Imidacloprid disrupts larval molting regulation and nutrient energy metabolism, causing developmental delay in honey bee *Apis mellifera*

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Abstract

Imidacloprid is a global health threat that severely poisons the economically and ecologically important honeybee pollinators, *Apis mellifera*. However, its effects on developing bee larvae remain largely unknown. Our pilot study found that imidacloprid causes developmental delay in bee larvae, but its toxicological mechanisms are not yet fully understood. In this study, we exposed bee larvae to imidacloprid at environmentally relevant concentrations of 0.7, 1.2, 3.1, and 377 ppb. A significant dose-dependent delay in the larval development was observed, including reductions in body mass, width, and growth index; however, imidacloprid did not affect larval survival and food consumption. Predominant toxicological effects induced by high concentrations of imidacloprid (377 ppb) included disruption of neural transmission, induction of oxidative stress and gut structural damage and apoptosis, inhibition of

developmental regulatory hormones and genes, disruption of proteolysis, amino acid transport, protein synthesis, carbohydrate catabolism, oxidative phosphorylation, and glycolysis energy production. In parallel, we observed that the larvae employed antioxidant defense and P450 detoxification mechanisms to counteract imidacloprid. Ultimately, this study provides the first evidence that environmental exposure to imidacloprid can retard bee larval growth and development by disrupting molting regulation and limiting the metabolism and utilization of dietary nutrients and energy. These findings have broader reference implications for studies assessing pesticide hazards to other juvenile animals.

Keywords: Imidacloprid; *Apis mellifera*; Toxicity effects; Developmental retardation; Juvenile animal;

1. Introduction

Hazardous pesticides are extensively used worldwide to safeguard crop production, particularly in developing countries that depend on agriculture. However, exposure to these chemicals has become a global health concern. Imidacloprid, introduced in 1991, is known for its high animal toxicity and has received international attention (Jeschke et al., 2011). The World Health Organization and the U.S. Environmental Protection Agency have listed it as an acute oral and dermal toxicant for animals. Overuse of imidacloprid can lead to environmental contamination, endangering human and animal health through food chains, poisoning non-target pollinators, and disrupting ecological balances (Tudi et al., 2021). Adults exposed to high doses of imidacloprid experience dizziness, apathy, movement disorders, labored breathing, and temporary growth retardation. Imidacloprid also causes adverse congenital, hematological, hepatic, and renal effects, along with degenerative changes in various organs (Khan et al., 2010; Shaw et al., 2014; Yang et al., 2014). In rats and rabbits, imidacloprid causes severe liver,

53 thyroid, and body weight problems, reproductive toxicity, developmental retardation,
54 and neurobehavioral deficits, even at low doses(Anatra-Cordone M, 2005). Imidacloprid
55 also affects the singing ability of birds, making it difficult for them to attract mates and
56 reproduce. Imidacloprid represents a significant contributing factor to the decline in the
57 number of terrestrial insects since 1925, which has declined by an average of 9% per
58 decade (Cardoso et al., 2020; Sánchez-Bayo and Wyckhuys, 2019).

59 The development and health status of juvenile animals play a critical role in
60 population structure and future growth (Gill et al., 2012), but they are more susceptible
61 to the toxic effects of pesticides than adults. However, there is a paucity of information
62 on the assessment of toxic effects of imidacloprid on juvenile animals. Available reports
63 primarily describe the epistatic traits influenced by toxicity, with limited investigation
64 into the biochemical mechanisms involved. Studies have shown that imidacloprid poses
65 a potential health risk to children through dietary exposure (Anderson and Harmon-
66 Threatt, 2021) and can lead to developmental abnormalities in amphibians (Samojeden
67 et al., 2022) and fish (Islam et al., 2019) as well as negative effects on insect pupal
68 development (Jia and Li, 2023). In insects, the honeybees (*Apis mellifera* L.) are valuable
69 model organisms with significant economic and ecological importance as pollinators of
70 crops and maintenance of ecological balance (Zheng and Fu-Liang, 2009). However, the
71 widespread use of pesticides, including imidacloprid, has resulted in colony collapse
72 disorder (CCD), causing serious impacts on beekeeping industry and ecological balance
73 (Mahé et al., 2021). Studies have shown that Imidacloprid is applied as seed coating
74 (Simon-Delso et al., 2015) and accumulate in the plant (Dively and Kamel, 2012; Rocha
75 et al., 2022), exposing non-target bees to imidacloprid residues during pollen and nectar
76 collection (Tong et al., 2018). Even low levels of systemic imidacloprid cause sublethal
77 effects in adult bees, including neurotoxicity (Belzunces et al., 2012), behavioral changes
78 (Karahan et al., 2015; Medrzycki et al., 2003), reproductive impairment (Chaimanee et
79 al., 2016), immunity (Pettis et al., 2013), apoptosis and autophagy (Carneiro et al., 2022),
80 and shortened lifespan (Anderson and Harmon-Threatt, 2021). Larvae is the future of

colony growth and development, yet the broader toxic effects of imidacloprid on larvae remain unknown. Limited reports suggest that developing bee larvae are exposed to imidacloprid through contact with contaminated hives or ingesting contaminated food (Bhme et al., 2018), leading to delayed development, reduced rates of capping, pupation, and eclosion (Yang et al., 2012), and impaired olfactory learning behavior (Peng and Yang, 2016). In the pilot study, we found that imidacloprid exposure caused obvious developmental delay in bee larvae, but the molecular and biochemistry mechanisms remain to systematically investigated. To this end, this study comprehensively evaluated the toxicity of imidacloprid on honey bee larvae at multiple levels including histology, neurotoxicity, molting regulation, oxidative stress, detoxification, nutrition, and energy metabolism, and provides first-hand evidence for imidacloprid causing developmental delay in bee larvae. Because the mechanisms involved are relatively conserved in animals, our findings have broader implications for understanding and assessing the potential risk of continuous exposure to imidacloprid during animal development.

Results

Imidacloprid causes bee larval developmental retardation

During the 96-hour observation period, larval survival in the four imidacloprid-exposed groups was not significantly different from that in the control group. However, with increasing exposure concentration and duration, larval developmental progress, weight and width were significantly reduced (Fig. 1A-1E). Further analysis focused on the most adverse exposure level of 377 ppb, which did not affect survival, feeding (Fig. 1C, 1F, and 1G), and the time taken for larvae to successfully enter the pupal stage (Fig. 1H), but resulted in slow developmental obstacles and lower initial rate of successful pupation and growth index. In addition, imidacloprid also caused consistently lower weight (Fig. 1I) and width (Fig. 1J) of larvae up to the pupal stage (Fig. 1B).

109 **Imdacloprid neurotoxicity in bee larvae**

110 Under imidacloprid environmental stress at 377 ppb, AChE activity, which is closely
111 related to the disruption of neurotransmission, was significantly inhibited in larvae (Fig.
112 2A). Neither acetylcholine receptor alpha 1 (*Alph1*) nor acetylcholinesterase 2 (*Ace2*)
113 gene expression was significantly different from the control. In contrast, acetylcholine
114 receptor alpha 2 (*Alph2*) and acetylcholinesterase 1 (*Ace1*) gene expression was
115 significantly higher than that in the control group (Fig. 2B).

116 **Imidacloprid disrupts the homeostasis of developmental regulation in larvae**

117 In imidacloprid-exposed larvae, the developmental regulatory gene juvenile hormone
118 acid methyl transferase (*JHAMT*) and the transcription factor broad complex (*Br-c*) were
119 downregulated, while vitellogenin (*Vg*) gene expression was upregulated by seven-fold
120 in comparison to control groups (Fig. 3A). In parallel, imidacloprid exposure decreased
121 the major regulator of insect metamorphosis 20-hydroxyecdysone (20E) titers but had
122 no effect on preventing premature metamorphosis juvenile hormone (JH-3) titers (Fig.
123 3B).

124 **Imidacloprid induces detoxification in larvae**

125 According to the qRT-PCR analysis, the expression levels of cytochrome P450
126 monooxygenase family members such as CYPq1, CYPq2, CYP450, CYP6AS14, CYP4G11,
127 and CYP306A1, except for CYPq3, were observed to be significantly upregulated by
128 several fold in imidacloprid-induced developmental retardation larvae. Among them,
129 CYPq1 showed an abnormal 94-fold increase (Fig. 4).

130 **Imidacloprid toxicity causes oxidative stress and induces antioxidant defense in larvae**

131 Larvae exposed to imidacloprid exhibited a significant increase in reactive oxygen
132 species (ROS) levels (Fig. 5A) and upregulation of the antioxidant genes GPX and Trx
133 expression (Fig. 5C), as well as increased activities of the antioxidants CAT, SOD and GSH

134 (Fig. 5B), resulting in a significant increase in total antioxidant capacity (T-AOC) (Fig. 5B)
135 compared to the control group. Under these conditions, MDA levels, used as a marker of
136 lipid peroxidation, were observed to increase, while protein carbonylation damage
137 indicator PCO levels remained unchanged (Fig. 5A).

138 **Imidacloprid induces gut apoptosis and tissue damage in larvae**

139 Hematoxylin-eosin staining revealed a significant reduction in the number of cells in
140 the muscular layer of the larval gut exposed to imidacloprid with clear apoptotic
141 characteristics compared to the standard feeding control groups (Fig. 5D). This was
142 evidenced by increased nuclear staining and compacted chromatin, as well as disrupted
143 cell arrangement in the basal layer of the gut, increased nuclear staining of basal lamina
144 cells, and apoptotic signals. The integrity of the peritrophic membrane structure of the
145 gut was better in the control group, while imidacloprid-exposed larvae were still in the
146 formation stage, resulting in the accumulation of undigested food residues in their guts
147 (Fig. 5D).

148 **Imidacloprid impairs larval digestion and breakdown of dietary nutrients**

149 Exposure to imidacloprid did not significantly affect the daily food intake of
150 developmentally delayed larvae compared to controls (Fig. 1F and 1G). However,
151 imidacloprid toxicity suppressed the expression of nutrient catabolism genes, including
152 alpha-amylase (*α-Am*) and alpha-glucosidase (*α-Glu*), which are involved in
153 carbohydrate catabolism (Fig. 6A), carboxypeptidase (*CPs*) and aminopeptidase (*APs*)
154 involved in proteolysis (Fig. 6B), and glycine tRNA ligase (*Aats-Gly*) and tyrosine tRNA
155 ligase (*Aats-Tyr*) involved in amino acid transport (Fig. 6C).

