditakis et al., 2011). Additionally, *B.*
535 *tabaci* CYP6CX4 has been found to contribute to flupyradifurone (butenolide) and
536 imidacloprid (neonicotinoid) resistance (Wang et al., 2020). The *L. migratoria* CYP303A1 is
537 implicated in pyrethroid (deltamethrin), organophosphate (malathion, chlorpyriphos) and
538 carbamate (carbaryl) resistance (Wu et al., 2020) while CYP9AQ2 from the same species has
539 been functionally associated with detoxification of pyrethroids (Guo et al., 2015). Also,
540 CYP6FD1 has been shown to metabolize deltamethrin (pyrethroid)(Liu et al., 2019) (Table
541 2B). *T. castaneum* CYP4BN6 and CYP6BQJ have been shown to contribute to essential oil
542 (from *Artemisia vulgaris*) metabolic detoxification (Gao et al., 2020; Zhang et al., 2021).

543 *5.3 Mite pests*

544 Several more specialized P450s of the CYP392 family in *T. urticae* are capable of metabolizing
545 specific acaricides showing narrower range metabolism in comparison to other arthropod
546 P450s. For instance, CYP392A16 and CYP392E10 are metabolizing abamectin and ketoenols,
547 respectively (Demaeght et al., 2013; Riga et al., 2014) (Table 2C). *T. urticae* CYP392A11 is
548 able to detoxify certain acaricides b- ketonitrile (cyenopyrafen) and METI (fenpyroximate),
549 while RNAi studies indicated the involvement of the specific P450 in abamectin resistance
550 (Riga et al., 2015; Xu et al., 2021). Resistance to abamectin (avermectin) has also been
551 attributed to some *T. urticae* P450s: CYP392D8, CYP392A11, CYP392A12, CYP389C10 (Xu
552 et al., 2021). *T. cinnabarinus* P450s have been validated *in vivo* to be implicated in
553 fenpropathrin (pyrethroid) resistance: CYP389B1, CYP392A26, CYP391A1, CYP384A1,
554 CYP392A11 and CYP392A28 (Shi et al., 2016), while CYP389C16 is able to metabolize b-
555 ketonitrile (cyflumetofen and its de-esterified metabolite) and METI (pyridaben) acaricides
556 (Feng et al., 2020)(Table 2C). Recently, *T. urticae* CYP392A16 has been found to metabolize
557 a metabolite of pyflubumide (carboxanilide metabolite) (Fotoukkiaii et al., 2021).

558 *5.4 Pollinators and Papilio sp.*

559 The *A. mellifera* P450s 9Q1, 9Q2, 9Q3 have all demonstrated metabolic activity against tau-
560 fluvalinate (pyrethroid), thiacloprid, acetamiprid (neonicotinoids), coumaphos
561 (organophosphate) and flupyradifurone (butenolide) as well as natural compounds of the
562 flavonoid group (quercetin) (Haas et al., 2021; Manjon et al., 2018; Mao et al., 2011) (Table
563 2D). Also, *A. mellifera* P450s 6AS1, 6AS3, 6AS4 and 6AS10 have been implicated in natural
564 compound metabolism, from the group of flavonoids (quercetin) (Mao et al., 2009). The eastern
565 honey bee *A. cerana cerana* P450s 301A1, 303A1, 306A1 showed to be functionally associated

566 with a wider xenobiotic spectrum, involving thiamethoxam (neonicotinoid), dichlorvos
567 (organophosphate), deltamethrin (pyrethroid) and a herbicide, classified as viologen (paraquat)
568 (Liu et al., 2019). The *B. terrestris* P450s 9Q4, 9Q5, 9Q6 and the *O. bicornis* CYP9BU1 and
569 CYP9BU2 have also been found to be involved in neonicotinoid detoxification: thiacloprid,
570 acetamiprid (Manjon et al., 2018; Troczka et al., 2019) and imidacloprid, thiacloprid (Beadle
571 et al., 2019) respectively (Table 2D).

572 Among the most well characterized examples of P450-mediated detoxification of plant
573 allelochemicals is that of furanocoumarin metabolism within the genus *Papilio* (swallowtail
574 butterflies). *Papilio polyxenes* CYP6B1, can metabolize plant allelochemicals: the
575 fouranocoumarins xanthotoxin, psoralen, angelicin and flavone, the simplest member of the
576 class of flavones, as well as the OP diazinon (Li et al., 2003; Wen et al., 2003) Interestingly,
577 the comparison between the greater substrate range of the generalist *H. zea* CYP6B8 and the
578 narrower substrate range of the specialist *P. polyxenes* CY6B1 indicated that generalist
579 detoxification proteins have the ability to metabolize more structurally diverse compounds (Li
580 et al., 2003). *P. polyxenes* CYP6B3 was observed to metabolize alpha-naphthoflavone and
581 furanocoumarins (Li et al., 2003; Wen et al., 2003) (*P. glaucus*, CYP6B17, CYP6B21, *P.*
582 *canadensis* CYP6B25 and *P. multicaudatus* CYP6B33 have all been involved in
583 furanocoumarin metabolism (Hung, 1997; Li et al., 2003). Notably, P450s from *P. glaucus*,
584 which feeds occasionally on furanocoumarin-containing host plants, showed higher activities
585 against furanocoumarins than those from *P. canadensis*, which normally does not encounter
586 furanocoumarins. These P450s in turn catalyze a larger range of furanocoumarins at lower
587 efficiency than CYP6B1, a P450 from *Papilio polyxenes*, which feeds exclusively on
588 furanocoumarin-containing host plants (Li et al., 2003).

