The leafcutter bee, *Megachile rotundata* is more sensitive to *N*-cyano neonicotinoid and butenolide insecticides than other managed bees

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Abstract

Recent research has shown that several managed bee species have specific P450 enzymes that are preadapted to confer intrinsic tolerance to some insecticides including certain neonicotinoids. However, the universality of this finding across managed bee pollinators is unclear. Here we show that the leafcutter bee, *Megachile rotundata*, lacks such P450s, and is >2500-fold more sensitive to the neonicotinoid thiacloprid and 170-fold more sensitive to the butenolide flupyradifurone than other managed bee pollinators. These findings have significant implications for the safe use of insecticides in crops where *M. rotundata* is used for pollination, and ensuring regulatory pesticide risk assessment frameworks are protective of this species.

Main

In common with other insects, bees have evolved biotransformation systems to metabolize many of the natural xenobiotics encountered in their environment into non-toxic compounds [1]. Recent research on three managed bee species, namely western honey bees, *Apis mellifera*, buff-tailed bumblebees, *Bombus terrestris*, and red mason bees, *Osmia bicornis*, has demonstrated that specific enzymes within these metabolic pathways can also be critically important in determining the sensitivity of bees to insecticides [2-4]. Specifically, cytochrome P450 enzymes belonging to the CYP9Q and CYP9BU subfamilies have been shown to provide protection to certain insecticides from three different classes including *N*-cyanoamidine neonicotinoids [2,3]. This leads to an important question – is the presence of insecticide-degrading P450s universal to all bee species, and if not, what are the implications for insecticide sensitivity in species that lack these enzymes? To address this question we used phylogenetic, toxicological and biochemical approaches to characterise the phenotypic and metabolic response of the alfalfa leafcutter bee*, M. rotundata*, to select insecticides. *M. rotundata* is one of the most economically important managed solitary bee pollinators worldwide [5]. This species is principally used as a commercial pollinator in alfalfa seed production (*Medicago sativa*), with secondary uses in the pollination of canola (*Brassica napus*) and lowbush blueberries (*Vaccinium angustifolium*) [5-7].

We first asked if the genome of *M. rotundata* encodes P450s belonging, or closely related to, the CYP9Q and CYP9BU subfamilies that metabolise certain insecticides in other managed bee species. Forty-nine full length P450 genes were curated from the sequenced genome of *M. rotundata* and named by the P450 nomenclature committee (Table S1). Phylogenetic analyses revealed that the CYPome of *M. rotundata*, *A. mellifera*, *B. terrestris* and *O. bicornis* have 1:1 orthologs for all members of the mitochondrial CYP clan, and clans 2 and 4 are almost identical (Table S2). The major differences in the CYPomes of the species are found in clan 3, with the CYP9 family showing the greatest interspecies variation (Table S2, Fig. 1a). A Maximum Likelihood phylogeny of the CYP9 family using P450s derived from the

genome sequences of 12 bee species [2,8] is shown in Fig. 1b. All 9 species from the Apidae family have CYP9Q genes, with the most basal of the species, *Habropoda laboriosa*, having only one gene member (CYP9Q9). *O. bicornis* has 2 CYP9BU genes and *Dufourea novaeangliae* 4 CYP9DL genes that share a common ancestor with the Apidae CYP9Q subfamily, with the relative time for divergence of these subfamilies to the CYP9Q subfamily estimated as 0.32 and 0.72 respectively (Fig. 1b). *M. rotundata* is the only species that has no CYP9Q or closely related gene (Fig. 1b).

The finding that *M. rotundata* lacks P450s belonging, or related to, the CYP9Q subfamily raises important questions about the capacity of this species to metabolise and, by extension tolerate, certain pesticides. To explore this we examined the sensitivity of *M. rotundata* to the *N*-nitroguanidine neonicotinoid imidacloprid, the *N*-cyanoamidine neonicotinoid thiacloprid and the butenolide insecticide flupyradifurone. While imidacloprid is highly toxic to honey bees, bumblebees and mason bees, thiacloprid and flupyradifurone are classified as practically non-toxic to all three species in acute contact bioassays [2,3,9] (Fig. 2a). In the case of the two neonicotinoids previous work has demonstrated that this differential toxicity results from marked differences in the efficiency of their metabolism by honey bee and bumblebee P450s belonging to the CYP9Q subfamily, and red mason bee P450s belonging to the CYP9BU subfamily [2,3].