156 **Imidacloprid causes restriction of larval protein synthesis and energy metabolic**
157 **dysfunction**

158 RT-qPCR analysis revealed that imidacloprid exposure reduced the expression of
159 critical genes involved in energy metabolism, including cytochrome c oxidase copper

160 chaperone (*COX17*) and NADH dehydrogenase [ubiquinone] 1β subcomplex subunit 7
161 (*NDUFB7*), in developmentally delayed larvae (Fig. 6D). Imidacloprid exposure also
162 inhibited the expression of genes related to glycolysis and energy production, such as
163 glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) and glucosamine-6-phosphate
164 isomerase (*Oscillin*) (Fig. 6E). In addition, the expression of transcription initiation
165 factors related to protein synthesis, including translation initiation factor 3 subunit M
166 (*Tango7*), translation initiation factor 3 subunit B (*eIF3-S9*), and translation initiation
167 factor 3 subunit A (*eIF3-S10*), was significantly suppressed by imidacloprid toxicity (Fig.
168 6F). Finally, the total levels of ATP, glycogen and protein, which are closely related to
169 energy production and reserves, were significantly lower in developmentally delayed
170 larvae than in controls (Fig. 6G-6I).

171 **Correlation between developmental phenotypic changes and molecular features**
172 **caused by imidacloprid toxicity**

173 To better understand the mechanism of imidacloprid-induced larval developmental
174 retardation, we performed a comprehensive correlation analysis between epigenetic
175 growth traits and molecular characteristics (Fig. 7). The results showed that in larvae
176 with imidacloprid-induced developmental retardation, body weight, width, growth
177 index, and developmental rate were positively correlated with energy metabolism and
178 reserves, nutrient catabolism, protein synthesis, and developmental regulation.
179 Conversely, negative correlations were observed between these growth traits and
180 parameters associated with detoxification and antioxidant defense processes that
181 require additional energy expenditure. Moreover, oxidative stress and damage,
182 detoxification, and antioxidant defense all exhibited significant positive correlations (Fig.
183 7).

184

185 ## Discussion

186 **Toxic effects and the molecular basis of environmental concentrations of imidacloprid**
187 **on bee larvae**

188 Previous research has shown that bee larvae are more susceptible to the adverse
189 effects of pesticides than adult bees (Tomé et al., 2020). In this study, larvae were
190 exposed to environmental concentrations of imidacloprid, resulting in sublethal effects
191 such as decreased body weight, width, developmental rate, and growth index (Fig. 1).
192 Our results support the notion that pesticides can affect larval development (Dai et al.,
193 2018; Zhu et al., 2014). However, current research has primarily focused on
194 characterizing the effects of imidacloprid on larval phenotype and behavior, and the
195 molecular mechanisms underlying its ecotoxicological effects remain poorly understood.

196 To obtain molecular evidence of imidacloprid toxicity to bee larvae, we first examined
197 the expression of acetylcholine receptors and AChE activity. The results showed that
198 imidacloprid exposure increased the expression of the *Alph2* gene and inhibited AChE
199 activity (Fig. 2). Normally, acetylcholine stimulates nerve impulses by binding to the Alph
200 receptor, and AChE degrades acetylcholine to terminate nerve conduction. However,
201 imidacloprid competitively binds to AChE receptors, inhibits AChE activity, and causes
202 continuous excitation of nerve conduction, ultimately leading to paralysis and death
203 (Katić et al., 2021; Shan et al., 2020). Therefore, our results strongly suggest that
204 imidacloprid is neurotoxic to bee larvae.

205 The growth and development of juvenile animals depends on the digestion,
206 absorption and utilization of food nutrients. Therefore, we next investigated whether
207 imidacloprid affects the digestion and utilization of dietary by larvae. The results
208 indicate that exposure to imidacloprid suppressed the hydrolysis genes *CPs* and *APs* and
209 the amino acid transport pathway genes *Aats-Gly* and *Aats-Tyr* in larvae (Fig. 6B and 6C).
210 Since these genes are responsible for cleaving dietary proteins into absorbable peptides
211 and amino acids and transporting them for metabolism (2019), inhibiting the expression
212 of these genes would severely affect larval digestion, resulting in protein deficiency and

213 impaired metabolic activity. In addition, imidacloprid also affected the expression of *α-*
214 *Am* and *α-Glu* genes (Fig. 6A), which are involved in carbohydrate digestion, indicating
215 that imidacloprid also affects the digestion of dietary carbohydrates. Indeed, histological
216 observations also confirmed that a large amount of undigested food remained in the
217 intestine of larvae exposed to imidacloprid (Fig. 5D). In conclusion, imidacloprid inhibits
218 the digestion and utilization of dietary proteins and carbohydrates by larvae, which is
219 extremely detrimental to their growth and development.

220 In addition to nutrition, energy metabolism is critical for the maintenance of all life
221 activities. In this study, imidacloprid exposure inhibited the expression of genes related
222 to mitochondrial oxidative phosphorylation (*COX17*, *NDUFB7*) and its alternative
223 glycolytic pathway (*Gapdh*, *Oscillin*). Mitochondrial oxidative phosphorylation is the
224 central pathway for ATP generation during energy metabolism (Tian et al., 2020a), while
225 glycolysis is an alternative mechanism for ATP synthesis (Jiang et al., 2020). Since both
226 biochemical processes are essential for energy production, their inhibition resulted in
227 reduced ATP levels in bee larvae (Fig. 6G). These results strongly suggest that
228 imidacloprid induces mitochondrial dysfunction leading to insufficient energy
229 production in larvae. Since larval energy production is insufficient, this is likely to lead to
230 a depletion of energy reserves. Indeed, further analysis revealed a significant decrease
231 in glycogen and protein energy reserves in larvae exposed to imidacloprid (Fig. 6H and
232 6I). These findings are consistent with previous reports that exposure to pesticides such
233 as endosulfan, chlorpyrifos, insecticides, and fipronil can lead to a reduction in energy
234 reserves in organisms (Bouayad et al., 2012a; Dutra et al., 2009; Radwan et al., 2008;
235 Rambabu and Rao, 1994; Ribeiro et al., 2001). In conclusion, our results suggest that
236 imidacloprid induces mitochondrial dysfunction and affects energy production and
237 reserves in bee larvae.

238 Insects rely primarily on cytochrome P450 detoxification to resist toxic compounds (Li
239 et al., 2007), and the level of CYP450 expression indicates the severity of pesticide

240 toxicity. While adult worker bees have shown a positive response to imidacloprid (Mao
241 et al., 2009), it is uncertain whether bee larvae can respond to pesticide toxicity. In this
242 study, we found that seven cytochrome P450 family transcripts, including CYPq1, CYPq2,
243 CYP450, CYP6AS14, CYP4G11, and CYP306A1, were significantly upregulated in bee
244 larvae, with CYP9q1 being 94-fold higher than the control group (Fig. 4). These results
245 are consistent with previous findings that the CYP450 family genes CYP6AS3, CYP6AS4,
246 and CYP9S1 were significantly upregulated in adult worker bees exposed to quercetin,
247 coumaphos, and fluvalinate (Mao et al., 2011) and that CYP9Q1, CYP9Q2, and CYP9Q3
248 help bees detoxify fluvalinate and coumaphos (Gregorc et al., 2018). This suggests that
249 the P450 detoxification system is already established in bee larvae and they respond
250 positively by activating the detoxification function of CYP450 upon exposure to
251 imidacloprid.

252 Oxidative stress is the excessive production of ROS in aerobic organisms (Felton, 1995;
253 Ozcan and Ogun, 2015). Pesticides, including coumaphos (Gregorc et al., 2018), fipronil
254 (Paris et al., 2017), paraquat (Li-Byarlay et al., 2016), organophosphorus, pyrethroid,
255 organochlorinated (Chakrabarti et al., 2015), and chlorpyrifos (Shafiq-ur-Rehman et al.,
256 2012), have been shown to cause oxidative stress in bees. In this study, exposure to
257 imidacloprid resulted in increased levels of ROS and MDA in larvae (Fig. 5A), indicating
258 severe oxidative stress and lipid damage. Organisms have evolved antioxidant defense
259 mechanisms to scavenge excessive ROS and resist oxidative stress, including increasing
260 levels of antioxidants such as CAT, SOD, GSH, TrxR, and GSH (Dong et al., 2013; Tian et
261 al., 2020b). In this regard, we have recently confirmed this in worker bees (Li et al.,
262 2022), which is also well reflected in the present study. We observed a significant
263 increase in the activities of antioxidant enzymes SOD and CAT, gene expression of Trx
264 and Gpx, and GSH levels in larvae after exposure to imidacloprid (Fig. 5B and 5C). These
265 results show that the antioxidant system in developing larvae is established and used to
266 resist oxidative stress caused by imidacloprid, similar to that in adult worker bees.
267 Notably, compared to our recent study in adult bees (Li et al., 2022), the larvae in this

268 study exhibited more robust antioxidant activity when exposed to higher concentrations
269 of imidacloprid toxicity than worker bees. However, the underlying mechanism requires
270 further investigation. The underlying mechanisms involved require further study.

271 Notably, in addition to routine antioxidants, imidacloprid induces a 7-fold increase in
272 Vg expression (Fig. 3A). Vg is known to provide nutrition to developing embryos
273 (Jorgensen et al., 2009) and to help animals cope with pollution-induced stress (Matozzo
274 et al., 2008). In bees, Vg plays an important antioxidant role by protecting other
275 hemolymph molecules from ROS and increasing cellular oxidative stress tolerance
276 (Corona et al., 2007). Previous studies have shown a correlation between high Vg
277 expression in adult worker bees and resistance to oxidative stress resulting from
278 exposure to the pesticide paraquat (Seehuus et al., 2006). In this study, our results
279 support this observation and suggest that larvae may also resist oxidative stress caused
280 by imidacloprid through increased Vg production.
281

282 **Mechanisms of imidacloprid-induced developmental retardation in bee larvae**

283 Impaired juvenile development can have a significant impact on the growth and
284 sustainability of entire populations. Deviations in juvenile development due to
285 environmental stress have recently received considerable attention. Drosophila
286 melanogaster exposed to heat stress exhibit developmental delays due to the failure of
287 early embryos to fully inhibit the synthesis of non-heat shock proteins (Bergh and Arking,
288 1984). Similarly, exposure to nutrient and heavy metal stress can cause delayed or
289 arrested development in *Caenorhabditis elegans* due to altered gene expression and
290 disruption of developmental signaling pathways (Carranza-García and Navarro, 2020;
291 Rashid et al., 2021). In the present study, we also observed typical developmental delays
292 in honeybee larvae exposed to imidacloprid, but the underlying mechanisms remain
293 unclear.

