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Population density modulates insect progenitive plasticity through the regulation of dopamine biosynthesis

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Abstract

Insect fecundity is a quantitative phenotype strongly affected by genotypes and the environment. However, the interaction between genotypes and the environment factors in modulating the insect fecundity remains largely unknown. In this study, we investigated the density-dependent population dynamics in the brown planthopper (BPH), *Nilaparvata lugens*, by using two populations respectively carrying homozygous high-fecundity (HFG) and low-fecundity (LFG) genotypes. Under low population densities, the fecundity and population growth rate of the two genotypes showed increasing trends across generations, while the trends between HFG and LFG under high population densities were opposite. RNA-seq was applied to unveil the key factors for these density-dependent progenitive phenotypes. Combined with the temporal analysis and weighted gene co-expression network analysis, we identified two gene modules that simultaneously enriched in four pathways related to the neural system. Among these pathways, the gene *Nlpale* encoding a tyrosine hydroxylase was identified as the key gene. The RNA interference of this gene and manipulation of its downstream product dopamine could significantly impact the basic and density-dependent

progenitive phenotypes of BPH. This study shows that the dopamine biosynthesis is the key regulatory factor for the determination of fecundity in response to density changes in different genotypes of BPH, which gives an insight into the interaction of a typical environmental factor and insect genotype during the process of population regulation.

Key works: Brown planthopper · Fecundity · Population density · Genotype · Dopamine biosynthesis

Key Message

- The interaction of environment factors and genotypes on the fecundity of the brown planthopper *Nilaparvata lugens*, has not been reported.
- We investigated the density-dependent population dynamics in *N. lugens* by using two populations with high- and low-fecundity genotype.
- Under high population densities, the fecundity and population growth rate of the two genotypes of *N. lugens* showed opposite trends across generations.
- The key gene *Nlpale* encoding a tyrosine hydroxylase was identified using RNA-seq and bioinformatics analysis.
- RNAi study confirmed the dopamine biosynthesis plays a vital role for the two genotypes of *N. lugens* in response to density changes.

Introduction

Insects comprise the most diverse class of animals due to their excellent adaptability and high reproductive capacity (Grimaldi and Engel 2005). The reproductive fitness of insects is a quantitative phenotype determined by both of genotypes and the environmental factors. Genotypic factors usually refer to genetic variants that change the function of fecundity-related proteins (Watt 1992; Edward et al. 2014). And environmental factors associated with insect fecundity usually include predators (Rosenheim 1998), temperature (Geister et al. 2008), xenobiotics (Ge et al. 2011), nutrition (Winkler et al. 2006), and the density of insect populations (Stiling 1988). However, how genotypes interact with the environment to modulate the fecundity in a specific insect is largely unknown, due to the lack of appropriate experimental materials.

Population density acting as a dynamic factor that closely related to insect diet and the available nutrition, has great impact on the population growth of insects (Hassel 1975; Stiling 1988). Population density is critical to the survival and development of the larvae, and consequently impact the fecundity of the emerged adults in a variety of insect species. *Aedes aegypti* reared in higher larval density showed a greater number of eggs laid (Silva et al. 2020), while *Chilo partellus* moths at lower pairing density had higher fecundity (Hari et al. 2008). Increased initial density resulted in prolonged nymphal development duration, lower emergence rate, shorter longevity and lower female fecundity in the rice planthopper (Mori and Nakasuji 1991; Horgan et al. 2016). Besides, population density can influence many other processes, such as predator and prey, parasite and host interactions, disease transmission, and competition (Arcese and Smith 1988; Pacala and Hassell 1991; Berg 1992). Thus, the density stress is an ideal factor to study the interaction between insect genotypes and environment during the regulation of population growth.

Previous studies have indicated that population dynamics of insects could be influenced by individual variations (Hanski and Saccheri 2006; Sun et al. 2015). However, it is still a mystery that whether the same population density has equal or diverse impact on insect populations carrying different fitness-determined genotypes. To address this issue, we selected two genotypes of the brown planthopper (BPH), *Nilaparvata lugens*, a serious rice insect pest. Based on our previous studies, we obtained two homozygous BPH populations [a high-fecundity genotype (HFG) and a low-fecundity genotype (LFG) population] from the same initial population, with the ACE_{-862} and VgR_{-816} genotypes CCGG and AAAA, respectively (Sun et al. 2015; Liu et al. 2020). In this study, we described the population dynamic of the two genotypes BPH in different initial nymph densities by designing a density-dependent experiment. After that, we applied RNA-seq and a series of bioinformatic analysis to unveil the key factors for density-dependent progenitive phenotypes in this insect. Overall, we aimed to uncover the potential mechanisms behind the insect genotypes and population densities interaction in the modulation of insect fecundity.

Materials and methods

Insect populations and density-dependent bioassays

The BPH populations carrying homozygous high- (HFG) and low-fecundity (LFG) genotypes were previously obtained in our lab (Zhai et al. 2013; Sun et al. 2015), and were reared on a susceptible rice variety (Fenghuazhan) in the greenhouse at 26±2 ℃ with 70–90% relative humidity and a light-dark cycle of 16L: 8D h.

10, 50, 90, 130 and 170 newly (<12 h) hatched nymphs of the two BPH populations were individually reared in the 45-day old rice plants, then the rice plants for each treatment were transferred into a pot covered with insect-proof nets. Ten replicative treatments were conducted for each group. The nymphs' development and survival were recorded daily until becoming adults. The rice plants were replaced timely to keep fresh and sufficient for feeding with each treatment. Two-day-old female adults from each treatment were collected for weighting using electronic balance, then placed immediately in liquid nitrogen and stored at -80℃ for subsequent transcriptome sequencing. Those planthoppers in the same density treatment were then mate randomly. When nymphs emerged, we count the number of offsprings from each mate until no nymph hatched for three consecutive days. Those newly emerged nymphs taken from corresponding density treatment were used to conduct the same density-dependent bioassays as the next generation. The density-dependent bioassays were conducted for continuous 6 generations. The population growth rate was calculated as follows: P = (N*S*R*F*E)/N (P: Population growth rate, N: Initial nymph number, S: Nymph survival rate, R: Female ratio, F: Fecundity for per female, E: Egg hatchability) (Yu et al. 2013).

RNA extraction and sequencing

The insect samples of HFG and LFG reared under low density (10 nymphs per clump) and high density (170 nymphs per clump) at generations F1, F3 and F5 were collected. Total RNA was subsequently extracted from insect samples with TRIzol Reagent according to the manufacturer's instructions. The RNA samples were utilized for tag library preparation using Illumina gene expression sample preparation kit (Illumina, San Diego, CA, USA), following the manufacturer's instructions. The total RNA integrity and quality were assessed using the RNA 6000 Nano LabChip kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The poly(A)-containing mRNA was purified using magnetic oligo(dT) beads and the Oligotex mRNA kit (Qiagen, Hilden, Germany). The transcriptome sequencing library

was prepared according to a modified method (Su et al. 2012). The mRNA strands were cleaved into short fragments by fragmentation buffer and reverse-transcribed for cDNA synthesis.

