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Syntaxin 1A modulates the sexual maturity rate and progeny egg size related to phase changes in locusts

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The migratory locust (*Locusta migratoria*) exhibits clear phenotypic plasticity depending on its population density. Previous studies have explored the molecular mechanisms of body colour, behavior, immunity, and metabolism between high population density gregarious (G) and low population density solitarious (S) locusts. However, the molecular mechanisms underlying differences in reproductive traits remain unknown. G locusts reach sexual maturation much faster and lay larger eggs compared with S locusts. The traits of G locusts decreased significantly with isolation, whereas those of S locusts increased with crowding. Analysis of gene expression in female adults indicated that *syntaxin 1A* (*Syx1A*) was expressed significantly higher in G locusts than in S locusts. After silencing *Syx1A* expression in G locusts by RNA interference (RNAi), their sexual maturity rate and progeny egg size changed towards those of S locusts. Similarly, increment in the traits of S locusts with crowding was blocked by *Syx1A* interference. Changes in the traits were also confirmed by decrease in the level of vitellogenin, which is regulated by *Syx1A*. In conclusion, plasticity of the sexual maturity rate and progeny egg size of G and S locusts, which is beneficial for locusts to adapt to environmental changes, is regulated by *Syx1A*.

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1. Introduction

Phenotypic plasticity, which includes changes in behavior, physiology, morphology, growth, life history and demography, helps organisms adapt to different combinations of abiotic and biotic environments without a time lag (Miner et al., 2005; Schlichting and Smith, 2002). These changes greatly contribute to organism diversification at various levels of biological organization (Moczek, 2010). The migratory locust (*Locusta migratoria*) is a globally distributed pest species (Ma et al., 2012) that displays clear population density-dependent phenotypic plasticity called phase polyphenism (Wang and Kang, 2014). Evident differences in the body colour, behavior, immunity, metabolism and egg size exist between high-density gregarious (G) and low-density solitarious (S) locusts. We have previously explored the differences in gene expression and biological characteristics between two phase individuals as well as the molecular mechanism of locust phase changes in behavior, metabolomics and immunity response (Guo et al., 2011; Guo et al., 2013; Jiang et al., 2012; Kang et al., 2004; Ma et al., 2011; Wang et al., 2014; Wang et al., 2013; Wu et al., 2012; Yang et al., 2014). However, the molecular mechanism of reproductive trait changes in response to change in population density of migratory locusts is unknown.

Sexual maturity rate and egg size are the most obvious phase-related reproductive traits in locusts. Female sexual maturity rate is species- or even population-specific. In desert locusts (*Schistocerca gregaria*), female G locusts mature earlier than S locusts (Norris, 1952). In African migratory locusts (*L. m. migratorioides*), S locusts mature faster than G locusts (Norris and Richards, 1950). However, in migratory locusts from northern China, female G locusts mature earlier than S locusts (Guo, 1956). In addition, many studies have reported differences in progeny egg size between S and G locusts (Pener and Simpson, 2009; Wang et al., 2012). This trait is determined by the population density experienced by the female parents as adults (Hunter-Jones, 1958; Islam et al., 1994). The majority of studies on phase-related egg biology have examined desert locusts (Hunter-Jones, 1958; Islam et al., 1994; Maeno and Tanaka, 2008), although a few studies have also investigated

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differences in egg weights of migratory locusts (Hunter-Jones, 1958). In migratory locusts, the progeny eggs from S and G adults differ not only in size but also in cold tolerance (Wang et al., 2012). However, the correlation between female sexual maturity rate and progeny egg size in response to population density and the underlying mechanism are unclear.

In general, the egg size of insects is determined in the ovary. Previous studies have suggested that insulin and TOR signaling pathways regulate terminal oocyte development by regulating expression of vitellogenin (Vg), a precursor of yolk proteins (Parthasarathy and Palli, 2011). Vg expression affects egg size in birds. Female zebra finches (*Taeniopygia guttata*) treated with an estrogen receptor antagonist lay smaller but more eggs, with 50% decrease in two yolk precursors in the plasma (Williams, 2001). This suggests that Vg and its upstream regulators may contribute to regulation of egg size in animals. Recently, deep RNA-sequencing in migratory locusts has revealed that the pathways of metabolism and genetic information processing are up-regulated in G locusts at adult stage (Chen et al., 2010). In addition to up-regulation at the adult stage, genes related to metabolism are also up-regulated in eggs, as well as first, second, and fifth instar stages (Chen et al., 2010). However, genes related to genetic information processing, including SNARE interactions in vesicular transport, proteasome, DNA replication, RNA polymerase and ribosome, are only up-regulated at adult stage (Chen et al., 2010). SNARE proteins, such as vti1A, vti1B, NSF and SNAP-25, have been found to be involved in regulating reproduction in ticks (Browning and Karim, 2013; Villarreal et al., 2013). Among these SNARE protein genes, *Syx1A* has been reported to be up-regulated the most strongly in G locusts (Chen et al., 2010). This suggests that *Syx1A* may play an important role in regulating sexual maturity rate and egg size in locusts.

In the present study, we compared changes in the sexual maturity rate and progeny egg size between S and G locusts. Transcriptomic analyses of fat body genes revealed differential expression of many genes including Vg and *Syx1A*. Results of RNAi experiments indicate that *Syx1A* can modulate the sexual maturity rate and progeny egg size in G and S locusts by regulating Vg levels.