As shown in Fig. 2a all three compounds were found to be highly toxic to *M. rotundata* (contact-LD_{50} < 2µg/bee) in acute insecticide bioassays, with uncertainty in the actual endpoints, given the non-standardized nature of the employed test method. Notably, this included thiacloprid and flupyradifurone, with *M. rotundata* >2500-fold more sensitive to the former and 170-fold more sensitive to the latter than honey bees, bumblebees and mason bees tested using comparable methodologies. Furthermore, while the three latter species exhibit marked differences (500-2000-fold) in their sensitivity to thiacloprid and imidacloprid [2,3], only a 15-fold difference is seen in the sensitivity of *M. rotundata* to these two

84 neonicotinoids. These results clearly demonstrate that the intrinsic tolerance of other
85 managed bee pollinators to thiacloprid and flupyradifurone is not observed in *M. rotundata*.
86
87 One possible explanation for the lack of tolerance of *M. rotundata* to thiacloprid and
88 flupyradifurone is an increased affinity of these compounds for the target receptor, the
89 nicotinic acetylcholine receptor (nAChR), of this species relative to other bee pollinators. To
90 explore this we conducted radioligand binding studies of *M. rotundata* head membrane
91 preparations and examined the displacement of tritiated imidacloprid by unlabeled
92 imidacloprid, thiacloprid and flupyradifurone. All three compounds reversibly bind with
93 nanomolar affinity generating similar IC_{50} values to those reported previously for honey bees
94 (Fig. 2b). Furthermore, in common with prior studies on other managed bee pollinators [2,3] no
95 significant difference was observed in the specific binding of the three compounds at the
96 receptor (Fig. 2b). Thus, the lack of tolerance of *M. rotundata* to thiacloprid and
97 flupyradifurone is not a consequence of an enhanced affinity of these compounds for the *M.*
98 *rotundata* nAChR relative to other bee species.
99
100 To investigate the functional significance of the absence of CYP9Q and CYP9BU P450s in
101 *M. rotundata* on insecticide metabolism, we examined the capacity of native microsomes (a
102 source of total cytochrome P450s localized in the endoplasmic reticulum) to metabolise
103 imidacloprid, thiacloprid and flupyradifurone. We also included *tau*-fluvalinate in this
104 analysis, as it is metabolised by CYP9Q P450s in honey bees [4], and the alkaloid nicotine - a
105 potent natural insecticide also acting on nAChRs. Incubation of microsomal preparations
106 from *M. rotundata* with each compound and analysis of parent compound depletion by liquid
107 chromatography tandem mass spectrometry (LC-MS/MS) revealed no significant
108 metabolism of any of the four synthetic insecticides (Fig. 2c). In contrast, the alkaloid
109 nicotine was significantly and rapidly metabolised by *M. rotundata* microsomes (~40% of
110 parent compound depleted in 1h; $p<0.0001$). These findings demonstrate that while P450s
111 of this species have the capacity to break down a natural xenobiotic they appear to lack the

112 capacity to break down synthetic insecticides of different chemical classes that are
113 effectively metabolised by other bee species.
114
115 In summary our data demonstrate that P450 enzymes that are preadapted to detoxify certain
116 insecticides are not ubiquitous across all managed bee species. The absence of P450s
117 belonging to, or closely related to, the CYP9Q subfamily in *M. rotundata* is correlated with an
118 inability of microsomal P450s of this species to metabolise these insecticides *in vitro* and
119 high sensitivity to these compounds *in vivo*. These results have important practical
120 implications for assessing the compatibility of insecticides in crops where *M. rotundata*
121 provides an important pollination service. Specifically, they demonstrate that direct contact
122 with thiacloprid and flupyradifurone poses a greater hazard to this species than other
123 managed bees. Thus, the application method and timing of these insecticides to crops
124 dependent on this species for pollination should be considered. In alfalfa, where *M.*
125 *rotundata* is a principal commercial pollinator used for seed production, thiacloprid is not
126 registered, whereas flupyradifurone has been registered for forage, fodder, hay and straw
127 production. In these uses the alfalfa is harvested before flowering, which effectively mitigates
128 the risk to *M. rotundata*; moreover, alfalfa for seed production is not on the commercial
129 flupyradifurone label. Further work is required to establish if *M. rotundata* is also highly
130 sensitive to other insecticides that show low toxicity to other managed bee species. In this
131 regard we show that microsomal preparations of this species do not metabolise the
132 pyrethroid *tau*-fluvalinate, suggesting it may also have high acute toxicity to this species.
133 Furthermore, as *Megachile* is one of the largest genera of bees (1,500 species worldwide),
134 further research is required to establish if other wild species within this genus also lack
135 P450s that provide protection against certain insecticides. In this regard our study illustrates
136 the utility of phylogenetic analyses of enzyme superfamilies in combination with targeted
137 functional analyses to predict the capability of bee pollinator species to break down synthetic
138 insecticides, and hence to predict their likely sensitivity. Finally, since the use of other
139 managed bee species as a proxy for *M. rotundata* in risk assessment appears to be

