Metabolomic analysis reveals that carnitines are key regulatory metabolites in phase transition of the locusts

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Edited by David L. Denlinger, Ohio State University, Columbus, OH, and approved January 13, 2012 (received for review November 22, 2011)

Phenotypic plasticity occurs prevalently and plays a vital role in adaptive evolution. However, the underlying molecular mechanisms responsible for the expression of alternate phenotypes remain unknown. Here, a density-dependent phase polyphenism of *Locusta migratoria* was used as the study model to identify key signaling molecules regulating the expression of phenotypic plasticity. Metabolomic analysis, using high-performance liquid chromatography and gas chromatography–mass spectrometry, showed that solitarious and gregarious locusts have distinct metabolic profiles in hemolymph. A total of 319 metabolites, many of which are involved in lipid metabolism, differed significantly in concentration between the phases. In addition, the time course of changes in the metabolic profiles of locust hemolymph that accompany phase transition was analyzed. Carnitine and its acyl derivatives, which are involved in the lipid β-oxidation process, were identified as key differential metabolites that display robust correlation with the time courses of phase transition. RNAi silencing of two key enzymes from the carnitine system, carnitine acetyltransferase and palmitoyltransferase, resulted in a behavioral transition from the gregarious to solitarious phase and the corresponding changes of metabolic profiles. In contrast, the injection of exogenous acetylcarnitine promoted the acquisition of gregarious behavior in solitarious locusts. These results suggest that carnitines mediate locust phase transition possibly through modulating lipid metabolism and influencing the nervous system of the locusts.

aggregation | metabolic profiling | phase change

Phenotypic plasticity is the ability of an organism with a single genotype to change its phenotype in response to different environments and is currently seen as an important aspect of adaptation (1–3). Several regulatory mechanisms associated with phenotypic plasticity, including hormonal activity, gene expression, alternative splicing, and DNA methylation, have recently been identified in a number of genetic-model organisms (4, 5). However, the key signaling circuits or regulatory networks mediating the differential expression of plastic phenotypes triggered by environmental cues are still largely unknown, especially in noncanonical study models (6).

The migratory locust, *Locusta migratoria*, a worldwide agricultural pest, displays remarkable phase polyphenism, in which the expression of numerous physiological, morphological, and behavioral traits occurs in response to changes in local population density (7). As one of the striking examples of phenotypic plasticity, locusts provide an ideal model system to study the mechanisms underlying phenotypic plasticity (3). Locust crowding induces the aggregating, more active, and conspicuously colored gregarious phase, whereas isolation leads to the shy, sedentary, and cryptically colored solitarious phase. In addition, phase polyphenism involves variation in many physiological traits, such as immunity, reproduction, and endocrine and energy metabolism, which do not all share the same underlying mechanisms (7). The transition between solitarious and gregarious phases is a reversible process and continues even across generations. Behavioral change has been regarded as a key step for the establishment of a positive-feedback loop that can drive an initially solitarious phase to the gregarious phase, which is triggered by multiple sensory inputs from other individuals (7). The neuromodulation of CNS circuits and the changes of olfactory sensitivity have been recently proposed to be involved in mediating the initiation of behavioral phase change (8–10). However, the process in which a set of varied phase traits are subsequently expressed during the development of locusts is not as well understood.

Metabolomics has been widely applied to uncover biomarkers and metabolic fingerprints in drug discovery and clinical toxicology, as well as to reveal active pathways and signaling metabolites (11–13). Metabolomic analysis can provide a "closer" glimpse into the phenotypic state of an organism because it is the downstream of both transcriptomics and proteomics compared with that of functional genomic analysis (14). Although previous studies have uncovered critical information about the gene regulation underlying locust phase polyphenism (15–17), detailed analysis regarding the metabolic state of different phase groups is still lacking. Therefore, metabolomic analysis is a very promising strategy to elucidate the key signaling molecules regulating the development of phase polyphenism in locusts.

In the current study, methods based on high-performance liquid chromatography and gas chromatography–mass spectrometry (HPLC/MS and GC/MS) were used to determine the comprehensive multimetabolite profile of the hemolymph associated with phase polyphenism and to identify the key pathways and metabolites involved in phase transition. Results showed that lipid metabolites display the most significant differences between the two phase locusts. In addition, concentrations of both carnitine and acylcarnitine, which are essential for energy metabolism and neuromodulation, were found to be higher in gregarious locusts. The role of carnitines as key contributors underlying behavioral phase change was confirmed by RNA interference (RNAi) and pharmacological intervention.

