RESEARCH ARTICLE

Phase-related differences in egg production of the migratory locust regulated by differential oosorption through microRNA-34 targeting activinβ

Lianfeng Zhao1,2*, Wei Guo2,3*, Feng Jiang1,2, Jing He2,3, Hongran Liu2,3, Juan Song2,3, Dan Yu3, Le Kang1,2,3*

1 Beijing Institutes of Life Science, Chinese Academy of Sciences, Beijing, China, 2 CAS Center for Excellence in Biotic Interactions, University of Chinese Academy of Sciences, Beijing, China, 3 State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing, China

☯ These authors contributed equally to this work.
* lka@ioz.ac.cn

Abstract

Outbreaks of locust plagues result from the long-term accumulation of high-density egg production. The migratory locust, Locusta migratoria, displays dramatic differences in the egg-laid number with dependence on population density, while solitarious locusts lay more eggs compared to gregarious ones. However, the regulatory mechanism for the egg-laid number difference is unclear. Herein, we confirm that oosorption plays a crucial role in the regulation of egg number through the comparison of physiological and molecular biological profiles in gregarious and solitarious locusts. We find that gregarious oocytes display a 15% higher oosorption ratio than solitarious ones. Activinβ (Actβ) is the most highly upregulated gene in the gregarious terminal oocyte (GTO) compared to solitarious terminal oocyte (STO). Meanwhile, Actβ increases sharply from the normal oocyte (N) to resorption body 1 (RB1) stage during oosorption. The knockdown of Actβ significantly reduces the oosorption ratio by 13% in gregarious locusts, resulting in an increase in the egg-laid number. Based on bioinformatic prediction and experimental verification, microRNA-34 with three isoforms can target Actβ. The microRNAs display higher expression levels in STO than those in GTO and contrasting expression patterns of Actβ from the N to RB1 transition. Overexpression of each miR-34 isoform leads to decreased Actβ levels and significantly reduces the oosorption ratio in gregarious locusts. In contrast, inhibition of the miR-34 isoforms results in increased Actβ levels and eventually elevates the oosorption ratio of solitarious locusts. Our study reports an undescribed mechanism of oosorption through miRNA targeting of a TGFβ ligand and provides new insights into the mechanism of density-dependent reproductive adaption in insects.
Author summary

The continuous accumulation of high-density eggs laid by flying swarms of adults results in huge populations of flightless juveniles, which contributes to the outbreaks of locust plagues. An interesting phenomenon is that locusts have the phenotypic plasticity of reproduction. The gregarious locusts lay fewer big eggs than do solitarious phase locusts. In contrast, the solitarious phase locusts lay more small eggs compared to the gregarious locusts. We find the egg-laid number is not only regulated by the phase status of parents but also controlled by oosorption, a type of oocyte death. Further studies confirmed the phase-related ratio of oocyte death in the mother is regulated by a microRNA, which posttranscriptionally influences the expression level of a TGFβ ligand. This maternal effect on progeny size is especially critical for gregarious locusts to control the population size and maintain population fitness, and for solitarious locusts to enhance chance for gregarization and further enlargement of population size. This is the first study to reveal the molecular mechanism underlying the regulation of a microRNA-gene circuit for locust oocyte death to determine the offspring number. These findings can provide some important clues to develop potential drugs to prevent vast locust reproduction from a plague upsurge.

Introduction

Locusts can cause serious economic losses in agriculture upon population explosion. The accumulation of high-density egg numbers is one of the main causes of locust plague outbreaks. The migratory locust Locusta migratoria displays remarkable reproductive plasticity in the trade-off between egg size and clutch size (number of eggs per egg pod) between gregarious and solitarious ones [1, 2]. The high-density gregarious locusts lay larger but a little bit fewer eggs, while low-density solitarious locusts lay smaller but relatively more eggs [1, 3–5]. The egg-laid number is limited by the number of ovarioles and ratio of oocyte resorption, while solitarious females and their progeny hatchlings have higher numbers of ovarioles than their gregarious counterparts of L. m. migratoroides and Schistocerca gregaria [6, 7]. Meanwhile, oosorption (oocytes resorption), a process by which oocytes stop yolk deposition and are resorbed instead of being laid in response to behavioral, ecological or physiological factors, decreased the number of matured oocytes for egg production [8]. However, whether the egg-laid number difference is associated with ovariole number and/or oosorption in the migratory locust is still unclear.

Follicle degeneration, known as oosorption or follicular atresia, commonly occurs in insects and mammals. In mammals, follicular atresia is mainly regulated by the transforming growth factor β (TGFβ) superfamily members-initiated cascades. Several ligands, such as activin, bone morphogenetic proteins and Nodal have key roles in multiple aspects of follicle development [9]. These ligands are a large class of evolutionarily conserved polypeptides, exerting a wide range of biological effects by regulating cell growth, differentiation and cell fate determination [10]. In insects, follicle degeneration is mediated by several cellular processes, such as autophagy, apoptosis and necrosis [11, 12]. However, the upstream regulatory factors of oosorption are still unknown.

Our previous studies confirm that the phase transition of the migratory locust is regulated at the transcriptional and posttranscriptional levels. Several phase-core genes specifically regulate phenotypic plasticity in the behavior, body color, immunity and reproduction of the locusts [1, 13–18]. Expression differences of some coding genes and miRNAs confirm the
The gregarious behavior of the locust is regulated by dopamine and the genes in its synthesis pathway including henna, pale and dop1 [14, 15]. Further study found that low miR-133 levels in gregarious locust resulted in high expression of henna and pale to synthesize more dopamine, maintaining locust aggregation [18]. As a dopamine receptor, Dop1 activation enhanced adenylyl cyclase 2 expression by inhibiting miR-9a maturation to initiate olfactory attraction among locust individuals [13]. In particular, individual aggregation can promote the accumulation of more miR-276 in gregarious ovaries, synchronizing egg-hatching by upregulating brm by trans-generational inheritance [23]. Thus, the interactions between genes and miRNAs play critical roles in reproductive plasticity. However, whether gene and miRNA interactions control the egg-laid number difference between gregarious and solitarious locusts remains unclear.