140 unreliable for some insecticides it is important to invest in further bee toxicogenomic
141 research so that crop-pollination, managed pollination activities and bee safety is not
142 impaired.
143

144 **Methods**

145 **Phylogenetic analysis**

146 Sequences encoding *M. rotundata* P450s were identified and assembled using three
147 separate interrogations of the NCBI protein database. G1: assembled all annotated CYPs
148 from the database; G2: assembled CYP gene clusters or orthogroups and G3: assembled
149 the results of a BLASTp search of the *M. rotundata* proteome using annotated *A. mellifera*
150 CYPs as query sequences. The resulting sequences were manually curated and aligned to
151 those of 11 other bee species (*A. cerana, A. dorsata, A. florea, A. mellifera, B. impatiens, B.*
152 *terrestris, D. novaeangliae, E. mexicana, H. laboriosa, M. quadrifasciata and O. bicornis*)
153 using CYPcam (camphor hydroxylase from *Pseudomonas putida* (>gi|117297|sp|P00183.2|
154 Cytochrome CYP-cam; CPXA_PSEPU) [10] as an outgroup in Geneious (Version 10.2.3,
155 Biomatters) using MUSCLE [11] (default settings). Parameters including proportion of variable
156 sites (I) and gamma rate (G) were optimized using amino acid substitution matrices LG
157 based on minimum Bayesian information criterion [12]. Phylogeny was estimated using
158 Maximum likelihood (branch lengths in relative time) and Bayesian inference algorithms [13,14].

159 **Acute contact insecticide assays**

160 All acute contact insecticide assays were performed on female bees following the general
161 guidance of the Organisation for Economic Cooperation and Development (OECD) guideline
162 No. 214 for *A. mellifera* [15], with reference to the International Commission for Plant Pollinator
163 Relationships (ICPPR) solitary bee, acute contact toxicity test protocol [16]. Bioassays on *M.*
164 *rotundata* using the neonicotinoids thiacloprid and imidacloprid were performed in the UK
165 and compared with data for *A. mellifera*, *B. terrestris* and *O. bicornis* (Fig. 2a) generated in a

previous study (2, 3) using identical methods and conditions. This allowed robust comparisons to be made between and within these bee species for these compounds. Bioassays on *M. rotundata* and *Osmia bicornis* using the butenolide insecticide flupyradifurone were conducted in Germany, and compared with data for *A. mellifera* and *B. terrestris* (Fig. 2a) generated in a previous study [17] using analogous methods and conditions to allow robust comparisons between bee species for this compound. Commercially available *M. rotundata* cocoons were obtained from Canada through Bayer AG CropScience Division, Monheim, Germany. *O. bicornis* cocoons were obtained from Dr. Schubert Plant Breeding (Landsberg, Germany). Cocoons were stored on arrival at 4°C in constant darkness. To trigger emergence, cocoons were warmed for 24 h (24°C± 2°C; 55% RH; L16:D8). Cocoons were then transferred to an incubator (*O. bicornis*: 25°C± 1°C; 55% RH; L0:D24; *M. rotundata*: 30°C± 1°C; 55% RH; L0:D24) to allow bees to emerge. Males (which are usually first to emerge) were removed and discarded to allow only non-mated females to populate the test cages. Emerged females were removed from the emerging boxes and placed into test cages and kept under test conditions (24 ± 2°C; 55% RH and L16:D8) until enough bees were collected to populate a test replicate (60-70). Bees were fed *ad libitum* with sucrose solution in water with a final concentration of 500 g/L (50% w/v). Female bees (24-48 h old) were anaesthetised with CO_2 for 10-15 seconds to allow for the application of insecticide (technical grade imidacloprid or thiacloprid (Sigma), or formulated flupyradifurone (commercial product: Sivanto®Prime/Sivanto™ 200 SL)), or insecticide diluent alone (in the case of controls) by topical application with a hand held microapplicator (Hamilton repeating dispenser PB600-1). A volume of 1 µl of test substance solution was applied to the dorsal side of the thorax of each bee. Six concentrations of each insecticide were tested (spanning 0.000256 µg ai/bee – 0.05 µg ai/bee) with three replicates of 10 bees tested for each concentration. Mortality was assessed at 48 h post application for thiacloprid and imidacloprid and at 72 h for flupyradifurone. Bioassays with control mortality >10% were excluded from analysis. The relationship between concentration and mortality was determined using probit analysis with LD_{50} values and their respective 95% confidence