Author contributions: R.W., Z.W., X.W., G.X., and L.K. designed research; R.W., Z.W., X.W., D.Y., and C.Z. performed research; R.W., Z.W., X.W., P.Y., G.X., and L.K. analyzed data; and R.W., Z.W., X.W., G.X., and L.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1119155109/-/DCSupplemental.

AGRICULTURAL SCIENCES

Results

Differential Metabolite Analysis Between Gregarious and Solitarious Locusts. HPLC/MS and GC/MS analyses were performed to determine the relative levels of metabolites across gregarious and solitarious hemolymph samples (Fig. 1 and *SI Appendix*, Figs. S1–S4). A total of 319 metabolites (252 by HPLC/MS and 67 by GC/MS) displayed significantly different levels between the two sample groups (Wilcoxon rank-sum test: $P < 0.05$; *SI Appendix*, Table S1 for HPLC/MS; *SI Appendix*, Table S2 for GC/MS). Orthogonal projection to latent structures–discriminant analysis (OPLS-DA) showed clear differences in the hemolymph metabolome between the two phases. These data points were clustered into two distinct groups in the plot map, obviously separating the gregarious and solitarious samples (Fig. 1*A* and *SI Appendix*, Fig. S5).

Overall, several metabolites displayed considerable differences between gregarious and solitarious hemolymph, including multiple lipids, carbohydrates, amino acids, free carnitine, and their derivatives (Fig. 1*B* and *SI Appendix*, Tables S1 and S2). In gregarious locusts, the metabolites at relatively higher levels mainly included lysophosphatidylcholines (LysoPCs), diacylglycerols (DGs) with linoleic acid acyl (18:2), and carnitines [free carnitine, oleoylcarnitine, and acetylcarnitine (ALC)]. The most differentially elevated metabolites in solitarious locusts were DGs with linolenic acid acyl (18:3) and phosphatidylethanolamines (PEs) (Fig. 1*B*). Most of the differential amino acids and carbohydrates, such as proline and glucose, were more abundant in gregarious locusts (Fig. 1*B* and *SI Appendix*, Tables S1 and S2).

The construction of a network model clearly revealed three separate structures containing two major networks and one small network, where the largest network (cluster II) included two lipid metabolites, DGs and PEs. These lipid metabolites were present in higher levels in solitarious locusts (*SI Appendix*, Fig. S6). Thus, the metabolites in lipid metabolism exhibited notable differences between the two phases of locusts (*SI Appendix*, Fig. S7).

Metabolite Changes During the Time Courses of Phase Transition. The principal component analysis (PCA) score trajectories showed that metabolic profiles of solitarization (IG) groups had a more extensive response than gregarization (CS) groups (16 h vs. 32 h) compared with their corresponding time-series controls. A notable transformation of metabolic phenotypes was observed in IG groups at the last time point of treatment. In addition, a plot of scores at different time points on the direction of the first principal component (PC1) accounted for a sizable portion of variance in the data set (Fig. 1*C*).

During the time courses of CS and IG, 341 metabolites in CS and 355 metabolites in IG were significantly up- or down-regulated, but 231 metabolites were equally shared by both groups (*SI Appendix*, Fig. S8*A*). To visualize the relationship between these metabolites, hierarchical clustering was used to arrange the metabolites based on their relative levels across samples (*SI Appendix*, Fig. S8*B*). A subset of lipid metabolite categories including carnitines, DGs, and PEs significantly changed during CS or IG processes (Fig. 2). Among these metabolites, carnitine and ALC showed robust consistency of changing patterns during the time course of treatments (Pearson correlation: $r = 0.76$ and $r = 0.74$ in CS; $r = -0.46$ and $r = -0.42$ in IG; $P < 0.01$; *SI Appendix*, Table S5). These findings suggest that carnitines can have a potential role in regulating phase change of the locusts.

