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Molecular cloning and temporal–spatial expression of I element in gregarious and solitary locusts

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ABSTRACT

It has been reported that many genes and small RNAs are associated with density-dependent polyphenism in locusts. However, the regulatory mechanism underlying gene transcription is still unknown. Here, by analysis of transcriptome database of the migratory locust, we identified abundant transcripts of transposable elements, which are mediators of genetic variation and gene transcriptional regulation, mainly including CR1, I, L2 and RTE-BovB. We cloned one I element, which represents the most abundant transcripts in all transposable elements, and investigated its developmental and tissue-specific expression in gregarious and solitary locusts. Although there are no significant differences of I element expression in whole bodies between gregarious and solitary locusts at various developmental stages, this I element exhibits high expression level and differential expression pattern between gregarious and solitary locusts in central and peripheral nervous tissues, such as brain, antenna and labial palps. These results suggest that I element is potentially involved in the response of neural systems to social environmental changes in locusts.

1. Introduction

The migratory locust, *Locusta migratoria*, is an important agricultural pest worldwide and displays intriguing phase polyphenism depending on environmental conditions. At low population density, locusts develop cryptically coloured, relatively inactive and mutually aversive individuals (called "solitary" phase). At high population density, they become brightly coloured and actively aggregate-forming individuals (called "gregarious" phase). The gregarious and solitary locusts exhibit many differences in morphology, body colour, behavior, longevity, reproduction and immune response (Pener and Simpson, 2009). However, locusts can change reversibly in response to population density. This phenotypic plasticity has been regarded as a result of transcriptional regulation rather than genomic DNA mutation, because the genetic structures for producing the two phases are packaged within a single genome (Simpson and Sword, 2008). Although it was reported recently that many genes and small RNAs are related to phase polyphenism (Kang et al., 2004; Wang et al., 2007; Wei et al., 2009), the underlying mechanism of gene transcriptional regulation in response to environmental conditions remains largely unknown.

It has been suggested that transposable elements (TEs) are involved in orchestrating the environment-induced transcriptional regulation (Capy et al., 2000). Environmental stresses, such as high temperature (Bucheton, 1978) and infection (Raghavan et al., 2003), affect the expression of TEs. Eukaryotic TEs are categorized into DNA-mediated (Class II) or RNA-mediated (Class I) elements according to their transposition mechanisms (Finnegan, 1989). The Class I elements include LTR (long terminal repeats), SINE (short interspersed elements) and LINE (long interspersed elements). TEs constitute a substantial proportion of eukaryotic genomes (>40% of human genome) and sculpt the genome by innovating genes and creating new regulatory networks during evolution (Muotri et al., 2007). Although most of these sequences have lost their ability to transpose, some TE fragments can still be transcribed when integrated into mRNA coding regions (Caras et al., 1987; Gotea and Makalowski, 2006). These insertions contribute to alternative splicing and protein coding (Tamura et al., 2007; Wu et al., 2007). Recent research indicated that TEs are important sources of small RNAs, such as piRNA and endogenous siRNA in both germline and soma (Ghildiyal et al., 2008; Obbard and Finnegan, 2008; Thomson and Lin, 2009).

Our previous studies on the small RNA transcriptome of the migratory locust suggested that gregarious and solitary locusts have different expression profiles and that TEs are the sources of ~20% small RNAs (Wei et al., 2009). Thus, we hypothesized that TEs might have important roles in regulating gene transcription

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involved in phase polyphenism in migratory locusts. Here, we analyzed distribution patterns of TE transcripts in nine insect species. Furthermore, we investigated the developmental and tissue-specific expression of one I element, which is one of the most abundantly transcribed TEs. Our results suggested a possible role for these elements on neural systems in response to population density in migratory locusts.

2. Materials and methods

2.1. Insects

Locust individuals of the migratory locust (*L. migratoria*) were from the gregarious and solitary colonies in the Institute of Zoology, CAS, China. Gregarious cultures were reared in large, well-ventilated cages (25 cm × 25 cm × 25 cm) at densities of 200–300 insects per cage. Solitary-reared insects were kept in physical, visual and olfactory isolation that was achieved by ventilating each cage (10 cm × 10 cm × 25 cm) with charcoal-filtered compressed air. Rearing conditions of both colonies were under a 14 h/10 h light/dark photo regime at 30 ± 2 °C on a diet of fresh greenhouse-grown wheat seedlings and wheat bran.