interval values calculated in Le Ora Software PoloPus (Version 1.0) and IBM SPSS (Version 24; Table S3). In relation to the use of bioassay guidelines developed for *A. mellifera* in this study, a previous meta-analysis of insecticide sensitivity data generated from 19 bee species (including *M. rotundata*), using different bioassay methods, revealed that the sensitivity ratio of non-honey bee species to the honey bee was below 10 for 95% of the cases [18].

Receptor binding studies

[^{3}H]imidacloprid (specific activity 1.406 GBq μmol^{-1}) displacement studies were conducted using membrane preparations isolated from frozen (-80°C) *M. rotundata* heads, following previously published protocols [19]. Briefly, bee heads weighing ~10 g were homogenized in 200 ml ice-cold 0.1 M potassium phosphate buffer, pH 7.4 containing 95 mM sucrose using a motor-driven Ultra Turrax blender. The homogenate was then centrifuged for 10 min at 1200 g and the resulting supernatant filtered through five layers of cheesecloth with protein concentration determined using Bradford reagent (Sigma) and bovine serum albumin (BSA) as a reference. Assays were performed in a 96-well microtitre plate with bonded GF/C filter membrane (Packard UniFilter-96, GF/C) and consisted of 200 μl of homogenate (0.48 mg protein), 25 μl of [^{3}H]imidacloprid (576 pM) and 25 μl of competing ligand. Ligand concentrations used ranged from 0.001 to 10, 000 nM and were tested in triplicate per competition assay. The assay was started by the addition of homogenate and incubated for 60 min at room temperature. Bound [^{3}H]imidacloprid was quantified by filtration into a second 96-well filter plate (conditioned with ice-cold 100 mM potassium phosphate buffer, pH 7.4 (including BSA 5 g litre-1)) using a commercial cell harvester (Brandel). After three washing steps (1 ml each) with buffer the 96-well filter plates were dried overnight. Each well was then loaded with 25 μl of scintillation cocktail (Microszint-O-Filtercount, Packard) and the plate counted in a Topcount scintillation counter (Packard). Non-specific binding was determined using a final concentration of 10 μM unlabelled imidacloprid. All binding experiments were repeated twice using three replicates per tested ligand concentration. Data were analysed using a 4 parameter logistic non-linear fitting routine (GraphPad Prism

version 7 (GraphPad Software)) in order to calculate IC_{50}-values (concentration of unlabelled ligand displacing 50% of [^{3}H]imidacloprid from its binding site).

Metabolism assays and UPLC-MS/MS analysis

Microsomes were prepared from approximately 60 adult female *M. rotundata* following a standard protocol of homogenisation and differential centrifugation [20]. The protein concentration of microsome preparations was determined using Bradford reagent (Sigma) and bovine serum albumin (BSA) as a reference. Native microsomes (160 µg/well) were incubated for 1 hour (with shaking) with insecticide substrates (10 µM) in a total assay volume of 200 µl at 30±1^{o}C, in the presence or absence of an NADPH regeneration system. Three replicates were performed for each data point. Samples incubated without NADPH served as controls. The reactions were terminated by the addition of ice-cold acetonitrile (to 80% final concentration), centrifuged for 10 min at 3,000 g and the supernatant subsequently analysed by tandem mass spectrometry as described previously [21]. LC-MS/MS analysis was performed on a Waters Acquity UPLC coupled to a Sciex API 4000 mass spectrometer and an Agilent Infinity II UHPLC coupled to a Sciex QTRAP 6500 mass spectrometer utilizing electrospray ionization. Recovery rates of parent compounds using microsomal fractions without NADPH were normally close to 100%. Substrate turnover was determined using GraphPad Prism version 7.

Data availability

Accession numbers of the *M. rotundata* P450 genes analysed in this study are shown in Table S1. All other data generated or analysed during this study are included in this published article (and its supplementary information files).