Functional Validation of Carnitines in Phase Change. In animals, carnitines do not participate in metabolism by itself; instead, they exert metabolic functions by forming esters with a wide range of fatty acyl groups (18). To reveal the role of carnitines in phase change of the locusts, RNAi was used to knock down two key enzymes that are involved in carnitine system, namely, carnitine

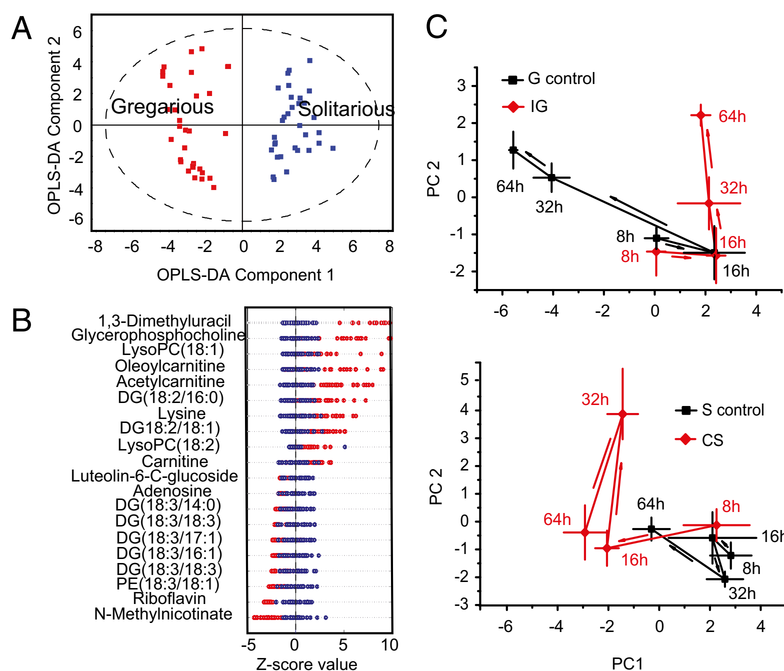

Fig. 1. Differential metabolic profiles in fourth instar-nymph hemolymph between gregarious and solitarious locusts and during the time courses of gregarization and solitarization processes. (*A*) OPLS-DA score plot separating gregarious (red squares) and solitarious (blue squares) samples. (*B*) Z-score plot of the top 10 named metabolites that have higher relative levels in gregarious (*Upper*) and solitarious locusts (*Lower*), respectively. Each point represents one metabolite in one sample, colored by phase type (blue, solitarious; red, gregarious). (*C*) 2D PCA score plot depicting time-dependent trajectory of metabolic profiles during the IG (up) and CS (down) processes. The treatment groups are marked by red diamonds, and pairwise controls are marked by black squares at a series of time course (8, 16, 32, and 64 h). Each dot represents an averaged metabolic status of 8 biological replicates; bar lines indicate SE of PC1 and PC2.

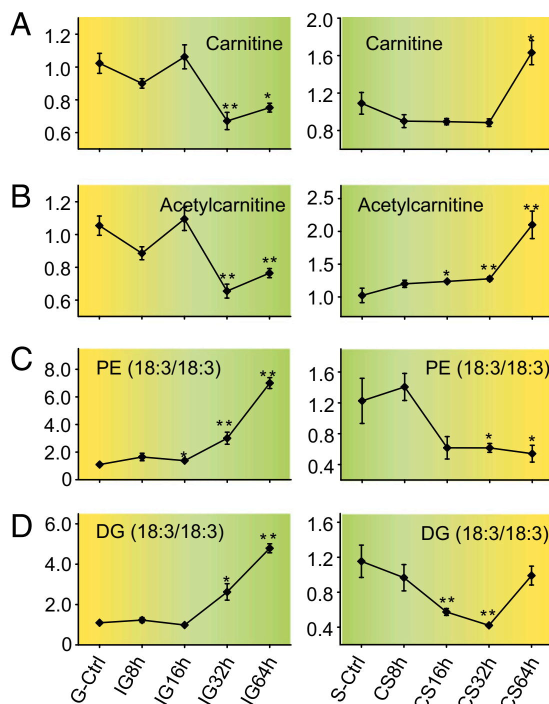

Fig. 2. Abundance of metabolites related to lipid metabolism with the time course of IG and CS. (*A*) Relative levels of carnitine during IG and CS. (*B*) Relative levels of ALC during IG and CS. (*C*) Relative levels of PE (18:3/18:3) during IG and CS. (*D*) Relative levels of DG (18:3/18:3) during IG and CS. $^{*}P <$ 0.05 and $^{**}P < 0.01$ comparing with control (G/S-Ctrl).