2. Materials and methods

2.1. Insects

All G and S migratory locusts were obtained from the same stock at the Institute of Zoology, Chinese Academy of Sciences. G locusts were reared in a well-ventilated metal case (25 cm × 25 cm × 25 cm) (large) at a density of approximately 400 individuals per case. S locusts were derived from G locusts and were reared individually in cages (10 cm × 10 cm × 25 cm) (small) ventilated with charcoal-filtered air for at least 10 generations. Both G and S locusts were reared at 30 ± 2 °C with a 14:10 light:dark photo regime. Greenhouse-cultured wheat seedlings and wheat bran were the primary food of the locusts. For isolation of gregarious locusts (IG), each female G locust was reared alone in a small case on the day after its emergence as an adult. For crowding of solitarious locusts (CS), approximately 16 female S locusts were reared with 30 male G locusts in a large case when they emerged as adults.

2.2. Sampling

On the 8th day after emergence of adults, their fat bodies were dissected and placed immediately in liquid nitrogen. At the same time, one half of each ovary was placed in liquid nitrogen for RNA isolation, and the other half was placed in locust saline for measuring the lengths of terminal oocytes. The ovarioles were separated from each other and fixed in 4% paraformaldehyde overnight.

2.3. Egg collection and measurement

Egg pods were collected every day. Two egg pods were placed in a plastic cup, which was filled with sterilized sand containing 10% pure water. The cups were kept in an incubator at 30 ± 1 °C. Seven days later, one egg pod from each cup was removed for weight and length measurements. The other pod was left for hatching count. The egg weight was measured with a Mettler microbalance (AE240, Switzerland), while egg length and terminal oocyte length were measured under a microscope (Leica DFC490; Leica, Wetzlar, Germany).

2.4. Gene expression data and sequences from transcriptome and genome of the migratory locust

Expression data on genes related to SNARE interactions in vesicular transport were from obtained from Chen et al. (2010). Protein sequences of the syntaxin gene family of *Drosophila melanogaster*, *Tribolium castaneum* and *Apis mellifera* were downloaded from NCBI and used as queries sequences to determine their orthologous genes from the locust genome (Wang et al., 2014). Protein sequences of the syntaxin gene family from the above four species were used to construct a phylogenetic tree using maximum likelihood algorithms in MEGA 6.06 (Tamura et al., 2013). Protein sequence identities, positives and gaps were measured using the BLAST tool at the NCBI website. Protein sequences of insulin and TOR signaling pathways of *D. melanogaster* were downloaded from the KEGG database. These protein sequences were used as query sequences to determine their orthologous genes in the locust genome (Wang et al., 2014).

2.5. Quantitative real-time PCR

Fat bodies or ovaries from 3 individuals were pooled together as one replicate, and 8 biological replicates were performed for each treatment. Total RNA was isolated from the samples using miRNeasy Mini Kit (Qiagen, Hilden, Germany) based on the manufacturer's protocol with DNase digestion. cDNA was reverse-transcribed with 2 μg of total RNA using MMLV Reverse Transcriptase (Promega, Madison, USA). Expression at the mRNA level was measured using a Real Master mix Kit (Tiangen, Beijing, China) and normalized to ribosome protein 49. PCR cycling conditions were based on the manufacturer's recommendations. PCR amplification was conducted using a Roche Light cycler 480. Melting curve analysis was performed to confirm the specificity of amplification. The primers used for qRT-PCR are presented in Table S1.

2.6. RNAi

Considering that *Syx1A* is a member of the *syntaxin* gene family, dsSyx1A was designed against its 3′ UTR to ensure specificity (Fig. S1A). Double stranded RNA corresponding to green fluorescent protein (dsGFP) and *Syx1A* (dsSyx1A) were synthesized *in vitro* using the T7 RiboMAX Express RNAi system (Promega, Madison, USA) as recommended by the manufacturer. A total of 5 μg (1.25 μg/μl) of corresponding dsRNA was injected into each female locust through the second segment of the abdomen on the 3rd day after emergence of adults. Five days later, females were sampled for silencing efficiency analysis and terminal oocyte length measurement. Females were injected every 6 days to achieve continuous knockdown and to explore the role of *Syx1A* in regulating egg size. RNAi against Vg was performed similarly by mixing 5 μg dsVgA and

5 μg dsVgB and then injecting into locusts. The primers used for RNAi are presented in Table S1.

2.7. Quantification of yolk protein titer in the hemolymph

Hemolymph was extracted from the neck of the locusts using micropipettes after puncturing first with forceps. A total of 10 μl of hemolymph was diluted in 240 μl TRIS-buffer (20 mM Tris–HCl, 150 mM NaCl, 5 mM EDTA, 1 mM protease inhibitor mixture, pH 7.5) (Amdam et al., 2006). The solution was kept on ice to avoid protein degradation and was mixed using micropipettes before centrifugation at 1000 × *g* for 10 min to remove hemolymph cells. The supernatant (16 μl) was mixed with 4 μl 5 × loading buffer and then boiled at 99 °C for 10 min. Then, 10 μl of the boiled protein was separated by 8% SDS-PAGE and stained with coomassie brilliant blue. Migratory locust yolk proteins have 6 subunits, namely, 126.9, 115.9, 103.4, 85.5, 79.1, and 71.4 kDa (Chen et al., 2002). The 103.4 and 85.5 kDa bands were selected to quantify the yolk proteins. ImageJ software was used for densitometric analysis.

2.8. Statistical analysis

Statistical analysis was done using the SAS 9.0 software. Normality and equality of data variances were checked using the Shapiro–Wilk test. For data that showed normal and homogenous distribution, *t*-test was used for two-group comparison, whereas one-way ANOVA was used for comparison of three or more groups. The Student-Newman-Keuls test was used for multiple comparisons. For data that did not conform to normal distribution and equality of variances, the Kruskal–Wallis test was used for comparisons. Differences were considered significant if $P < 0.05$. Data are presented as mean ± SEM (standard error of mean).