In the present study, we investigate the relationships among the number of ovarioles, oosorption of ovary and egg-laid number in gregarious and solitarious migratory locusts, Locusta migratoria migratoria, and examine the differential regulation mechanism of egg production in response to population density changes. Our results demonstrated that different oosorption ratios in gregarious and solitarious locusts contributed to the differentiation of egg-laid number through miR-34 to target Actβ, regulating oosorption. Therefore, our study elucidated a molecular mechanism of egg number differences in response to population density in migratory locusts.

**Results**

**The oosorption ratio is different in gregarious and solitarious locusts**

Because the difference in the egg number in L. m. migratorioides and S. gregaria is mainly attributed to different ovariole numbers [6, 7], we investigate the differences in ovariole number and oosorption ratio between gregarious and solitarious L. m. migratoria under the same rearing conditions except for density. We found that the ovariole number showed no significant differences at 0, 2, 4, 6, 8, 10 and 12 days post adult eclosion (PAE0, 2, 4, 6, 8, 10, 12) between gregarious and solitarious locusts (Fig 1A and 1B). Then, we discriminated the oosorption into five stages in the locusts (Fig 1A) based on specific criteria [24, 25]. Compared to solitarious oocytes, gregarious ones showed significantly higher oosorption ratios of 21%, 31%, 24% and 21% from PAE6, 8, 10 and 12, respectively, during oocyte maturation (Fig 1C). Corresponding to the higher oosorption ratio, gregarious locusts laid 17% fewer eggs than solitarious locusts (Fig 1D). Thus, egg number variation is mainly affected by the oosorption ratio but not ovariole number in the migratory locust.

**Actβ promotes oosorption**

To investigate which genes are involved in oosorption differences, we performed high-throughout RNA-seq to compare the gene expression profiles of gregarious terminal oocytes (GTO) and solitarious terminal oocytes (STO). In total, 676 genes were differentially expressed, and of these, 328 and 348 genes were upregulated in GTO and STO, respectively (S1 Dataset, Fig 2A). The top 5 genes that were upregulated in GTO or STO were selected as candidates and validated by qRT-PCR (Table 1). Gram-negative bacteria binding protein 3 (GNBP3), Activinβ (Actβ), Cytochrome P450 307a1 (spo), Cholesterol desaturase daf-36 (daf-36) and PI-PLC X domain-containing protein DDB_G0269228 (DDB_G0269228) were significantly upregulated in GTO (Fig 2B), and Acyl-CoA-binding protein homolog (Dbi), Cytochrome P450 6k1 (CYP6K1), Serine protease easter (ea), Sushi, nidogen and EGF-like domain-containing protein 1 (Sned1) and Epithelial chloride channel protein (CLCN) were significantly upregulated in STO (Fig 2C). Then, we compared the expression patterns of these
9 differentially expressed genes between normal (N) and resorption body 1 (RB1) oocytes. 
*Actβ* and *Sned1* showed significant increases of 4.9- and 1.7-fold in RB1 compared to N, respectively (Fig 2D). In combination with the respective expression patterns of *Actβ* and *Sned1* between GTO and STO, *Actβ* is considered a key candidate for regulation of the oosorption ratio in the locust.

*Actβ* belongs to the activin subfamily of TGFβ (Fig 3A). To confirm the function of *Actβ* in oosorption, we injected dsActβ in the gregarious female adults. The expression levels of *Actβ* mRNA, proprotein and mature dimer were significantly reduced to 6%, 60% and 27% of the dsGFP-injected controls in the terminal oocytes, respectively (Fig 3B and 3C). Consistently, the oosorption ratio was significantly decreased by 13% after dsActβ injection when compared to dsGFP-injected controls (Fig 3D). However, the terminal oocyte length was not significantly different between the two groups (Fig 3E), implying no significant effect on the egg size of the locusts.

**Actβ** is a target of miR-34

Since posttranscriptional regulation plays variant roles in locust phase trait determination [13, 18, 23], we speculate that the expression difference of *Actβ* between gregarious and solitary adult locusts is regulated by miRNA. Previously, 689 lineage-specific miRNAs and 144 evolutionarily conserved miRNAs were identified in the genome of *L. migratoria* [26]. Conserved miRNAs show sequence and target conservation among the entire metazoan kingdom [27].

---

Fig 1. Oosorption differences between gregarious and solitary locusts. (A) Normal and resorbed terminal oocytes at different stages. N, normal terminal oocyte; RB, resorption body. Scale bar, 2 mm. (B) Ovarirole number of gregarious or solitary adults at PAE0, 2, 4, 6, 8, 10 and 12. n = 15. (C) Oosorption ratio of gregarious or solitary adults at PAE0, 2, 4, 6, 8, 10 and 12. n = 10–18. (D) The number of resorbed terminal oocytes and eggs in the first egg pod in the gregarious or solitary adults. n = 15–16. **P < 0.01, ***P < 0.001.

https://doi.org/10.1371/journal.pgen.1009174.g001
Fig 2. Transcriptomic analyses of gregarious (G) and solitary (S) terminal oocytes (TO). (A) The number of differentially expressed genes between GTO and STO. (B and C) qRT-PCR verification of the top 5 genes upregulated in GTO (B) or STO (C). n = 7–12. (D) The expression levels of nine verified genes at N and RB1 stages. n = 4–7. *P < 0.05, **P < 0.01, ***P < 0.001.