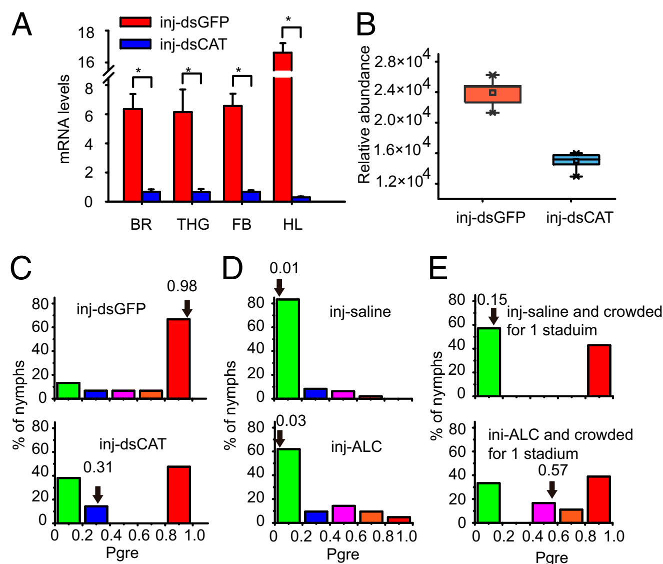

Fig. 3. Functional analysis of ALC in behavioral phase change. (*A*) Relative expression levels of CAT mRNA in various tissues of fourth-instar gregarious locusts after ds*GFP* and ds*CAT* injection. B, brain; FB, fat body; HL, hind leg; TH, thorax ganglia. (*B*) Relative abundance of ALC in hemolymph of fourth-instar gregarious locusts after ds*GFP* and ds*CAT* injection. (*C*) Behavioral changes induced by RNAi of CAT in fourth-instar gregarious locusts. (*D*) Behavioral responses of fourth-instar solitarious locusts induced by direct injections of saline and ALC. (*E*) Behavioral responses of the third-stadium solitary nymphs exposed to one stadium of crowding after injection with ALC. $^{*}P < 0.05$. Arrows indicate median P_{gre} values of the population. All statistical analyses were conducted with a Mann–Whitney *U* test relative to control groups ($P < 0.05$).

O-acetyltransferase (CAT) and carnitine palmitoyltransferase I (CPT). Subsequently, pharmacological intervention experiments were performed in the fourth-instar nymphs. *CAT* was used as the interfering target gene to control ALC levels and *CPT* was used as a target gene to control acyl-carnitine levels in the cells of gregarious nymphs (Figs. 3 and 4).

In comparison with double-stranded (ds)*GFP*-injected controls (green fluorescent protein), the relative expression levels of *CAT* mRNA in various tissues, such as the brain, thoracic ganglia, fat body, and hind leg, significantly decreased after injection of ds*CAT* ($P < 0.01$; Fig. 3*A*). The interference of *CAT* also resulted in a significant, approximately twofold decrease in the hemolymph ALC levels compared with ds*GFP*-injected nymphs ($P < 0.01$; Fig. 3*B*). Analysis of PCA plots showed clear differences in the hemolymph metabolome between ds*CAT* injection and ds*GFP* control (*SI Appendix*, Figs. S10 and S12). The levels of free carnitine, DGs, PE, glycerol, and proline were significantly affected by ds*CAT* treatment. Among the differential metabolites that were analyzed, metabolites with higher levels in the gregarious phase declined to a similar degree as ALC by ds*CAT* treatment, whereas most of the metabolites that were more abundant in the solitarious phase were not affected (*SI Appendix*, Figs. S11 and S13). As a consequence, the behavior of *dsCAT*-injected gregarious nymphs changed toward the solitarious state, with ~48% falling into the P_{gre} interval of 0.8–1 compared with 67% in the ds*GFP*-injected group (Fig. 3*C*).

To further investigate the role of ALC in phase change, the behavioral changes after pharmacological intervention in solitarious nymphs were examined. Fig. 3*D* shows that ALC-injected solitarious nymphs did not significantly change their behavioral state, with ~62% falling into the P_{gre} interval of 0–0.2, compared with 83% of the saline-injected control nymphs. However, the behavior of third-instar solitarious nymphs significantly shifted toward the gregarious state when they were exposed to a stadium of crowding and were injected with ALC at three consecutive 48-h time intervals, compared with the nondrugged controls (Fig. 3*E*).

In addition to CAT, the levels of carnitines are also regulated by CPT, an enzyme that transfers the acyl group on CoA to carnitine. *CPT* knockdown resulted in a 10-fold decrease in CPT mRNA levels (*t* test, $P < 0.05$) (Fig. 4*A*) and a significant decrease in long-chained carnitine levels (*t* test, $P < 0.05$) (Fig. 4*C* and *SI Appendix*, Table S6) compared with the controls (ds*GFP* injection) when similar RNA interference experiments of CPT were performed in gregarious locusts. As a consequence, the behavior of ds*CPT*-injected gregarious nymphs changed toward the typical solitarious state, with ~21% falling into the P_{gre} interval of 0.8–1 compared with 67% in the ds*GFP*-injected group (Fig. 4*B*).

