New Insights Into the Biological Interaction Between Unripe Citrus Fruits and the Tephritid Fly *Bactrocera Minax* Based on Omics

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Abstract

The adaptation of phytophagous insects to host defence is an important aspect of plant-insect interactions. The reciprocal adaptability between specialist insects and their hosts have been adequately explored; however, the mechanisms underlying the adaptation of tephritid fruit fly specialists, a group of notorious pests worldwide, to unripe host fruits remain elusive. Here, plant metabolomes and insect transcriptomes were analysed for the first time to explore the interaction between unripe citrus fruits and the Chinese citrus fly *Bactrocera minax*. Seventeen citrus secondary metabolites, mainly flavones, alkaloids and phenylpropanoids, were identified in the unripe citrus fruit metabolome and they accumulated during larval feeding. Three detoxification genes (1 P450 gene, 2 ABCs genes) were highly expressed in *B. minax* larvae collected from unripe citrus fruits compared with the ones fed on artificial diets and ripe citrus fruits. Based on omics data, a novel ABC gene was screened through plant allelopathy tests and the gene was significantly upregulated in *B. minax* larvae treated with defensive secondary metabolites; additionally, the mortality rate of the larvae reached 51% after silencing the ABC gene by RNAi technique. Overall, these results shed light on the mechanisms underlying the biological interactions between tephritid fruit fly specialists and host fruits.

Key Message

- Little was known about the molecular mechanisms underlying the adaptation of *B. minax* to unripe citrus fruits.
- Fifty-four differential expression metabolites were detected in the unripe citrus fruit metabolome and 17 citrus secondary metabolites significantly upregulated during *B. minax* larval feeding.
- Three detoxification genes were highly expressed in the *B. minax* larvae collected from unripe citrus fruits, and *BmOGS12791* knockdown decreased *B. minax* larval survival rate in unripe citrus fruits.
- This work shed light on the mechanism of the biological interactions between tephritid fruit fly specialists and host fruits.

Introduction

Herbivorous insects closely interact with their host plants, which provide food resources, oviposition sites and habitat throughout their life cycles (Futuyma et al., 2009). In the long arms race with herbivorous insects, plants have evolved complex defence systems to resist infestation (Kessler et al., 2002; Chuang et al., 2014). Constitutive defence involves physical and chemical defensive traits, such as cuticles and plant secondary metabolites such as nicotine (Wu et al., 2010). The inducible defence of plants occurs after being attacked by herbivorous insects and is attributed to the phenylpropanoid and octadecanoid pathways mediated by salicylic acid (SA) and jasmonic acid (JA), respectively (Hagenbucher et al., 2013). These pathways produce massive plant secondary metabolites to reduce the development and survival of herbivores (Zavala et al., 2004; Howe et al., 2008). For example, the “mustard oil bomb” compound released in brassicaceous plants after herbivorous insect damage exhibits direct toxicity to insects

and/or acts as a feeding deterrent (Hopkins et al., 2009; Müller et al., 2010; Dixit et al., 2017). Additionally, the concentrations of certain plant secondary metabolites increase in response to herbivory, as reported in cotton, tomato, and coffee, to inhibit herbivorous insect growth and development (Balkema et al., 2003; Magalhães et al., 2008; Bhonwong et al., 2009).

To counteract these plant allelochemicals and other toxic compounds, herbivorous insects have evolved a complete detoxification system, including three important types of detoxifying enzymes (cytochrome P450 monooxygenases, esterases and glutathione *S*-transferases) and two functional gene families, UDP-glucosyltransferases (UGTs) and ATP binding cassette (ABC) transporters (Francis et al., 2005; Jin et al., 2019). These enzyme families have been reported in herbivorous insects such as *Aphis gossypii*, *Helicoverpa armigera*, and *Spodoptera frugiperda* in response to plant secondary metabolites such as gossypol, tannic acid, and nicotine (War et al., 2013; Zou et al., 2016; Li et al., 2019). Enhanced metabolism caused by detoxification genes allows herbivorous insects to survive and complete their development on their host plants. In *Myzus persicae nicotianae*, the increased expression of *CYP6CY3* and homologous *CYP6CY4* genes is related to tolerance to nicotine (Bass et al., 2013). Furthermore, in aphids, some ABC transporters and UGTs showed a dramatic increase in mRNA expression levels after feeding on barley, suggesting that these genes could be critical for detoxification metabolism (Huang et al., 2019).