589 Although the aforementioned studies provide an overview of the number of substrates
590 catalyzed by each P450, drawing conclusions concerning their substrate specificity (narrow or
591 broad metabolizer) should be determined on the basis of the range of substrates explored in
592 each study. For instance, *T. urticae* CYP392A16 has been shown to metabolize abamectin only,
593 although other insecticides belonging to different classes were also tested without detecting
594 metabolism. On the other hand, other P450s may show metabolism to specific xenobiotics,
595 while may or may not be tested for their catalytic activity against other compounds, i.e.
596 CYP6A51 from *C. capitata*, CYP6BQ23 from *B. (M.) aeneus* and others.

597 **6. Conclusions**

598 Arthropods have expanded the limits of P450 sequence diversity, with many variations on the
599 sequences diverse approaches have been used to characterize P450s in arthropods. Each
600 approach comes with unique strengths and weaknesses and most of the time frequently a clear
601 picture can only be drawn upon successful completion of parallel efforts. In principle the P450
602 characterization approaches can be divided into *in situ* approaches e.g. genome editing or RNAi
603 in the species of interest and *ex situ* approaches e.g. *in vivo* ectopic expression in model
604 organisms or heterologous expression *in vitro*. The toolbox in biology has never been richer
605 than it is today. To decipher the contribution of P450s (and other players in pathways)
606 multidisciplinary approaches should be combined. While the *in situ* approaches offer the
607 advantages of the context the danger maybe to overlook compensatory processes (regulation
608 of other genes as an unintended consequence of the primary desired changes). On the other
609 hand, the *ex situ* approaches may be less convincing e.g. low resistance ratios of transgenic *D.*
610 *melanogaster* because the relevant context is missing. Rarely are measured turnover rates *in*
611 *vitro* put in a clear context to what it means for the toxicokinetics *in vivo*. The toolbox that
612 allows us to study single genes/enzymes in detail may distract us with describing the principal
613 component (in the absence of certainty about how many there are) in detail.

614 Studying the evolution and function of arthropod P450s could inspire genomics-based
615 ecotoxicology and pest control research, while the catalytic activity of unusual P450s
616 could serve as an inspiration for green chemistry pesticide leads (Figure 5). For example, bees
617 and pollinators are equipped with P450-based defense that define their sensitivity to
618 insecticides and this knowledge can be exploited to avoid negative off-target effects (Manjon
619 et al., 2018). Indeed, previous studies demonstrated that honeybees exhibit differences in their
620 sensitivity to pesticides, and that certain compounds display very low toxicity to bees,
621 even when used as in-hive treatments against *Varroa* mites (Tomizawa and Casida, 2005). The
622 negative cross-resistance between various insecticide/pro-insecticide classes, due to
623 overproduction of P450s in resistant insects which detoxify certain active ingredients
624 but activates others is another example that has also been explored (Adolfi et al., 2019). P450
625 -biotechnology based applications, including testing pipelines for screening the selectivity and
626 liability of active compounds against insecticidal leads have been considered and/or developed
627 at certain extend, for pest and vector control. These include robust pipelines for standardized
628 microsomal preparations for the monooxygenase blend based metabolism, libraries of
629 recombinant insect (pests and pollinators) P450s, as well as transient and stable reverse genetic
630 approaches in insects and/or insect cell lines. However, the potential of those tools for

631 industrial applications needs to be further validated and can only be realized if sufficient and
632 consistent yields of recombinant proteins are achieved or robust reverse genetic systems or cell
633 based assays are established. With a toolbox as rich as today's we should aim at fusing the
634 knowledge of principle components with the bigger picture. Knocking out a P450 and running
635 a bioassay is a good step, preparing microsomes form the knock-out strain and measuring
636 metabolism vs. wildtype microsomes (and/or *in vivo* metabolism) should become a logical
637 second step to put a finger on the rate of metabolism in the relevant context (where no reliable
638 knock-outs can be achieved a similar principle may be applied to model species). If more
639 holistic studies are conducted in tandem to heterologous expression, it may allow translation
640 of results and bring us closer to extrapolation of toxicity thus enabling *in vitro* screens for
641 desired selectivity based on differential metabolism.

642

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648

649 **Competing interests' statement**
650 The authors have no competing interests to declare
651
652 **Declarations of interest**
653 None

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1330 **Tables**

1331 **Table 1. Species used for the phylogenetic analysis.**

Species	**Insect order**	**Number of P450s**	**Source**
Helicoverpa armigera	Lepidoptera	112	Publicly available; manually curated
Spodoptera littoralis	Lepidoptera	137	Private Syngenta genome; manually curated
Plutella xylostella	Lepidoptera	88	Private Syngenta genome; manually curated
Apis mellifera	Hymenoptera	49	Publicly available; manually curated
Megachile rotundata	Hymenoptera	50	Publicly available; manually curated
Osmia bicornis	Hymenoptera	60	Publicly available; manually curated
Bombus terrestris	Hymenoptera	6*	Publicly available; manually curated
Nasonia vitripennis	Hymenoptera	90	Publicly available; manually curated

1332 * only partially curated

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1344 **Table 2 A-D:** P450s from agricultural insect pest species that are involved in xenobiotic
1345 metabolism (synthetic or natural compounds) and the validation systems (*in vitro*/ *in vivo*/
1346 genome modification) identified in the literature. A. Lepidoptera pest species, B. Non-
1347 Lepidoptera insect pest species, C. Mite and D. Pollinator/*Papilio sp.* species.