3. Results

3.1. Sexual maturity rate

The length of terminal oocyte (Fig. 1A) is an ideal phenotype for determining the sexual maturity rate of female locusts (Guo, 1956). On the 4th day after female adult emergence, the terminal oocyte length of G locusts was 1.3 mm, which was significantly longer than that of S locusts (Fig. 1A; $n = 15$ for each group; *t*-test $P < 0.0001$). From the 4th to the 8th day after adult emergence, the terminal oocyte length of G locusts increased by 3.6-fold and reached 4.6 mm, whereas that of S locusts increased by only 1.8-fold and reached 1.6 mm. Consequently, the terminal oocyte length of G locusts was 2.8 times longer than that of S locusts (Fig. 1A; $n = 13$ and 9 for G and S locusts, respectively; Kruskal–Wallis test $P = 0.0007$). G locusts reached a maximum length of 5.6 mm on the 12th day after adult emergence, which was significantly longer than that of S locusts (Fig. 1A; $n = 9$ and 13 for G and S locusts, respectively; *t*-test $P = 0.0046$). And most G locusts produced their first egg pod at this time. On the 16th day after adult emergence, the second oocyte of the G locusts had length similar to that of the first oocyte of S locusts (Fig. 1B; $n = 16$ and 5 for G and S locusts, respectively; Kruskal–Wallis test $P = 0.6797$).

With isolation of G locust (IG) and crowding of S locust (CS) during adult emergence, evident changes in terminal oocyte length were observed. The terminal oocyte length of G locusts decreased by 57% after IG, whereas that of S locusts increased up to thrice the control length after CS (Fig. 1C; $n = 17$, 10, 10 and 6 for G, IG, S and CS, respectively; Kruskal–Wallis test $P < 0.0001$).

3.2. Change in egg size

We compared changes in the egg sizes of S and G locusts to investigate whether the terminal oocyte maturity rate in the

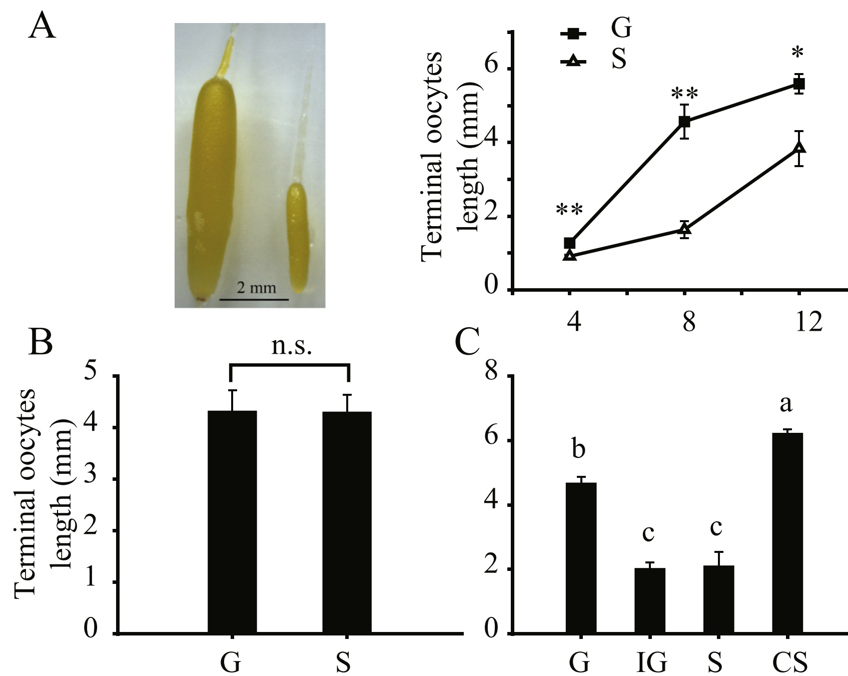

Fig. 1. Effects of population density on terminal oocyte development. (A–C) Terminal oocyte morphology and length. Entries labeled with different letters indicate significantly different means, and those labeled with similar letters indicate non-significantly different means. Error bars represent SEM. *, $P < 0.05$, **, $P < 0.01$, n.s., non-significant. These labels are the same as in the other figures.

locusts could affect progeny egg size. Eggs laid by G locusts were much larger and heavier than those of S locusts (Fig. 2A, B; $n = 18$ and 9 for G and S, respectively; t-test $P = 0.0013$). The egg sizes of G locusts decreased by 22% after IG, whereas those of S locusts increased 1.2-fold after CS (Fig. 2B; $n = 18$, 36, 9 and 16 for G, IG, S and CS, respectively; ANOVA $P < 0.0001$). Contrary to the changes observed in egg size, clutch size (total egg number per egg pod) was smaller in G locusts than that in S locusts (Fig. 2C; $n = 16$ and 14 for G and S, respectively; t-test $P = 0.0173$). However, no difference was observed in the egg pod weights between the two locust types (Fig. 2D; $n = 16$ and 21 for G and S, respectively; t-test $P = 0.5373$). Egg weight was found to be positively correlated with terminal oocyte length (Fig. 2E; $n = 63$; $y = 0.7613x + 9.577$, $r^2 = 0.4316$, $P < 0.0001$). Given that egg weight and length are positively correlated (Fig. 2F; $n = 31$; $y = 1.8311x$, $r^2 = 0.4482$, $P < 0.0001$), both factors can be used as criteria for morphological traits of eggs. In this study, we adopted egg weight to examine changes in egg traits.