Two replicates of each treatment were used for RNA sequencing. The cDNA library (~300 base pairs-long) was sequenced using Illumina HiSeq™ 4000 sequencing platform (Illumina, San Diego, CA, USA). The adapter sequences, empty reads, and low-quality sequences (i.e. reads with a ratio of unknown sequences 'N' > 5%) were removed to obtain clean reads. All clean reads were mapped to the *N. lugens* genome (Accession No. GCF_000757685.1) with Hisat2 software (Kim et al. 2015). Read counts for all transcripts were extracted with HTSeq-count (Anders et al. 2015) and then transfer to FPKM (Fragments Per Kilobase Million) with R software GenomicFeatures (https://git.bioconductor.org/packages/GenomicFeatures).

Temporal analysis

We generated a FPKM matrix of all transcripts in all samples. Then the samples were divided into four groups: HFG under low density (HFG-LD), HFG under high density (HFG-HD), LFG under low density (LFG-LD), and LFG under high density (HFG-HD). For each group, the temporal expression pattern from F1 to F5 of all genes was calculated with the corresponding FPKM matrix by using the Short Time-series Expression Miner (STEM) software (Ernst and Bar-Joseph 2006). The cluster number was set to 12 for each analysis.

Identification and annotation of candidate co-expression gene modules

Genes with FPKM lower than 0.3 in a sample were considered as "too lowly expressed" (Kang et al. 2013; Bai et al. 2015). We filtered the FPKM matrix by removing the transcripts that showed too low expression in more than half of the tested samples. The filtered matrix was then used for inferring the co-expression gene network modules by using weighted gene co-expression network analysis (WGCNA) in R (Langfelder and Horvath 2008). The parameters were set as "power $\beta = 6$, min module size = 50, ME miss thread = 0.25".

The candidate co-expression gene modules were selected according to the temporally analyzed gene list. The genes in candidate modules were annotated to the KEGG pathway

database, and the pathway enrichment analysis was performed based on the Fisher's exact test with Benjamini and Hochberg (BH) adjustment by using the KEGG annotation of all genes in the BPH as background.

Differential expression analysis and quantitative real-time PCR validation

The read counts generated by HTseq-count were normalized using R package DESeq2 (Love et al. 2014). Then the differentially expressed genes (DEGs) between pairwise comparisons were estimated by DESeq2 according to the threshold of |log2 ratio| > 1 and adjusted *P*-value < 0.05 (BH adjustment).

To support the analysis, twenty DEGs were randomly selected for measurement of expression. Total RNA (1 μg) reverse-transcribed into first-strand cDNA using a PrimeScript™ RT reagent kit (Takara Bio, Inc. Otsu, Shiga, Japan). qPCR was performed using a 10-μL reaction containing 1 μL cDNA, 0.3 μL each of 10 μmol·L^{-1} forward and reverse primers, and 5 μL SYBR® FAST Universal qPCR mix (KAPA Biosystems, Woburn, MA, USA) and LightCycler 480 system (Roche Diagnostics GmbH, Mannheim, Germany), with the following amplification conditions: 5 min at 95°C, followed by 45 cycles at 95°C for 10 s, at 60°C for 20 s, and at 72°C for 20 s. The qPCR experiments were performed for each sample using three biological and three technical replicates. The expression levels of selected genes were normalized to the expression levels of *N. lugens β-actin* (Chen et al. 2013). The differential gene expression was calculated using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001).

RNA interference of *Nlpale*

Gene *Nlpale* encoding tyrosine hydroxylase [(TH; the rate-limiting enzyme in dopamine production metabolic pathway] was selected as the key regulatory factor, and its function was validated by RNA interference (RNAi). For double-stranded RNA (dsRNA) synthesis, a 466-bp fragment (*Nlpale*) were amplified by PCR using *Nlpale* cDNAs. All primers contained the T7 promoter sequence at each end (Table S1). The dsRNA of *Aequorea victoria* green fluorescent protein (GFP, Accession No. ACY56286) was used as a control (Qiu et al. 2016; Pang et al. 2017). The T7 RiboMAX Express RNAi System (Promega, USA) was used to

synthesize dsRNA according to the manufacturer's instructions. The concentrations of dsRNA were quantified spectrophotometrically with a NanoDrop 2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the quality and size of the dsRNA were further verified via 1% agarose gel electrophoresis.

The 1-day-old female adults of HFG and LFG were used for RNAi. The dsRNA injection experiment was mainly conducted as previously described (Chen et al. 2013). Briefly, approximately 250 ng dsRNA was injected into the mesothorax of the individuals. Three females were randomly collected to test the RNAi efficiency by quantitative real-time PCR (qRT-PCR) at 24 h after injection. Then the injected females were mated with male adults at the ratio 1:1, and were released to potted rice according to the densities of 10, 90, and 170 individuals respectively. The method for qRT-PCR and fecundity bioassay of the treated insects was as described above. The total number of offsprings from each treatment was counted.

Manipulation of dopamine in the BPH

The Insect Dopamine elisa kit (Boshen Biotechnology, Jiangsu, China) was applied to detect the dopamine content in the BPH following the manufacturer's instruction. In brief, every five 1-day-old female adults was used as one repeat and six repeats was conducted for each treatment. The homogenate, standard substance and horse radish peroxidase (HRP) labeled antibody of dopamine were successively added to the micropores that are precoated with the dopamine antibody, and were incubated in 37°C. Then the incubation was washed, and the substrate 3', 3', 5', 5', -tetramethyl benzidine (TMB) is used for color rendering. The absorbance (OD value) was measured at the wavelength of 450 nm to reflect the dopamine content in the samples.

Dopamine hydrochloride (Sigma-Aldrich), a dopamine activator, and chlorpromazine (CPZ), which was a dopamine receptor antagonist, were dissolved in ringer's solution. The pharmacological injection experiment was mainly conducted as previously described (Ma et al. 2011; Chen et al. 2013). The injection metering of dopamine hydrochloride is 100 nl of 80μg/μl while CPZ is 100 nl of 20μg/μl solution. They were injected into the mesothorax of the BPH. Ringer's solution was used as the control. The qRT-PCR and fecundity bioassay

after injection was the same as described above.

Detection of the hormone 20‐hydroxyecdysone content

The content of hormone 20‐hydroxyecdysone (20E) in the BPH was detected with the Insect Ecdysone ELISA Assay kit (Boshen Biotechnology) according to the manufacturer's instruction. Briefly, every five 1-day-old female adults was used as one repeat and six repeats was conducted for each treatment. The homogenate, standard substance and horse radish peroxidase (HRP) labeled antibody of ecdysone were successively added to the micropores that are precoated with the ecdysone antibody, and were incubated in 37°C. Then the incubation was washed, and the substrate 3', 3', 5', 5', -tetramethyl benzidine (TMB) is used for color rendering. The absorbance (OD value) was measured at the wavelength of 450 nm to reflect the ecdysone content in the samples.