Discussion

In the current study, the differential metabolic profiles associated with phase polyphenism were characterized and carnitines were identified as the key signaling regulator in the behavioral phase change of *L. migratoria*. The high proportion of metabolites displaying significant abundance changes (45.5% of total detected metabolites) in response to population density is consistent with the results of previous transcriptomic investigations (10). Together, these results suggest that locust-phase polyphenism may involve many different regulatory components, similar to other study models of phenotypic plasticity, such as honey bees (19). The current study reports that a small group of intermediary metabolites appears to play a regulatory role in the expression of phase characteristics. It represents a mechanism of plasticity regulation, distinct from traditional regulators, such as developmental hormones (7) and biogenic amines (9).

The results from metabolomic analyses and functional investigation highlighted the roles of carnitines in regulating behavioral phase change of the migratory locust. The carnitine

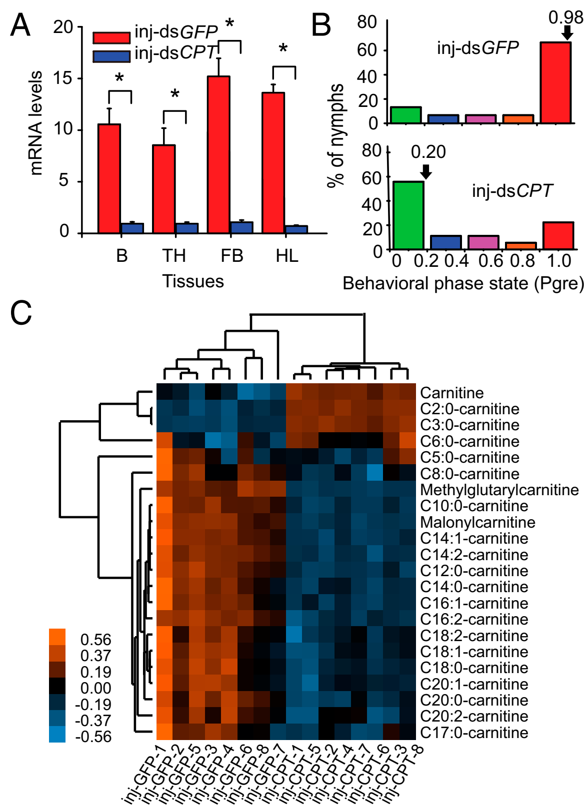

Fig. 4. RNAi effects of CPT in fourth-instar gregarious locusts. (*A*) Relative expression levels of CPT mRNA in various tissues of fourth-instar gregarious locusts after ds*GFP* and ds*CPT* injection. B, brain; FB, fat body; HL, hind leg; TH, thorax ganglia. *$P < 0.05$. (*B*) Behavioral changes induced by RNAi of CPT in fourth-instar gregarious locusts (Mann–Whitney *U* test: $P < 0.01$, relative to ds*GFP*-injected controls). Arrows indicate median P_{gre} values of the population. (*C*) Heat map representation of unsupervised hierarchical clustering of the data using a complete linkage algorithm in the hemolymph of fourth-instar gregarious locusts after ds*GFP* and ds*CPT* injection. Shades of red and blue represent an increase and decrease of a metabolite, respectively, relative to the metabolite levels in paired controls (see color scale).

system, including free carnitine and acylcarnitines, is essential for cell energy metabolism as a carrier of long-chain fatty acids for β-oxidation or as a reservoir pool of acyl-CoA (20). The difference in the abundance of carnitines and their precursors, lysine and methionine, between both phases and the dynamic changes during the time course of IG or CS might reflect the alternative energy requirement associated with phase polyphenism (Figs. 1 and 2). Silencing of key enzymes involved in carnitine metabolism and the pharmacological manipulation of carnitines both resulted in the change of locust behavioral state (Figs. 3 and 4). The change can be indicated by a single probabilistic metric of gregariousness P_{gre} (the value from 0 to 1) that encompassed two different variables, attraction and activity (21). These variables not only suggested the important role of carnitines in modifications to activity levels but also their attraction behavior. In addition, the silencing of *CAT* and *CPT* also caused more widespread effects on other connected differential metabolites in the metabolomic network, especially membrane phospholipids and carbohydrates (*SI Appendix*, Figs. S11 and 13). Hence, the effect of carnitines not only is restricted to activity and metabolic rate but suggests that carnitines, perhaps in conjunction with other coregulated differential metabolites, are also involved in signaling pathways that translate crowding-associated sensory inputs into behavioral phase change.