3.3. Gene expression related to reproduction

KEGG enrichment analysis of deep RNA-sequencing data indicated that the genes related to metabolism and genetic information processing were up-regulated in G adult locusts (Chen et al., 2010). At the same time, genes related to metabolism were also up-regulated at the egg and first, second and fifth instar stages. However, genes related to genetic information processing, including SNARE interactions in vesicular transport, proteasomes, DNA replication, RNA polymerase and ribosome, were only up-regulated at the adult stage. Compared with S locusts, most SNARE genes were up-regulated in adult G locusts (Fig. S2). Notably, *Syx1A* expression showed the most significant difference between S and G locusts (Table S2). Results of qRT-PCR experiments indicated that *Syx1A* expression in the fat body of G locusts was three times as high as that in S locusts from the 8th to 12th day after adult emergence (Fig. S3). In addition to fat body, *Syx1A* was also found to be expressed in other tissues, such as oocyte and head, but no significant difference was detected in *Syx1A* expression in oocyte and head between G and S locusts (Fig. S4). However, genes in the insulin and TOR signaling pathways were expressed differentially between G and S locusts only at one time point (8th or 12th day after adult emergence) (Fig. S3).

We investigated *Syx1A* expression from the 4th to 16th day after female adult emergence. On the 4th day after adult emergence, no significant difference was found in *Syx1A* mRNA levels between the fat body of G and S locusts (Fig. 3A; $n = 8$ for each group;

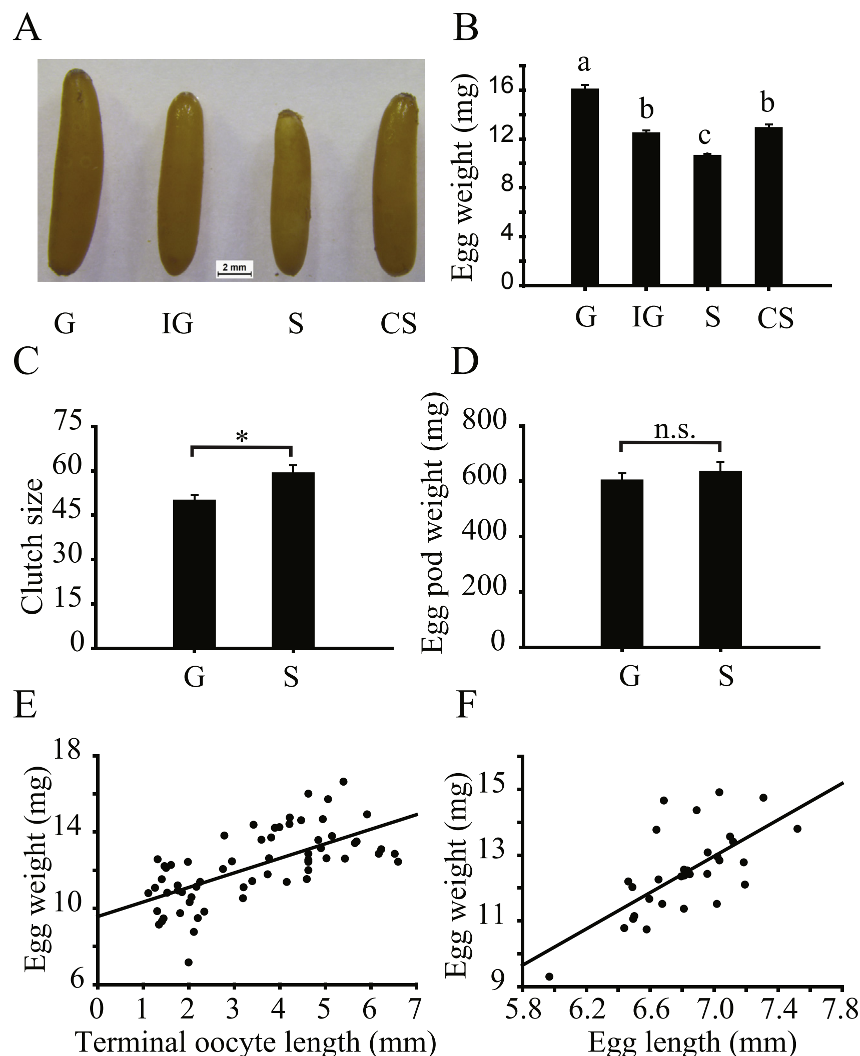

Fig. 2. Effects of population density on progeny egg size. (A, B) Egg morphology (A), egg weight (B), (C) clutch size (total number of eggs per egg pod) and (D) egg pod weights. (E) Egg weight was significantly positively correlated with terminal oocyte length. (F) Egg weight was correlated positively with egg length.

$P = 0.5042$). However, *Syx1A* mRNA level in G locusts increased rapidly and peaked on the 8th day after adult emergence. By contrast, *Syx1A* expression in S locusts did not increase during the same period. Thus, the *Syx1A* mRNA level in G locusts was three times the level observed in S locusts (Fig. 3A; $n = 8$ for each group; *t*-test $P < 0.0001$, 0.0375 and 0.0012 for 8th, 12th and 16th day, respectively). The *Syx1A* mRNA level in G locusts decreased by half after IG, and its expression in S locusts increased up to 4-fold after CS (Fig. 3B; $n = 8$ for each group; Kruskal–Wallis test $P = 0.0012$). Therefore, *Syx1A* expression in locusts correlated well with changes in their population density in both directions.