Statistical analysis

The impact of density, generation, genotype and their combinations on insect fecundity and population growth rate was analyzed with multi-factor analysis of variance (multi-way ANOVA) and Tukey's test using SPSS 18.0 statistical software. The differences in pairwise comparison on gene expression levels and offspring number of the BPH were analyzed with student's *t*-test using SPSS 18.0. Statistical differences were considered significant at $P < 0.05$ or very significant at $P < 0.01$. The differences amongst multiple comparisons were tested by one-way ANOVA, followed by Duncan's least significant ranges (LSR) test for multiple comparisons ($P < 0.05$).

Results

Population density modulates the fecundity of BPH

The population growth trends and dynamics between HFG and LFG under the densities of 10, 50, 90, 130 and 170 nymphs were measured for 6 continuous generations. The density, generation, genotype and the combinations of these factors all had significant impacts on the number of offspring per female (Table S2). Both genotypes showed a lower fecundity in a higher individual density (Fig. 1A). Under the densities of 90, 130 and 170 nymphs, the

241 offspring number of HFG showed a decreased trend across generations, while the tendencies
242 under low densities (10 and 50 nymphs) were opposite. In contrast, the LFG showed higher
243 fecundity as the generation increasing in all tested densities except for the highest one (170
244 nymphs). There was no significant difference between most adjacent generations in LFG
245 under the density of 170 nymphs.
246 The differential response to the population density between these two genotypes of BPH
247 was also seen from statistics of the population growth rate (Table S2). The population growth
248 rate of HFG presented a significantly rising trend under low population densities (10 and 50
249 nymphs) and a significantly decreasing trend under high population densities (90, 130 and
250 170 nymphs) (Fig. 1B). The LFG demonstrated similar change in the population growth rate
251 under low densities, however, the changing of the population growth rate was not obvious for
252 LFG under high densities (Fig. 1B). These findings suggested that BPH populations with
253 different fecundity-determined genotypes were quite different in their strategies of population
254 regulation to density changes.
255
256 **Temporal expression trends revealed by RNA-seq**
257 RNA-seq experiments were conducted to explore the key factors responsible for different
258 response to density changes between HFP and LFP. Insects reared on the densities of 10 (low
259 density, LD) and 170 (high density, HD) nymphs were selected, and samples were collected at
260 F1, F3 and F5. The RNA-seq produced an average 33,320,239 clean paired-end reads per
261 sample with Q30 value higher than 92% (Table S3). The average mapping rate of all samples
262 to the reference genome of the BPH was 54.43% (Table S3), and a FPKM matrix of 24,306
263 transcripts in all samples was generated based on the mapping results (Table S4). After
264 filtering the “too lowly expressed” transcripts, 15,147 transcripts were used for subsequent
265 analysis.
266 We applied temporal analysis with the filtered FPKM matrix to each treatment (HFG-LD,
267 HFG-HD, LFG-LD, and LFG-HD) independently. According to the fecundity phenotype, the
268 key factors in both HFG and LFG should show a linear correlation to the fecundity under low
269 density, thus the transcripts showing continuous increasing or decreasing trend along with
270 generations in group HFG-LD and LFG-LD were selected (Fig. 2A). For both genotypes

under high density, the key factors should show differential expression patterns to those under low density. Additionally, the expression patterns of key factors between HFG and LFG under high density should also be inconsistent. By using these considerations as filter parameters, we finally identified 31 candidate genes (Fig. 2B). The expression levels of these genes demonstrated significantly high correlation between HFG-LD and LFG-LD, while most of them only show low correlation in the other comparisons (HFG-LD to HFG-HD, LFG-LD to LFG-HD, and HFG-HD to LFG-HD) (Fig. 2C).

Identification of the key regulatory factor for density-dependent progenitive phenotypes

To further determine the key factors for density-dependent progenitive phenotypes, we conducted weighted gene co-expression network analysis (WGCNA). All the filtered transcripts were used for this analysis. The analysis resulted in 16 distinct gene modules (Fig. 3A, Table S5). The 31 candidate genes were distributed in eight of the modules. Among them, the turquoise and darkred modules accounted for most of the candidate genes (21/31, Table S6). The KEGG pathway enrichment of the genes in these two modules was analyzed. The genes in turquoise module were significantly enriched in a total of 69 pathways, while the number of enriched pathways for the genes in darkred module was 25 (Table S7). Remarkably, four pathways were identified to be enriched for both modules, which included Endocrine and other factor-regulated calcium reabsorption, Axon regeneration, Dopaminergic synapse, and Regulation of actin cytoskeleton (Fig. 3B). This finding suggested that these pathways might play important roles in regulation of fecundity between different population densities.

According to the phenotypic difference, the key factors should show significantly differential expression between HFG-HD and HFG-LD at F5. Thus, a differential expression analysis was conducted on these two datasets. The analysis identified a total of 464 DEGs in HFG-HD compared to HFG-LD, including 311 up-regulated and 153 down-regulated genes (Fig.4A, Table S8). Among the DEGs, only six were involved in the above four pathways. And importantly, only one of the 6 DEGs belonged to the candidate transcripts obtained from the temporal analysis. This candidate gene, LOC111049383 (*Nlpale*), encodes tyrosine hydroxylase that is the key rate-limiting enzyme for dopamine synthesis in organisms (Morgan et al. 1996; Ma et al. 2011) (Fig. 4B). The decreased expression trend of gene *Nlpale*

in low population density was irrelevant to the fecundity genotypes of BPH (Fig. 4B). However, the expression level of *Nlpale* was continuously elevated in HFG under high population density with the generations increasing, but similar trend was not seen in LFG under the same treatment. Overall, the expression changes of *pale* were negatively correlated to the phenotypic changes of fecundity in both genotypes of BPH.

The modulation of gene *Nlpale* and dopamine in the progenitive plasticity of BPH

The insects of HFG and LFG under low population fecundity (10 nymphs) were used to determine the basic expression level of gene *Nlpale* and content of dopamine in different genotypes of BPH. Based on the qRT-PCR analysis, the mRNA level of *Nlpale* was much higher in LFG than that in HFG (Fig. 5A). Accordingly, the LFG showed higher content of dopamine than the HFG (Fig. 5B). These results were corresponding to the transcriptomic analysis that the expression of gene *Nlpale* and the fecundity of BPH were negative correlation. This negative regulatory relationship was further confirmed by RNAi of *Nlpale*. The expression of *Nlpale* in insects of both genotypes was significantly decreased from 24 to 72 h after injection of dsRNA, compared with the GFP control (Fig. 5C). Interestingly, the expression of *NlVg*, an important molecular marker for insect fecundity, were significantly increased in insects of both genotypes at 48 and 72 h after RNAi of *Nlpale* (Fig. 5D). Correspondingly, the offspring number of HFG and LFG was significantly elevated after RNAi of *Nlpale* (Fig. 5E). In addition, when the female adults were supplied with extra dopamine hydrochloride by micro-injection, their offspring number was significantly declined. And the injection of CPZ, a dopamine inhibitor, resulted in the increasing of offspring number in corresponding female adults (Fig. 5F). The negative correlation between dopamine content and fecundity had no difference between insects of HFG and LFG, indicating the intrinsic regulatory role of dopamine biosynthesis in the fecundity of BPH.