Numerous studies have suggested that carnitines can influence a variety of human and animal behaviors, including ambulatory (22), aggressive (23), impulsive (24), and learning (25). Carnitines have been proposed to act as important neuromodulators by regulating carbohydrate and lipid metabolism in the animal central nervous system (21). ALC also promotes the biosynthesis and release of several key neurotransmitters involved in the regulation of multiple animal behaviors, such as acetylcholine, GABA, melatonin, and dopamine, or facilitates the neuronal response to these neurotransmitters (26). In addition, a chronic ALC treatment can change the expression levels of many genes in rat brains (27). On the other hand, the expression of genes related to dopamine and serotonin pathways in the migratory locust have been demonstrated to play important roles in the initiation of behavioral phase change (9). Several metabolites involved in dopamine pathways, such as tyrosine and acetyldopamine, have been observed in the differential metabolic profiles during the time course of phase change. Thus, the influence of carnitines on behavioral phase change might be achieved by modulating the nervous system in the migratory locust.

Recently, several studies in honey bees have proven that epigenetic regulation might generally play an important role in the development of phenotypic plasticity (28). Interestingly, ALC has been found to influence phenotypic changes through epigenetic modulation, for example, histone acetylation and DNA methylation in *Drosophila melanogaster* and humans (29). In particular, density-dependent phase polyphenism shows transgenerational effects in *L. migratoria* and may be epigenetically regulated (7). The presence of several candidate genes that may be involved in epigenetic control, including two DNA methyltransferases (Dnmts), Dnmt1 and Dnmt2, in the transcriptome of *L. migratoria* has been recently found (16). However, further experiments are warranted to elucidate whether, and at what level in the molecular cascade, carnitines influence epigenetic modulation involved in phase change.

The current analysis of overall metabolic profiles also suggested that the shift of metabolic homeostasis in locust hemolymph occurs together with population density changes. Solitarious locusts accumulate more DGs, whereas gregarious locusts have high abundances of free amino acids and sugars, particularly proline and glucose. DG is the primary lipid component of insect hemolymph, and glucose is the major energy source for locusts at the resting level (30). This shift may reflect differences in energy requirements for phase-related behavioral and physiological state between gregarious and solitarious locusts, especially regarding locomotor activities (7) and lipid storage (31). The evidence from previous studies suggests that two hormones (adipokinetic and juvenile hormones) might be involved in the regulation of the metabolism of these lipids and sugars (30). In honey bees, various castes also display differential abundance of free amino acids in the hemolymph (32), suggesting the common existence of metabolic regulation associated with behavioral plasticity.

Evidently, the major difference of metabolic profiles between solitarious and gregarious nymphs is the abundance and the unsaturated fatty acid composition of lipid metabolites. As two major membrane phospholipids, the phosphatidylcholine (PC)/PE ratio and their fatty acid composition are responsible for the regulation of membrane fluidity and permeability (33). Higher PC/PE ratio and low unsaturated fatty acid composition in gregarious locusts indicate that the formation of a compact and more stable membrane bilayer might be induced during gregarization (34). Moreover, membrane fluidity plays an important role in maintaining optimal cell physiology and has been reported to be involved in various kinds of plasticity, such as thermal, hypoxia, toxic, infection, and oxidative stresses (35). The change of membrane fluidity between phases might be an adaptive mechanism under-

lying the resistance of oxidative stress or the regulation of neuronal plasticity in response to varied population density (7). In addition, linoleic and linolenic acids are key precursors of many signaling metabolites, such as arachidonic acid and sex pheromones (31). The shift in the ratio between linoleic and linolenic acids may also induce a difference of hormonal regulation or olfactory signals between solitarious and gregarious locusts (7). A previous transcriptomic analysis performed by our group suggests that gregarious locusts have low expression levels of most desaturase genes, which increase the unsaturated degree of fatty acids (16). This phenomenon is consistent with the current observation of linoleic acid and linolenic acid levels.

Transition from the solitarious phase to the gregarious phase is essential for locust swarm formation and migration (7). In the current study, clear evidence shows that carnitines play key roles in behavioral phase change, possibly through modulating lipid metabolism, the nervous system, or epigenetic modeling in *L. migratoria*. The present study provides insights into the mechanisms underlying phase polyphenism and highlights *L. migratoria* as the potential model system for addressing the molecular basis of phenotypic plasticity.