2.2. Experimental samples

To investigate temporal expression profiles of I element, samples were collected at different developmental stages, including eggs incubated at 30 °C for 7 days (middle developmental stage of eggs), 1–5th instar nymphs reared for 2 days after ecdysis (middle developmental stage of nymphs) and adults reared for 7 days after ecdysis (middle developmental stage before sexual maturation) of gregarious and solitary phases. For each developmental stage, six biological replicates were used, each including four eggs or whole bodies of nymphal or adult individuals.

To investigate the tissue-specific expression profiles of I element, tissue samples were collected from seven different organs, including antenna, labial palp, brain, wing, hindleg, ovary and fat body, in fourth instar nymphs of gregarious and solitary phases. The function of these organs have been suggested to be related to the difference between gregarious and solitary phases in olfactory perception (Despland, 2001), food selection (Despland and Simpson, 2005), behavioral change (Anstey et al., 2009), morphological change, nutrition and reproduction (Pener, 1991). For each organ, tissues from six individuals were dissected and pooled into one biological replicate and four biological replicates were sampled. The sexual ratio of all samples from nymphs and adult is 1:1. All these samples were stored in liquid nitrogen.

2.3. Total RNA extraction, cDNA synthesis and genomic DNA extraction

Total RNA was extracted from various tissues using RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. cDNA was reverse-transcribed from 2 μg DNase-treated total RNA using MMLV reverse transcriptase (Promega). DNeasy Blood & Tissue Kit (Qiagen) was used to extract total DNA following the manufacturer's instructions.

2.4. Molecular cloning of locust I element

First, the Clontech SMART RACE cDNA amplification kit was used to prepare 3′-RACE cDNA template and the BD Advantage™ 2 PCR Kit was used for PCR amplification following the manufacturer's protocols using a GeneAmp PCR system 9700 (Applied Biosystems Inc.). 3′-RACE primer (3′RACE-contig63 in Table 1) was designed according to one locust EST sequence (contig63). One cDNA fragment, including part of ORF2 and 3′NCR, was cloned. Then, the genomic sequence between ORF1 and ORF2 was cloned by PCR, which initiated with a 2 min incubation at 94 °C, followed by 30 cycles of 94 °C, 30 s; 50 °C, 30 s; 72 °C, 2 min, and terminated with 72 °C, 10 min (forward primer, rgrhc0_003970F and reverse primer, contig63R in Table 1). Finally, three primers (GW-sp1, GW-sp2 and GW-sp3 in Table 1) were designed according to the acquired sequence, and part of ORF1 and 5′NCR was cloned using the Genome Walking Kit (Takara) according to the manufacturer's protocol.

Table 1
Listing of primers used in molecular cloning of I element (1) and qRT-PCR (2).

	Primer names	Primer sequences (5′ → 3′)
1	3′RACE-contig63	CCTATGGGGCTCTCCGGCACCTTG
	rgrhc0_003970F	CTTCGCAATGTCTTCCTTC
	contig63R	CCAAGGGACCTAGACACGAC
	GW-sp1	CCAACAACTTACGAGCGGCAGAT
	GW-sp2	GGGCGGACTGAGATAACTTCGTA
	GW-sp3	TGTCCACTTCCAGCAACAATGAG
2	QLmIF	CGGGTATTCTCCGCACATT
	QLmIR	CTGAAAGGCAGTCCACGAT
	Qcontig480F	GCACACTGTCCATTCCGT
	Qcontig480R	GTTGTAGCCTTTCCCCAA
	Qcontig2936F	GAGTCGGTTTGTATGGCG
	Qcontig2936R	CAGTTGGTTTGCGTGGCG

2.5. qRT-PCR analysis

The stand curve method (Wang et al., 2006) was used to measure the relative RNA expression level (RNA REL). PCR amplifications were conducted using an MX3000P spectrofluorometric thermal cycler (Stratagene) and RealMasterMix (SYBR Green) kit (Tiangen), initiated with a 2 min incubation at 95 °C, followed by 40 cycles of 95 °C, 20 s; 58 °C, 20 s; 68 °C, 20 s. Melting curve analysis was performed to confirm the specificity of amplification. RNA expression levels were normalized by β-actin which has been suggested to be the most suitable internal control gene for migratory locusts in our previous studies (Wang et al., 2006).