294 Insect development is regulated by the hormones JH and 20E, which control
295 morphological remodeling events during molting. 20E promotes pupation (Yuan et al.,
296 2020), while JH maintains juvenile characteristics and prevents metamorphosis by
297 antagonizing the effects of 20E (Luo et al., 2021). To elucidate the mechanism
298 underlying imidacloprid-induced larval developmental delay, we first focused on the
299 hormonal regulation of molting development and found that imidacloprid inhibits the
300 expression of JHAMT (Fig. 3A), a positive regulator of JH synthesis (Liu et al., 2018), but
301 did not affect JH titer levels (Fig. 3B). This suggests that the antagonistic effect of JH still
302 restricts the developmental process, thereby maintaining larval morphology. In parallel,
303 imidacloprid toxicity also resulted in reduced ecdysteroid 20E titer and Br-c expression
304 (Fig. 3). The role of 20E is to induce larval molting and pupation by triggering
305 downstream response factors, such as Br-c (Deng et al., 2012). Therefore, our results
306 suggest that larval developmental delay may be caused by imidacloprid reducing 20E
307 titer and inhibiting *Br-c* expression, thereby blocking molting. Notably, we also observed
308 that a decrease in 20E levels was accompanied by a decrease in AChE activity (Fig. 2A),
309 which is known to cause delayed pupation (He et al., 2012) and developmental arrest
310 (Desneux et al., 2007) in insect larvae. Therefore, our results suggest that imidacloprid
311 neurotoxicity may cause developmental delay in bee larvae by inhibiting the molting
312 hormone 20E. This conclusion is supported by a correlation analysis showing that
313 decreases in 20E titer, *Br-c* expression and AChE activity were positively correlated with
314 decreased developmental rates and growth index, weight and width growth (Fig. 7). In
315 conclusion, imidacloprid neurotoxicity inhibits the 20E and *Br-c* genes, thereby blocking
316 molting, which may be an important cause of delayed larval development in honey bees.

317 Animals employ selective foraging as a survival strategy to avoid ingesting toxic foods
318 that may cause adverse physiological effects (Berenbaum and Johnson, 2015). Although
319 bees cannot actively reject toxins innately, postingestive malaise allows them to learn to
320 avoid ingesting toxic-containing foods with adverse physiological effects (Hurst et al.,
321 2014). Given this, we speculate that it is likely that the honey bee larvae in this study

322 reduced their intake of toxic imidacloprid-containing foods, making it difficult to meet
323 their nutritional and energy needs, which may have resulted in delayed development.
324 However, monitoring of daily food intake did not show a significant difference between
325 larvae exposed to imidacloprid and the control group (Fig. 1F and 1G), indicating that
326 larval food intake was not reduced and larvae did not initiate avoidance behavior
327 toward toxic food. Therefore, food intake was not a critical factor in the delayed larval
328 development caused by imidacloprid.

329 Nutrition and energy are critical for the growth and development of juvenile animals.
330 Exposure to heavy metals and neonicotinoid pesticides has been shown to affect
331 digestion, energy reserves, production, and utilization in animals (Jiang et al., 2020; Li et
332 al., 2021). Given this, since the lack of the necessary correlation between delayed larval
333 development and food intake in this study prompts us to consider potential problems in
334 larval food and energy utilization that led to delayed development. Consequently, our
335 focus shifted to the investigation of larval nutrition and energy metabolism.
336 Interestingly, we found severe pathological changes in the gut tissues of
337 developmentally delayed larvae exposed to imidacloprid (Fig. 5D). Imidacloprid caused a
338 significant reduction in the tightness of the cell arrangement in the basal layer of the gut,
339 and a drastic decrease in the number of cells. The peritrophic membrane was
340 incompletely formed, and undigested food residues were present in the gut. qRT-PCR
341 analysis revealed a significant decrease in larval digestion and catabolism of dietary
342 proteins and carbohydrates, amino acid transport, oxidative phosphorylation, and
343 glycolysis (Fig. 6D-6F). Biochemical assays showed that imidacloprid stress also
344 decreased ATP levels and total glycogen and protein content, resulting in a lack of
345 energy reserves and increased energy expenditure (Fig. 6G-6I). Correlation analysis
346 indicated a positive relationship between impaired catabolism and dietary nutrient
347 utilization caused by imidacloprid and larval developmental delay (Fig. 7). These
348 evidence strongly suggest that imidacloprid exposure affects the digestion and
349 utilization of nutrients and energy in larvae, resulting in insufficient nutrients and energy

350 for growth and development, which may be another important factor contributing to
351 imidacloprid-induced larval developmental delay.

352 The Dynamic Energy Budget (DEB) theory suggests that environmental stress can
353 significantly affect the energy balance of organisms, leading to increased energy
354 expenditure (Kooijman, 2009). To cope with stress, animals may deplete their energy
355 reserves, such as glycogen and protein (Bouayad et al., 2012b; Matsukura et al., 2008).
356 In this study, we found that larvae with delayed development caused by imidacloprid
357 had lower energy reserves (ATP, total protein, and total glycogen), and these energy
358 reserves were negatively correlated with larval antioxidant and detoxification (Fig. 7),
359 suggesting that the reduction in energy reserves caused by imidacloprid is related to the
360 energy-consuming activities of CYP450 detoxification and antioxidant defense. To meet
361 the high energy cost demands of environmental stress, animals often have to allocate
362 most of their food intake to defense (Guedes et al., 2006), and this redistribution of
363 energy resources will inevitably lead to an insufficient energy supply for growth and
364 development (Beyers et al., 1999). Therefore, the P450 detoxification and antioxidant
365 defense used by the larvae in this study to resist imidacloprid toxicity will inevitably
366 force them to consume more food resources that should have been used for growth and
367 development, resulting in a severe lack of energy needed for development. This may
368 also be another important reason why imidacloprid causes delayed development in bee
369 larvae.

370

371 **Conclusion**

372 Imidacloprid toxicity caused growth and developmental delay in A. mellifera larvae.
373 The toxic mechanism may include imidacloprid disrupting the regulatory balance of molt
374 development, resulting in restricted development. In addition, the gut damage caused
375 by imidacloprid restricts the metabolism and utilization of food nutrients and energy in
376 the larvae. Third, the additional energy consumed by larval P450 detoxification and

377 antioxidant defenses further reduces the supply of nutrients and energy for growth and
378 development (Fig. 8). This study is the first multi-level investigation of the toxic effects
379 of imidacloprid on A. mellifera larvae. These findings have broader implications for
380 understanding and assessing the threat of harmful pesticides to animal larvae.

381

382 **Materials and Methods**

383 **Larval rearing**

384 The Animal Bioethics Committee at Chongqing Normal University, China approved all
385 experimental protocols. The chosen colonies were healthy and not exposed to
386 pathogens or pesticides. Two-day-old larvae were individually transferred to sterile 24-
387 well cell culture plates filled with a standard diet consisting of royal jelly, glucose,
388 fructose, water, and yeast extract (Aupinel et al., 2005). The larvae were cultured in dark
389 conditions at 35°C and 96% RH, with daily feeding volumes adjusted according to their
390 age (20 µL for three-day-old larvae, 30 µL for four-day-old larvae, 40 µL for five-day-old
391 larvae, and 50 µL for six-day-old larvae). Any remaining food was recorded and removed.

392

393 **Experimental design**

394 This study employed four different concentrations of imidacloprid, which were
395 residual concentrations found in honey (0.7 ppb) (Chauzat et al., 2009), bees (1.2 ppb)
396 (Chauzat et al., 2011), pollen (3.1 ppb) (Mullin et al., 2010), and beeswax (377 ppb)
397 (Kapoor et al., 2014). The experiment was conducted with three treatment groups:
398 control group (CK), solvent control group (CKac), and imidacloprid-treated group (IMI),
399 each with three replicates. Each replicate contained 32 bees that were distributed in
400 standard 24-well cell culture plates (1 bee/well). The CK group consisted of larval bees
401 fed acetone-royal jelly glucose-fructose-yeast extract-distilled water (0.00377%-50%-
402 6%-6%-1%-36.99623%, v/w/w/w/w/v). The IMI group consisted of larvae-fed control
403 solution containing imidacloprid (99% active ingredient, Hubei Norna Technology Co. Ltd,
404 Hubei, China) at 0.7 ppb, 1.2 ppb, 3.1 ppb, and 377 ppb. The CKac group served as a

405 solvent control and contained only acetone at a concentration of 0.00377%. A total of
406 312 larvae were used for survival statistics (96 bees), body weight and width (96 bees),
407 and gene expression, enzyme activity, histochemical content, and histopathologic
408 analysis. Food was administered daily according to age (20 μL for three-day-old larvae,
409 30 μL for four-day-old larvae, 40 μL for five-day-old larvae, and 50 μL for six-day-old
410 larvae). Dead individuals and food remain were recorded daily, and incubation
411 conditions were 35°C and 96% RH. Surviving larvae were collected 72 h after
412 consumption of the test solution and stored at −80°C for further analysis, including
413 evaluations of gene expression, enzyme activity, and chemical content evaluations.
414 Furthermore, HE-stained sections were used to evaluate any imidacloprid-induced
415 tissue damage and apoptosis.
416
417 **Developmental phenotypic traits**
418 Larval individuals were randomly selected daily from the CK and IMI groups to
419 measure body weight and width. Food residues, larval mortality, developmental
420 progression, and the amount of time spent in the larval developmental stage (from the
421 first imidacloprid exposure to before pupa) were recorded daily to calculate food
422 consumption, survival rates, developmental rates, and development times. The growth
423 index (GI) was calculated for larvae exposed to imidacloprid from the three-day-old to
424 the six-day-old using the equations described by Zhang (Zhang et al., 1993). The
425 developmental rate was measured as the number of larvae successfully reaching the
426 pupal stage as a percentage of the total initial sample size.
427
428 **RNA extraction, cDNA synthesis, and real-time quantitative PCR analysis**
429 Total RNA was extracted from larvae using TRIzol reagent (Thermo Fisher Scientific)
430 and RNAex Pro Reagent (Accurate Biology, China) as instructed by the manufacturer.
431 The RNA quality was evaluated by gel electrophoresis and absorbance determination
432 using a NanoDrop 2000 spectrophotometer (Thermo Scientific, New York, USA).