Table 1. Top 5 genes upregulated in GTO or STO.

| Gene ID  | GTO FPKM | STO FPKM | Log_{2} FC (GTO/STO) | P value          | Gene Name                                                   |
|----------|----------|----------|-----------------------|------------------|-------------------------------------------------------------|
| GTO UP   | LOCM00582 | 33.87    | 1.07                  | 4.98             | 4.58E-02 Gram-negative bacteria binding protein 3           |
|          | LOCM116458 | 5.26    | 0.24                  | 4.45             | 2.52E-02 Actinβ                                              |
|          | LOCM16206 | 751.97   | 40.24                 | 4.22             | 3.70E-02 Cytochrome P450 307a1                               |
|          | LOCM100790 | 230.22   | 13.49                 | 4.09             | 7.58E-03 Cholesterol desaturase daf-36                      |
|          | LOCM05839 | 47.92    | 3.01                  | 3.99             | 3.25E-02 PI-PLC X domain-containing protein DDB_G0269228    |
| STO UP   | LOCM11326 | 7.09     | 163.51                | -4.53            | 1.52E-02 Acyl-CoA-binding protein homolog                   |
|          | LOCM16169 | 0.47     | 5.25                  | -3.48            | 3.73E-02 Cytochrome P450 6k1                                |
|          | LOCM17544 | 0.92     | 9.86                  | -3.42            | 1.51E-04 Serine protease easter                             |
|          | LOCM14056 | 0.50     | 4.68                  | -3.22            | 4.30E-03 Sushi, nidogen and EGF-like domain-containing protein 1 |
|          | LOCM10397 | 4.19     | 35.99                 | -3.10            | 1.03E-02 Epithelial chloride channel protein                |

https://doi.org/10.1371/journal.pgen.1009174.g002
https://doi.org/10.1371/journal.pgen.1009174.t001
Thus, based on these 144 conserved miRNAs, we predicted the potential miRNAs to target Act\_\beta in the CDS and 3’ UTR sequences by using the algorithms miRanda [28] and RNAhybrid [29]. In total, we obtained 11 predicted miRNAs, of which miR-6494, miR-3900-3p, miR-6012, miR-34a, miR-34b and miR-34c can bind to the CDS sequence, and miR-283, miR-980, let-7, miR-275 and miR-275-3p can bind to the 3’UTR sequence (Fig 4A, S1 Fig). In luciferase reporter assays in Drosophila S2 cells, we confirmed only miR-34a, miR-34b and miR-34c significantly decreased the luciferase activity by 66%, 43%, and 57% compared to the negative control miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducts and showed no difference compared with S2 cells cotransfected with miR-NC (Fig 4B). After mutating the complementary sequences of Act\_\beta to the "seed region" of three miR-34 isoforms, the respective luciferase activities significantly recovered compared to S2 cells cotransfected with the wild-type psiCHECK-2 conducting...
miR-34 suppresses oosorption by downregulating Actβ

The expression levels of miR-34a, miR-34b and miR-34c were 1.5-, 1.4- and 1.5-fold higher in the solitarious locusts than in the gregarious ones in the N oocytes (Fig 5A). In contrast, the expression levels of miR-34a, miR-34b and miR-34c significantly decreased by 29%, 32%, and 28% in the RB1 when compared to N oocytes, respectively (Fig 5B). Thus, miR-34 showed a contrary expression pattern with the Actβ gene from N to RB1.

We injected miR-34a, miR-34b and miR-34c agomirs in the gregarious females to enhance their expression levels to 189-, 716- and 182-fold, respectively, compared to the agomir-NC control in the terminal oocytes (Fig 6A). After injection with the three agomirs, the Actβ mRNA levels significantly decreased to 55%, 31% and 43% of the agomir-NC control, respectively (Fig 6B). The Actβ proprotein levels significantly decreased to 44%, 27% and 11% of the agomir-NC control, respectively (Fig 6C), and the Actβ mature dimer levels significantly decreased to 25%, 28% and 20% of the agomir-NC control, respectively (Fig 6C). Consistently, the oosorption ratios significantly decreased by 13%, 10% and 8% compared to the agomir-NC control, respectively (Fig 6D). However, the length of terminal oocytes was not significantly different from the agomir-NC control (Fig 6E).

In the solitarious female adults, we injected miR-34a, miR-34b and miR-34c antagomirs. The abundance of miR-34a, miR-34b and miR-34c showed no significant changes compared to antagomir-NC control in the normal terminal oocytes (Fig 7A). However, after injection with the three antagomirs, the Actβ mRNA levels significantly increased to 2.9-, 4.3- and 4.5-fold of the antagomir-NC control, respectively (Fig 7B). The Actβ proprotein levels significantly increased to 1.4-, 2.2- and 1.7-fold of the antagomir-NC control, respectively (Fig 7C), and the Actβ mature dimer levels significantly increased to 3.4-, 3.4- and 3.1-fold of the antagomir-NC control, respectively (Fig 7C). Correspondingly, the oosorption ratios significantly increased by 10%, 14% and 13% compared to the antagomir-NC control, respectively (Fig 7D). Similarly, the length of terminal oocytes was not significantly different from that of the
antagomir-NC control (Fig 7E). Taken together, the oosorption ratios in the locust determine that the egg-laid number is regulated by the $\text{Act}\beta$ expression difference, which is a consequence of posttranscriptional modification due to miR-34 involvement.