Materials and Methods

Insect Rearing and Treatments. Gregarious and solitarious locusts were reared under a 14-h/10-h light/dark cycle at 30 ± 2 °C and on a diet of fresh greenhouse-grown wheat seedlings and wheat bran (10). The fourth-instar nymphs that had developed for 3 d from previous molt were used. Hemolymph samples were obtained from both gregarious and solitarious nymphs (with 32 replicates of each), and the time-course treatment of solitarization and gregarization was 8, 16, 32, and 64 h. Each treatment group consisted of 8 replicates, and a corresponding control group with same replicates was also used.

Metabolomic Profiling. The metabolomic profiling analysis included sample extraction, metabolite separation and detection, metabolomic data preprocessing (e.g., metabolite feature extraction, chromatographic peak alignment, data reduction), and, finally, statistical analysis. Details are included in *SI Appendix, Materials and Methods*.

RNAi and Pharmacological Intervention. dsRNA of GFP, CAT, and CPT was prepared using the T7 RiboMAX Express RNAi system (Promega) according to the instructions of the manufacturer. ALC was supplied for pharmacological intervention. Details of the methods are included in *SI Appendix, Materials and Methods*.

Behavioral Assay. An arena assay was performed in a rectangular Perspex arena with opaque walls and a clear top. Three parameters, namely, entry frequency in the stimulus area (EFISA), mean distance to the stimulus group (MDTSG), and total distance moved (TDM), were included in the final binary logistic regression model. The methods of behavioral assay are included in *SI Appendix, Materials and Methods*.

Data Analysis. The data matrix was analyzed by PCA and orthogonal projection to latent structures–discriminant analysis (OPLS-DA), a regression extension of PCA, using SIMCA-P software (version 11.0; Umetrics) (36, 37). Samples were clustered using the Cluster program and visualized using Treeview.

A nonparametric test, the Wilcoxon rank-sum test, was used for two-sample tests of association between phases, and Mann–Whitney *U* test was used for multivariate comparisons between all time-course treatment groups (in R program). False discovery rates were calculated using the q-value conversion algorithm. Moreover, comparisons involving multiple RNA expression in time-series-treated groups used repeated-measures ANOVA to adjust for the multiple measures per group. Fold change was estimated using independent *t* tests. The threshold for significance was $P < 0.05$ or $P < 0.01$. The software used for other statistical analysis was SPSS 15.0 (SPSS) unless specifically mentioned otherwise.

ACKNOWLEDGMENTS. We thank Dr. Stephen J. Simpson (University of Sydney) for commenting on and revising the early version of the manuscript. We are grateful to Yi Zhang for help with data analysis of metabolomic data. This research was supported by National Basic Research Program of China 2012CB114102; National Natural Science Foundation of China Grants 30830022, 30970407, and 20835006; Creative Research Group Project 21021004; and Chinese Academy of Sciences Grant KSCX3-EW-N-05.