433 Genomic DNA was removed, and cDNA was synthesized using a reverse transcription kit
434 (Evo M-MLV RT Mix Kit with gDNA Clean for qPCR, Accurate Biology, China) according to
435 the manufacturer's instructions. Briefly, 1 μg of RNA reacted with the 5X gDNA Clean
436 Reaction Mix provided in the kit at 42°C for 5 min to remove residual genomic DNA to
437 ensure the accuracy of the quantitative results. After the reaction, 4 μL of 5X Evo M-
438 MLV RT Reaction Mix and 4 μL of RNase-free water were added to the reaction tube
439 for 20 μL. The mixture was incubated at 37°C for 15 min and 85°C for 5 sec to generate
440 cDNA. Then, quantitative RT–PCR was performed with a Bio-Rad CFX96™ Real-time PCR
441 Detection System (Bio-Rad, Hercules, CA, USA) according to standard protocols and
442 cycling conditions. RP49 (ribosomal protein 49, Accession no. AF441189) (Lourenco et al.,
443 2008) was used as the endogenous control. The primers for target genes and RP49 are
444 listed in Table S1. qPCR system (20 μL): EvaGreen Express 2X qPCR MasterMix (10 μL),
445 each forward and reverse primer (0.4 μL, 0.2 μmol/L), cDNA (1 μL, 100 ng/μL), ddH_2O
446 (8.2 μL). The thermal cycling program was as follows: 95°C for 30 sec, followed by 40
447 cycles of 95°C for 5 s and 60°C for 30 s. The qPCR products were sequenced by Shanghai
448 Biological Engineering Technology Services Co., Ltd. to confirm whether the amplified
449 fragments were the target sequences. Relative mRNA expression levels of target genes
450 were calculated using the $2^{-\Delta\Delta Ct}$ method.
451
452 **Histochemical measurement**
453 Juvenile hormone 3 (JH-III), 20-hydroxyecdysone (20E) titers, and reactive oxygen
454 species (ROS) levels in larvae were determined using ELISA enzyme-linked
455 immunosorbent assay kits (Jining, Shanghai, China) according to the manufacturer's
456 instructions. Briefly, the larva was homogenized on ice in 5 volumes (M: V) of
457 phosphate-buffered saline (PBS) buffer, and the supernatant was collected after
458 centrifugation at 12,000 x g for 20 min. Subsequently, 50 μL of reference standard
459 solution or 10 μL of sample supernatant plus 40 μL of dilution were added to the
460 microtiter wells and incubated at 37°C for 60 min with 100 μL of HRP-labeled detection

461 antibody. Each well was washed five times with 400 µL of PBS buffer containing 0.05%
462 Tween 20, pat dried, and left to stand at room temperature for 5 min. TMB chromogen
463 solution A (50 µL) and solution B (50 µL) were added to each well, gently mixed, and
464 incubated at 37°C for 15 min in the dark. Then, 50 µL of H_2SO_4 (4 mol/L) was added to
465 each well to terminate the reaction. Finally, the OD value was measured at 450 nm with
466 a NanoDrop 2000 spectrophotometer (Thermo Scientific, New York, USA), and the
467 content of the detected substance was calculated from a calibration curve constructed
468 by reference standards.

469 To prepare samples for analysis of protein carbonylation (PCO), acetylcholinesterase
470 (AchE), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (T-AOC),
471 superoxide dismutase (SOD), and glutathione (GSH), larvae was weighed and
472 homogenized on ice in 5 volumes (M: V) of PBS buffer, then centrifuged at 12,000 x g for
473 15 min to collect the supernatant. Subsequently, PCO content was measured using the
474 2,4-dinitrophenyl-hydrazine (DNPH) method with slight modifications. The above-
475 prepared supernatant was mixed with streptomycin sulfate at a 9:1 volume (V: M), kept
476 at room temperature for 10 min, and then centrifuged at 12,000 x g for 15 min. Then, 80
477 µL of supernatant was mixed with 160 µL of 10 mM DNPH in 2 M HCl and reacted at
478 37°C for 30 min. Sample controls were also prepared by adding an equal volume
479 (500 µL) of 2 M HCl. Then, 200 µL of 20% TCA was added, mixed thoroughly, and
480 centrifuged at 4°C for 10 min at 12,000 x g, and the precipitate was collected. Add 400
481 µL of ethanol: ethyl acetate mixture (1:1), vortex and mix, centrifuge at 4°C 12,000 x g
482 for 10 min, and collect the precipitate to remove unbound DNPH. This DNPH removal
483 step was repeated once. The pellet was mixed with 400 µL of 6 M guanidine
484 hydrochloride, kept at room temperature for 15 min, and then centrifuged at 4°C 12,000
485 x g for 10 min to collect the supernatant. The carbonyl and protein contents were
486 determined by reading the absorbance at λ = 370 nm and 280 nm with a SpectraMax
487 190 spectrophotometer (Molecular Devices, California, USA) for each sample against an

488 appropriate blank. Finally, the carbonyl content per unit of protein was calculated based
489 on the protein content.

490 AChE activity was measured by the Ellman method as described by Orhan et al.
491 (Orhan et al., 2007) with slight modifications. Briefly, 20 μL of the prepared sample
492 supernatant was mixed with 160 μL of 0.1 mM PBS buffer (pH 8.0) and 10 μL of an AChE
493 (0.28 U mL^{-1}) and incubated for 15 min at room temperature. Then, 10 μL of ATChI
494 (acetylthiocholine iodide, 4 mg mL^{-1}) was added together with 20 μL of DTNB (5,5-
495 dithiobis-2-nitrobenzoic acid, 1.2 mg mL^{-1}) and immediately mixed, and the absorbance
496 was read at 412 nm. The results were expressed as activity percentages relative to the
497 sample solvent negative control.

498 The CAT activity was determined using a kit (Beyotime, Shanghai, China) according to
499 the manufacturer's instructions with slight modifications. Briefly, 10 μL of the prepared
500 sample supernatant was mixed with 30 μL of Tris-HCl buffer (pH 7.0, 50 mmol L^{-1}), 10 μL
501 of 250 mM H_2O_2 was added, and the mixture was immediately mixed and kept at 25°C
502 for 5 min. Then, 450 μL of 1.8 mol L-1 H_2SO_4 was added, and the mixture was vortexed
503 to terminate the reaction. Subsequently, 10 μL of this termination reaction solution was
504 mixed with 40 μL of Tris-HCl buffer (pH 7.0, 50 mmol L^{-1}). Then, 10 μL of this mixture
505 was added to a 96-well plate mixed with 200 μL of color development working solution
506 containing peroxidase and incubated for 15 min at 25°C. Finally, the absorbance was
507 measured at 520 nm, and the CAT activity was calculated using a calibration curve of 5
508 mM H_2O_2.

509 T-AOC was measured using the total antioxidant capacity assay kit with the ABTS
510 (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) method based on a previous report
511 (Chen et al., 2019). Add 10 μL of the prepared sample supernatant to 200 μL of ABTS
512 working solution. After 6 min of reaction, the absorbance was measured at 734 nm.
513 Finally, the antioxidant activity was displayed by Trolox equivalents antioxidant capacity
514 as mmol Trolox equivalents/g extract. The ABTS working solution was prepared as
515 follows: 400 μL of potassium persulfate was added to 400 μL of ABTS to generate an

516 ABTS stock solution, which was then incubated for 16 h at room temperature in the dark.
517 Before use, the stock solution was diluted with phosphate buffer, and the absorbance at
518 734 nm was adjusted to 0.70 ± 0.05.

519 Additionally, MDA, SOD, and GSH levels were detected using ELISA enzyme-linked
520 immunosorbent assay kits (Beyotime, Shanghai, China) according to our recent study (Li
521 et al., 2022).

522 **Histopathological analysis**

523 Microsectioning and hematoxylin-eosin (HE) staining was performed according to
524 standard histological protocols (Khan et al., 2010) with some modifications. Briefly,
525 entire fresh larvae were fixed in 10% neutral-buffered formalin solution for 12 h at 4°C
526 after 72 h of imidacloprid exposure. After isopropyl alcohol dehydration, samples were
527 embedded in paraffin and sectioned. Three-micron-thick sections were stained with a
528 Hematoxylin and Eosin Staining Kit (Beyotime, Shanghai, China), observed with an
529 Olympus BX51 microscope, and photographed with an Olympus digital camera.

530

531 **Statistical analysis**

532 Data were obtained from three independent experiments performed in triplicate and
533 are presented as the mean ± SEM. SPSS statistical package was used for statistical
534 analysis using Student's t-test and one-way ANOVA followed by Tukey's multiple
535 comparison test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ were considered statistically
536 significant). GraphPad software was used for data normalization, Pearson correlation
537 coefficient calculation was analyzed using SPSS, and a heat map was generated.

538

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Additional information

Author contributions

Zhi Li: Supervision, Designed research, Conceptualization, Methodology, Funding acquisition, Writing-original draft, Writing-review & editing. **Yuedi wang:** Investigation, Methodology, Validation, Formal analysis, Project administration. **Lanchun Chen:** Investigation, Data curation. **Qiqian Qing:** Investigation, Data curation. **Xiaoqun Dang:** Data curation. **Zhengang Ma:** Data curation. **Zeyang Zhou:** Conceived the research, Research guidance. All the authors read, corrected, and approved the manuscript.

Ethics

Animal experimentation: This study was performed in strict accordance with the guidelines of the Animal Bioethics Committee at Chongqing Normal University, China approved all experimental protocols.

Data Availability

All the data needed to evaluate the conclusions in this paper are present in the paper and/or Supplementary Materials.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional files

571 **Supplementary files：Table S1. Primers used in real-time quantitative PCR.**

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769

770 ## Figure legends

771 **Figure 1.** Imidacloprid causes larval developmental retardation in *Apis mellifera*. Larvae
772 were orally administered imidacloprid (IMI) or artificial food (CK) beginning at three-
773 day-old, followed by developmental monitoring for 96 hours. (A) Larval developmental
774 phenotypes at 0.7, 1.2, 3.1, and 377 ppb environmental concentrations of imidacloprid
775 exposure; (B) Larval developmental phenotype at 377 ppb imidacloprid exposure; (C)
776 Effect of 0.7, 1.2, 3.1, and 377 ppb imidacloprid on larval survival; (D) and (E) body width
777 and weight of larvae exposed to 0.7, 1.2, 3.1, and 377 ppb imidacloprid for 96 hours; (F)
778 and (G) statistics for daily (F) and three-day cumulative feeding (G) for larvae exposed to
779 377 ppb imidacloprid; (H) larval development time from three-day-old to pupal stage; (I)
780 The number of larvae that successfully reached the pupal stage as a percentage of the
781 total initial sample size; (J) The growth index of larvae. Statistical significance was set at
782 $*P < 0.05$ and $**P < 0.01$.
783