The Chinese citrus fruit fly *Bactrocera minax* (Enderlein) (Diptera: Tephritidae) is an oligophagous pest whose host range is almost exclusively restricted to citrus species such as *Citrus limon*, *Citrus aurantium* and *Citrus sinensis* (Wang et al., 2019; Rashid et al., 2021). The female oviposits eggs in green unripe citrus fruits, differing from *Bactrocera dorsalis* which prefers to oviposit in mature fruits (Zhou et al., 2012; Xu et al., 2019). As an oligophagous insect, *B. minax* larvae face two challenges: coping with plant secondary metabolites in unripe fruits and completing development within two months. How larvae handle adversity and how citrus fruit metabolites change remain unclear. Previous studies indicated that the adaptation of *Rhagoletis pomonella* to Rosaceae fruits was most notably related to detoxification-related genes such as cytochrome P450s (Ragland et al., 2015). Moreover, the fitness ability of *Bactrocera dorsalis* is mostly attributed to its complex detoxification system (Shen et al., 2013). Therefore, the identification and analysis of the detoxification enzyme genes of *B. minax* will aid in better understanding the molecular mechanism underlying adaptation to unripe citrus fruits.

The only host of *B. minax*, *Citrus* spp., is the most produced fruit crop in the world and is cultivated worldwide (Mabberley et al., 2004; Barreca et al., 2011). Citrus fruits are rich dietary sources of flavonoids, which decrease the weight gain of silkworm larvae (Zhang et al., 2012; Dugo et al., 2005). In addition, large amounts of bitter compounds have been detected in citrus fruits, especially limonin and nomilin, and a peak in their contents was observed at the unripe fruit or fruit expansion stage, which corresponds to the *B. minax* larval development stage (Huang et al., 2019). *Aedes albopictus* are even killed when exposed to different concentrations of limonoids (Hafeez et al., 2011). Additionally, the changes of metabolites in unripe citrus fruits responding to biological stress remains unclear. Exploring

the composition of metabolites in unripe citrus fruits under biological stress helps to understand the defence mechanism of citrus fruits.

In the current study, we carried out metabolomic analyses of citrus fruits to investigate metabolite changes in response to *B. minax* larvae feeding. Moreover, corresponding *B. minax* larvae were collected for RNA-Seq to determine the differentially expressed detoxification genes that potentially contribute to host adaptation. These results are expected to provide new insights into the interaction mechanisms between unripe citrus fruits and *B. minax*.

Materials And Methods

Insects and plants

B. minax larvae and citrus fruits were collected on 31st July, 2018 from San Douping (N 30°821, E 111°051), Hubei Province, China. Healthy and active *B. minax* larvae were reared in unripe citrus fruits at 23°C in the laboratory.

Citrus fruits with and without infestation by *B. minax* larvae were considered treatment and control samples, respectively. These samples were collected on Sept 1st and Oct 1st, corresponding to *B. minax* first instar larvae and second instar larvae, respectively. The pulp was separated from citrus fruits, immediately placed into liquid nitrogen and then stored at -80°C.

RNA isolation, cDNA synthesis and qRT-PCR

Total RNA was isolated using RNA TRIzol (Takara, Japan) following the manufacturer's instructions. First-strand single-strand cDNA was prepared using a PrimerScriptTM RT Reagent Kit (TaKaRa Bio, Dalian, China) according to the manufacturer's instructions. Samples of 1st, 2nd and 3rd-instar larvae were used for stage-specific expression profiles, while different tissues of the 2nd-instar larvae were used for tissue-specific expression profiles.

The mRNA levels were measured by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR® Premix Ex TaqTM II (Tli RNaseH Plus, TaKaRa Bio) with StepOnePlusTM (Thermo Fisher Scientific). Real-time PCRs were performed in technical triplicates under the following procedures: a holding cycle of 95 °C for 30 s, followed by 40 cycling stages of 95°C for 5 s, 55°C for 30 s, and 72°C for 31 s. The relative expression was calculated based on the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

Analysis of citrus fruit metabolomics based on LC-MS data

The citrus pulp was ground by zirconia beads in a Mixer mill (MM 400) for 1.5 min at 30 Hz. One hundred milligrams of powder and 1.0 ml of 70% aqueous methanol were mixed and incubated overnight at 4°C for extraction. Before LC-MS analysis, the extracts were absorbed by centrifugation at 10000 g for 10 min and filtered through a microporous membrane (0.22 µm pore size).