We further investigated that whether *Nlpale* and dopamine were the key factors to determine the difference in response to population density changes between HFG and LFG insects. A significant correlation between the content of dopamine and the fecundity were observed on the insects from both genotypes under different population densities across 6 generations (R=-0.88 and *P*-value $=1.52\times10^{-6}$ for HFG, and R=-0.90 and *P*-value $=3.45\times10^{-7}$

for LFG, Fig. S1). Then the fecundity of *Nlpale* RNAi-treated insects of both genotypes was compared to those of GFP control. In the control traits, the average fecundity of both genotypes was significantly decreased as the population density increasing (Fig. 6A). When the *Nlpale* was knockdown, the density-dependent change of fecundity in LFG were almost disappeared (Fig. 6B). However, the absence of density effect only found in the HFG under high population density (130 and 170 nymphs). We subsequently modulated the content of dopamine in the insects of HFG and LFG. Interestingly, the injection of dopamine hydrochloride and CPZ all eliminated the density effect on fecundity of LFG, and similar result was only observed on high population density-treated samples of the HFG (Fig.6C and 6D). These results suggested that the disruption of dopamine biosynthesis has great impact on the density-dependent progenitive plasticity of BPH.

Previous studies have showed that the dopamine can regulate the biosynthesis of other insect hormones (Gruntenko et al. 2005a; 2005b; Rauschenbach et al. 2007). Thus, the relationship between the hormone 20E and dopamine in this density-dependent experiment was assessed. Accordingly, the content of 20E was negatively correlated to the dopamine content but positively correlated to the fecundity in BPH samples under different densities across different generations (Fig. S1). Additionally, the RNAi of gene *Nlpale* lead to the increase of 20E in the insect of both genotypes (Fig. S2), indicating the intrinsic regulation relationship between dopamine and 20E in this species.

Discussion

In the present study, we validated the progenitive plasticity in the BPH populations with two different fecundity-related genotypes. Under the continuous condition of low density, the insect population continuously expanded by the elevation of individual fecundity, and this elevation was independent of the genotypes. When under the continuous high density, the HFG insect continuously reduced its individual fecundity as well as the population growth rate, but this decreasing trend was not observed on the LFG (Fig. 1).

Our findings can be largely explained by the traditional theory of density-dependent population regulation. Nicholson (1933) proposed that the population could adjust density according to its own natural and environmental conditions. All species would balance their

population density with the resources which they need to live. Accordingly, a population density higher than the balance can be restricted by a series of morphological, physiological, behavioral, and genetic factors to prevent the population from infinite growing (Chitty 1960). In turn, the factors suppressing population growth will be weakened if the population density is lower than the balance (Murdoch 1994; Turchin 1999). The changes in the population growth rate of treatments LFG-LD, HFG-LD and HFG-HD were basically conformed with this hypothesis. For the treatment of LFG under high density, the fecundity showed increased trends but the population growth rate was overall stable across different generations. One possibility was that there exist some thresholds for population balance under different densities in this species. For high density, the threshold was to the advantage of LFG. Thus, the LFG under these densities could rapidly reach the state of balance, as was observed from the stabilized population growth rates.

RNA-seq and a series of bioinformatic analysis were utilized to unveil the determinant for the phenotypic difference between HFG and LFG under high population density. The analyzed results centralized at four pathways (Fig. 3B). Interestingly, all these pathways were closely related to the neural system. The Regulation of actin cytoskeleton is an essential process during axonogenesis, because that the neuroblast growth must undergo an engorgement that involves depolymerization of actin (Bradke and Dotti 1999) followed by the penetration of the microtubules into the central and peripheral domains (Kunda et al. 2001). Endocrine and other factor-regulated calcium reabsorption is a pathway associated with calcium regulation in organism, while calcium ion plays an important role in the activity of neural synaptotagmin (Li et al. 1995; Tucker et al. 2004). Importantly, the pathways of Axon regeneration and Dopaminergic synapse were exactly the components of the neural system (Neve et al. 2004; Hammarlund and Jin 2014).

Our subsequent research found that the dopamine was the key factor in modulating the fecundity of BPH. Interestingly, it seems that the fecundity was negative correlated to its dopamine content in the BPH (Fig. 4 and Fig. 5). Dopamine is a catecholamine neurotransmitter that is synthesized in both neuronal and non-neuronal tissues. It plays an important role in insect development and is known to be involved in insect's stress responses (Neckameyer 1996; Neckameyer and Weinstein 2005). Dopamine can act as a

391 neuromodulator to stimulate behavior like arousal, reproductive and locomotor activity
392 (Neckameyer et al. 2000; Pendleton et al. 2002; Pfeiffenberger and Allada 2012).
393 Environmental stress, such as heat, parasite infection can affect dopaminergic signaling
394 pathways in insects, then in turn, insects can benefit from dopamine to adapt to external
395 environment by modulating ontogenesis and development (Hirashima et al. 2000; Gruntenko
396 et al. 2000, 2004; Lavista-Llanos et al. 2014). Actually, it has been previously reported that
397 dopamine can also regulate insect reproduction by modulating female sexual receptivity,
398 oogenesis, and oviposition (Neckameyer 1998; Boulay 2001). Meanwhile, the change in
399 dopamine content can cause the changes in other hormones like 20E and JH, indicating its
400 potential regulative role to these hormones (Gruntenko et al. 2005a, 2005b; Rauschenbach et
401 al. 2007). Our data also suggested that the 20E content was negatively regulated by the
402 dopamine (Fig. S2). In BPH, the 20E content in female adults is significant associated with
403 their progenitive capacity (Yu et al. 2014; Zhou et al. 2020). Thus, the regulation of dopamine
404 biosynthesis on the fecundity of BPH may go through the 20E signal pathway. Further
405 research will be conducted to investigate this regulative approach.
406 More importantly, our study showed that dopamine exactly contributed to the
407 density-dependent progenitive plasticity of BPH. Both RNAi of the tyrosine hydroxylase
408 coding gene (*Nlpale*) and modulating of the dopamine content in insects significantly
409 disturbed the density effect on the fecundity of BPH (Fig. 6). In *Locusta migratoria*, the
410 transitions between solitary and gregarious phases in response to population density changes
411 are closely related to dopamine biosynthesis (Ma et al. 2011; Zhang et al. 2020). Here, we
412 assumed that the different population densities would release different signals to the brain of
413 insects, which differentially regulate the pathway of dopamine synthesis and thus result in the
414 different changes in female fecundity. The trend of phenotypic change across generations is
415 finally determined by the population genotype that is associated to the distance to population
416 balance under a specific density.
417 In summary, we demonstrated that the dopamine biosynthesis is the key regulatory factor
418 for density-dependent progenitive plasticity in the BPH. The findings in this study provide
419 insights into the interaction of genotypes and environmental factors in regulating insect
420 population dynamics, and will facilitate the understanding of the mechanism behind the

421 population regulation.