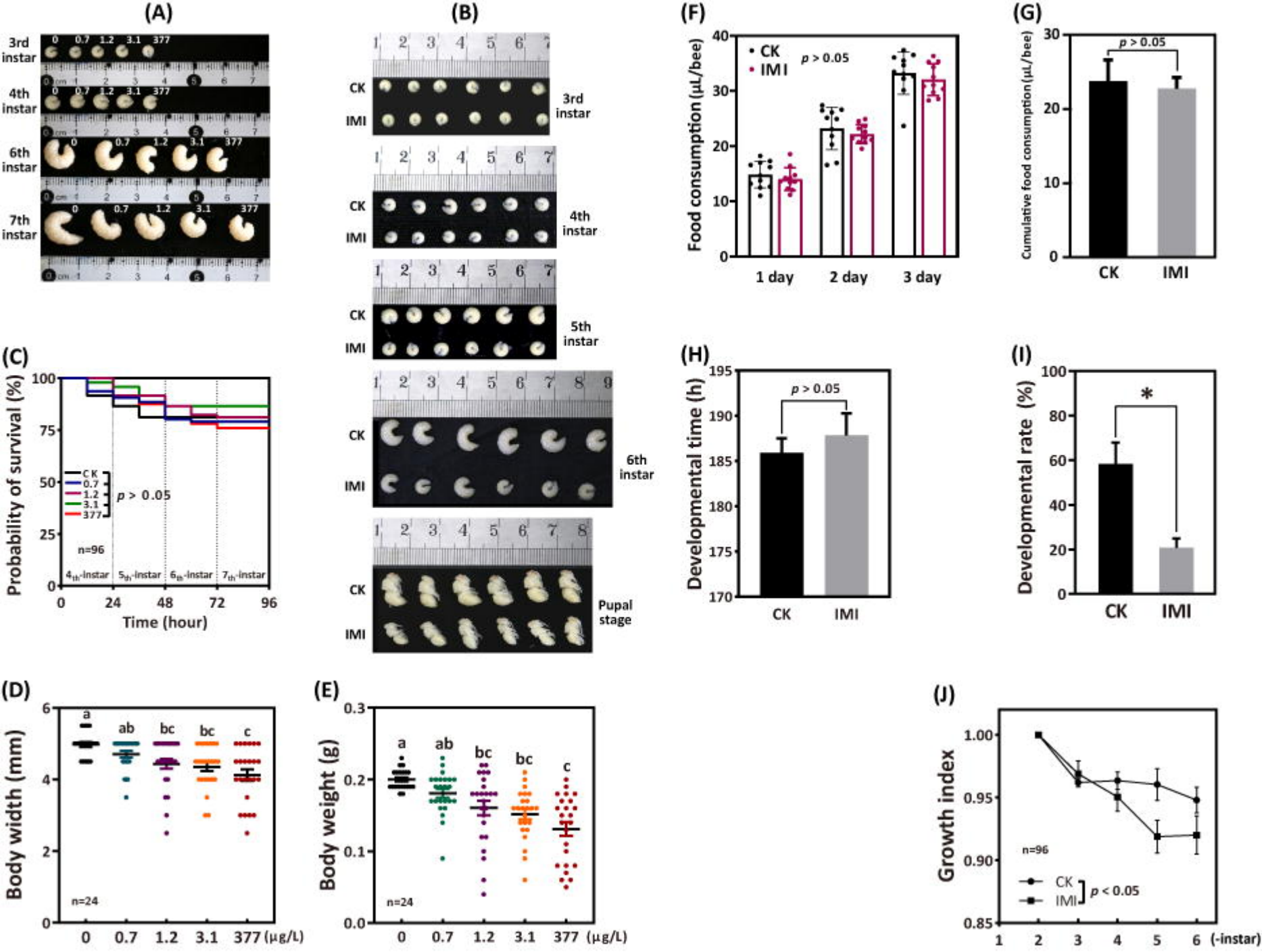
784 **Figure 2.** Imidacloprid neurotoxicity in *Apis mellifera* larvae. (A) AChE activity and (B)
785 nerve conduction-related gene expression analysis in larvae exposed to 377 ppb
786 imidacloprid for 72 hours or a control group. IMI is the larvae exposed to imidacloprid
787 for 72 hours. CK is the artificial food control group. Statistical significance was set at $*P <$
788 0.05 and $**P < 0.01$.
789

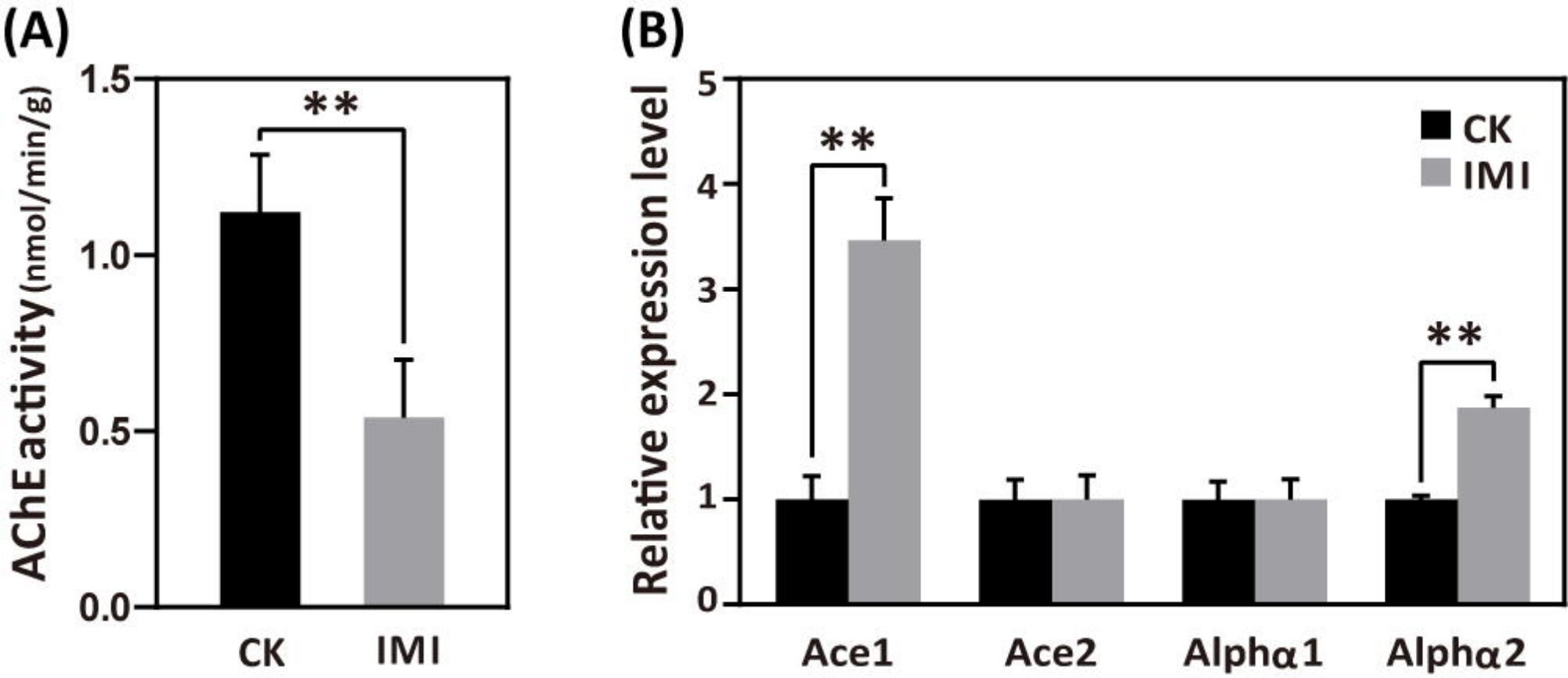
790 **Figure 3.** Effects of imidacloprid on the homeostasis of developmental regulation in *Apis*
791 *mellifera* larvae. (A) Relative gene expression and (B) hormone levels of developmental
792 regulatory-related genes in larvae exposed to 377 ppb imidacloprid for 72 h or a control
793 group. Statistical significance was set at $*P < 0.05$ and $**P < 0.01$.
794

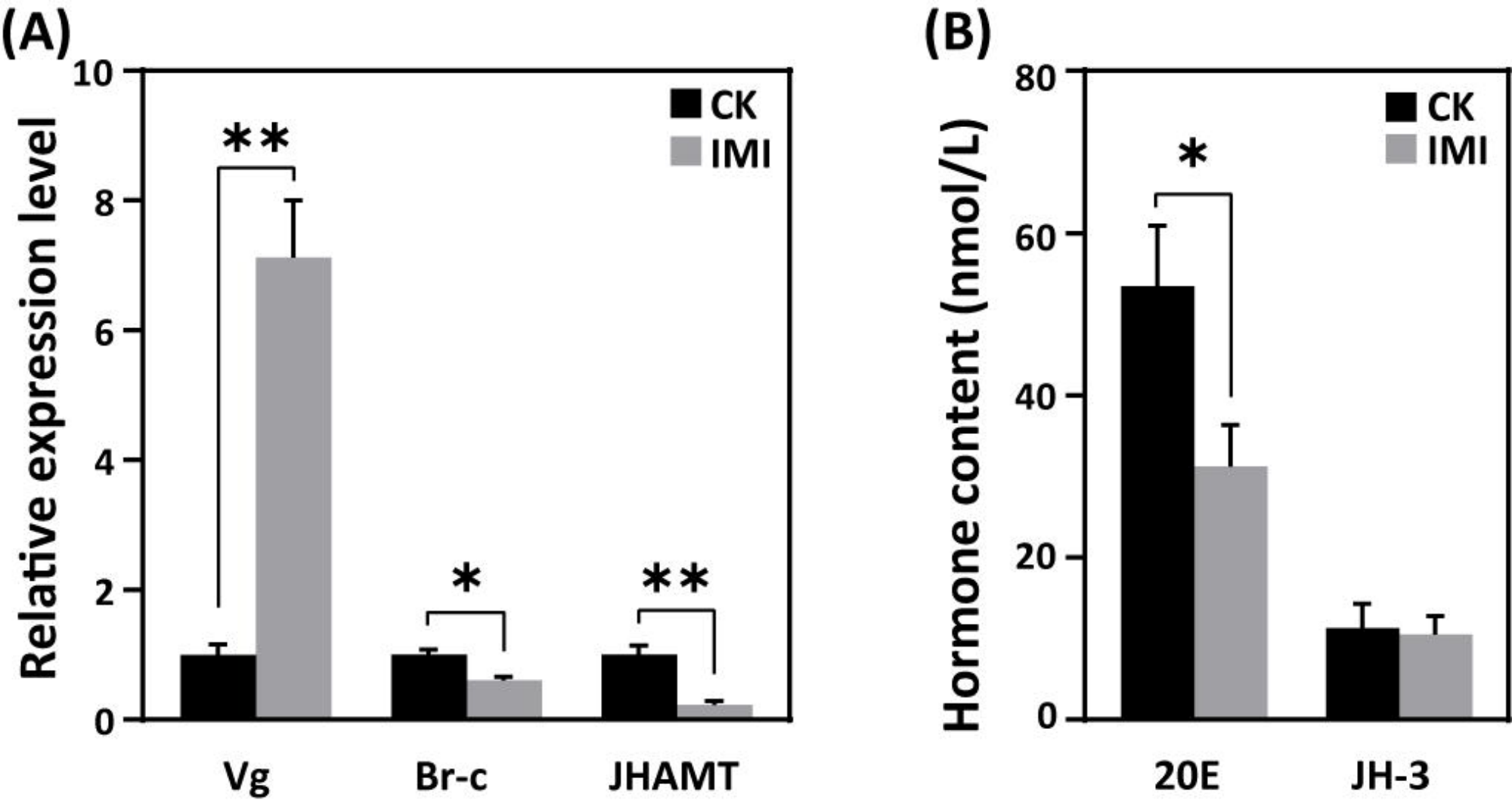
795 **Figure 4.** Imidacloprid induced a detoxification response in *Apis mellifera* larvae. Relative
796 expression levels of cytochrome P450 monooxygenase family genes in larvae exposed to
797 imidacloprid for 72 hours. IMI is the larvae exposed to imidacloprid for 72 hours. CK is