1. Pigliucci M (2001) *Phenotypic Plasticity: Beyond Nature and Nurture* (Johns Hopkins University Press, Baltimore).
2. West-Eberhard MJ (2003) *Developmental Plasticity and Evolution* (Oxford University Press, New York).
3. Whitman DW, Ananthakrishnan TN (2009) *Phenotypic Plasticity of Insects: Mechanisms and Consequences* (Science Publishers, Enfield, NH).
4. Garland T, Jr., Kelly SA (2006) Phenotypic plasticity and experimental evolution. *J Exp Biol* 209:2344–2361.
5. Gilbert SF, Epel D (2009) *Ecological Developmental Biology: Integrating Epigenetics, Medicine, and Evolution* (Sinauer Associates, Sunderland, MA).
6. Robinson GE, Fernald RD, Clayton DF (2008) Genes and social behavior. *Science* 322: 896–900.
7. Pener MP, Simpson SJ (2009) Locust phase polyphenism: An update. *Adv In Insect Phys* 36:1–272.
8. Anstey ML, Rogers SM, Ott SR, Burrows M, Simpson SJ (2009) Serotonin mediates behavioral gregarization underlying swarm formation in desert locusts. *Science* 323:627–630.
9. Ma Z, Guo W, Guo X, Wang X, Kang L (2011) Modulation of behavioral phase changes of the migratory locust by the catecholamine metabolic pathway. *Proc Natl Acad Sci USA* 108:3882–3887.
10. Guo W, et al. (2011) CSP and takeout genes modulate the switch between attraction and repulsion during behavioral phase change in the migratory locust. *PLoS Genet* 7: e1001291.
11. Griffin JL, Bollard ME (2004) Metabonomics: Its potential as a tool in toxicology for safety assessment and data integration. *Curr Drug Metab* 5:389–398.
12. Bino RJ, et al. (2004) Potential of metabolomics as a functional genomics tool. *Trends Plant Sci* 9:418–425.
13. Sreekumar A, et al. (2009) Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 457:910–914.
14. Al-Rubeai M, Fussenegger M (2007) *Systems Biology* (Springer, Heidelberg), pp 237–273.
15. Kang L, et al. (2004) The analysis of large-scale gene expression correlated to the phase changes of the migratory locust. *Proc Natl Acad Sci USA* 101:17611–17615.
16. Chen S, et al. (2010) De novo analysis of transcriptome dynamics in the migratory locust during the development of phase traits. *PLoS ONE* 5:e15633.
17. Wei Y, Chen S, Yang P, Ma Z, Kang L (2009) Characterization and comparative profiling of the small RNA transcriptomes in two phases of locust. *Genome Biol* 10:R6.
18. Steiber A, Kerner J, Hoppel CL (2004) Carnitine: A nutritional, biosynthetic, and functional perspective. *Mol Aspects Med* 25:455–473.
19. Whitfield CW, et al. (2006) Genomic dissection of behavioral maturation in the honey bee. *Proc Natl Acad Sci USA* 103:16068–16075.
20. Jones LL, McDonald DA, Borum PR (2010) Acylcarnitines: Role in brain. *Prog Lipid Res* 49:61–75.
21. Roessingh P, Simpson SJ, James S (1993) Analysis of phase-related changes in behaviour of desert locust nymphs. *Proc R Soc Lond B Biol Sci* 252:43–49.
22. Hagen TM, et al. (1998) Acetyl-L-carnitine fed to old rats partially restores mitochondrial function and ambulatory activity. *Proc Natl Acad Sci USA* 95:9562–9566.
23. Van Oudheusden LJ, Scholte HR (2002) Efficacy of carnitine in the treatment of children with attention-deficit hyperactivity disorder. *Prostaglandins Leukot Essent Fatty Acids* 67:33–38.
24. Adriani W, et al. (2004) Acetyl-L-carnitine reduces impulsive behaviour in adolescent rats. *Psychopharmacology (Berl)* 176:296–304.
25. Ristori C, et al. (2006) Acetyl-L-carnitine affects nonassociative learning processes in the leech Hirudo medicinalis. *Neuroscience* 142:931–939.
26. Scafidi S, et al. (2010) Metabolism of acetyl-L-carnitine for energy and neurotransmitter synthesis in the immature rat brain. *J Neurochem* 114:820–831.
27. Traina G, Federighi G, Brunelli M, Scuri R (2009) Cytoprotective effect of acetyl-L-carnitine evidenced by analysis of gene expression in the rat brain. *Mol Neurobiol* 39: 101–106.
28. Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of reproductive status in honeybees via DNA methylation. *Science* 319:1827–1830.
29. Pascale E, et al. (2003) Modulation of methylation in the FMR1 promoter region after long term treatment with L-carnitine and acetyl-L-carnitine. *J Med Genet* 40:e76.
30. Applebaum SW, Heifetz Y (1999) Density-dependent physiological phase in insects. *Annu Rev Entomol* 44:317–341.
31. Canavoso LE, Jouni ZE, Karnas KJ, Pennington JE, Wells MA (2001) Fat metabolism in insects. *Annu Rev Nutr* 21:23–46.
32. Hrassnigg N, Leonhard B, Crailsheim K (2003) Free amino acids in the haemolymph of honey bee queens (Apis mellifera L.). *Amino Acids* 24:205212.
33. Li Z, et al. (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab* 3:321–331.
34. Bangur CS, Howland JL, Katyare SS (1995) Thyroid hormone treatment alters phospholipid composition and membrane fluidity of rat brain mitochondria. *Biochem J* 305:29–32.
35. Sajbidor J (1997) Effect of some environmental factors on the content and composition of microbial membrane lipids. *Crit Rev Biotechnol* 17:87–103.
36. Bijlsma S, et al. (2006) Large-scale human metabolomics studies: A strategy for data (pre-) processing and validation. *Anal Chem* 78:567–574.
37. Wiklund S, et al. (2008) Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal Chem* 80:115–122.