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Competing interests

This study received funding from Bayer AG, a manufacturer of neonicotinoid and butenolide insecticides. N.E., M.Z., M.-T.A, A.N., C.G., J.G. and R.N. are employees of Bayer AG.

Contributions

C.B. and R.N. conceived and directed the study, A.H., K.B., K.S.S., and J.G. performed experiments and analysis. N.E., M.Z., M.-T.A., A.N., C.G. analysed and interpreted data. A.H., C.B. and R.N. wrote the paper with contributions from all authors.

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320

321 **Figure legends**

322 **Fig. 1: Distribution and phylogeny of the CYP9 family of P450 genes in bee**
323 **pollinators. a,** Distribution of the CYP9 family across four species of managed bee
324 pollinators. **b,** Maximum likelihood phylogeny of CYP9 family sequences from 12 species of
325 bee (*Apis mellifera*, *Apis florea*, *Apis dorsata*, *Apis cerana*, *Bombus terrestris*, *Bombus*
326 *impatiens*, *Melipona quadrifasciata, Dufourea novaeangliae, Eufriesea mexicana,*
327 *Habropoda laboriosa, Osmia bicornis* and *Megachile rotundata*). Branches show relative
328 time.

329

330 **Fig. 2: Biological and biochemical characterization of the response of *M. rotundata* to**
331 **select insecticides. a,** Contact LD_{50} (48h) values for topical application of thiacloprid (TCP,
332 a.i.), imidacloprid (IMI, a.i.) and LD_{50} (72h) flupyradifurone (FPF, commercial product:
333 Sivanto®/Sivanto™ 200 SL) in four managed pollinator species [error bars indicate 95%
334 confidence limits]. Sensitivity thresholds are depicted according to the EPA toxicity ratings [22].
335 Neonicotinoid data for *A. mellifera, B. terrestris* and *O. bicornis* are taken from [2,3,19]; data for
336 flupyradifurone (200 SL) for *A. mellifera* and *B. terrestris* are taken from [17]. **b,** Binding affinity
337 (IC_{50} values) of selected insecticides to nAChR head membrane preparations of *A. mellifera*
338 and *M. rotundata*. Data for *A. mellifera* is taken from [3]. **c,** Metabolism of thiacloprid (TCP),
339 imidacloprid (IMI), flupyradifurone (FPF), *tau*-fluvalinate (τ-FLV) and nicotine (NCT) by native
340 microsomal preparations of *M. rotundata* as measured by tandem mass spectrometry (1h
341 incubation at 30°C, ± NADPH [error bars indicated 95% confidence limits]). Analysis
342 performed using a Welch's t-test (two-tailed; df=3.581) with significant differences
343 ($p<0.0001$) marked with ****.