Similar to most of insects, vitellogenin (Vg) was mainly expressed in fat body in locust. The mRNA levels of VgA and VgB in fat bodies were twice as those in whole bodies, 30 times as those in ovaries and more than 30, 000 times as those in eggs ($n = 4$ for each group; ANOVA VgA, $P < 0.0001$; VgB, $P < 0.0001$) (Fig. 3C). Like *Syx1A* expression, both VgA and VgB (Fig. 3D) were expressed at much higher level in the fat body of G locusts than in that of S locusts ($n = 8$ for each group; VgA, Kruskal–Wallis test $P = 0.0023$, 0.0375, 0.0347 and 0.0063 for 4th, 8th, 12th and 16th day, respectively; VgB, Kruskal–Wallis test $P = 0.0033$, 0.0209, 0.0185 and 0.0081 for 4th, 8th, 12th and 16th day, respectively). The mRNA levels of both VgA and VgB in the fat body of G locusts decreased after IG, whereas those of S locusts increased after CS (Fig. 3E; $n = 8$ for each group; Kruskal–Wallis test $P < 0.0001$ for both VgA and VgB). Coinciding with mRNA level, Vg protein levels in fat bodies of G locusts were twice as those in S locusts ($n = 8$ for each group; *t*-test $P = 0.0008$) (Fig. 3F). Correspondingly, yolk protein levels in hemolymph ($n = 8$ for each group; *t*-test $P = 0.0012$), ovaries ($n = 8$ for each group; Kruskal–Wallis test $P = 0.0357$) and eggs ($n = 8$ for each group; *t*-test $P = 0.0044$) of G locusts were significantly higher than in those of S locusts.

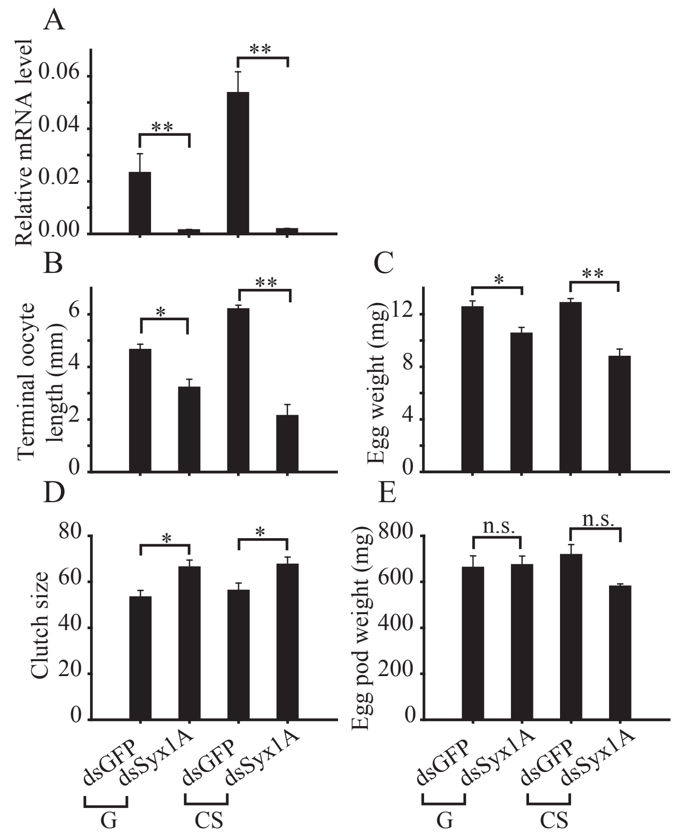

Fig. 4. Function of *Syx1A* in reproduction. *Syx1A* mRNA level (A), oocyte length (B), egg weight (C), clutch size (D) and egg pod weight (E) after silencing *Syx1A* in G and CS locusts.

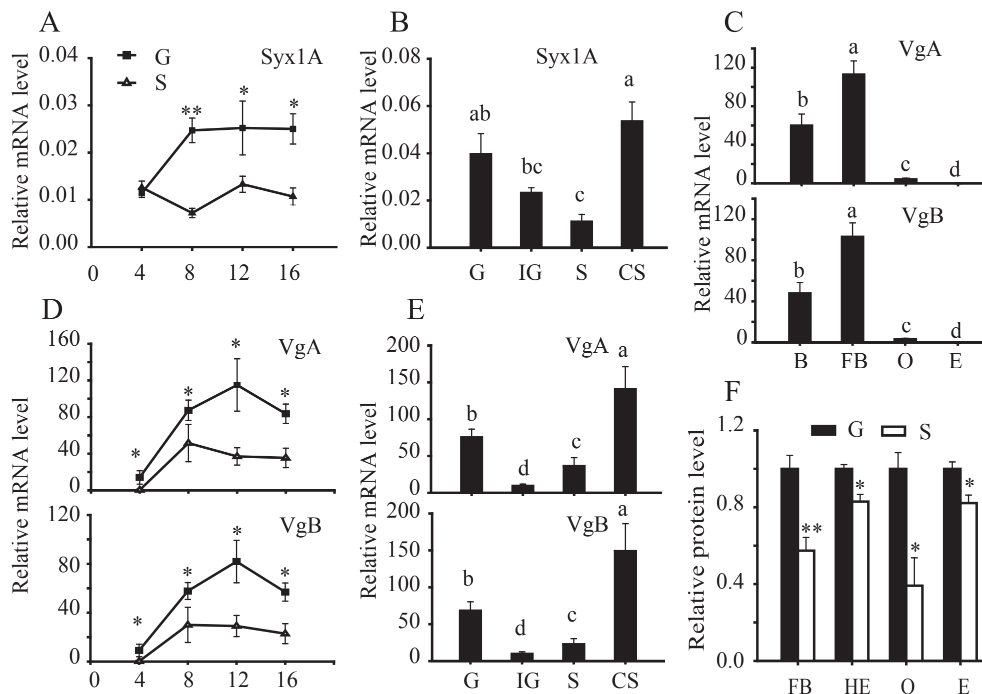

Fig. 3. Effects of population density on expression of *Syx1A* and Vgs in the fat body. (A, B) *Syx1A* expression. (C–E) Relative mRNA level of VgA and VgB. (F), protein level of Vg and yolk protein. B, whole body, FB, fat body, O, ovary, E, egg, HE, hemolymph. These labels are the same as in the other figures.