**Discussion**

Previous studies have shown that the difference in the number of ovarioles is phase-related and contributes to the larger clutch size in solitarious females than in gregarious ones in L. m. migratorioides and S. gregaria [6, 7]. However, our results found that the number of ovarioles was not different between gregarious and solitarious adult females of the migratory locust, L. m. migratoria. Nevertheless, fewer oocytes were resorbed in the solitarious adults, which resulted in a higher number of eggs laid by solitarious adults than gregarious adults. Our previous study confirmed that Syx1A indirectly determined progeny egg size and clutch size by regulating yolk protein in the hemolymph and ovary [1]. In this study, we showed that density-dependent regulation of the miR-34-$\text{Act}\beta$ circuit had a direct effect on oosorption but not the oocyte size in the terminal oocytes. Based on the adaptive investment hypothesis, progeny egg size is determined by maternal generation in response to environmental cues [30]. The adjustment of egg size inclusively leads to egg number changes for reallocation of limited nutritional resources under high population density [1]. The molecular signal miR-34-$\text{Act}\beta$, possibly as an alternative for condition-dependent trade-off in reproductive plasticity, regulates the respective high or low oosorption ratio in gregarious or solitarious locusts for environmental adaptation.
The *L. m. migratoria* adopted the oosorption adjustment strategy but not both the oosorption and ovariole number adjustment strategy as in the *L. m. migratorioides* and *S. gregaria* to control progeny number [6, 7]. The causes of the differential adjustment strategies might reside in three aspects. First, some phase traits displayed species-specific characteristics, such as how aggregation behavior is controlled by dopamine in *L. m. migratoria* [14, 15] but by serotonin in *S. gregaria* [31]. In the grasshopper *Melanoplus sanguinipes*, parental crowding did not affect the ovariole number [32]. In the grasshopper *Romalea microptera*, the ovariole number was influenced by genetic variation and nutrition during development, but not maternal environment [33]. Second, differential internal physiological states might affect the final strategy. In *S. gregaria*, studies revealed contrasting oocyte development rates and JH titers between gregarious and solitarious adults [34, 35]. Third, habitat may contribute to the different strategies. The African migratory locust, *L. m. migratorioides*, and desert locust, *S. gregaria*, live in tropical and subtropical zones; however, *L. m. migratoria* is mainly distributed in the temperate zone. The study of 17 populations of coho salmon (*Oncorhynchus kisutch*) in North America showed a significant latitudinal increase in their egg number, but this increase was accompanied by significant latitudinal decreases both in egg size and total biomass of eggs produced [36]. The tropical butterfly *Bicyclus anynana* exhibited temperature-mediated plasticity in egg size and number, laying higher numbers of smaller eggs at the higher temperature but fewer and larger eggs at the lower temperature [37]. We inferred that the differential strategies adopted by variant species or subspecies might be the results of regulation by different genes or pathways.
Our study showed that Actβ acted as a regulator of oosorption in the migratory locust. Although the TGFβ ligand-initiated pathways are conserved from Caenorhabditis elegans (C. elegans) to Homo sapiens, they function in variant aspects of reproduction [9, 38]. The regulatory roles of TGFβ ligands in folliculogenesis are rather complex among species because abundant family members are involved in [9]. In C. elegans, reduced TGFβ Sm/Mab signaling from dbl-1 positively regulates germine/oocyte quality maintenance through sma-2 [39]. In Drosophila, gbb and dpp are essential for GSC maintenance in the ovary [40] and dpp also functions in the formation of eggshell structure [41]. In mammals, activin A fosters granulosa cell proliferation, preantral follicle growth and antral formation, as well as increase in the survival of preantral follicles by decreasing the proportion of atretic follicles [42, 43]. Another ligand, Nodal, was found to promote granulosa cell apoptosis and follicular atresia [44]. In migratory locusts, Actβ, which is a homolog of mammalian activin, fly Actβ and worm DAF-7 (Fig 3A), has special function in regulating oocyte death during oosorption.

Furthermore, we found that Actβ was regulated by the three miR-34 isoforms in the locusts. miR-34 belongs to a markedly conserved miRNA family and has three mammalian homologs and a single ortholog in invertebrate species with identical seed sequence [45]. In Drosophila, miR-34 displays three isoforms (miR-34a, miR-34b and miR-34c) that have a uniform 5’ terminus but differ at the 3′ end resulting from nibbler-mediated trimming, where nibbler trims the 24 nt mR-34a for the generation of shorter isoforms of 22 nt miR-34b and 21 nt miR-34c with preferred lengths for Ago1 binding [46]. The sequences of the locust miR-34 isoforms are identical to that of Drosophila. With agomir-34a treatment in the locust, the miRNA abundance of miR-34a as well as the shorter miR-34b and miR-34c isoforms all increased significantly (Fig 4F). Additionally, treatment with agomir-34b in the locust resulted in significant increases in miR-34c abundances in micr-RNA-34c abundance (S2 Fig). Our results suggested the mechanism of miR-34 isoforms production might be similar to that in Drosophila. The three isoforms of miR-34 jointly downregulated Actβ expression.

Because of the different physiological and behavioral characteristics, gregarious and solitary locusts present obviously different allocation of resources as a life-history trait trade-off. Gregarious locusts allocate more resources in flying and migration rather than reproduction like in other migratory insects [47–49]. However, non-migratory solitary locusts tend to invest more resources in reproduction through more egg-laying for enlargement of population size. Therefore, gregarious and solitary locusts balance the resources allocation through differential oosorption ratios. Oosorption determines the clutch size between gregarious and solitary locusts, which provides alternative strategy for environmental adaptation. In addition, vitellogenesis-driven oocyte maturation is energy-consuming for massive syntheses and transportation of vitellogenin and other proteins [50, 51], thus oosorption also provides alternatives for the reallocation of resources in variant insect species under varied conditions such as food stress [8, 52]. The elucidation of miRNA-mediated regulation on TGFβ signaling in locusts is particularly crucial for understanding the reproduction plasticity and reallocation of body energy of insects and potentially provides new targets for pest control.