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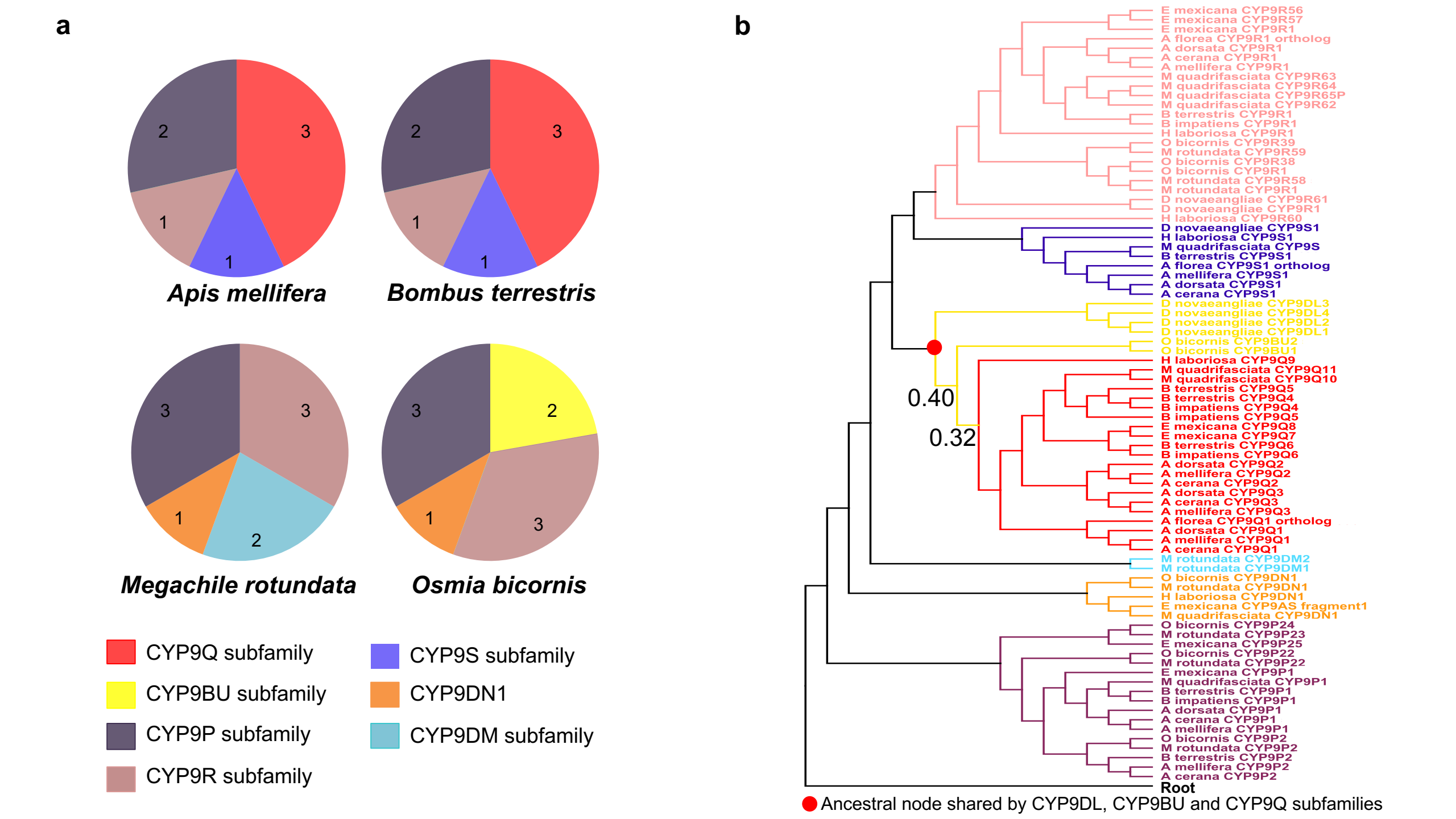
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Apis mellifera
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Bombus terrestris
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Megachile rotundata
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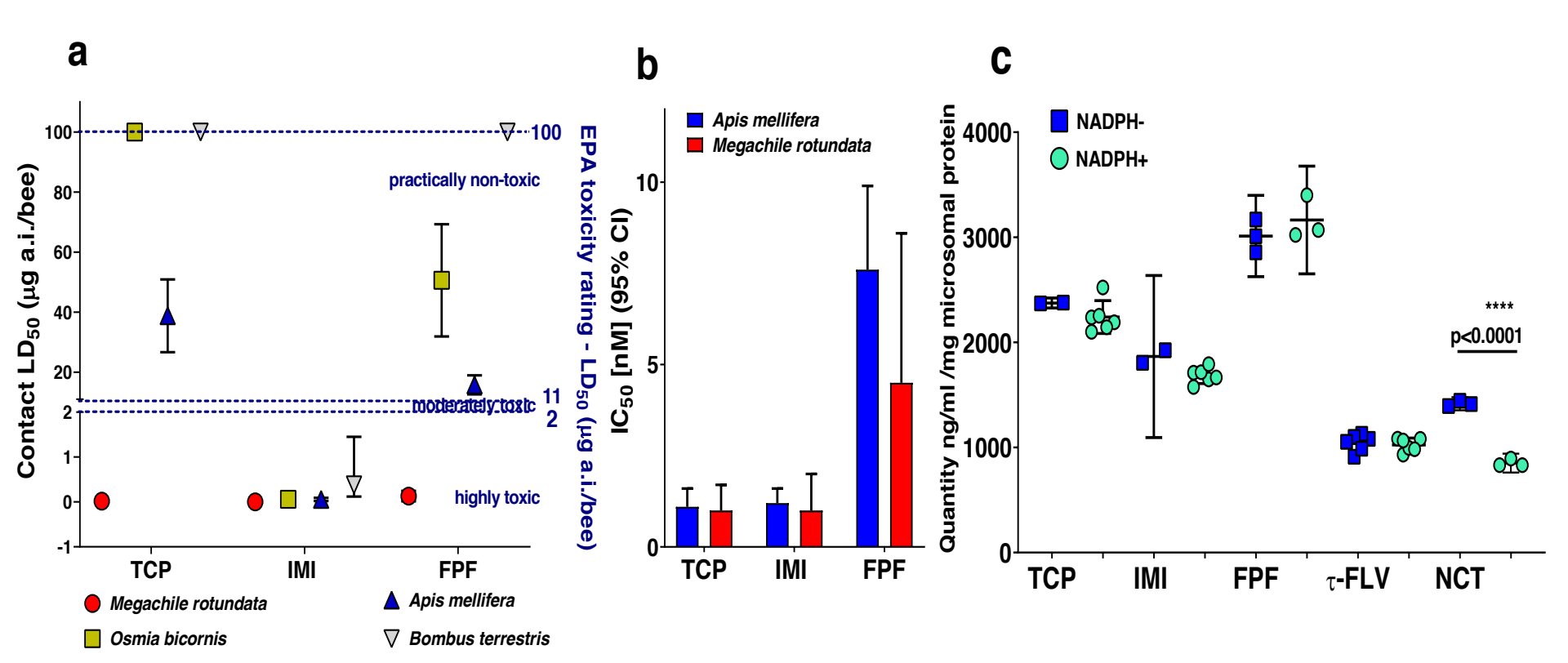
Osmia bicornis
CYP9Q subfamily
CYP9BU subfamily
CYP9P subfamily
CYP9R subfamily
CYP9S subfamily
CYP9DN1
CYP9DM subfamily
b
E mexicana CYP9R56
E mexicana CYP9R57
E mexicana CYP9R1
A florea CYP9R1 ortholog
A dorsata CYP9R1
A cerana CYP9R1
A mellifera CYP9R1
M quadrifasciata CYP9R63
M quadrifasciata CYP9R64
M quadrifasciata CYP9R65P
M quadrifasciata CYP9R62
B terrestris CYP9R1
B impatiens CYP9R1
H laboriosa CYP9R1
O bicornis CYP9R39
M rotundata CYP9R59
O bicornis CYP9R38
O bicornis CYP9R1
M rotundata CYP9R58
M rotundata CYP9R1
D novaeangliae CYP9R61
D novaeangliae CYP9R1
H laboriosa CYP9R60
D novaeangliae CYP9S1
H laboriosa CYP9S1
M quadrifasciata CYP9S
B terrestris CYP9S1
A florea CYP9S1 ortholog
A mellifera CYP9S1