3.4. Function of *Syx1A*

In order to test whether *Syx1A* expression could affect terminal oocyte maturity rate and progeny egg size, we designed dsSyx1A against its 3′UTR (Fig. S1A). Injection of dsSyx1A led to 93% and 97% decreases in the mRNA level in the fat bodies of G and CS locusts, respectively (Fig. 4A; $n = 8$ for each group; Kruskal–Wallis test $P = 0.0012$ and 0.0016 for G and CS, respectively). However, dsSyx1A injection had no significant effect on the relative mRNA levels of other members of the *syntaxin* gene family, such as *syntaxin 5* ($n = 8$ for each group; *t*-test $P = 0.2876$), *syntaxin 18* ($n = 8$ for each group; *t*-test $P = 0.0746$) (Fig. S1B), and *syntaxin 1B* (Fig. S1C; $n = 8$ for each group; Kruskal–Wallis test $P = 0.2207$) even though *Syx1A* is a member of the *syntaxin* gene family (Fig. S5).

Injection of dsSyx1A decreased the terminal oocyte length by 31% in G locusts (Fig. 4B; $n = 17$ and 16 for dsGFP and dsSyx1A, respectively; *t*-test $P = 0.0010$) and blocked the acceleration of terminal oocyte maturity rate in CS (Fig. 4B; $n = 6$ and 8 for dsGFP and dsSyx1A, respectively; *t*-test $P < 0.0001$). Correspondingly, progeny egg size of G locusts decreased by 8% (Fig. 4C; $n = 17$ and 13 for dsGFP and dsSyx1A, respectively; Kruskal–Wallis test $P = 0.0079$). In CS, the increase in egg size of S locusts was blocked (Fig. 4C; $n = 16$ and 6 for dsGFP and dsSyx1A, respectively; *t*-test $P < 0.0001$). With decrease in egg size, clutch size increased significantly (Fig. 4D; G, $n = 25$ and 12 for dsGFP and dsSyx1A, respectively; *t*-test $P = 0.0146$; CS, $n = 16$ and 6 for dsGFP and dsSyx1A, respectively; Kruskal–Wallis test $P = 0.0392$). However, the egg pod weight remained unchanged (Fig. 4E; G, $n = 17$ and 12 for dsGFP and dsSyx1A, respectively; *t*-test $P = 0.8747$; CS, $n = 16$ and 6 for dsGFP and dsSyx1A, respectively; Kruskal–Wallis test $P = 0.2688$). These eggs had normal embryonic development and hatching (Fig. S6). Thus, change in *Syx1A* expression modulated terminal oocyte maturity rate and progeny egg size in locusts.

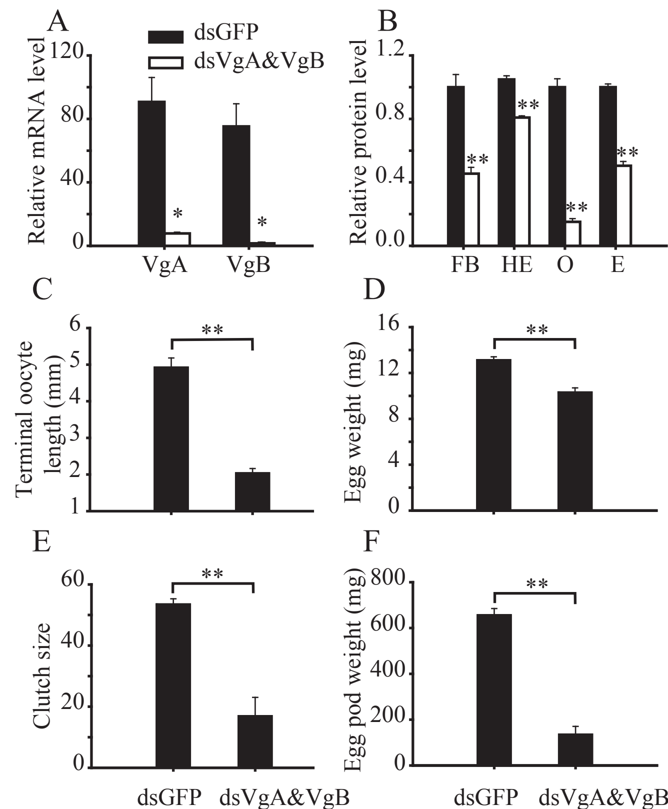

Fig. 5. Vg-regulated terminal oocyte development and progeny egg size. Relative mRNA levels of VgA and VgB (A), protein level (B), terminal oocyte length (C), egg weight (D), clutch size (E) and egg pod weight (F) after silencing VgA and VgB.

3.5. Function of Vgs

As mentioned above, VgA and VgB expressed at much higher level in the fat body of G locusts than in that of S locusts. We designed dsVgA and dsVgB to explore the role of Vgs in terminal oocyte maturity rate and progeny egg size. The relative mRNA level of VgA decreased by 91%, while that of VgB decreased by 97% upon co-injection of dsVgA and dsVgB (dsVgA&VgB) in G locusts (Fig. 5A; $n = 8$ for each group; *t*-test $P = 0.0010$ and 0.0013 for VgA and VgB, respectively). Similarly, Vg protein level in fat body decreased by 50% ($n = 8$ for each group; *t* test $P < 0.0001$) (Fig. 5B). Correspondingly, yolk protein level in hemolymph, ovary and egg decreased by 20%, 85% and 50%, respectively ($n = 8$ for each group; *t* test, hemolymph $P < 0.0001$, ovary, $P < 0.0001$, egg, $P < 0.0001$) (Fig. 5B). As a result, the terminal oocyte length decreased by 51% (Fig. 5C; $n = 11$ and 13 for dsGFP and dsVgA&VgB, respectively; Kruskal–Wallis test $P < 0.0001$), while the egg weight decreased by 22% (Fig. 5D; $n = 20$ and 10 for dsGFP and dsVgA&VgB, respectively; *t*-test $P < 0.0001$). The clutch size (Fig. 5E; *t*-test $P < 0.0001$) and egg pod weight (Fig. 5F; *t*-test $P < 0.0001$) both decreased following the reduction in egg size.