Materials and methods

Insects

Gregarious and solitary locusts were from the same colonies maintained at the Institute of Zoology, Chinese Academy of Sciences, China. Approximately 200–300 gregarious locusts were kept in a cage (25 cm×25 cm×25 cm), and solitary locusts were cultured alone in small metal cages. Both colonies were reared under a 14:10 light/dark photo regime at 30±2˚C. The locusts were fed with fresh wheat seedlings and bran every day. The gregarious male locusts
were removed from the cage immediately post adult eclosion, and the females were continually kept at high density.

Transcriptomic sequencing and data processing

Fifteen terminal oocytes from three individuals of gregarious or solitarious locusts were pooled together respectively as one biological replicate and three biological replicates were collected for transcriptomic sequencing. Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Approximately 3 μg of total RNA was used to establish a paired-end RNA-seq library for transcriptome sequencing on an Illumina HiSeq 2000 platform (Tiangen). After the low-quality reads had been trimmed and adapters had been removed, the clean RNA-seq reads were mapped to the reference genome using TopHat2 (version 2.0.13) with default parameters [26, 53, 54]. The unique mapped reads were used to calculate the number of reads that mapped to an aviary gene model using HTSeq [55]. The influence of differences in RNA output size between samples was reduced by the trimmed mean of M-values (TMM) method [56]. Gene expression level was measured as fragments per kilobase million (FPKM). Student’s t test was used for the significance test. Genes with significance levels of $P < 0.05$ and fold change $\geq 2$ were considered differentially expressed. The RNA-seq data was deposited in the Sequence Read Archive database (accession no. PRJNA659804).

qRT-PCR verification

Each sample of total RNA was isolated from five normally developed terminal oocytes or resorption body 1 oocytes per female adult respectively as one biological replicate using TRIzol reagent (Invitrogen). For mRNA quantification, Oligo (dT)-primed cDNA was reverse transcribed from total RNA using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega). qRT-PCR was carried out using a LightCycler 480 instrument (Roche) and Talent qPCR PreMix (SYBR Green) (Tiangen) according to the manufacturer’s instructions. For miRNA quantification, the miRcute Plus miRNA First-Strand cDNA Synthesis Kit (Tiangen) was used to synthesize the cDNA. The cDNA of miRNA was subjected to qRT-PCR using the miRcute Plus miRNA qPCR Detection Kit (SYBR Green) (Tiangen) according to the manufacturer’s instructions on a LightCycler 480 instrument (Roche). The PCR data were analyzed by the $2^{-\Delta\Delta C_t}$ method of relative quantification with the internal control rp49 and U6 for mRNA and miRNA, respectively. All the primers for qRT-PCR are listed in S1 Table.

RNA interference (RNAi)

Double stranded RNAs of Actβ and green fluorescent protein (GFP) were synthesized in vitro using the T7 RiboMAX Express RNAi System (Promega). For RNAi in locusts, a total of 8 μg (1 μg/μL) of Actβ or GFP dsRNA dissolved in a mixture of acetone and H₂O (2:1 ratio) was intra-abdominally injected into each female adult within 12 h after eclosion and boosted on day 5 [57]. Upon maturation, the oosorption ratio was calculated by the total number of RB1 to RB5 oocytes divided by the total number of terminal oocytes in an individual locust, and five terminal oocytes per female adult were sampled as one biological replicate and stored in liquid nitrogen for qRT-PCR analysis. The primers for dsRNA synthesis are listed in S1 Table.

Luciferase reporter assays

The 507 bp, 551 bp and 658 bp sequences of CDS and 3’UTR surrounding the predicted target sites of miR-6494, miR-3900-3p, miR-6012, miR-34, miR-283, miR-980, let-7, miR-275 and miR-275-3p in Actβ were separately cloned into the psiCHECK-2 vector (Promega) using the
Xho I and Not I sites. Site mutation in the Actβ DNA sequence complementary to the miR-34 seed region was performed by using the KOD-Plus-Mutagenesis Kit (TOYOBO). The mimic of C. elegans miRNA, cel-miR-67-3p (5’ to 3’: UCACAACCUCUAGAAAGAGUAGA), was used as the negative control (miR-NC) [23]. Drosophila S2 cells with complete Schneider’s Drosophila medium were cotransfected with the luciferase reporter vector (WT or MT) and miRNA mimics or miR-NC using lipofectamine 3000 (Invitrogen). Luciferase activity was determined using the Dual-Luciferase Reporter Assay System and a GloMax 96 Microplate Luminometer (Turner Biosystems Instrument). Normalized luciferase activity was calculated by dividing Renilla luciferase activity by firefly luciferase activity and normalizing to the mean of the control miR-NC group.

RNA immunoprecipitation (RIP) assay

The RIP assay was performed using a Magna RIP Quad kit (Millipore) with slight modifications. Briefly, approximately 40 terminal oocytes were collected as one biological duplication and homogenized in ice-cold RIP lysis buffer. The homogenates were stored at -80˚C overnight. After centrifugation for 15 min at 14,000 × g, the supernatant was incubated at 4˚C overnight with magnetic beads preincubated with a monoclonal antibody against locust Ago1 [18] or normal mouse IgG. The precipitated RNA was quantified by qRT-PCR.