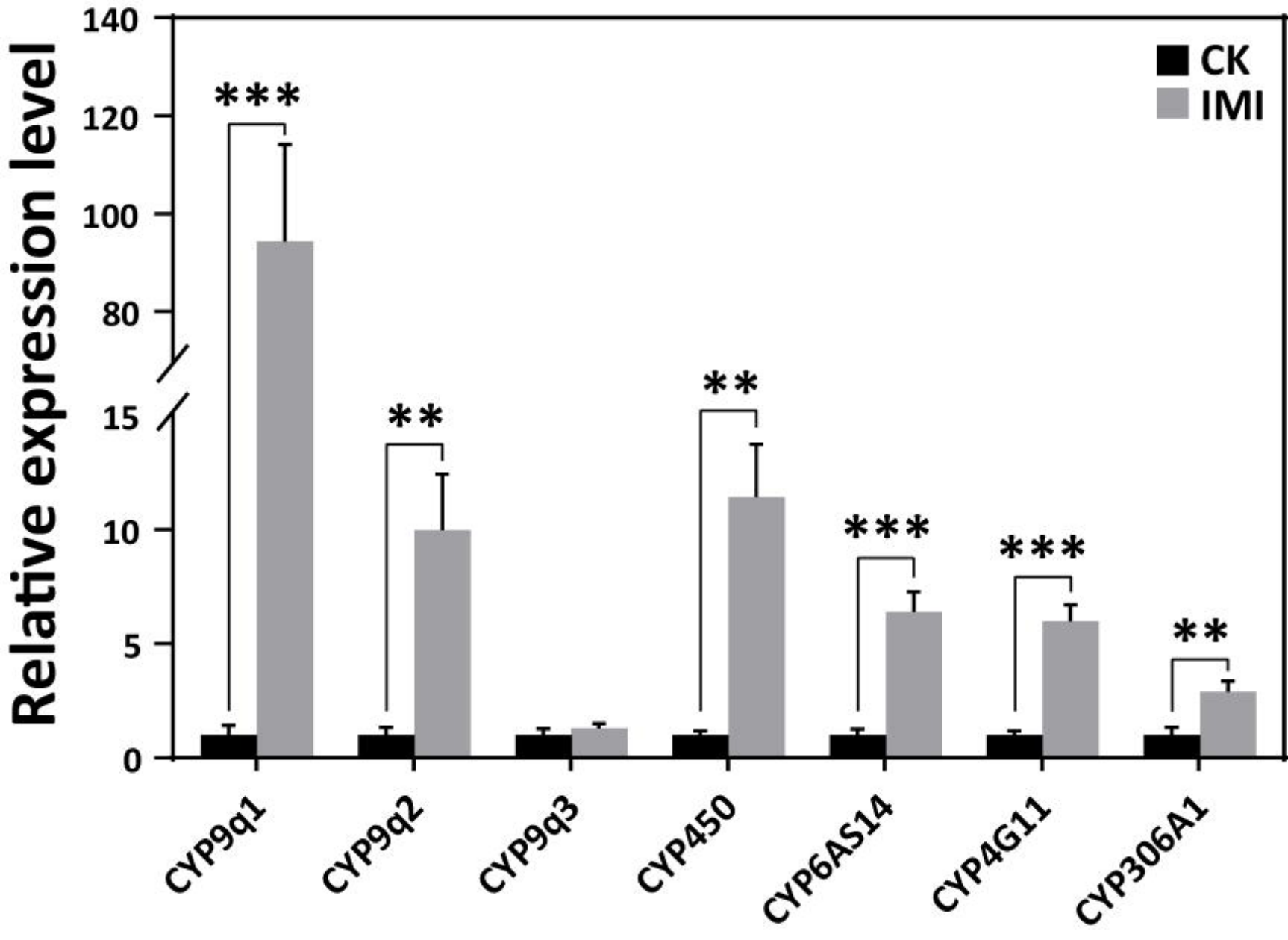
798 the artificial food control group. Statistical significance was set at *$P < 0.05$, **$P < 0.01$,
799 and ***$P < 0.001$.
800
801 **Figure 5.** Imidacloprid exposure causes oxidative stress, gut apoptosis, and tissue
802 structural damage while inducing antioxidant defenses in *Apis mellifera* larvae. (a)
803 Analysis of oxidative stress and damage in larvae exposed to imidacloprid for 72 hours.
804 ROS levels indicate the degree of oxidative stress. MDA is a marker for lipid peroxidation.
805 PCO levels reflect the extent of protein carbonylation damage; (b) Activity of
806 antioxidants in larvae exposed to imidacloprid for 72 hours; (c) The relative expression
807 levels of antioxidant genes in larvae exposed to imidacloprid for 72 hours; (d)
808 Histological sections of the larval gut stained with H&E. The circled inserts in the figure
809 show magnified views of the muscle layer. The blue arrow indicates the cell nucleus of
810 the muscle layer. The boxed inset in the figure is a magnified view of the basal layer
811 (black arrow), peritrophic membrane (red arrow), and food residues (green arrow). IMI is
812 the larvae exposed to imidacloprid for 72 hours. CK is the artificial food control group.
813 Statistical significance was set at *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.
814
815 **Figure 6.** Imidacloprid toxicity inhibited the expression of genes involved in nutrient
816 catabolism, protein synthesis, and energy metabolism and reserves. (A) Carbohydrate
817 catabolism; (B) Proteolysis; (C) Amino acid transport; (D) Mitochondrial oxidative
818 phosphorylation; (E) Glycolysis; (F) Protein synthesis. (G) The total contents of ATP,
819 glycogen (H), and protein (I). IMI is the larvae exposed to imidacloprid for 72 hours. CK is
820 the artificial food control group. Statistical significance was set at *$P < 0.05$, **$P < 0.01$,
821 and ***$P < 0.001$.
822
823 **Figure 7.** Correlation between phenotypes and molecular characteristics in imidacloprid-
824 exposed larvae with developmental retardation. Data were normalized using GraphPad
825 software, followed by a Pearson correlation coefficient calculation using SPSS, and a
826 heatmap was generated.
827

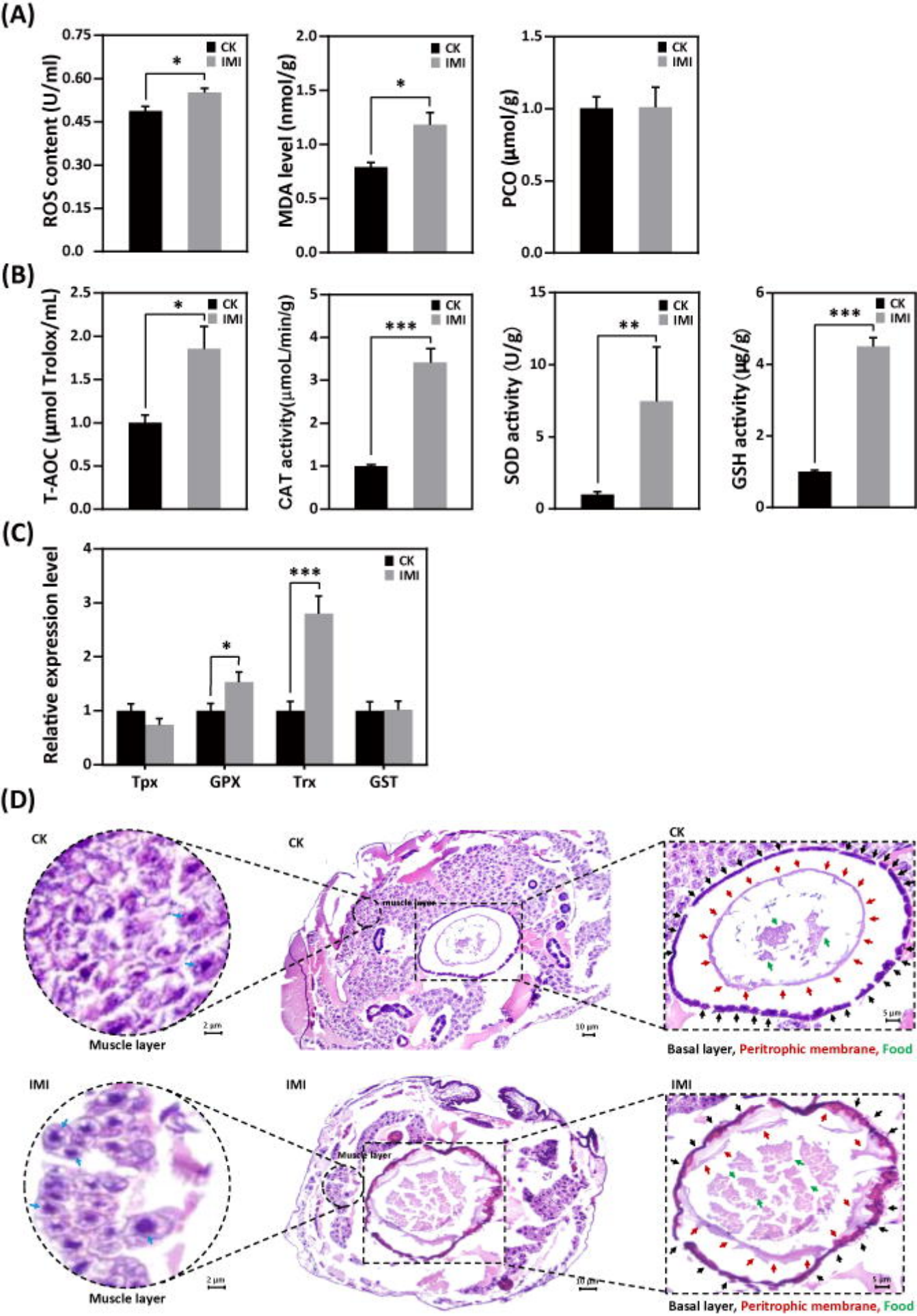
828 **Figure 8.** A model interpreting the ecotoxicological effects of imidacloprid on *Apis*
829 *mellifera* larvae and the mechanisms of imidacloprid-induced developmental
830 retardation. Imidacloprid toxicity caused growth and developmental delay in *A. mellifera*
831 larvae. It disrupts the regulatory balance of molt development, resulting in restricted
832 development. In addition, the damage caused by imidacloprid restricts the metabolism
833 and utilization of food nutrients and energy in larvae. The additional energy consumed
834 by larval P450 detoxification and antioxidant defenses further reduces the supply of
835 nutrients and energy supply for growth and development. The arrows indicate induction
836 or promotion, the straight lines indicate inhibition, and the green dotted lines indicate
837 speculative inhibition.
838
839
840
841
842