2.6. Data analysis

Unigene data of migratory locusts (Kang et al., 2004) and eight other insect species downloaded from FTP of EBI (European Bioinformatics Institute) were analyzed using the protein-based RepeatMasking program, which is based on WU-BLAST algorithm (http://www.repeatmasker.org/). The eight insect species are representative from Orthoptera, Hemiptera, Lepidoptera, Coleoptera, Hymenoptera and Diptera. The protein hits are reported as transcripts of TEs. We chose the sequences with a cutoff value of $p < 1E{-}05$.

For phylogenetic analyses, 19 sequences were obtained from previously reported alignments (Permanyer et al., 2003), and the mosquito (*Aedes aegypti*) sequence mosqI (GenBank accession No. AF134900), pea aphid (*Acyrthosiphon pisum*) sequence ApI (GenBank accession No. XM_001946744) were added. A freshwater snail (*Biomphalaria glabrata*) I element, nimbus (GenBank accession No. EF413180), was also added because it showed the highest BLAST hit (e value = 0) with LmI, and *LINE1-bg*, a fragment of nimbus, was reported closely related with mosqI (Tu and Hill, 1999). Clustal W 2.0.12 was used for sequence alignments. Neighbor joining method (implanted in MEGA 4) was used to reconstruct the phylogenetic tree using reverse transcriptase domain.