A			Synthetic compounds									Natural compounds										Validation systems	
Order	Species	P450	PYR	NN	OC	OX	AV	CAR	OP	DM	DCH	FC	KT	PA	FLV	IA	AFL	CR	CA	CN	GA	*in vitro*	*in vivo/ Genome mod.*
Lepidoptera	*Amyelois transitella*	CYP6AB11										✓										X	
	Chilo suppressalis	CYP6CV5								✓													R
		CYP9A68								✓													R
		CYP321F3								✓													R
		CYP324A12								✓													R
	Cydia pomonella	CYP9A61	✓																			X	
		CYP6B2	✓						✓														R
	Depressaria pastinacella	CYP6AB3										✓										X	
		CYP6AB3v2										✓										X	
		CYP6AE89										✓										X	
	Helicoverpa armigera	CYP337B3v1	✓																			X	
		CYP6B6	✓						✓											✓		X	R
		CYP6B7	✓						✓													X	
		CYP6AE11	✓	✓	✓	✓		✓				✓	✓									X	C
		CYP6AE12	✓		✓			✓				✓										X	C
		CYP6AE14	✓	✓	✓	✓		✓				✓	✓	✓								X	R/C
		CYP6AE15	✓		✓			✓				✓										X	C
		CYP6AE16	✓	✓	✓			✓				✓										X	C
		CYP6AE17	✓	✓	✓	✓		✓				✓										X	C
		CYP6AE18	✓	✓	✓	✓		✓				✓										X	C
		CYP6AE19	✓	✓	✓	✓		✓				✓	✓									X	C
		CYP6AE20	✓			✓						✓	✓										C
		CYP6AE24	✓	✓	✓																	X	
		CYP9A12	✓											✓						✓		X	
		CYP9A14	✓																	✓		X	R
		CYP337B1	✓																			X	
		CYP9A16	✓																			X	
		CYP9A3	✓																			X	
		CYP9A23	✓																			X	
		CYP6B2	✓																			X	
		CYP9A17	✓																	✓		X	
	Helicoverpa zea	CYP6B8	✓		✓			✓	✓			✓			✓	✓			✓			X	
		CYP321A1	✓		✓				✓			✓			✓		✓					X	
		CYP6B27	✓		✓			✓	✓													X	
	Plutella xylostella	CYP6BG1	✓							✓													R/D
		CYP321E1								✓													R
		CYP340W1					✓																R
	Spodoptera frugi-perda	CYP321B1								✓													R
		CYP321A8								✓													R
		CYP321A9								✓													R
	Spodoptera exigua	CYP9A105	✓																				R
		CYP6AE10	✓												✓								R
		CYP6AB14	✓																				R
		CYP9A98	✓																				R
		CYP9A10	✓																				R
		CYP9A21v3								✓													R
		CYP332A1							✓													X	D
		CYP321A8	✓						✓													X	D
		CYP321A16							✓													X	D

		CYP9A186					✓															X	C
	Spodoptera litura	CYP321B1	✓						✓														R
		CYP6B50										✓											R
		CYP6AB14										✓			✓			✓					R
		CYP9A40	✓								✓				✓				✓				R
		CYP6AB60																✓			✓		R
		CYP6AB12	✓																				R

PYR:Pyrethroids; NN:Neonicotinoids;; OC: Organochlorines; OX: Oxadiazines; AV:Avermectins; CAR: Carbamates; OP:Organophosphorus pesticides; DM:Diamides DCH: Diacylhydrazine ; FC: Furanocoumarins; KT: Ketones; PA: Phenolic aldehydes ; FLV: Flavonoids; IA: Indolyl alcohols; AFL: Aflatoxins; CR:Coumarin; CA: Cinnamate ester; CN: Capsaicinoids; GA: Glycoalcanoids X: validated with in vitro systems; C: Validated with the use of CRISPR; D : Validated with the use of D. melanogaster heterologous expression system; R: Validated with the use of the RNAi system