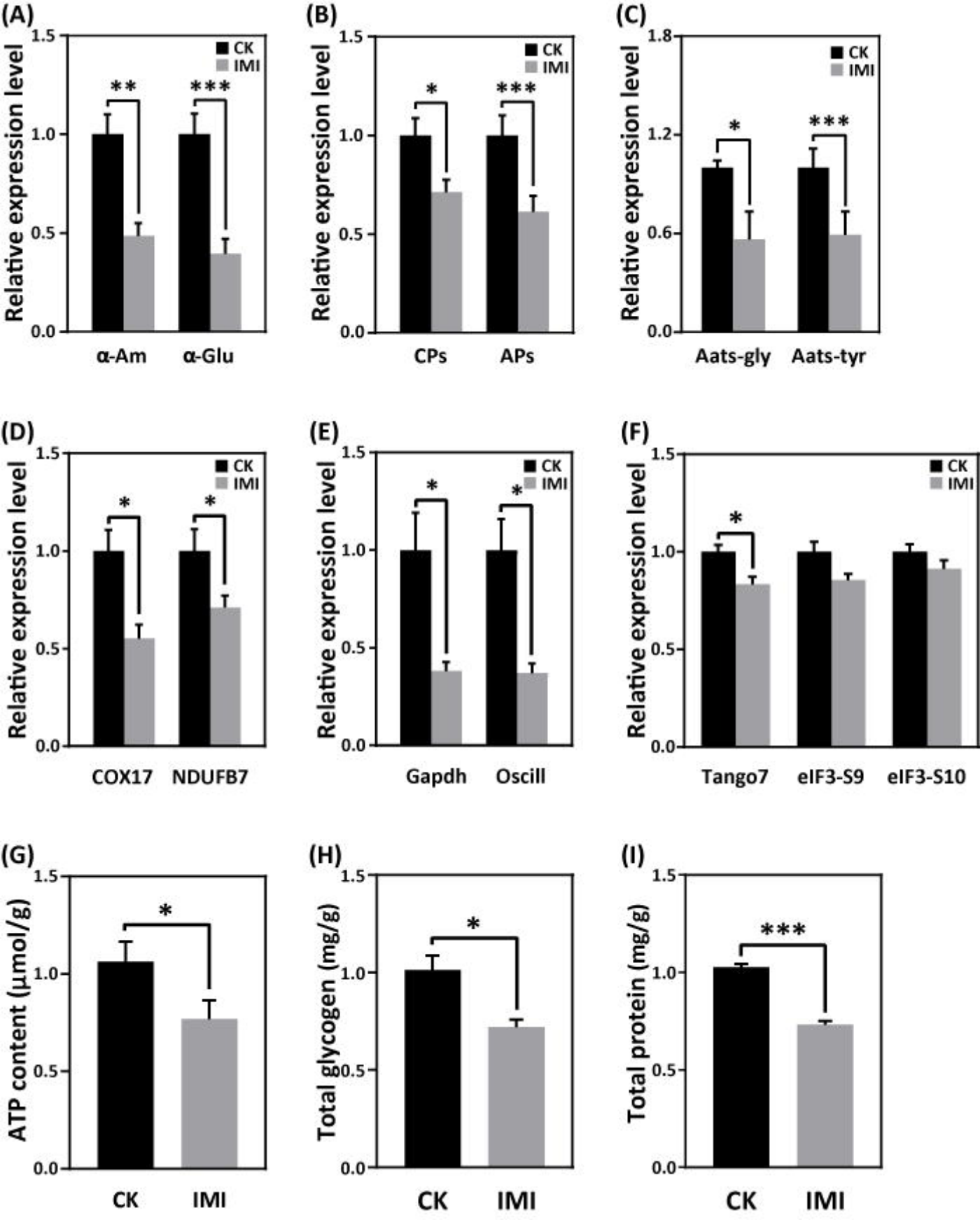

(A)
3rd instar
4th instar
6th instar
7th instar
0
0.7
1.2
3.1
377
(B)
CK
IMI
3rd instar
4th instar
5th instar
6th instar
Pupal stage
(C)
Probability of survival (%)
Time (hour)
CK
0.7
1.2
3.1
377
p > 0.05
n=96
4th-instar
5th-instar
6th-instar
7th-instar
(D)
Body width (mm)
a
ab
bc
bc
c
n=24
0
0.7
1.2
3.1
377 (μg/L)
(E)
Body weight (g)
a
ab
bc
bc
c
n=24
0
0.7
1.2
3.1
377 (μg/L)
(F)
Food consumption (μL/bee)
CK
IMI
p > 0.05
1 day
2 day
3 day
(G)
Cumulative food consumption (μL/bee)
p > 0.05
CK
IMI
(H)
Developmental time (h)
p > 0.05
CK
IMI
(I)
Developmental rate (%)
*
CK
IMI
(J)
Growth index
n=96
CK
IMI
p < 0.05
(-instar)

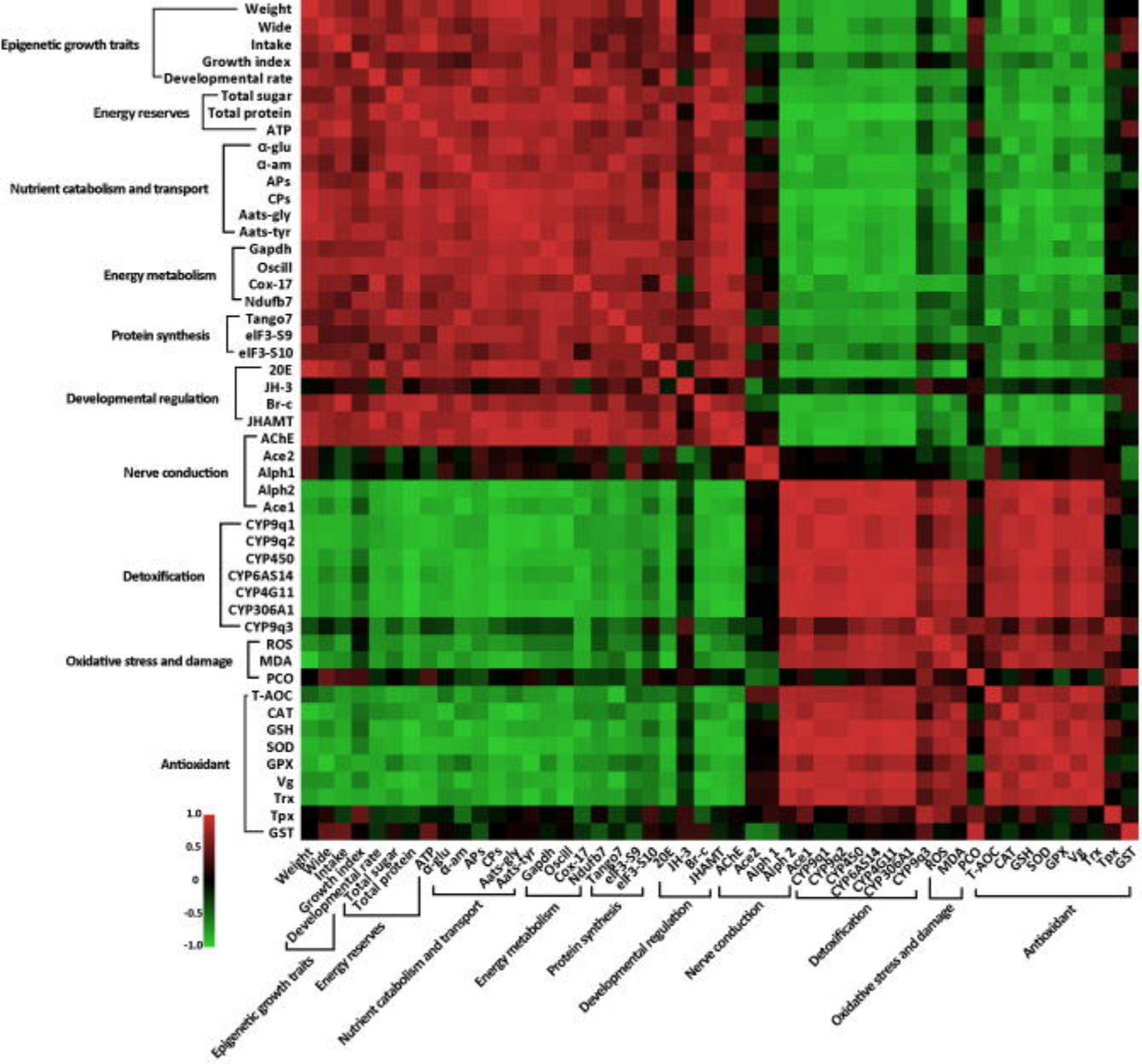

(A)
AChE activity (nmol/min/g)
1.5
1.0
0.5
0.0
**
CK
IMI
(B)
Relative expression level
5
4
3
2
1
0
**
**
CK
IMI
Ace1
Ace2
Alphα1
Alphα2