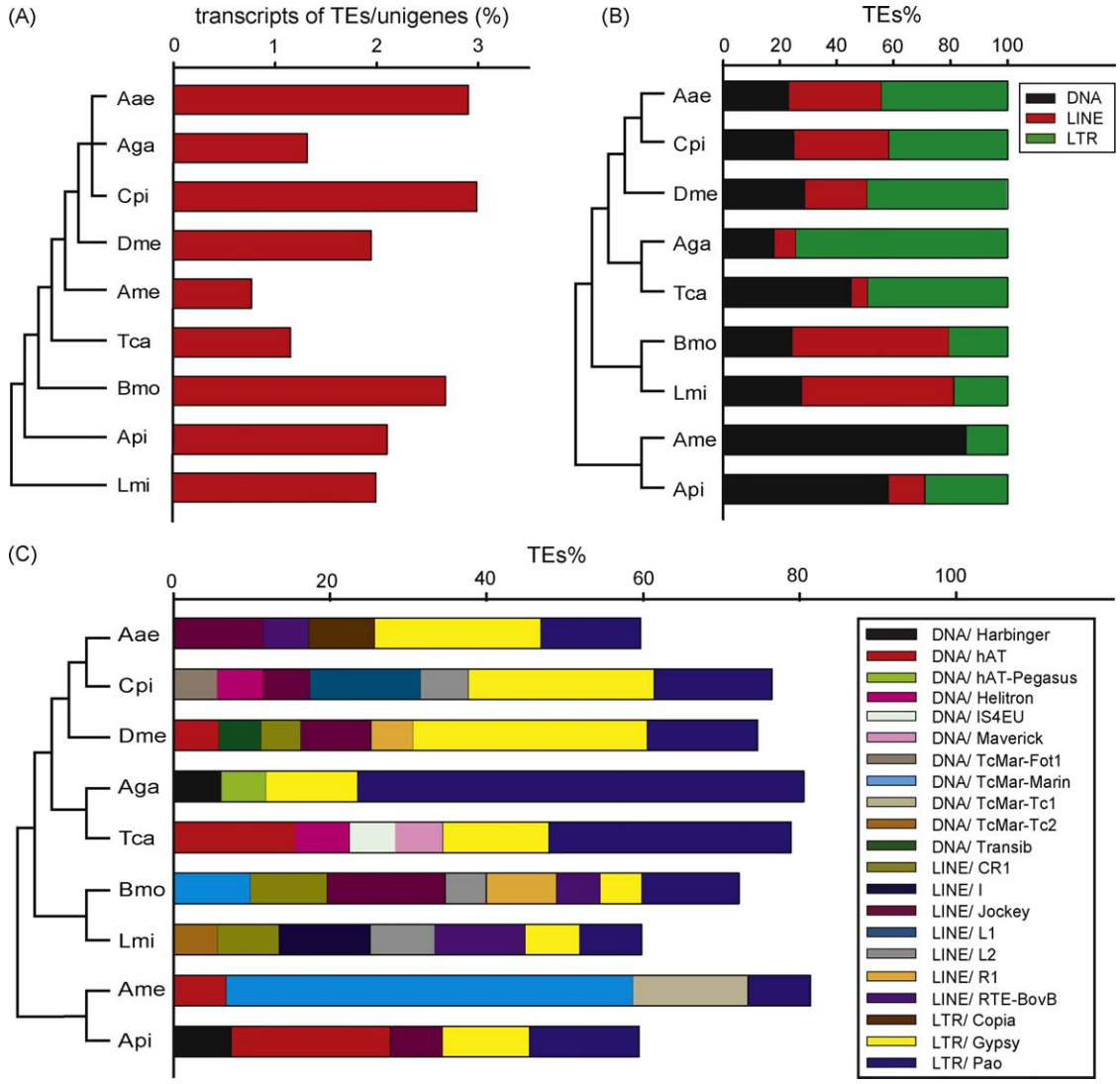

Fig. 1. Transcripts of TEs in nine insect species. (A) Percentage of TE transcripts in all unigenes. Phylogenetic tree indicates the evolutionary relationships of the species. (B) Percentage of four TE categories in all identified TEs transcripts. Hierarchical clustering was performed to identify insect species with similar expression pattern of TE transcripts. (C) Transcripts of TEs with more than 5% in different TE clades. Aae, *Aedes aegypti*; Aga, *Anopheles gambiae*; Api, *Acyrthosiphon pisum*; Ame, *Apis mellifera*; Bmo, *Bombyx mori*; Cpi, *Culex pipiens*; Dme, *Drosophila melanogaster*; Lmi, *Locusta migratoria*; Tca, *Tribolium castaneum*.

In statistical analysis, differences between treatments were compared either by Student's *t*-test, or by one-way analysis of variance (ANOVA) followed by a Tukey's test for multiple comparisons. Differences were considered significant at $p < 0.05$. Values are reported as mean ± SE. Data were analysed using SPSS 15.0 software (SPSS Inc., Chicago, IL).

3. Results

3.1. Transcripts of TEs in insect species

Transcripts of TEs in nine insect species were analyzed. The percentage of TE transcripts in the unigenes for all the species is <3%. The species that have higher percentage of TE transcripts are *Culex pipiens*, *A. aegypti* and *Bombyx mori*, and the species that have lower percentage were *Apis mellifera*, *Tribolium castaneum* and *Anopheles gambiae*. There was no clear correlation between phylogeny and the percentage of TE transcripts in insects (Fig. 1A). The annotated TE transcripts in the nine species can be classified into three categories: DNA, LINE and LTR. Cluster analysis of these three categories indicated four distribution patterns of TE transcripts. In *L. migratoria*, more LINEs were transcribed (Fig. 1B). The abundance of TEs in specific clades differs among insect species. Transcripts of clades CR1, I, L2 and RTE-BovB represent 7.9%, 11.6%, 8.3% and 11.6% of total TE transcripts in *L. migratoria* (Fig. 1C).

3.2. Molecular cloning of I element and phylogenetic analysis

We cloned one I element, LmI (GenBank Accession No. GU722580), with 5757 bp in length that contains several regions, including two ORFs (open reading frames), 5′NCR (non-coding region), 3′NCR and one short connection NCR in migratory locusts (Fig. 2A). A specific character of Class I non-LTR retrotransposons was found in LmI, namely that ORF1 encodes a nucleic acid binding protein, which contains three zinc finger domains of the CCHC type: $CX_2CX_4HX_4C$ (1), $CX_2CX_4HX_3C$ (2) and $CX_2CX_3HX_6C$ (3), and that ORF2 encodes the apurinic/apyrimidinic endonuclease (APE), reverse transcriptase (RT) and RNase H (RNH) domains.