B			**Synthetic compounds**													**Natural compounds**			**Validation systems**	
Order	**Species**	**P450**	**PYR**	**NN**	**SUL**	**CAR**	**OP**	**PPF**	**BF**	**TTA der**	**PA der**	**DM**	**PHP**	**BTL**	**PHO**	**T**	**AL**	**EO**	***in vitro***	***in vivo/ Genome mod.***
Homoptera	*Sogatella furcifera*	CYP6FD1			✓															R
		CYP6ER4			✓															R
		CYP4FD2			✓															R
Hemiptera	*Aphis gossypii*	CYP380C6								✓										R
		CYP6A2	✓							✓										R
		CYP6CY13		✓	✓														X	R
		CYP6CY14		✓																R
		CYP6CY19			✓															R
		CYP6CY22		✓															X	R
		CYP6UN1		✓																R
		CYP6DC1		✓																R
		CYP6CZ1		✓																R
	Bemisia tabaci	CYP6CM1*		✓				✓			✓			✓					X	R/D
		CYP6CX4		✓				✓			✓			✓						R
	Diaphorina citri	CYP4C67		✓																R
		CYP4DA1		✓																R
		CYP4C68		✓																R
		CYP4G70		✓																R
		CYP4DB1		✓																R
	Laodelphax striatellus	CYP6AY3v2		✓															X	D
		CYP6FU1	✓																X	
		CYP353D1v2		✓					✓										X	
		CYP417A2v2		✓															X	
		CYP439A1v3	✓																X	
		CYP6CW1							✓		✓									R
		CYP4DE1											✓							R
		CYP6CW3v2											✓							R
		CYP4C71		✓															X	
	Myzus persicae	CYP6CY3		✓													✓		X	D
	Nilaparvata lugens	CYP6AY1		✓			✓												X	R
		CYP6ER1**		✓	✓									✓					X	R/D
		CYP4CE1		✓															X	R
		CYP6CW1		✓															X	R
		CYP4DE1		✓			✓													R
		CYP353D1		✓			✓													R
		CYP439A1		✓			✓													R
		CYP6CS1		✓							✓									R/D
Diptera	*Bradysia odoriphaga*	CYP9b2		✓																R
		CYP49a1		✓																R
		CYP12b1		✓																R
		CYP6FV12		✓																R
		CYP3356A1	✓	✓																R
	Ceratitis capitata	CYP6A51	✓																X	D
Coleoptera	*Leptinotarsa*	CYP4Q3		✓																R

	decemlineata	CYP9e2-like		✓																R
		CYP6BJ[a/b]		✓															X	R
		CYP6BJ1v1		✓															X	R
		CYP9Z25		✓															X	R
		CYP9Z26		✓																R
		CYP9Z29		✓															X	R
		CYP350D1										✓								R
	Brassicogethes (M.) aeneus	CYP6BQ23	✓																X	D
	Sitophilus zeamais	CYP6MS1														✓				R
	Tribolium castaneum	CYP6BQ9	✓																X	R/D
		CYP346													✓					R
		CYP4BN6													✓			✓		R
		CYP6BQJ																✓		R
Orthoptera	*Locusta migratoria*	CYP6FD1	✓																X	
		CYP6FF1	✓																	R
		CYP6FD2				✓														R
		CYP6FE1				✓														R
		CYP4G102	✓			✓	✓													R
		CYP4G62	✓			✓	✓													R
		CYP9AQ2	✓																	R
		CYP409A1	✓																	R
		CYP408B1	✓																	R
		CYP9AQ1	✓																	R
		CYP9A3	✓																	R
		CYP6HC1	✓																	R
		CYP6HL1	✓			✓														R
		CYP6HN1	✓			✓														R
		CYP6HQ1	✓			✓														R
		CYP303A1	✓			✓	✓													R

*CYP6CM1 includes CYP6CM1vB/ CYP6CM1-B/ CYP6CM1vQ **CYP6ER1 includes CYP6ER1-del3 and CYP6ER1-T318Sdel3.

PYR:Pyrethroids; NN:Neonicotinoids; SUL: Sulfoximines; CAR: Carbamates; OP:Organophosphorus pesticides; PPF: Pyriproxifen; F:Bu-profezin; TTA-der:Tetronoc and tetramic acid derivatives; PA-der: Pyridine azomethine derivatives; DM: Diamides; PHP: Phenylpyrazoles; BTL: Butenolides; T: Terpineol; AL:Alkaloids; Phosph: Phosphine; EO: Essential oils X: validated with in vitro and/or in vivo systems; D : Validated with the use of D. melanogaster heterologous expression system; R: Validated with the use of the RNAi system.

C			**Synthetic compounds**						**Validation systems**	
Order	**Species**	**P450**	**PYR**	**AV**	**TTA der**	**b-KT**	**CRN**	**METI**	***in vitro***	***in vivo/ Genome mod.***
Trombidiformes	*Tetranychus cinnabarinus*	CYP389C16				✓		✓	X	R
		CYP389B1	✓							R
		CYP392A26	✓							R
		CYP391A1	✓							R
		CYP384A1	✓							R
		CYP392D11	✓							R
		CYP392A28	✓							R
	Tetranychus urticae	CYP392A16		✓			✓		X	D
		CYP392A11		✓				✓	X	R/D
		CYP392E10			✓				X	
		CYP392A12		✓						R
		CYP392D8		✓						R
		CYP389C10		✓						R

PYR:Pyrethroids; AV: Avermectins; TTA der:Tetronoc and tetramic acid derivatives; b-KT: beta ketonitrile derivatives; CRN: Carboxanilides; METI: Mitochondrial complex I electron transport inhibitors

X: validated with *in vitro* systems;; D : Validated with the use of *D. melanogaster* heterologous expression system; R: Validated with the use of the RNAi