3.6. Function of *CAT* and *rheb*

Since *CAT* is involved in the lipid β-oxidation process and plays an important role in regulation of behavior change between G and S locusts (Wu et al., 2012), it was selected as representative gene of lipid metabolism to test whether it was involved in modulating reproduction traits between G and S locusts. At the same time, *Rheb*, which regulates TOR directly, was also selected. Silencing of *CAT* expression had no significant effect on egg length and egg weight, but increased the clutch size (Fig. S7). Likewise, silencing of

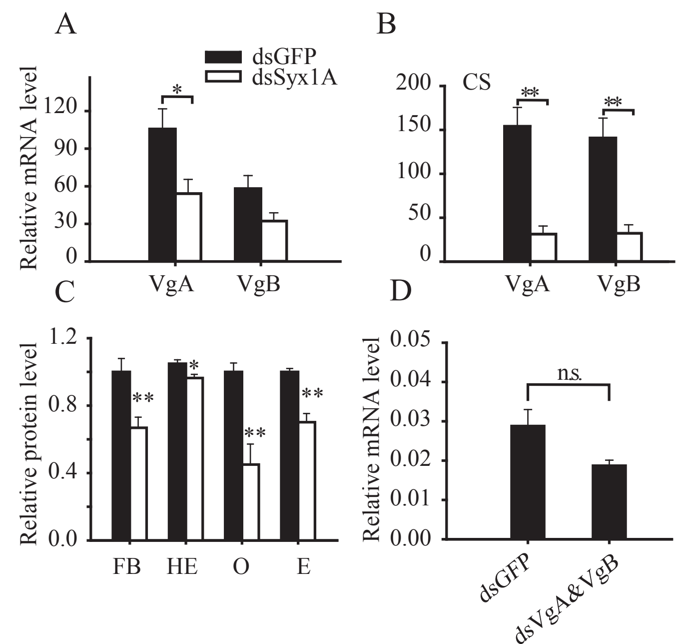

Fig. 6. Relationship between *Syx1A* and Vgs. Relative mRNA levels of VgA and VgB in G (A) and CS (B) locusts after dsSyx1A injection. (C) Protein levels of Vg and yolk protein in G locusts after dsSyx1A injection. (D) Relative mRNA level of *Syx1A* in G locusts after dsVgA&VgB injection.

Rheb expression had no effect on the length of terminal oocyte and the mRNA level of vitellogenin in fat bodies. Oviposition was blocked by continuous silencing of *Rheb* expression (Fig. S8). Thus, the two genes did not make direct contribution to different reproduction traits between G and S locusts.

3.7. Relationship between Syx1A and Vg

Considering that both *Syx1A* and Vg regulate terminal oocyte length and egg size between G and S locusts, we investigated the relationship between them by individually silencing each gene. The mRNA levels of VgA and VgB in the fat body of G locusts decreased significantly after dsSyx1A injection (Fig. 6A; $n = 8$ for each group; VgA, Kruskal–Wallis test $P = 0.0250$; VgB, Kruskal–Wallis test $P = 0.0782$). Further, their levels decreased more significantly in CS locusts (Fig. 6B; $n = 8$ for each group; Kruskal–Wallis test $P < 0.0001$ and 0.0003 for VgA and VgB, respectively). Coinciding with mRNA level, Vg protein levels in fat body decreased by 34% ($n = 8$ for each group; *t* test $P = 0.0057$) (Fig. 6C). Correspondingly, yolk protein levels in hemolymph ($n = 8$ for each group; *t*-test $P = 0.0179$), ovary ($n = 8$ for each group; *t*-test $P = 0.0022$) and egg ($n = 12$ for each group; *t*-test $P < 0.0001$) decreased by 9%, 55% and 30%, respectively (Fig. 6C). However, silencing Vg expression had no significant effect on *Syx1A* expression (Fig. 6D; $n = 8$ for each group; *t*-test $P = 0.0602$). These results indicate that *Syx1A* positively regulates Vg expression.

4. Discussion

In this study, we found that G locusts have faster sexual maturity rate and larger progeny egg size than S locusts. Analyses of expression of various genes indicated that Vg and *Syx1A* were expressed consistently at higher levels in G locusts than in S locusts during the entire adult stage. Expression of both genes decreased with IG and increased with CS. RNAi experiments confirmed that *Syx1A* modulated terminal oocyte maturity rate and progeny egg size by regulating Vg expression. Our findings uncover a relationship between several genes and phase-related reproductive traits in locusts.

4.1. Sexual maturity rate and egg size in locusts

Similar to desert locusts (Maeno and Tanaka, 2008; Norris, 1952), female migratory locusts develop and mature faster to generate larger eggs in high-density population. Previous studies have used terminal oocyte length and the time elapsed between adult emergence and deposition of the first egg pod to determine the female sexual maturity rate. Different female sexual maturity rates have been observed in different populations of migratory locusts (Guo, 1956; Norris, 1952; Norris and Richards, 1950). These differences may have been caused by race differentiation to adapt to different environments (Ma et al., 2012). Our results show that progeny egg size is positively correlated with terminal oocyte maturity rate (Fig. 2E).