Fluorescence In Situ Hybridization (FISH)

The RNA probe for Actβ was synthesized by a T7/SP6 RNA Transcription Kit (Roche) and was subsequently fragmented to approximately 250 bp by carbonate buffer. The primers used for probe synthesis of Actβ are in S1 Table. Ovarioles were separated from ovaries in locust saline and fixed in 4% (wt/vol) paraformaldehyde overnight. After digestion with proteinase K (20 μg/mL; Tiangen) at 37˚C for 15 min, these ovarioles were hybridized with miR-34a probe (2 pmol/mL) and Actβ probe (5 ng/μL) at 37˚C overnight. Then, the ovarioles were successively washed in 2× SSC, 1× SSC, and 0.2× SSC at 37˚C. Anti-DIG alkaline phosphatase-conjugated antibody (1:100) and anti-biotin antibody (1:100) were used for probe detection. Then, the fluorescent signal of digoxigenin (DIG) or biotin was obtained by HNPP/Fast Red (HNPP Fluorescent Detection Set, Roche) or Fluorescein-Tyramide (TSA Fluorescein System, Perkin Elmer). Images were captured on an LSM 710 confocal fluorescence microscope (Carl Zeiss) at a magnification of 63×.

miRNA agomir and antagomir treatments

Agomir-34a, agomir-34b and agomir-34c are chemically modified double-strand stable miRNA mimics. Antagomir-34a, antagomir-34b and antagomir-34c are chemically modified single-strand stable miRNA inhibitors whose sequences are reverse complementary to respective miR-34 isoforms. Agomir and antagomir of cel-miR-67-3p (agomir-NC and antagomir-NC, respectively) were used as negative controls. The agomirs and antagonirs were synthesized by GenePharma, Inc. (Suzhou). Adult females within 12 h after eclosion were intra-abdominally injected with 2.5 μg of miRNA agomir or agomir mixed with RNA Transfection Reagent (Engreen) and boosted at PAE5. Upon maturation, the oosorption ratio was calculated in each individual locust, and five terminal oocytes per female adult were sampled as one biological replicate and stored in liquid nitrogen for qRT-PCR analysis.

Western blotting

The polyclonal antibody for Actβ was raised against a synthetic peptide (CTPPLEERYRM DSLP) mapping at the C-terminus of Actβ and produced from rabbits. Total proteins were
extracted by TRIzol reagent (Invitrogen). The proteins were subjected to polyacrylamide gel (10%) electrophoresis and then transferred to polyvinylidene difluoride (PVDF) membranes (Millipore). Blocking was performed in 5% (wt/vol) skimmed milk at room temperature (RT) for 1 h. The membranes were incubated with primary antibody (anti-Actβ, 1 μg/mL; anti-GAPDH, 1:5000) in 5% (wt/vol) skimmed milk at 4˚C overnight. Secondary antibody (1:5,000) (CWBIO) was incubated at RT for 1 h. The immunoblot was detected using SuperSignal™ West Femto Maximum Sensitivity Substrate (Thermo Scientific). Protein band intensities were quantified using Image-Pro Plus 6.0 software (Media Cybernetics Inc.).

Statistical analysis
Statistical analysis was performed by Student’s t-test and the nonparametric Mann–Whitney U test using IBM SPSS Statistics v.19 software (SPSS Inc.). Differences were considered significant at $P<0.05$. Values are reported as the mean±SE.

Supporting information
S1 Table. Primers used for qRT-PCR, dsRNA synthesis and probe synthesis. (DOCX)
S1 Fig. Prediction of miRNAs that bind to Actβ. In red, the seed region of miRNA. (TIF)
S2 Fig. The relative miR-34c abundance in the terminal oocytes after injection with ago-mir-34b. n = 7–8. **$P < 0.01$. (TIF)
S1 Dataset. Differentially expressed genes between gregarious and solitary terminal oocytes. (XLSX)

Author Contributions
Conceptualization: Wei Guo, Le Kang.
Data curation: Lianfeng Zhao, Wei Guo.
Formal analysis: Lianfeng Zhao, Wei Guo, Feng Jiang, Hongran Liu, Dan Yu.
Funding acquisition: Wei Guo, Le Kang.
Investigation: Lianfeng Zhao, Wei Guo, Feng Jiang, Jing He, Juan Song.
Methodology: Lianfeng Zhao, Wei Guo, Feng Jiang, Jing He, Hongran Liu.
Supervision: Le Kang.
Visualization: Lianfeng Zhao, Wei Guo.
Writing – original draft: Lianfeng Zhao, Wei Guo.
Writing – review & editing: Le Kang.

References
1. Chen Q, He J, Ma C, Yu D, Kang L. Syntaxin 1A modulates the sexual maturity rate and progeny egg size related to phase changes in locusts. Insect Biochem Mol Biol. 2015; 56:1–8. https://doi.org/10.1016/j.ibmb.2014.11.001 PMID: 25446392.
2. Wang HS, Ma ZY, Cui F, Wang XH, Guo W, Lin Z, et al. Parental phase status affects the cold hardness of progeny eggs in locusts. Funct Ecol. 2012; 26(2):379–89. https://doi.org/10.1111/j.1365-2435.2011.01927.x PMID:20003021400009.

3. Maeno KO, Pieu C, Ghoul S. The desert locust, Schistocerca gregaria, plastically manipulates egg size by regulating both egg numbers and production rate according to population density. J Insect Physiol. 2020; 122:104020. https://doi.org/10.1016/j.jinsphys.2020.104020 PMID:3203952.

4. Pener MP. Locust Phase Polymorphism and Its Endocrine Relations. Adv In Insect Phys. 1991; 23:1–79. https://doi.org/10.1016/S0065-2806(08)60091-0 WOS:A1991MC40700001.

5. Pener MP, Simpson SJ. Locust Phase Polymorphism: An Update. Adv In Insect Phys. 2009; 36:1–272. https://doi.org/10.1016/s0065-2806(08)36001-9.

6. Injeyan HS, Tobe SS. Phase polymorphism in Schistocerca gregaria: reproductive parameters. J Insect Physiol. 1981; 27(2):97–102. https://doi.org/10.1016/0022-1910(81)90115-3 WOS: A1981LH34700003.

7. Albrecht FO, Verdier M, Blackith RE. Maternal control of ovariole number in the progeny of the migratory locust. Nature. 1959; 184(4680):103–4. https://doi.org/10.1038/184103a0 WOS:A1959ZQ01100018.