D			Synthetic compounds					Natural Compounds		Validation systems	
Order	**Species**	**P450**	**PYR**	**NN**	**OP**	**BTL**	**VL**	**FC**	**FLV**	***in vitro***	***in vivo/ Genome mod.***
Hymenoptera	*Apis cerana cerana*	CYP301A1	✓	✓	✓		✓				R
		CYP303A1	✓	✓	✓		✓				R
		CYP306A1	✓	✓	✓		✓				R
	Apis mellifera	CYP9Q1	✓	✓	✓				✓	X	
		CYP9Q2	✓	✓	✓	✓			✓	X	D
		CYP9Q3	✓	✓	✓	✓			✓	X	D
		CYP6AS1							✓	X	
		CYP6AQ1				✓				X	D
		CYP6AS3							✓	X	
		CYP6AS4							✓	X	
		CYP6AS10							✓	X	
	Bombus terrestris	CYP9Q4		✓						X	D
		CYP9Q5		✓						X	
		CYP9Q6		✓						X	D
	Osmia bicornis	CYP9BU1		✓						X	D
		CYP9BU2		✓						X	
Lepidoptera	*Papilio polyxenes*	CYP6B1			✓			✓	✓	X	
		CYP6B3						✓	✓	X	
	Papilio glaucus	CYP6B4						✓		X	
		CYP6B17						✓		X	
		CYP6B21						✓		X	
	Papilio canadensis	CYP6B25						✓		X	
	Papilio multicaudatus	CYP6B33						✓		X	

PYR:Pyrethroids; NN:Neonicotinoids;; OP: Organophosphates; BTL: Butenolides; VL:Viologen; FC: Furanocoumarins; FLV: Flavonoids
X: validated with *in vitro* systems;; D : Validated with the use of *D. melanogaster* heterologous expression system; R: Validated with the use of RNAi

1385 **Figure Legends**

1386 **Figure 1:** Heat map of the studied P450s per species and the number of scientific studies re-
1387 ferring to them. **A.** Lepidoptera pest species **B.** Non-Lepidoptera pest species. **C.** Mite pest
1388 species and **D.** Pollinators/*Papilio* sp. The scale bar indicates the amount of studies referring
1389 to each P450 within each insect category.
1390

1391 **Figure 2:** Phylogenetic analysis of Lepidoptera and Hymenoptera P450s. **A.** Phylogenetic
1392 analysis of Lepidoptera P450s from three major pest species; *H. armigera* (Harmi), *S. littoralis*
1393 (Slitt), and *P. xylostella* (Pxylo). Virtually all known insecticide metabolizers are found in
1394 Clan3. This is in contrast to the P450s implicated in physiological functions that are almost
1395 equally distributed across all four clans. **B.** Phylogenetic analysis of hymenopteran P450s from
1396 five species; *A. mellifera* (Amell), *B. terrestris* (Bterr), *O. bicornis* (Obico), *M. rotundata*
1397 (Mrotu), and *N. vitripennis* (Nvitr). Similarly to Lepidoptera, most insecticide metabolizers are
1398 found in Clan 3, whereas CYPs involved in physiological functions are found in the remaining
1399 clans. The shapes that are next to the genes indicate the following: black circles – P450s
1400 involved in the metabolism of insecticides; black triangles – P450s involved in known
1401 physiological functions; black stars – P450s belonging to families with few members, and are
1402 thus suspected to be involved in physiological functions; red stars – same as in the previous
1403 category, but these are also Lepidoptera or Hymenoptera-specific, respectively. Branch color
1404 denotes CYP clans: cyan – Clan2; yellow – Mito; orange – Clan4; green – Clan3.

1405 **Figure 3:** Venn graphs depicting the number of functionally characterized P450s with *in vivo*
1406 and *in vitro* systems. *In vivo* functional characterization has been categorized into RNAi and
1407 genome modification with the latter one including CRISPR and transgenic *Drosophila*
1408 *melanogaster*: **A.** Total species, **B.** Lepidoptera insect pest species, **C.** Non-Lepidoptera insect
1409 pest species, **D.** Mite pest species, **E.** Pollinator/ *Papilio sp.* species.

1410 **Figure 4:** Percentage of studies* that characterize P450s of insects and mites of economic and
1411 public health interest (including Lepidoptera species, other insect species, mite species,
1412 pollinators/*Papilio sp.* and mosquito species (*Anopheles* and *Aedes*)) using different *in vitro*
1413 systems (*E.coli*, insect cells- baculovirus mediated, yeast and stable insect cells). **A.** Total insect
1414 species, **B.** Lepidoptera insect pest species, **C.** non -Lepidoptera insect pest species, **D.** mite
1415 pest species, **E.** pollinator/ *Papilio sp.* species and **F.** mosquito species.

1416 *Studies that use two or more *in vitro* validation systems are considered as two or more
1417 different studies. Also a study for two or more P450s are considered as two or more different
1418 studies.

1419 **Figure 5:** *Left*: novel highly selective insecticides and synergists; **A**. **Synergists** (green
1420 structure with bar) that are not intrinsically toxic, will inhibit the target species CYPs (blue), to
1421 increase / maintain the effectiveness of the insecticides (green chemical); **B**. **Pro-drug** (green
1422 chemicals) will only become toxic, when enzymatically activated inside target insects (red
1423 dots), by specialized and resistance conferring P450s; **C. Safe chemicals**: synergists & pro-
1424 insecticides will be non-toxic to bees and non- target organisms, since they will be readily
1425 degraded by their P450s. *Right*: Innovative protein and cell biotechnology based tools for
1426 insecticide development; **D. High-throughput plate with immobilised enzymes, cells, or**
1427 **micro-patterned cells** for screening metabolic liability/activation, toxicity and selectivity of
1428 low risk/safe (pro)insecticides.