Egg size is one of the most intensely studied maternal traits because it affects the fitness of offsprings (Einum and Fleming, 2000; Fox and Czesak, 2000). For example, the survival and growth rate of offsprings of beetles are improved and accelerated, respectively, by increment in their egg size (Fox, 1993). Similarly, larger fish eggs generally result in larger larvae that can swim faster and longer than smaller conspecifics (Miller et al., 1988). In Sceloporus lizards, juveniles from larger eggs have faster sprint speeds than those from smaller eggs (Sinervo, 1990). Thus, larger eggs indicate higher survival rate in a broad array of animals. However, in contrast to G locusts, S locusts adopt an alternative strategy to generate smaller but more eggs that have enhanced resistance to cold stress (Wang et al., 2012). These smaller S locust eggs give rise to more offsprings in the following year. Given that no significant difference in the egg pod weight was found between G and S locusts, a trade-off between egg size and egg number must occur. Thus, with the help of plasticity in reproductive traits, locusts adapt to different environments to enlarge their distribution region (Ma et al., 2012).

4.2. Regulation of terminal oocyte maturity rate and progeny egg size by Syx1A and Vg

Generally, differential expression of coding genes and noncoding RNAs contribute to phenotype plasticity (Chen et al., 2010; Jiang et al., 2012; Kang et al., 2004; Wang and Kang, 2014; Wei et al., 2009). With an approximately 3-fold up-regulation, *Syx1A* is the most strongly up-regulated gene among those coding for SNARE proteins. *Syx1A* and SNAP-25 are located on the plasma membrane and function as t-SNAREs to regulate secretion. Previous studies have suggested that SNAP-25 plays an important role in the reproduction of ticks (Browning and Karim, 2013). However, *Syx1A*, rather than SNAP-25, is expressed at a consistently higher level in the fat body of G locusts than in that of S locusts. As an independent member of the vesicle transport machinery in cells, *Syx1A* plays important roles in many physiological processes such as protein secretion, nerve cell communication, and immunological responses (Mayer, 2002). In fact, it can regulate membrane potential and calcium entry through its interaction with voltage-gated K^+ and Ca^{2+} channels to enhance secretion (Leung et al., 2003). The amount of yolk protein in the hemolymph decreases significantly after silencing *Syx1A*, which suggests that yolk proteins require *Syx1A* for secretion into the hemolymph from the fat body. The *Syx1A* protein sequence exhibits a high degree of evolutionary conservation (Table S3), so the function of *Syx1A* in reproduction is likely conserved across the animal kingdom. However, members of the *syntaxin* family genes have low similarity with each other (Table S4), suggesting functional diversity of genes within this family.

We manipulated the expression of Vgs to explore their function in phase-related reproductive traits and to determine the relationship between Vgs and *Syx1A*. Our results suggest that *Syx1A* regulates terminal oocyte maturity rate through Vgs. RNAi of VgA and VgB decreased the terminal oocyte maturity rate and progeny egg size significantly. Consistent with these results, anti-estrogen tamoxifen-treated female zebra finches (*T. guttata*) have previously been found to lay smaller but more eggs with 50% decrease in two yolk precursors in the plasma (Williams, 2001). These results suggest that the role of Vgs in regulating female sexual maturity rate and egg size is evolutionarily conserved. Previous studies have also suggested that Vgs are regulated by insulin and TOR signaling pathways. However, in the current study, most regulators were not found to be consistently differentially expressed between G and S locusts (Fig. S3). Further, the length of terminal oocyte and the mRNA level of vitellogenin in fat bodies did not change significantly after silencing *Rheb* expression. Oviposition was blocked after silencing *Rheb* expression continuously (Fig. S8). These results indicate that *Rheb* does not make direct contribution to phase-related differences in sexual maturity rate and progeny egg size. The functions of other members of insulin and TOR pathways in phase-related differences in sexual maturity rate and progeny egg size need to be studied further. Additionally, the interactions between these genes and *Syx1A* in locust production need to be determined in the future.

In addition to decreasing yolk protein level in the hemolymph by impairing their secretion, silencing *Syx1A* can decrease the mRNA level of Vgs in the fat body. As an invertebrate equivalent of liver and white adipose tissue, the fat body is an important

endocrine organ that can sense the nutritional status of an animal and secrete humoral signals into the hemolymph to regulate body development (Geminard et al., 2009). For example, fat body can regulate the rate of secretion in insulin-producing cells in the *Drosophila* brain (Geminard et al., 2009). Thus, silencing *Syx1A* expression may lead to decrease in concentration of humoral signals in the hemolymph secreted from the fat body. As a result, the signal for vitellogenin expression from the brain may become weaker. Further studies are required to determine the molecular mechanism of transcriptional regulation of Vgs by *Syx1A*, which also distinguishes the reproductive traits of G and S locusts.

It is well-known that the physiological status of the mother and other environmental factors (e.g., host and temperature) can affect egg size (Fox, 1993; Steigenga et al., 2005). *Syx1A* can regulate the reproductive traits via changes in its expression level according to population density in the locusts. Our study sheds light on other environmental factors that mediate egg size plasticity in animals. We provide not only molecular evidence of the trade-off between egg and clutch sizes, but also a molecular basis of egg size plasticity in animals. Our study suggests that the mutual transition between S and G locusts has an important physiological basis. This condition is beneficial for locusts to adapt to changing environments by regulating their sexual maturity rate, egg size, and progeny population numbers.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmb.2014.11.001.

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