422

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427

428 **Data Accessibility**

429 All sequencing data were deposited to the NCBI Sequence Read Archive (SRA;
430 http://www.ncbi.nlm.nih.gov/sra/) under the BioProject PRJNA690237.

431

432 **Author Contributions**

433 KL, WZ and RP conceived and designed the study. KL, RP, WC and KK conduct the
434 experiments. KL and RP designed the RNA-seq analysis pipeline, and performed the data
435 analysis. LYY, LY, and JL contributed to materials. KL, WZ and RP wrote the manuscript.

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Figure legends

Fig. 1 The influence of population density on the fecundity (**a**) and population growth rate (**b**) of two BPH populations with different fecundity-related genotypes. HFG: High-fecundity genotype BPH; LFG: Low-fecundity genotype BPH. **a** F1-F6 represent the 1st to 6th generation of nymph density experiment, respectively. The values are presented as mean ± SE (n=10). The values sharing different lowercase letters are significantly different at $P < 0.05$ (one-way ANOVA and Duncan's multiple range test).

Fig. 2 Temporal expression trend analysis of the transcriptome data. **a** Clusters of gene expression trends by the Short Time-series Expression Miner (STEM) software. The gene clusters 1 and 10 of treatments HFG-LD and LFG-LD were selected. **b** Heatmap representing the expression patterns of 31 candidate genes in each treatment. The trends indicate the expression patterns in treatments HFG-LD and LFG-LD. **c** The Pearson correlation coefficient of pairwise comparison on the expression levels of candidate genes.

Fig. 3 Identification of co-expression genes related to candidate genes by Weighted gene co-expression network analysis (WGCNA). **a** Hierarchical cluster tree showing the co-expression modules identified by WGCNA. **b** The overlapped pathways that are enriched

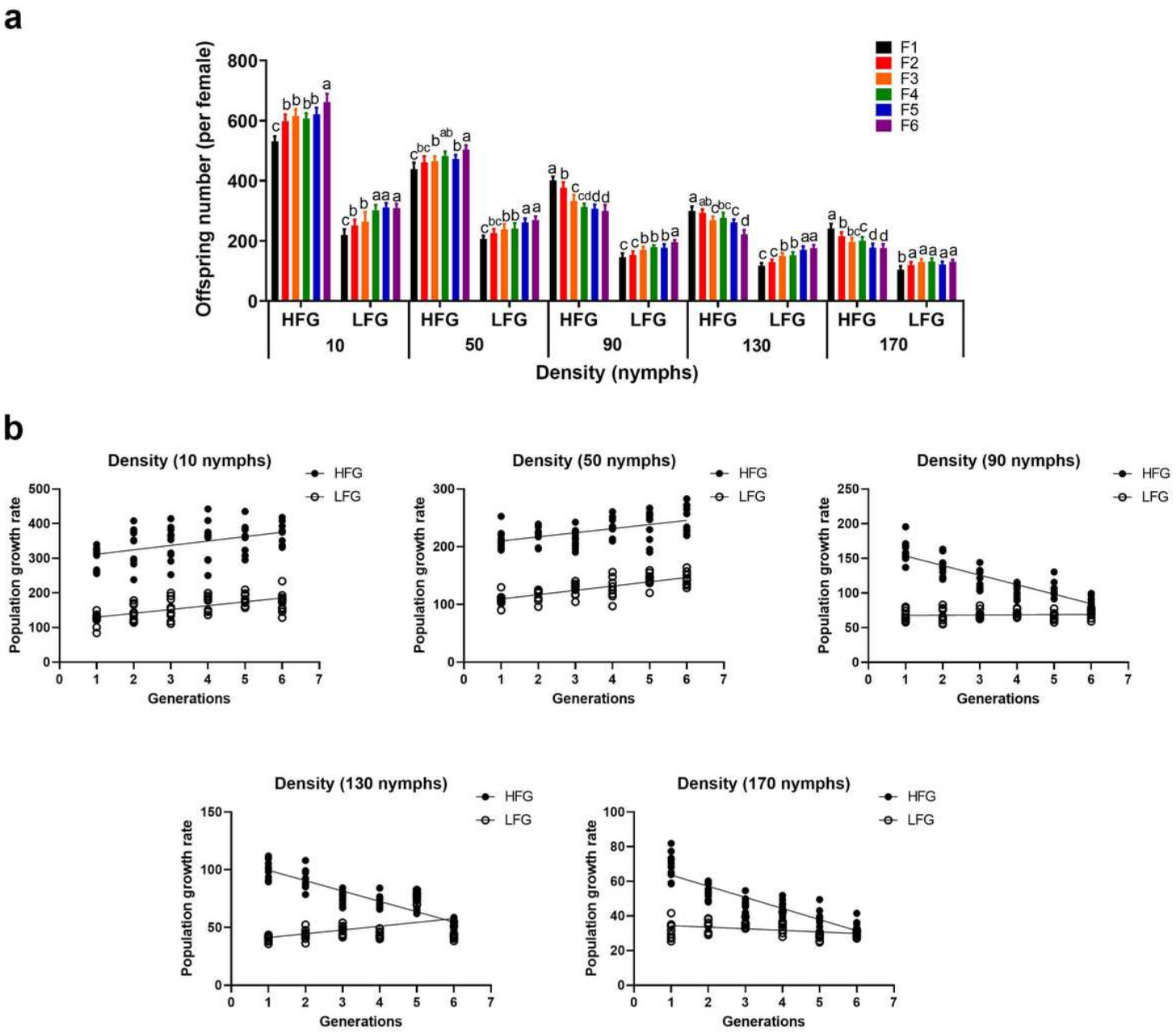
for gene modules turquoise and darkred.

Fig. 4 Targeting the key regulatory factors by differential expression analysis. **a** A volcano plot of the gene expression pattern between HFG-HD and HFG-LD at F5. The black, red and green points represent no difference in expression, up-regulated genes and down-regulated genes (FDR $\leq$ 0.05, Benjamini and Hochberg adjustment). **b** The pathway map of Dopaminergic synapse. The expression patterns of detected genes in this pathway are presented by heatmap.