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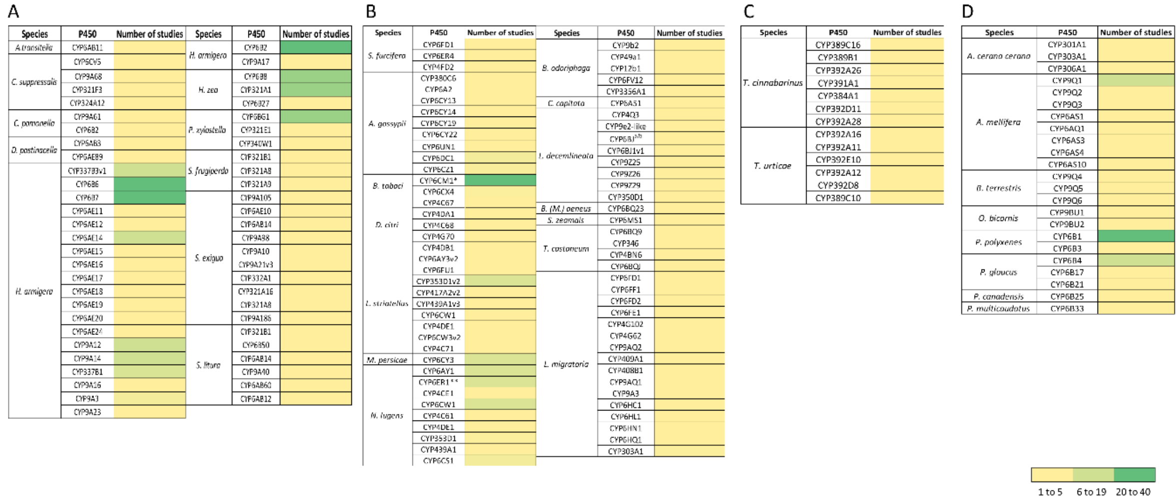
1443 **Figures**



A

Species	P450	Number of studies
A.transitella	CYP6AB11	
C. suppressalis	CYP6CV5	
	CYP9A68	
	CYP321F3	
	CYP324A12	
C. pomonella	CYP9A61	
	CYP6B2	
D. pastinacella	CYP6AB3	
	CYP6AE89	
H. armigera	CYP337B3v1	
	CYP6B6	
	CYP6B7	
	CYP6AE11	
	CYP6AE12	
	CYP6AE14	
	CYP6AE15	
	CYP6AE16	
	CYP6AE17	
	CYP6AE18	
	CYP6AE19	
	CYP6AE20	
	CYP6AE24	
	CYP9A12	
	CYP9A14	
	CYP337B1	
	CYP9A16	
	CYP9A3	
	CYP9A23	

Species	P450	Number of studies
H. armigera	CYP6B2	
	CYP9A17	
H. zea	CYP6B8	
	CYP321A1	
	CYP6B27	
P. xylostella	CYP6BG1	
	CYP321E1	
	CYP340W1	
S. frugiperda	CYP321B1	
	CYP321A8	
	CYP321A9	
S. exigua	CYP9A105	
	CYP6AE10	
	CYP6AB14	
	CYP9A98	
	CYP9A10	
	CYP9A21v3	
	CYP332A1	
	CYP321A16	
	CYP321A8	
	CYP9A186	
S. litura	CYP321B1	
	CYP6B50	
	CYP6AB14	
	CYP9A40	
	CYP6AB60	
	CYP6AB12	

B

Species	P450	Number of studies
S. furcifera	CYP6FD1	
	CYP6ER4	
	CYP4FD2	
A. gossypii	CYP380C6	
	CYP6A2	
	CYP6CY13	
	CYP6CY14	
	CYP6CY19	
	CYP6CY22	
	CYP6UN1	
	CYP6DC1	
	CYP6CZ1	
B. tabaci	CYP6CM1*	
D. citri	CYP6CX4	
	CYP4C67	
	CYP4DA1	
	CYP4C68	
	CYP4G70	
	CYP4DB1	
	CYP6AY3v2	
	CYP6FU1	
L. striatellus	CYP353D1v2	
	CYP417A2v2	
	CYP439A1v3	
	CYP6CW1	
	CYP4DE1	
	CYP6CW3v2	
	CYP4C71	
M. persicae	CYP6CY3	
N. lugens	CYP6AY1	
	CYP6ER1**	
	CYP4CE1	
	CYP6CW1	
	CYP4C61	
	CYP4DE1	
	CYP353D1	
	CYP439A1	
	CYP6CS1	

Species	P450	Number of studies
B. odoriphaga	CYP9b2	
	CYP49a1	
	CYP12b1	
	CYP6FV12	
	CYP3356A1	
C. capitata	CYP6A51	
L. decemlineata	CYP4Q3	
	CYP9e2-like	
	CYP6BJ[a/b]	
	CYP6BJ1v1	
	CYP9Z25	
	CYP9Z26	
	CYP9Z29	
	CYP350D1	
B. (M.) aeneus	CYP6BQ23	
S. zeamais	CYP6MS1	
T. castaneum	CYP6BQ9	
	CYP346	
	CYP4BN6	
	CYP6BQ	
L. migratoria	CYP6FD1	
	CYP6FF1	
	CYP6FD2	
	CYP6FE1	
	CYP4G102	
	CYP4G62	
	CYP9AQ2	
	CYP409A1	
	CYP408B1	
	CYP9AQ1	
	CYP9A3	
	CYP6HC1	
	CYP6HL1	
	CYP6HN1	
	CYP6HQ1	
	CYP303A1	