8. Bell WJ, Bohm MK. Oosorption in insects. Biol Rev Camb Philos Soc. 1975; 50(4):373–96. https://doi.org/10.1111/j.1469-185x.1975.tb01058.x PMID:1106789.

9. Knight PG, Glistner C. TGFB-β superfamily members and ovarian follicle development. Reproduction. 2006; 132(2):191–206. https://doi.org/10.1530/rep.1.01074 PMID:16885529.

10. Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol. 2007; 8(9):78–82. https://doi.org/10.1038/nrm2297 PMID:18000526.

11. Aguirre SA, Pons P, Settembrini BP, Arroyo D, Canavoso LE. Cell death mechanisms during follicular atresia in Dipetalogaster maxima, a vector of Chagas' disease (Hemiptera: Reduviidae). J Insect Physiol. 2013; 59(5):322–41. https://doi.org/10.1016/j.jinsphys.2013.03.001 PMID:23300993.

12. Nezis IP, Lamark T, Vellentzas AD, Rusten TE, Bjorkoy G, Johansen T, et al. Cell death during follicular atresia in Dipetalogaster maxima, a vector of Chagas' disease (Hemiptera: Reduviidae). J Insect Physiol. 2013; 59(5):322–41. https://doi.org/10.1016/j.jinsphys.2013.03.001 PMID:23300993.

13. Guo X, Ma Z, Dub L, Li T, Li W, Xu L, et al. Dop1 enhances conspecific olfactory attraction by inhibiting miR-9a maturation in locusts. Nat Commun. 2018; 9(1):1193. https://doi.org/10.1038/s41467-018-03437-z PMID:29567955.

14. Guo X, Ma Z, Kang L. Two dopamine receptors play different roles in phase change of the migratory locust. Front Behav Neurosci. 2015; 9:80. https://doi.org/10.3389/fnbeh.2015.00086 PMID:25873872.

15. Ma Z, Guo W, Guo X, Wang X, Kang L. Modulation of behavioral phase changes of the migratory locust by the catecholamine metabolic pathway. Proc Natl Acad Sci USA. 2011; 108(10):3882–7. https://doi.org/10.1073/pnas.1015098108 PMID:21325054.

16. Wang Y, Yang P, Cui F, Kang L. Altered immunity in crowded locust reduced fungal (Metarhizium anisopliae) pathogenesis. PLoS Pathog. 2013; 9(1):e1003012. https://doi.org/10.1371/journal.ppat.1003012 PMID:23326629.

17. Yang M, Wang Y, Liu Q, Liu Z, Jiang F, Wang H, et al. A beta-carotene-binding protein carrying a red pigment regulates body-color transition between green and black in locusts. eLife 2019; 8:e41362. https://doi.org/10.7554/eLife.41362.

18. Yang M, Wei Y, Jiang F, Wang Y, Guo X, He J, et al. MicroRNA-133 inhibits behavioral aggregation by controlling dopamine synthesis in locusts. PLoS Genet. 2014; 10(2):e1004206. https://doi.org/10.1371/journal.pgen.1004206 PMID:24586212.

19. Chen S, Yang P, Jiang F, Wei Y, Ma Z, Kang L. De Novo Analysis of Transcriptome Dynamics in the Migratory Locust during the Development of Phase Traits. PLoS One. 2010; 5(12):e15633. https://doi.org/10.1371/journal.pone.0015633 PMID:21209894.

20. Guo W, Wang X, Ma Z, Xue L, Han J, Yu D, et al. CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. PLoS Genet. 2011; 7(2):e1001291. https://doi.org/10.1371/journal.pgen.1001291 PMID:21304893.

21. Kang L, Chen X, Zhou Y, Liu B, Zheng W, Li R, et al. The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. Proc Natl Acad Sci USA. 2004; 101(51):17611–5. https://doi.org/10.1073/pnas.0407753101 PMID:15691108.

22. Wei Y, Chen S, Yang P, Ma Z, Kang L. Characterization and comparative profiling of the small RNA transcriptomes in two phases of locust. Genome Biol. 2008; 10(1):R6. https://doi.org/10.1186/gb-2008-10-1-r6 PMID:19146710.

23. He J, Chen Q, Wei Y, Jiang F, Yang M, Hao S, et al. MicroRNA-276 promotes egg-hatching synchrony by up-regulating brm in locusts. Proc Natl Acad Sci USA. 2016; 113(3):584–9. https://doi.org/10.1073/pnas.1521098113 PMID:26729868.
24. Davies P, King PE. The ultrastructure of oosorption in Locusta migratoria migratorioides. Z Zellforsch. 1972; 135(2):275–86. https://doi.org/10.1007/BF00315130 PMID: 4345191.

25. Lüsis O. The histology and histochemistry of development and resorption in the terminal oocytes of the desert locust, Schistocerca gregaria. Quart J Microsc Sci. 1963; 104:57–68.

26. Wang Y, Jiang F, Wang H, Song T, Wei Y, Yang M, et al. Evidence for the expression of abundant microRNAs in the locust genome. Sci Rep. 2015; 5(1):13608. https://doi.org/10.1038/srep13608 PMID: 26329925.

27. Lee CT, Risom T, Strauss WM. Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. DNA Cell Biol. 2007; 26(4):209–18. https://doi.org/10.1089/dna.2006.0545 PMID: 17465887.

28. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in Drosophila. Genome Biol. 2003; 5(1):R1. https://doi.org/10.1186/gb-2003-5-1-r1 PMID: 14709173.

29. Rehmsmeier M, Steffen P, Hochsmann M, Giegerich R. Fast and effective prediction of microRNA-target duplexes. RNA. 2004; 10(10):1507–17. https://doi.org/10.1261/ma.5248604 PMID: 15383676.