Samples (2 µl) were injected into an LC-ESI-MS/MS system (HPLC, Shim-pack UFLC SHIMADZU CBM30A), and a column (Waters ACQUITY UPLC HSS T3 C18, 1.8 µm, 2.1 µm*100 mm) was used to analyse the sample extracts. The solvent system included water (0.04% acetic acid) as solution A and acetonitrile (0.04% acetic acid) as solution B following the gradient program. The A:B (v/v) gradient was 95:5 at 0 min, 5:95 at 11.0 min, 5:95 at 12.0 min, 95:5 at 12.1 min, and 95:5 at 15.0 min. The flow rate was kept at 0.40 mL/min, and the temperature was maintained at 40°C.

A triple quadrupole-linear ion trap mass spectrometer (Q Trap), API 4500 QTrap LC/MS/MS system, equipped with an ESI Turbo Ion-Spray interface, was used to perform linear ion trap (LIT) and triple quadrupole (QQQ) scans. The ESI source operation parameters were carried out following Chen et al. (2013). In brief, the electrospray ionization temperature was 500°C, ion spray voltage (IS) was 5500 V, and ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) were set at 55, 60, and 25.0 psi, respectively. Ten and 100 µmol/L polypropylene glycol solutions under QQQ and LIT modes were used for instrument tuning and mass calibration, respectively. QQQ scans were acquired as MRM experiments with collision gas (nitrogen) set to 5 psi. DP and CE for individual MRM transitions were performed with further DP and CE optimization. A specific set of MRM transitions was monitored for each period according to the metabolites eluted within this period.

Plant allelochemical feeding assays

Second-instar *B. minax* larvae were used to detect the expression levels of detoxification genes. Synchronous larvae were fed artificial diets supplemented with 6 plant secondary metabolites. The control larvae were fed an artificial diet with the same amount of DMSO/H₂O. Twenty larvae were fed an artificial diet containing compounds. After 72 h, active larvae were collected for RNA extraction and qRT-PCR.

dsRNA preparation and microinjection

dsRNA was synthesized by a Transcript Aid T7 High Yield Transcription Kit (Thermo). The dsRNA DNA template (Table S1) was amplified by primers containing the T7 RNA polymerase promoter at both ends, and the purified DNA template (1 µg) was used to synthesize dsRNA. The quality and size of dsRNA were verified by 1% agarose gel electrophoresis and spectrophotometer (Thermo). Approximately 2 µg dsRNA was injected into the abdomens of the second instar larvae by the microinjection method. After injection, the *B. minax* larvae were placed in green citrus, which remained consistent with the natural environment. The survival rate was statistically significant after 72 h, and lively larvae were collected to detect RNAi efficiency. This experiment was replicated 6 times.

Statistical analysis

SPSS 22.0 and R software were used for statistical analysis. The heat maps and Venn diagrams of the transcriptome and metabolome were examined with an online R package (<http://www.ehbio.com/ImageGP/index.php/Home/Index/PHeatmap.html>). The gene expression level

and survival rate were analysed with SPSS 22.0 software by independent Student's t-test. The data are presented as the means $\pm$ SEM, and significance levels were set at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Results

Metabolite differences in unripe citrus fruits

A total of 820 metabolites were detected and quantified in four samples of citrus fruits. By mapping on the Metware database (MWDB), these metabolites were divided into seven classes, of which plant secondary metabolites were the most prevalent (62.8% of the metabolites), followed by amino acids (11.9%) and lipids (8%) (Fig 1).