C

Species	P450	Number of studies
T. cinnabarinus	CYP389C16	
	CYP389B1	
	CYP392A26	
	CYP391A1	
	CYP384A1	
	CYP392D11	
	CYP392A28	
T. urticae	CYP392A16	
	CYP392A11	
	CYP392E10	
	CYP392A12	
	CYP392D8	
	CYP389C10	

D

Species	P450	Number of studies
A. cerana cerana	CYP301A1	
	CYP303A1	
	CYP306A1	
A. mellifera	CYP9Q1	
	CYP9Q2	
	CYP9Q3	
	CYP6AS1	
	CYP6AQ1	
	CYP6AS3	
	CYP6AS4	
	CYP6AS10	
B. terrestris	CYP9Q4	
	CYP9Q5	
	CYP9Q6	
O. bicornis	CYP9BU1	
	CYP9BU2	
P. polyxenes	CYP6B1	
	CYP6B3	
P. glaucus	CYP6B4	
	CYP6B17	
	CYP6B21	
P. canadensis	CYP6B25	
P. multicaudatus	CYP6B33	

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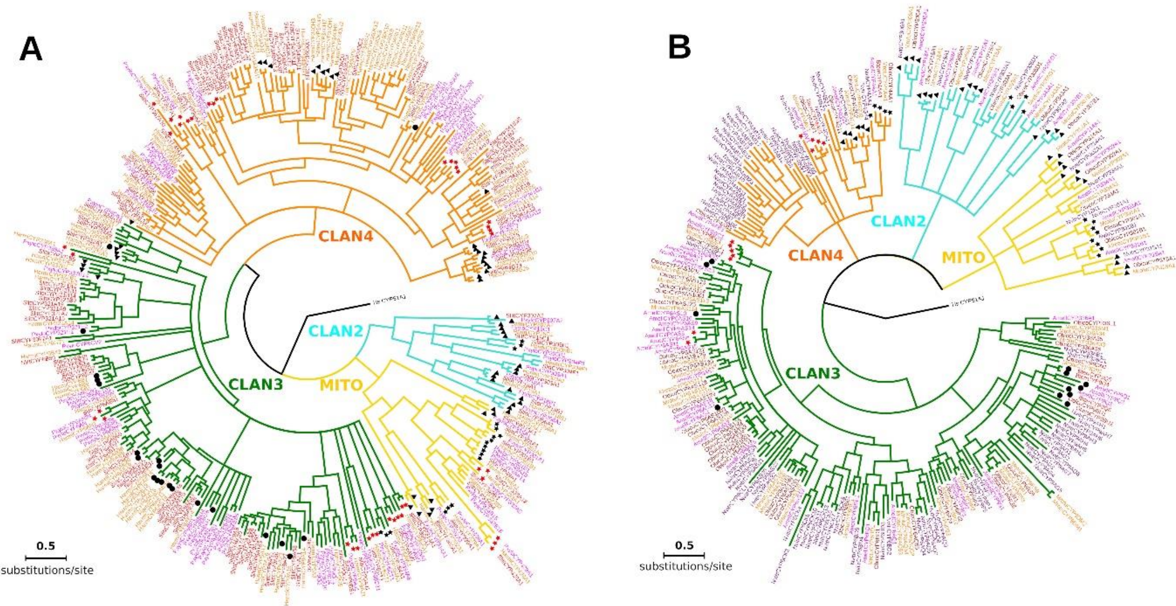
1445 **Figure 1**



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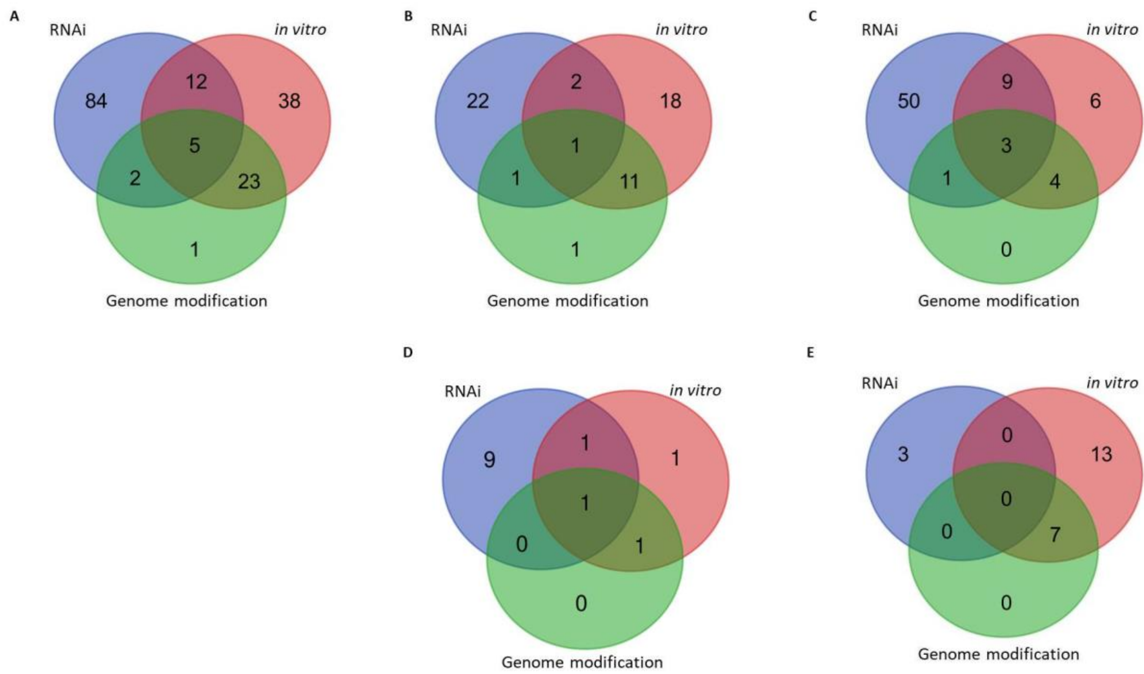
1447 **Figure 2**