Phylogenetic relationships between LmI and 22 other LINEs were analysed using the reverse transcriptase domain (Fig. 2B). LmI belongs to clade I together with nimbus of *B. glabrata*, mosqI

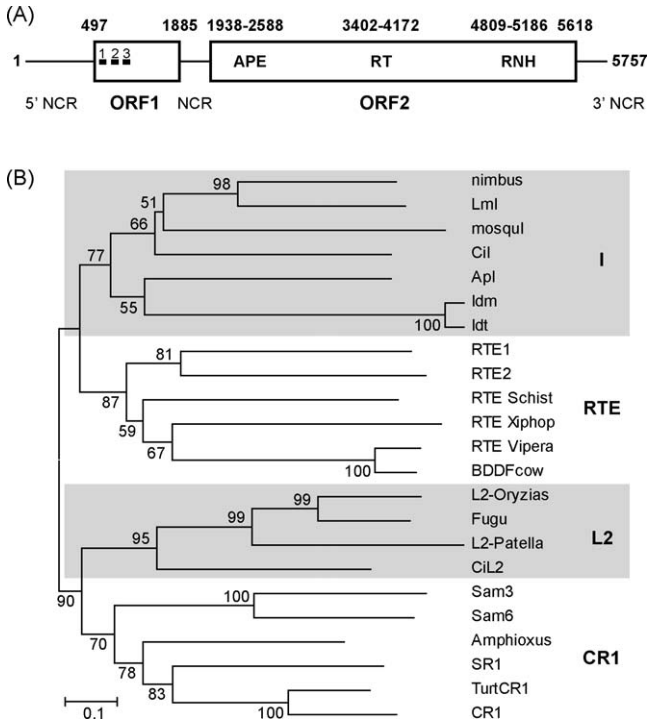

Fig. 2. Locust I element and its phylogenetic relationship. (A) Schematic diagram of locust I element. ORF1 encodes a nucleic acid binding protein, which includes three zinc finger domains. ORF2 encodes the apurinic/apyrimidinic endonuclease (APE), reverse transcriptase (RT) and RNase H (RNH) domains. (B) Phylogenetic tree reconstructed using the reverse transcriptase domain of long interspersed elements (LINE). The clades are boxed with LINE name at the end of each branch and name of each clade in bold text. The unrooted phylogenetic tree shown here is reconstructed using the neighbor-joining method with confidence of each node estimated by 1000 bootstrap replications. The bootstrap values are shown next to each branch with a cutoff value of 50. All characters are considered of equal weight and unordered.

of *A. aegypti*, CiI of *Ciona intestinalis*, ApI of *A. pisum* and two I elements of *Drosophila*. The reverse transcriptase domains of LmI and nimbus formed a subgroup. Since this is an unrooted tree, the relationships between the major groups are not certain.

3.3. Developmental expression profiles

The expression levels of LmI gene were not significantly different between various developmental stages for gregarious (ANOVA, Tukey's, $p = 0.613$) or solitary (ANOVA, Tukey's, $p = 0.549$) phases (Fig. 3). For each developmental stage, there was no significant difference of RNA REL of LmI gene between gregarious and solitary locusts (Fig. 3, Student's *t*-test, $p = 0.588$, 0.590, 0.182, 0.721, 0.780, 0.386 and 0.155 for egg, 1st instar, 2nd instar, 3rd instar, 4th instar, 5th instar nymphs and adult, respectively).

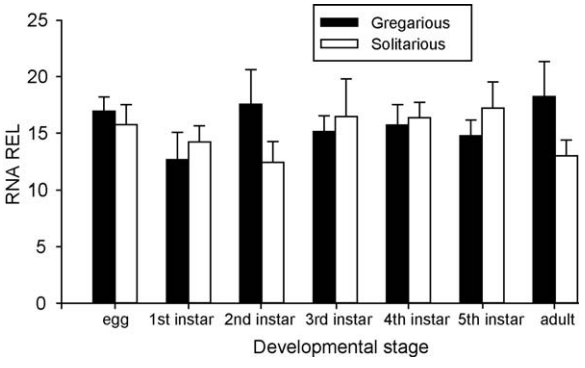

Fig. 3. Expression profiles of LmI gene in various developmental stages of gregarious and solitary locusts.