A dorsata CYP9S1
A cerana CYP9S1
D novaeangliae CYP9DL3
D novaeangliae CYP9DL4
D novaeangliae CYP9DL2
D novaeangliae CYP9DL1
O bicornis CYP9BU2
O bicornis CYP9BU1
H laboriosa CYP9Q9
M quadrifasciata CYP9Q11
M quadrifasciata CYP9Q10
B terrestris CYP9Q5
B terrestris CYP9Q4
B impatiens CYP9Q4
B impatiens CYP9Q5
E mexicana CYP9Q8
E mexicana CYP9Q7
B terrestris CYP9Q6
B impatiens CYP9Q6
A dorsata CYP9Q2
A mellifera CYP9Q2
A cerana CYP9Q2
A dorsata CYP9Q3
A cerana CYP9Q3
A mellifera CYP9Q3
A florea CYP9Q1 ortholog
A dorsata CYP9Q1
A mellifera CYP9Q1
A cerana CYP9Q1
M rotundata CYP9DM2
M rotundata CYP9DM1
O bicornis CYP9DN1
M rotundata CYP9DN1
H laboriosa CYP9DN1
E mexicana CYP9AS fragment1
M quadrifasciata CYP9DN1
O bicornis CYP9P24
M rotundata CYP9P23
E mexicana CYP9P25
O bicornis CYP9P22
M rotundata CYP9P22
E mexicana CYP9P1
M quadrifasciata CYP9P1
B terrestris CYP9P1
B impatiens CYP9P1
A dorsata CYP9P1
A cerana CYP9P1
A mellifera CYP9P1
O bicornis CYP9P2
M rotundata CYP9P2
B terrestris CYP9P2
A mellifera CYP9P2
A cerana CYP9P2
Root
0.40
0.32
Ancestral node shared by CYP9DL, CYP9BU and CYP9Q subfamilies