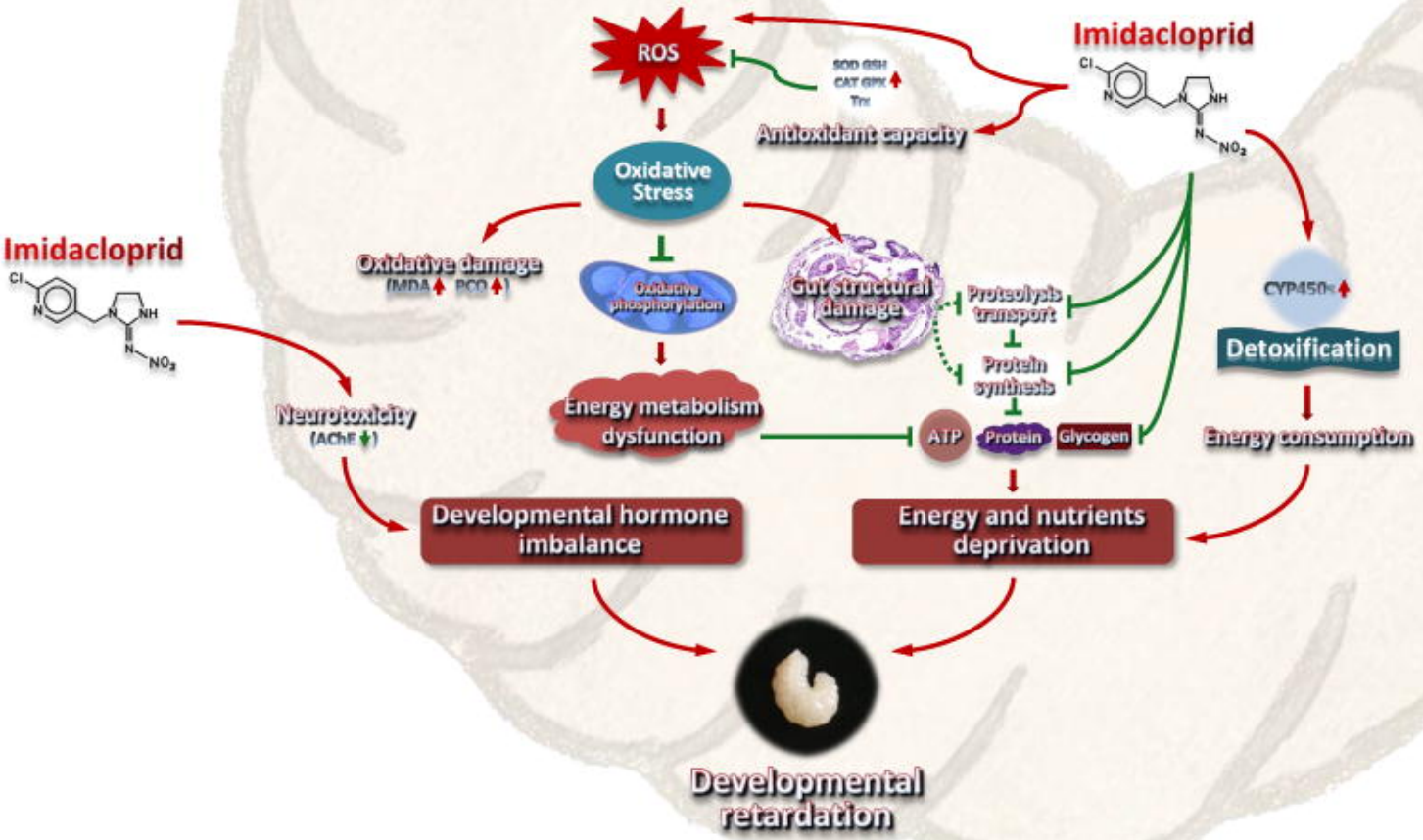

(A)
Relative expression level
10
8
6
4
2
0
CK
IMI
**
*
**
Vg
Br-c
JHAMT
(B)
Hormone content (nmol/L)
80
60
40
20
0
CK
IMI
*
20E
JH-3


Relative expression level
140
120
100
80
15
10
5
0
CK
IMI
***
**
**
***
***
**
CYP9q1
CYP9q2
CYP9q3
CYP450
CYP6AS14
CYP4G11
CYP306A1


(A)
ROS content (U/ml)
MDA level (nmol/g)
PCO (μmol/g)
CK
IMI
(B)
T-AOC (μmol Trolox/mL)
CAT activity(μmoL/min/g)
SOD activity (U/g)
GSH activity (μg/g)
(C)
Relative expression level
Tpx
GPX
Trx
GST
(D)
CK
IMI
Muscle layer
Basal layer, Peritrophic membrane, Food

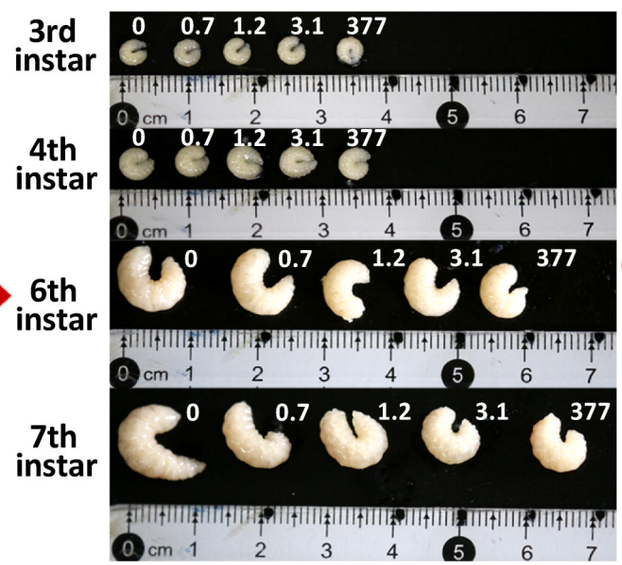

(A)
Relative expression level
CK
IMI
**
***
α-Am
α-Glu
(B)
Relative expression level
CK
IMI
*
***
CPs
APs
(C)
Relative expression level
CK
IMI
*
***
Aats-gly
Aats-tyr
(D)
Relative expression level
CK
IMI
*
*
COX17
NDUFB7
(E)
Relative expression level
CK
IMI
*
*
Gapdh
Oscill
(F)
Relative expression level
CK
IMI
*
Tango7
eIF3-S9
eIF3-S10
(G)
ATP content (μmol/g)
*
CK
IMI
(H)
Total glycogen (mg/g)
*
CK
IMI
(I)
Total protein (mg/g)
***
CK
IMI


Epigenetic growth traits
Weight
Wide
Intake
Growth index
Developmental rate
Energy reserves
Total sugar
Total protein
ATP
Nutrient catabolism and transport
α-glu
α-am
APs
CPs
Aats-gly
Aats-tyr
Energy metabolism
Gapdh
Oscill
Cox-17
Ndufb7
Protein synthesis
Tango7
eIF3-S9
eIF3-S10
Developmental regulation
20E
JH-3
Br-c
JHAMT
Nerve conduction
AChE
Ace2
Alph1
Alph2
Ace1
Detoxification
CYP9q1
CYP9q2
CYP450
CYP6AS14
CYP4G11
CYP306A1
CYP9q3
Oxidative stress and damage
ROS
MDA
PCO
Antioxidant
T-AOC
CAT
GSH
SOD
GPX
Vg
Trx
Tpx
GST
1.0
0.5
0
-0.5
-1.0

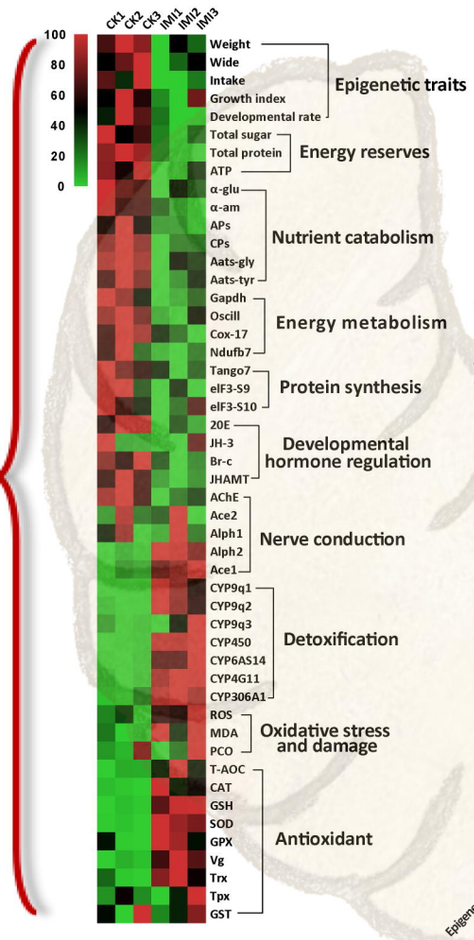

ROS
SOD GSH
CAT GPX
Trx
Antioxidant capacity
Imidacloprid
Oxidative Stress
Oxidative damage
(MDA PCO)
Oxidative phosphorylation
Gut structural damage
Proteolysis transport
Protein synthesis
CYP450
Detoxification
Energy consumption
Imidacloprid
Neurotoxicity
(AChE)
Energy metabolism dysfunction
ATP
Protein
Glycogen
Developmental hormone imbalance
Energy and nutrients deprivation
Developmental retardation

Imidacloprid toxicity and the underlying mechanism causing larval development delay

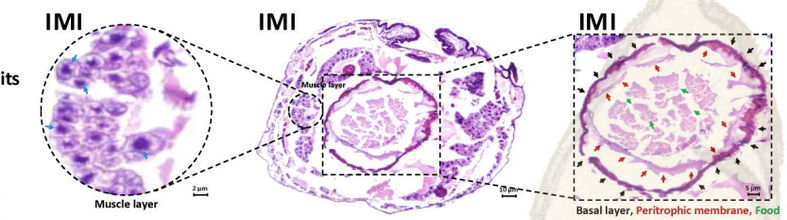
72 hours Exposure



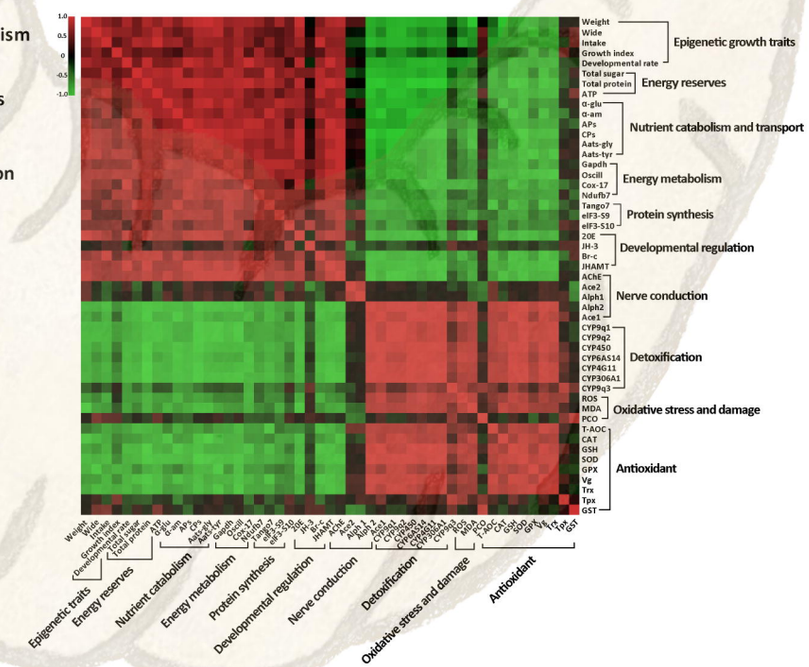
Developmental retardation



Toxic effects



Gut tissue damage



Correlation Analysis