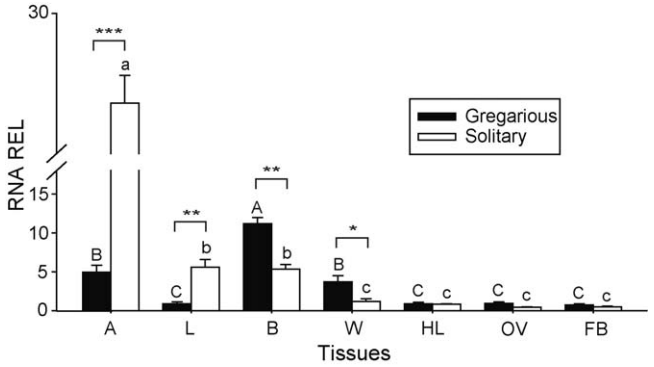

Fig. 4. Tissue-specific expressions of LmI gene in gregarious and solitary fourth instar nymphs. A, antenna; L, labial palp; B, brain; W, wing; HL, hindleg; OV, ovary; FB, fat body. Means labeled with the same letter within each treatment are not significantly different. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

3.4. Tissue expression profiles

The expression level of LmI gene exhibited a tissue-specific manner in fourth instar nymphs (Fig. 4). In gregarious nymphs, LmI gene was highly expressed in antenna, labial palp and brain (ANOVA, Tukey's, $p < 0.000$). In solitary nymphs, LmI gene was highly expressed in antenna, brain and wing (ANOVA, Tukey's, $p < 0.000$). In antenna and labial palp, RNA RELs of solitary nymphs were 5.7 and 6.3 folds, respectively, higher than in gregarious nymphs (Student's *t*-test, $p < 0.000$ and $p = 0.003$, respectively). In contrast, in brain and wing, RNA RELs of gregarious nymphs were 2.1 and 3.1 folds higher than in solitary nymphs (Student's *t*-test, $p = 0.001$ and $p = 0.032$, respectively). We also investigated the tissue-specific expressions of another two transcriptional fragments of Class I TEs: contig480 that belongs to LINE/RTE-BovB clade (Fig. 5A, ANOVA, Tukey's, $p = 0.002$ and $p < 0.000$ in gregarious and solitary nymphs, respectively) and contig2936 that belongs to LTR/gypsy clade (Fig. 5B, ANOVA, Tukey's, $p < 0.000$ and $p < 0.000$ in gregarious and solitary nymphs, respectively). In antenna and labial palps, fold changes of contig480 were 17.6 (Student's *t*-test, $p < 0.000$) and 54 (Student's *t*-test, $p = 0.028$), respectively, and fold changes of contig2936 were 5.7 (Student's *t*-test, $p = 0.017$) and 8.6 (Student's *t*-test, $p = 0.016$), respectively.

4. Discussion

The present study describes the identification and temporal–spatial expression of I element in the migratory locust, *L. migratoria*. First, we analyzed the expression of TE transcripts in nine insect species with available genomic or transcriptomic sequence information, and found that the abundance of TE transcripts is not correlated with the genome size of insect species, but the types of TEs exhibit species-specific traits. These results indicate that TEs are involved in evolutionary adaptation of species to different environmental stresses (Capy et al., 2000). In the migratory locust, the transcripts of several TEs, such as CR1, I, L2 and RTE-BovB, are abundant. Interestingly, our previous studies suggested that these TEs contribute to the production of locust small RNAs (Wei et al., 2009), implying that these TEs might have important roles in regulating gene expression.

One I element, one of the most abundant TEs in locust, was obtained. I element is a well-acknowledged LINE retrotransposon that was initially identified in *Drosophila melanogaster* (Fawcett et al., 1986). Full-length I elements have been identified in several