a
Contact LD50 (µg a.i./bee)
EPA toxicity rating - LD50 (µg a.i./bee)
100
practically non-toxic
11
2
moderately toxic
highly toxic
TCP
IMI
FPF
Megachile rotundata
Osmia bicornis
Apis mellifera
Bombus terrestris
b
IC50 [nM] (95% CI)
Apis mellifera
Megachile rotundata
c
Quantity ng/ml /mg microsomal protein
NADPH-
NADPH+
****
p<0.0001
TCP
IMI
FPF
τ-FLV
NCT

Supplementary Table 1

Description of the CYPome of *Megachile rotundata*

Clan	Name	Protein accession number	Nucleotide accession number	Size
CYP2	CYP15A1	XP_012138027.1	XM_012282637.1	503
	CYP18A1	XP_012142657.1	XM_012287267.1	538
	CYP303A1	XP_012153072.1	XM_012297682.1	564
	CYP305D1	XP_003702175.1	XM_003702127.2	485
	CYP307B1	XP_012139366.1	XM_012283976.1	507
	CYP343A1	XP_012138467.1	XM_012283077.1	515
	CYP369A1	XP_003703633.1	XM_003703585.2	505
CYP3	CYP6AQ52	XP_012136078.1	XM_012280688.1	515
	CYP6AQ53	XP_012136079.1	XM_012280689.1	515
	CYP6AQ54	XP_003701310.1	XM_003701262.2	515
	CYP6AS108	XP_012146067.1	XM_012290677.1	370
	CYP6AS109	XP_012150537.1	XM_012295147.1	502
	CYP6AS110	XP_012136792.1	XM_012281402.1	499
	CYP6AS111	XP_012143066.1	XM_012287676.1	501
	CYP6AS112	XP_003704416.1	XM_003704368.2	510
	CYP6AS113	XP_012143067.1	XM_012287677.1	501
	CYP6AS114	XP_012143073.1	XM_012287683.1	499
	CYP6AS115	XP_012143072.1	XM_012287682.1	501
	CYP6AS116	XP_003704412.1	XM_003704364.2	512
	CYP6AS117	XP_012143062.1	XM_012287672.1	502
	CYP6AS118	XP_003704278.1	XM_003704230.2	499
	CYP6AS119	XP_012140372.1	XM_012284982.1	501
	CYP6AS120	XP_003704414.1	XM_003704366.2	499
	CYP6BC1	XP_003703200.1	XM_003703152.2	517
	CYP6BD1	XP_003699913.1	XM_003699865.2	503
	CYP6BE1	XP_012153915.1	XM_012298525.1	511
	CYP6BE1P	XP_012153916.1	XM_012298526.1	440
	CYP9DN1	XP_003703411.1	XM_003703363.2	522
	CYP9P2	XP_012145771.1	XM_012290381.1	515
	CYP9P22	XP_012145774.1	XM_012290384.1	506
	CYP9P23	XP_012145773.1	XM_012290383.1	512
	CYP9R1	XP_003705491.1	XM_003705443.2	516
	CYP9R58	XP_003705489.1	XM_003705441.2	516
	CYP9R59	XP_012145777.1	XM_012290387.1	518
	CYP9DM1	XP_003705488.1	XM_003705440.2	498
	CYP9DM2	XP_003705490.2	XM_003705442.2	531
	CYP336A33	XP_003702293.1	XM_003702245.2	501
	CYP336A34	XP_003702219.1	XM_003702171.2	501
	CYP336M1	XP_012138246.1	XM_012282856.1	412
CYP4	CYP4G11	XP_012145465.1	XM_012290075.1	556
	CYP4G202	XP_003700755.1	XM_003700707.2	561
	CYP4AA1	XP_012137854.1	XM_012282464.1	515
	CYP4AV1	XP_003699552.2	XM_003699504.2	514
MITOCHONDRIAL	CYP301A1	XP_003703366.2	XM_003703318.2	530
	CYP301B1	XP_003703365.1	XM_003703317.2	492
	CYP302A1	XP_012135500.1	XM_012280110.1	519
	CYP314A1	XP_012150013.1	XM_012294623.1	525
	CYP315A1	XP_003704053.1	XM_003704005.2	533
	CYP334A1	XP_003700083.1	XM_003700035.2	582

Supplementary Table 3

Table showing LD_{50} values generated in this study

Species	Thiacloprid LD_{50} (48h) µg a.i/bee [CI 95%]	Imidacloprid LD_{50} (48h) µg a.i/bee [CI 95%]	Flupyradifurone LD_{50} (72h) µg a.i/bee [CI 95%]
Megachile rotundata	0.015 (0.012-0.023)	0.001 (0.000-0.001)	0.092 (0.036-0.26)
Osmia bicornis			46.7 (34.2-70.9)