A comparison was performed to screen the metabolites involved in the citrus fruit defensive response to infection by 1st- and 2nd-instar *B. minax* larvae (Fig 2a, Fig S1). The results indicated that 105 and 236 metabolites were changed after 1st- and 2nd-instar larval feeding, including 88 and 196 upregulated metabolites, respectively (Fig 2b). Among these changed metabolites, plant secondary metabolites accounted for a substantial proportion (69.5% and 72.5%). Organic acids, phenylpropanoids and flavones were the main components in 1st-instar *B. minax* larvae damaging citrus fruits, followed by terpenes and alkaloids. More plant secondary metabolites were detected in citrus fruits infected by 2nd-instar *B. minax* larvae, in which flavones and organic acids accounted for a substantial proportion, approximately 19.4% and 15.2%, followed by phenylpropanoids at 11.4% and alkaloids at 7.2% (Fig S2).

Fifty-four metabolites were detected to be significantly altered during infection of both 1st- and 2nd-instar *B. minax* larvae, of which plant secondary metabolites accounted for 68.5%, including phenylpropanoids, flavones, alkaloids, terpenes and organic acids (Fig 2c, Tab 1). Most metabolites were upregulated in 54 metabolites, and plant secondary metabolites still accounted for a large proportion (65%). In particular, some metabolites, such as coumaraldehyde and N-methylcytisine, had weak or no signals because of their low contents; however, the signals were obviously enhanced after damage from *B. minax* larvae (Tab 1). Based on the KEGG analysis, 18 functional pathways were annotated, with most of these compounds concentrated on biosynthesis of secondary metabolites and metabolic pathways (Fig S3).

Differential expression of detoxification genes

A total of 61 P450s, 17 GSTs, 33 CarEs, 16 UGTs and 46 ABCs were identified to be expressed in the transcriptome of *B. minax* from unripe citrus fruits, with a substantial proportion of genes in these detoxification families being highly expressed in *B. minax* 1st- and 2nd-instar larvae, suggesting that these genes might play an important role in the larval development of *B. minax* (Fig 3a). In comparison with those of control larvae reared in unripe citrus fruits, the DEGs in detoxification families were screened to explore the adaptative mechanisms of *B. minax* larvae (date unpublished). Four comparison groups of

1st- and 2nd-larval transcriptomes were compared. Three detoxification genes (1 P450, 2 ABCs) were highly expressed in control group larvae in common compared to both treatment groups (Fig 3b-c, Tab 2).

Induced expression of DEGs by plant allelochemical and expression patterns

Based on the analysis of citrus fruit metabolism, 6 plant secondary metabolites were selected. N-Methylcytisine, tryptamine and coixol are alkaloids that were upregulated after *B. minax* larval damage. The qRT-PCR results indicate that the mRNA expression levels of 6 upregulated detoxification genes were differentially induced when *B. minax* larvae were fed on 6 plant allelochemicals. Interestingly, the expression of *BmOGS6416* and *BmOGS0653* in *B. minax* larvae was significantly increased after N-methylcytisine and nomilin treatment, respectively, which implied the potential interaction between these two metabolites and detoxification genes (Fig 4a). Moreover, a gene, *BmOGS12791*, belonging to the ABC transporter family, was simultaneously significantly increased in 6 plant allelochemical treatments ($P < 0.05$). Based on the results above, the expression patterns in different developmental stages and different tissues were determined via qRT-PCR. The results showed that *BmOGS12791* exhibited higher expression levels in the 1st- and 2nd-instar larvae (Fig 4b) and in the midgut and malpighian tubule (Fig 4c).

Knockdown of BmOGS12791 and phenotypic effects

Microinjection was applied for RNA interference, as shown in Fig 5a. The transcript level of *BmOGS12791* decreased by 50% compared to the control level after 72 h of injection of 1st-instar *B. minax* larvae ($P < 0.05$). The survival rate was also determined after RNAi, and a significant decrease was found in the *dsBmOGS12791*-treated *B. minax* larvae compared to that of the *dsEGFP*-treated larvae (Fig 5b).

Discussion

Plant-insect interactions are key for their coevolution (Futuyma et al., 2009). In nature, most herbivorous insects are specialists that are closely related to their host plants (Clarke, 2017). To unravel the molecular mechanisms underpinning these interactions, a combined analysis of plant metabolism and the insect transcriptome was performed to explore the interaction between unripe citrus fruits and *B. minax* larvae. Seventeen secondary metabolites were detected significantly upregulated in the unripe citrus fruit during *B. minax* larval feeding. Meanwhile, a novel ABC gene was screened through plant allelopathy tests which significantly reduced the survival rate of *B. minax* larvae in unripe citrus fruits after RNAi. Understanding how *B. minax* larvae adapt to host citrus fruits is crucial for exploring the evolution of specialized diets.