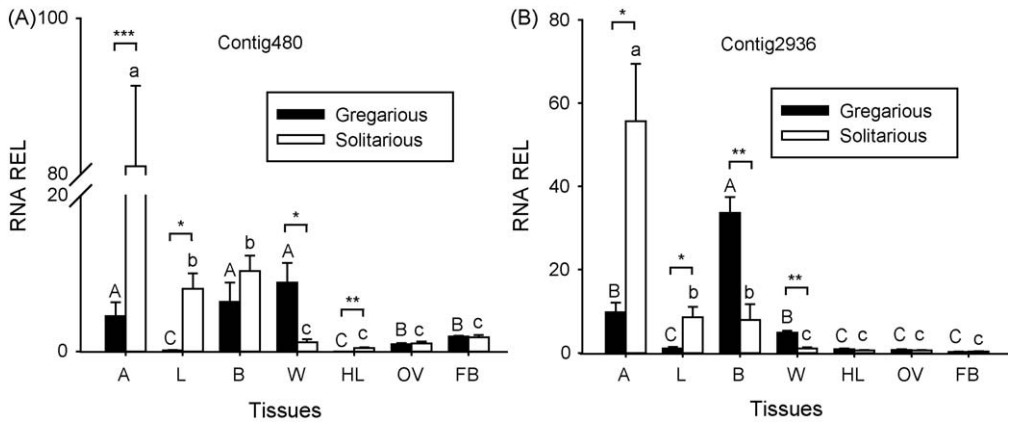

Fig. 5. Tissue-specific expressions of (A) contig480 and (B) contig2936 transcriptional fragments in fourth instar nymphs of gregarious and solitary locusts. A, antenna; L, labial palp; B, brain; W, wing; HL, hindleg; OV, ovary; FB, fat body. Means labeled with the same letter within each treatment are not significantly different. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$.

insect species with genomic information (Fawcett et al., 1986; Tu and Hill, 1999). However, we failed to obtain the full sequence of 5′NCR and 3′NCR because locust genomic information is not available. LmI has the typical characteristic of I clade elements (Fawcett et al., 1986; Tu and Hill, 1999). Phylogenetic analysis has shown that LmI forms a subgroup with nimbus, which is one I element from snail *B. glabrata* (mollusca). The result indicates that LmI is distantly related to the *Drosophila* I element and closely related to the *Biomphalaria* I element. Therefore, the LmI may be a paralog of the *Drosophila* I element and the LmI subgroup was separated before the latest split between ancestors of mollusca and arthropoda. Previous study also found that mosqI of *A. aegypti* forms a subgroup with *LINE1-Bg*, which is a fragment of nimbus (Tu and Hill, 1999).

Our results reveal that transcriptional expression of I element has similar expression levels at various developmental stages. Thus, it seems that I element does not impact the development of locust although many studies have demonstrated that reverse transcriptase (RT), which is expressed by activated TEs, is a mediator of cell proliferation and differentiation (Landriscina et al., 2005; Spadafora, 2004). However, we cannot exclude the tissue-specific patterns of I element at various developmental stages because we only analyzed the expression levels in whole bodies. In addition, we found that I element exhibits comparably high expression level to the house-keeping gene actin, implying that I element might have important roles in locusts.

Transposable elements, which can cause genetic variation and gene expression changes, are important in environmental adaptation (Capy et al., 2000; Feschotte, 2008). Expression of TEs can be activated at the transcriptional level in response to different biotic and abiotic stresses (Grandbastien, 1998). For example, infection (Raghavan et al., 2003), wounding (Kumar and Bennetzen, 1999), drought and salinity (Liu and Baird, 2003) can induce the expression of TEs. However, activation of TEs by social changes has not been reported. In this paper, we provided the first evidence that TEs display significant expression difference in antenna, labial palp and brain between gregarious and solitary fourth instar nymphs, although these genes have similar expression levels at whole body levels. Further characterization of these TEs will provide information on the role of TEs in response to social environmental changes in insects.

Present results indicated that several tissues that belong to peripheral (antenna and labial palp) or central nervous system (brain) displayed different expression levels of I element between gregarious and solitary locusts, implying its potential functional significance on neural plasticity. Previous studies have proved that TEs are involved in the development of nervous systems (Kania et al., 1995). In locusts, many differences in nervous systems have been found between gregarious and solitary locusts, such as the central olfactory system (Anton et al., 2002), the visuomotor pathway (Rogers et al., 2007), the sensory-motor pathway (Fuchs et al., 2003) and several neurochemicals (Rogers et al., 2004). These differences have been proposed to play important roles in behavioral adaptation to environmental stresses, including food selection (Despland and Simpson, 2005), responses to conspecies (Despland, 2001) and avoidance to natural enemies (Reynolds et al., 2009) in locusts. Therefore, the locust TEs may mediate the regulation of peripheral and central nervous systems by alternative splicing, protein coding, small RNA production and gene expression regulation (Muotri et al., 2005; Tamura et al., 2007; Thomson and Lin, 2009; Wu et al., 2007).

In conclusion, we have cloned one I element and revealed its phase-specific expression in neural systems that mediate behavioral phase change in response to social environment. Behavior is the most labile phase characteristic and provides motive force for phase change at population level (Simpson et al., 1999). Therefore, the present study about the locust TEs will provide further insights on the gene regulation mechanisms underlying phase changes of locusts.

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