Fig.5 The role of *Nlpale* mRNA level and dopamine content in the fecundity of BPH. The *Nlpale* mRNA levels (**a**) and dopamine contents (**b**) in two genotypes insects are presented. The mRNA levels of *Nlpale* (**c**) and *NlVg* (**d**) in the two genotypes of BPH were measured at 24, 48 and 72 h after RNA interference of *Nlpale*. The insects injected with ds*GFP* were used as control. **e** The fecundity of HFG and LFG after injection of *Nlpale* dsRNA. **f** The fecundity of HFG and LFG after injection of dopamine inhibitor chlorpromazine, dopamine hydrochloride (activator), and water control. All data are presented as mean ± SE. *: $P < 0.05$ level; **: $P < 0.01$ level (student's *t*-test). The values sharing different letters are significantly different at $P < 0.05$ (one-way ANOVA and Duncan's multiple range test).

Fig. 6 The role of dopamine biosynthesis in the density-dependent effects on the fecundity of BPH. The impact of injection of ds*GFP* (**a**), ds*Nlpale* (**b**), dopamine hydrochloride (**c**), and chlorpromazine (**d**) on the fecundity of two genotypes insects under the population densities of 10, 50, 90, 130 and 170 individuals. All data are presented as mean ± SE. The values sharing different capital letters are significantly different at $P < 0.05$ within the HFG, and the values sharing different lowercase letter are significantly different at $P < 0.05$ within the LFG (one-way ANOVA and Duncan's multiple range test).

Figures



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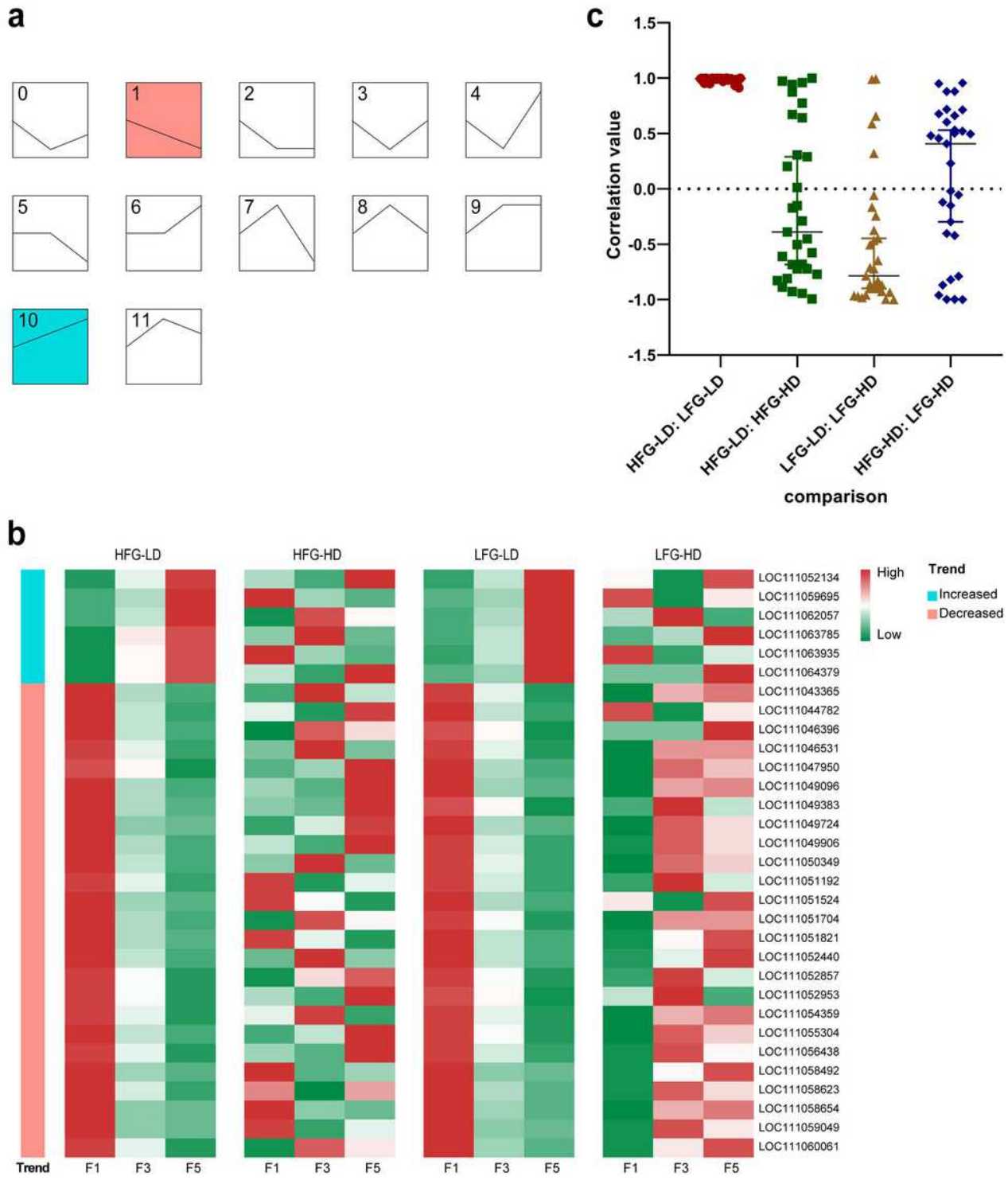