Plants produce hundreds of thousands of different specialized metabolites, which function as defences of plants, for example, nicotine in tobacco, gossypol in cotton, and mustard oil glycoside-black mustard enzyme in cruciferous crops (War et al., 2013; Zou et al., 2016; Li et al., 2019). In this study, we found many secondary metabolites in unripe citrus fruits by comparative metabolomics analysis. The

metabolite changes well explained the phenotypes when infested citrus fruits turned colour from green to yellow and then dropped to the ground before ripening. To date, few studies have focused on the response of unripe citrus fruits to biotic stress, with studies so far only focusing on citrus huanglongbing (Ferrarezi et al., 2020). Therefore, these differentially accumulated plant secondary metabolites identified herein may serve as important biochemical markers for induced resistance against insect herbivores. The plant secondary metabolites mainly contained phenylpropanoids, terpenoids and alkaloids, which were reported to be toxic in *Pieris rapae*, *Schizaphis graminum*, and *Ashbya gossypii* in a previous study (Hagenbucher et al., 2013; Fernandes et al., 2009; Yuling et al., 2019; Wang et al., 2018). Our results implied that these plant secondary metabolites were vitally important for unripe citrus fruit defence against *B. minax* larvae. Similarly, research on the interactions among *Anastrepha acris* and its highly toxic host plant *Hippomane mancinella* indicated that phenylpropanoids, flavonoids, chalcones and coumarins induced defence responses (Aluja et al., 2020). Taken together, these findings open a window for further study on the induced defence mechanisms of unripe citrus fruits.

Defence traits have been thought to be acquired by plants at the expense of other plant functions, such as growth and reproduction (Züst et al., 2017). Plant metabolism is usually reprogrammed to enhance specialized metabolism to ward off invaders, while primary metabolism is often suppressed (Zhao et al., 2020). For example, brown planthopper (BPH) infestation of rice upregulated the defensive response but downregulated primary metabolism (Kang et al., 2019). However, in this study, we found that *B. minax* larvae developed significantly with an increase in primary metabolites, including amino acids and vitamins, which may contribute to nutrition for the growth of larvae in unripe citrus fruits. More research is needed to explore the mechanisms by which larvae utilize these metabolites.

The detoxification system plays an important role in the host adaptation of phytophagous insects (Li et al., 2017; Ma et al., 2019). In this study, many differentially expressed detoxification genes belonging to the P450, GST, CarEs, ABC and UGT families were identified in the four comparison groups. Among them, a novel ABC transporter, *BmOGS12791*, decreased the survival of *B. minax* larvae in unripe citrus after RNA interference, which implied that *BmOGS12791* may play a critical role in detoxification and adaptation in *B. minax*. Moreover, *BmOGS12791* exhibited higher expression in the midgut and malpighian tubule, which are universally known to be important tissues associated with the metabolism of xenobiotics (Mao et al., 2007). Notably, ABC transporters have important roles in xenobiotic detoxification and *Bt* resistance (Wu et al., 2019). The expression of *HaABCG11* was upregulated after larvae were fed a diet supplemented with nicotine, and *HaABCB3* showed higher expression levels in the guts of *Helicoverpa armigera* larvae after they were fed a diet containing nicotine or tomatine (Huang et al., 2015; Bretschneider et al., 2016). ABCC genes were reported to be associated with resistance to Cry toxins from *Bacillus thuringiensis* (Bt) by reducing the binding affinity of Cry toxins to brush border membrane vesicles in different lepidopteran species (Pardol et al., 2013; Xiao et al., 2014; Chen et al., 2018). The effects of these detoxification enzymes on the metabolic ability of secondary metabolites and whether they affect the host adaptability of *B. minax* larvae are worth further study.