30. Fox CW, Czesak ME. Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol. 2000; 45:341–69. https://doi.org/10.1146/annurev.ento.45.1.341 PMID: 10761581.

31. Anstey ML, Rogers SM, Ott SR, Burrows M, Simpson SJ. Serotonin Mediates Behavioral Gregarization of Melanoplus sanguinipes1 (Orthoptera: Acrididae). Development. 2010; 137(19):341–69. https://doi.org/10.1038/nature10810 PMID: 22343898.

32. Smith DS. Crowding in Grasshoppers. II. Continuing Effects of Crowding on Subsequent Generations. J Insect Physiol. 1950; 24:1038–41. https://doi.org/10.1016/j.jinphys.2014.08.005 PMID: 1038/nat ure10810 PMID: 22343898.

33. Luo S, Kleemann GA, Ashraf JM, Shaw WM, Murphy CT. TGF-beta and insulin signaling regulate reproductive aging via oocyte and germline quality maintenance. Cell. 2010; 143(2):299–312. https://doi.org/10.1016/j.cell.2010.09.013 PMID: 20946987.

34. Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell. 1998; 94(2):251–60. WOS:000075020100013. https://doi.org/10.1016/s0092-8674(00)81424-5 PMID: 9695953.

35. Twombly V, Blackman RK, Jin H, Graff JM, Padgett RW, Gelbart WM. The TGF-β signaling pathway is essential for Drosophila oogenesis. Development. 1996; 122(5):1555–60. PMID: 8625842.

36. Coissigny DA, FINDLAY JK, Drummond AE. The effects of FSH and activin A on follicle development in vitro. Reproduction. 2012; 143(2):221–9. https://doi.org/10.1530/REP-11-0105 PMID: 22106407.

37. Guzel Y, Nur Sahin G, Sekero glu M, Deniz A. Recombinant activin A enhances the growth and survival of isolated preantral follicles cultured three-dimensionally in extracellular basement matrix protein (matrigel) under serum-free conditions. Gynecol Endocrinol. 2014; 30(5):388–91. https://doi.org/10.3109/09513590.2014.884111 PMID: 24665930.

38. Wang H, Jiang JY, Zhu C, Peng C, Tsang BK. Role and regulation of nodal/activin receptor-like kinase 7 signaling pathway in the control of ovarian follicular atresia. Mol Endocrinol. 2006; 20(10):2469–82. https://doi.org/10.1210/me.2005-0446 PMID: 16709598.

39. Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, et al. The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila. Nature. 2012; 482(7386):519–23. https://doi.org/10.1038/nature10810 PMID: 22343898.
46. Han BW, Hung JH, Weng Z, Zamore PD, Ameres SL. The 3’-to-5’ exoribonuclease Nibbler shapes the 3’ ends of microRNAs bound to Drosophila Argonaute1. Curr Biol. 2011; 21(22):1878–87. https://doi.org/10.1016/j.cub.2011.09.034 PMID: 22055293.

47. Dingle H. Migration strategies of insects. Science. 1972; 175(4028):1327–35. https://doi.org/10.1126/science.175.4028.1327 PMID: 17813822.

48. Gunn A, Gatehouse AG. The effect of adult feeding on lipid and protein reserves in African armyworm, Spodoptera exempta, moths before and during reproduction. Physiol Entomol. 1986; 11(4):423–31. https://doi.org/10.1111/j.1365-3032.1986.tb00433.x WOS:A1986F757300008.

49. Hunter DM, Mcculloch L, Wright DE. Lipid-accumulation and migratory flight in the Australian plague locust, Chortoicetes terminifera (Orthoptera, Acrididae). B Entomol Res. 1981; 71(4):543–6. https://doi.org/10.1017/S0007485300010051 PMID: WOS:A1981MV85500001.

50. Guo W, Wu Z, Song J, Jiang F, Wang Z, Deng S, et al. Juvenile Hormone-Receptor Complex Acts on Mcm4 and Mcm7 to Promote Polyploidy and Vitellogenesis in the Migratory Locust. PLoS Genet. 2014; 10(10):e1004702. https://doi.org/10.1371/journal.pgen.1004702 PMID: 25340846.

51. Guo W, Wu Z, Yang L, Cai Z, Zhao L, Zhou S. Juvenile hormone-dependent Kazal-type serine protease inhibitor Greglin safeguards insect vitellogenesis and egg production. FASEB J. 2019; 33(1):917–27. https://doi.org/10.1096/fj.201801068R PMID: 30063437.

52. Moore PJ, Attisano A. Oosorption in response to poor food: complexity in the trade-off between reproduction and survival. Ecol Evol. 2011; 1(1):37–45. https://doi.org/10.1002/ece3.4 PMID: 22393481.

53. Jiang F, Liu Q, Liu X, Wang XH, Kang L. Genomic data reveal high conservation but divergent evolutionary pattern of Polycomb/Trithorax group genes in arthropods. Insect Sci. 2019; 26(1):20–34. https://doi.org/10.1111/1744-7917.12558 PMID: 29127737.

54. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013; 14(4):R36. https://doi.org/10.1186/gb-2013-14-4-r36 PMID: 23618408.

55. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinformatics. 2015; 31(2):166–9. https://doi.org/10.1093/bioinformatics/btu638 PMID: 25260700.

56. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. Genome Biol. 2010; 11(3):R25. https://doi.org/10.1186/gb-2010-11-3-r25 PMID: 20196867.

57. Wu Z, Guo W, Xie Y, Zhou S. Juvenile Hormone Activates the Transcription of Cell-division-cycle 6 (Cdc6) for Polyploidy-dependent Insect Vitellogenesis and Oogenesis. J Biol Chem. 2016; 291(10):5418–27. https://doi.org/10.1074/jbc.M115.698936 PMID: 26728459.