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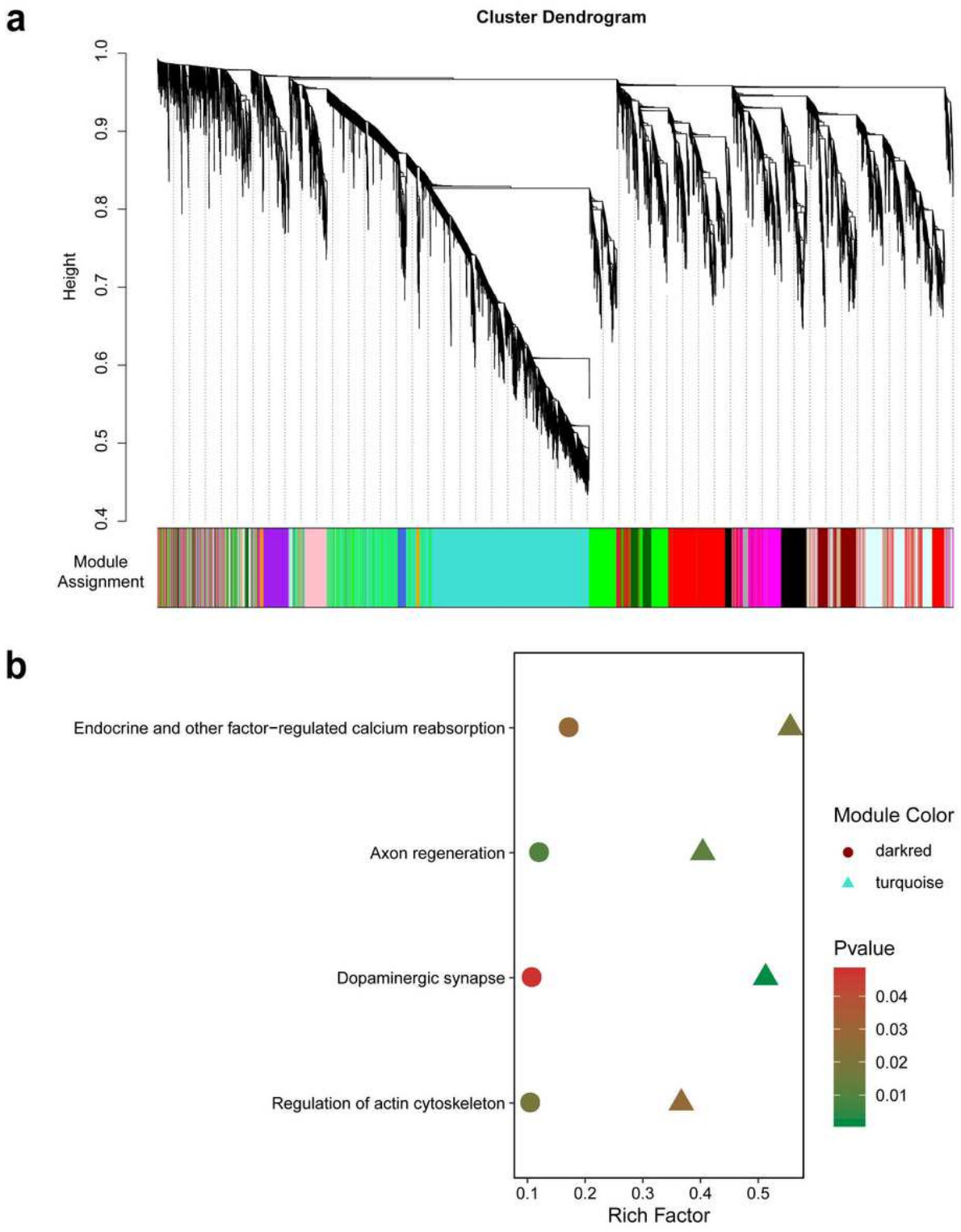

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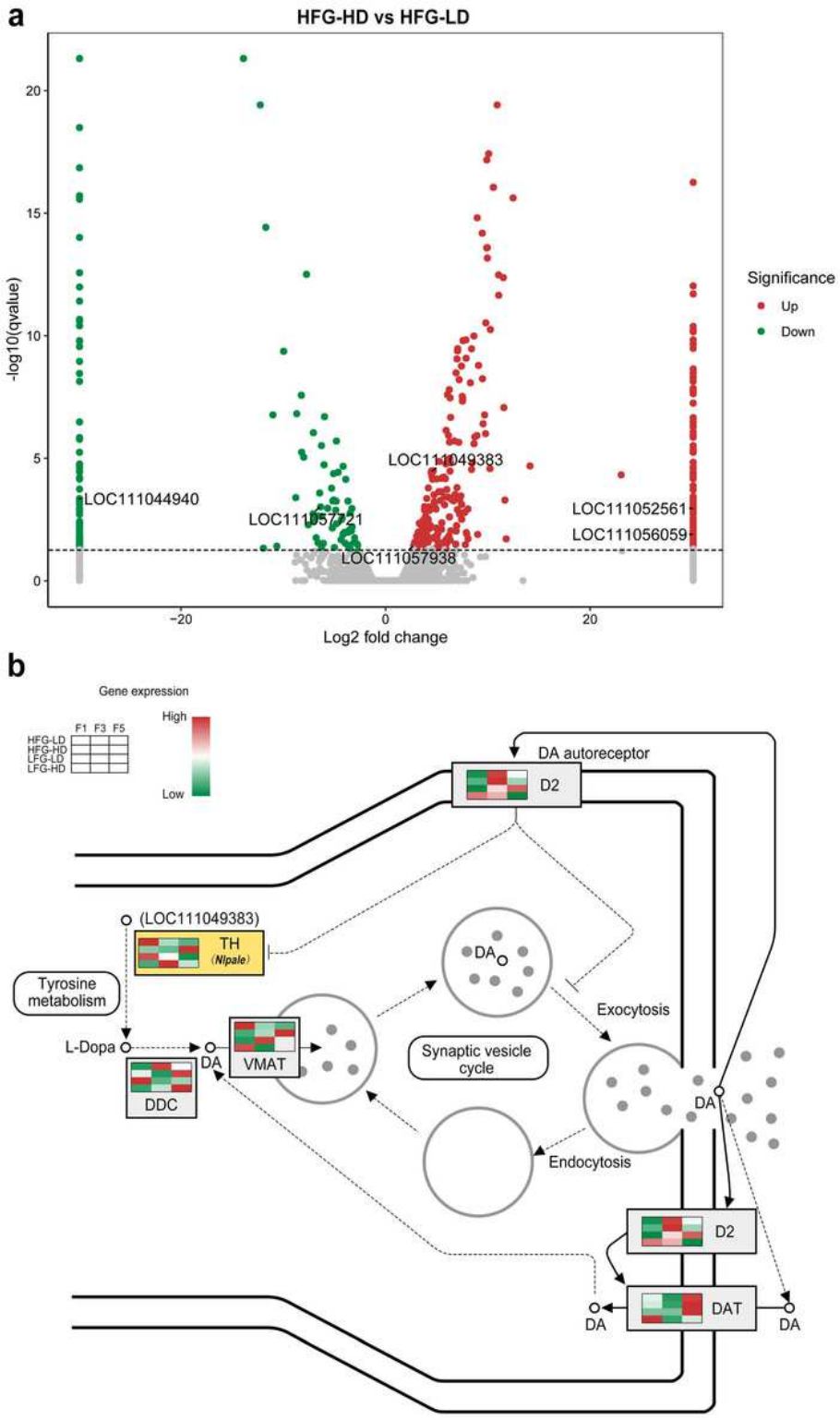