The interaction between *B. minax* larvae and unripe citrus fruits involves many metabolites and genotypic changes. Through the change in the number of differentially abundant metabolites, we speculated that the defence response of unripe citrus fruits gradually increased after the feeding of *B. minax* larvae, which was mainly due to the greater wound on citrus with the growth of larvae. However, *B. minax* larvae had access to nutrients from unripe citrus fruits and completed development within two months (Wang et al., 2019). There must be a specific gene expression system in *B. minax* larvae that contributes to their adaptation to and utilization of complex citrus metabolites as the adaptation of *Plutella xylostella* to crucifer (Sun et al., 2009). Here, we only explored the important role of the detoxification system in adapting to citrus, and future studies on the chemoreception system and digestion system in *B. minax* could better explain the host adaptability of the larvae. On the other hand, exploring the gene transcription level changes of unripe citrus fruits after larval infestation, especially jasmonic acid- and salicylic acid-related pathways based on transcriptomics, will comprehensively clarify the defence response of unripe citrus fruit upon feeding by *B. minax* larvae.

Overall, the results of current study are discussed with special emphasis on citrus fruits defence-responsive metabolites induced by *B. minax* larvae and the detoxification genes of *B. minax* involved in the response to citrus defence. Seventeen citrus secondary metabolites and three *B. minax* larval detoxification genes were screened to participate in the biological interaction between unripe citrus fruits and *B. minax* larvae. By linking the results of citrus metabolism and *B. minax* larval detoxification genes, we will better understand how coevolution between unripe citrus fruits and *B. minax* larvae shapes specialized diets traits. Moreover, we have screened a key detoxification gene that affects the *B. minax* larval adaptation to the unripe citrus fruits. However, the screening of specific defensive secondary metabolites in citrus fruits is still insufficient, which is the focus of further work. These studies will provide scientific basis for integrated management of *B. minax*.

Declarations

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Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Supporting information

Supporting information may be found in the online version of this article.

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Tables

Due to technical limitations, Table 1 is only available as a download in the Supplemental Files section.

Table 2 Detoxification genes in the *Bactrocera minax* with reference to the detoxification genes present in the *Drosophila melanogaster* genome

Genome ID	P value	Fly base ID	Best hit
BmOGS02200	0	FBgn0015033	Cyp4d8-PA
BmOGS03787	0	FBgn0030672	CG9281-PB
BmOGS12791	3.07E-25	FBgn0039890	CG2316-PA

Figures

Figure 1

Pie chart analysis of the metabolite composition of unripe citrus fruits.

Figure 2

Analysis of differential metabolites in citrus fruits in response to *B. minax* larval feeding. a: Heatmap of differential metabolites in citrus fruits. IF, Citrus fruits infected by 1st and 2nd instar *B. minax* larvae. CK, natural citrus fruits without *B. minax* larval feeding. b: Distribution of upregulated and downregulated metabolites of citrus fruits exposed to 1st- and 2nd-instar larval feeding in each comparison; c: Venn diagram analysis of DEGs in different comparisons.

Figure 3

Analysis of differentially expressed detoxification genes in *B. minax* larvae. a: Heatmap of detoxification genes in 1st-, 2nd- and 3rd- instar larvae using an average value of three replicates. b: Venn diagram analysis of DEGs in different comparisons among groups. G, Larvae reared in green citrus fruits; R, Larvae reared in ripe citrus fruits; Q, Larvae rearing on an artificial diet. c: Heatmap of 3 detoxification genes highly expressed in *B. minax* larvae reared in green citrus fruits.

Figure 4

Relative expression of differentially expressed detoxification genes. a: Confirmatory expression profiles of 6 genes screened in the transcriptome of wild-type *B. minax* by qRT-PCR. * $p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$ (t-test). b: Relative expression levels of *BmOGS12791* in different developmental stages of *B. minax*. c: Relative expression of *BmOGS12791* in various tissues of 2nd-instar *B. minax* larvae. Data are presented as the mean $\pm$ SE; different letters denote a significant difference among different samples ($p < 0.05$, one-way ANOVA with LSD test).

Figure 5

The mean influence of the *BmOGS12791* gene on host adaptation in *Bactrocera minax*. a: *BmOGS12791* mRNA levels of *B. minax* after dsRNA injection. b: Survival rate of *BmOGS12791* knockdown and control *B. minax* larvae. * $p < 0.05$, ** means $0.001 < p < 0.01$, *** $p < 0.001$ (t-test)

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