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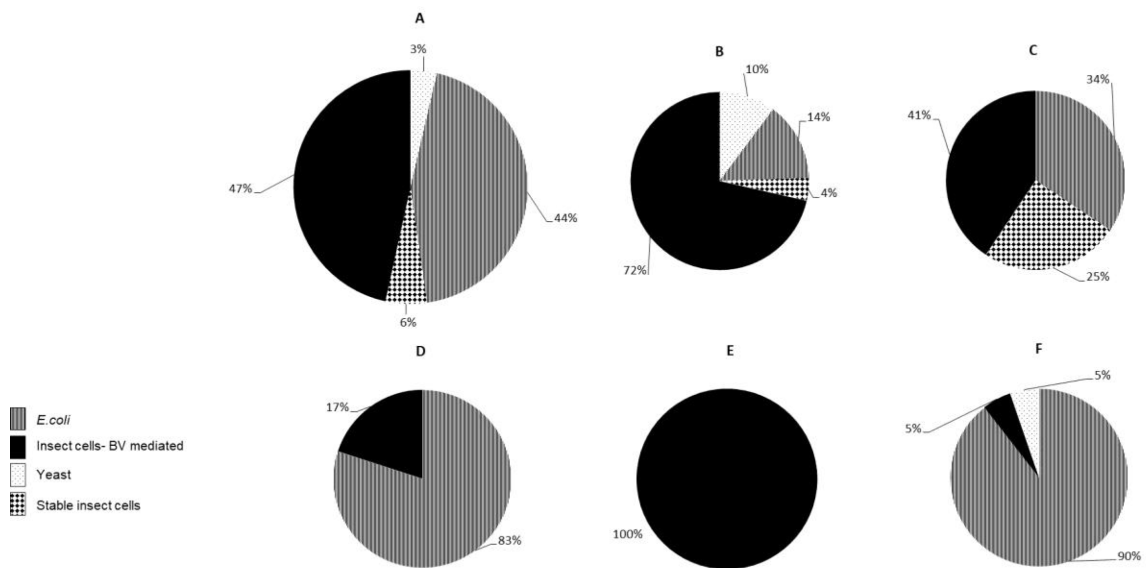
1449 **Figure 3**



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1451 **Figure 4**

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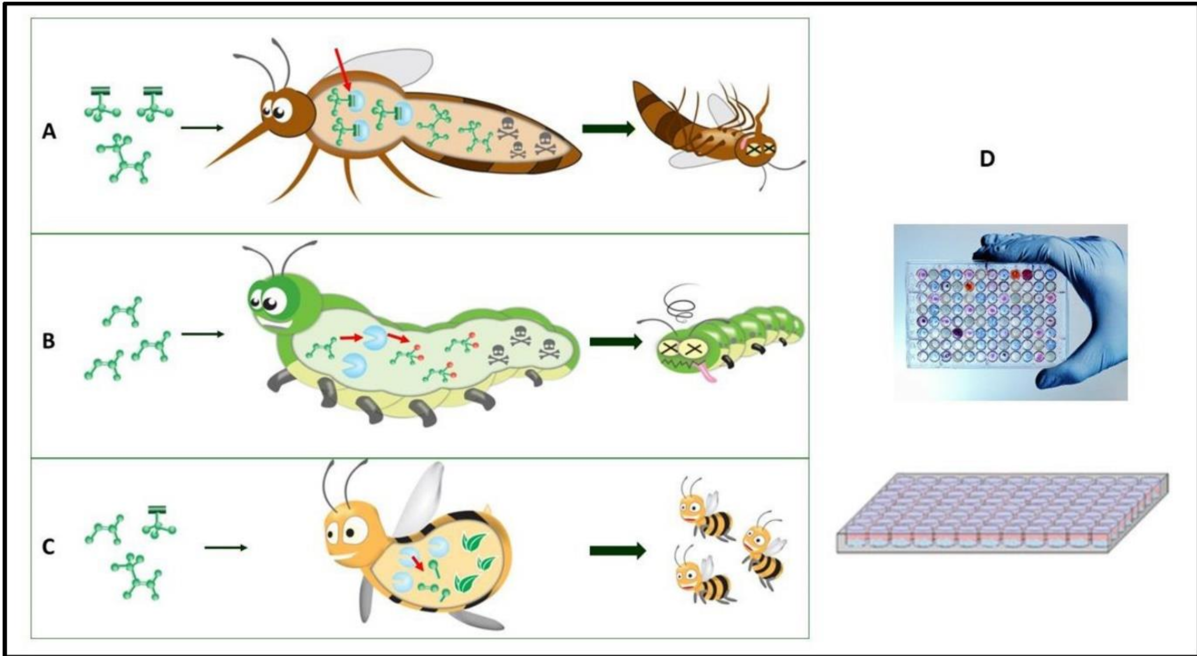

1453 **Figure 5**