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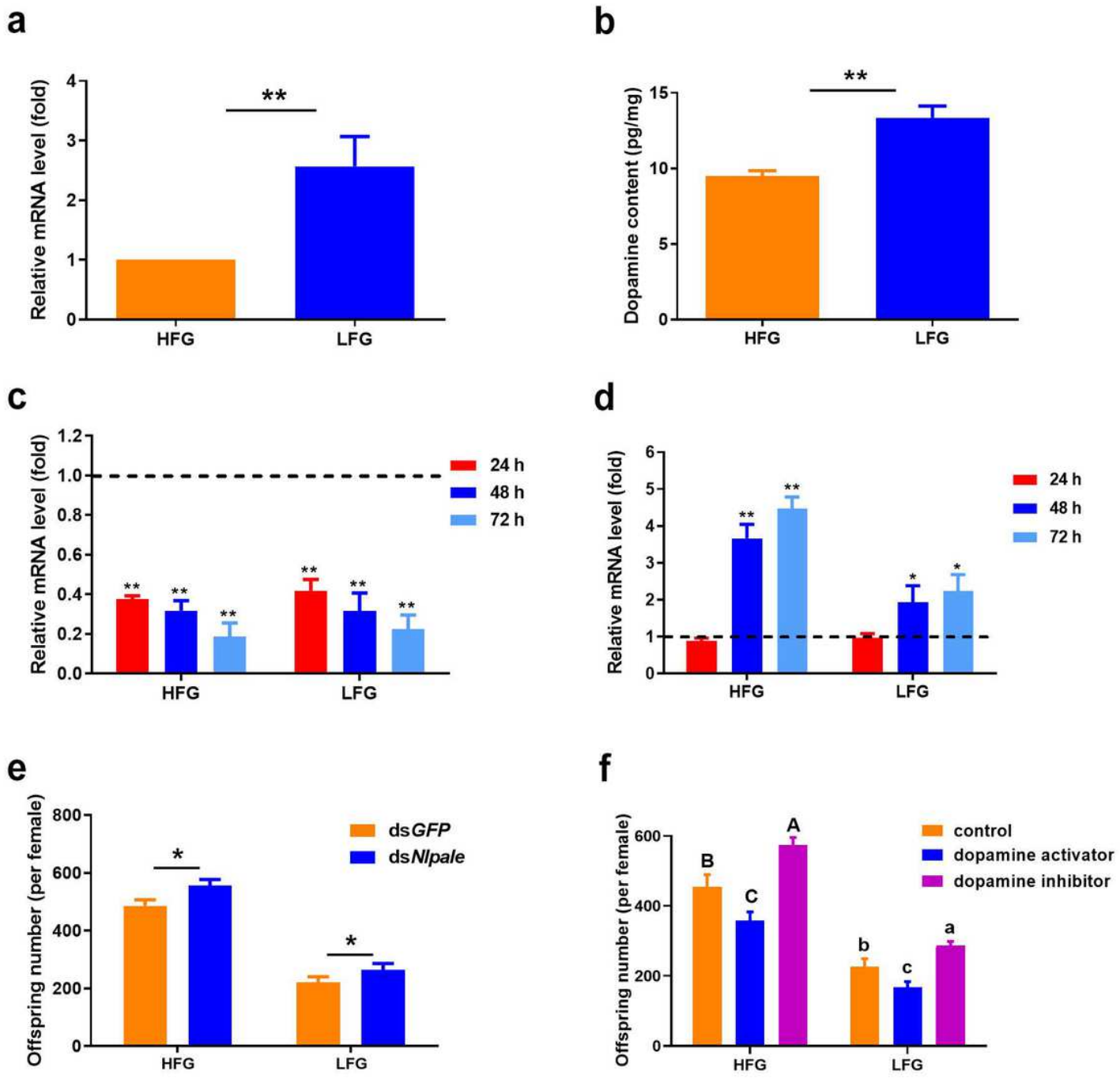

Figure 5

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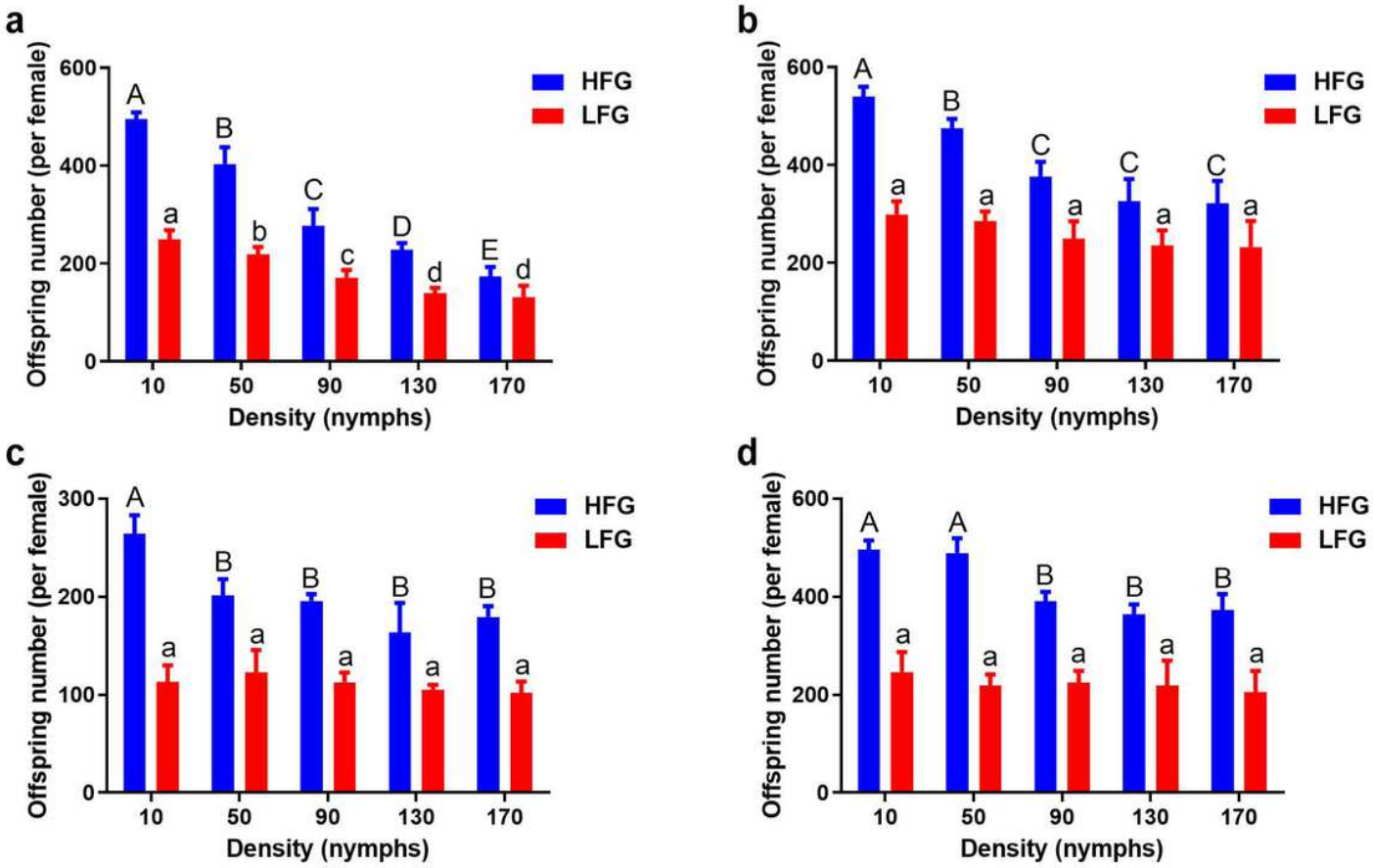

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