Molecular Mechanisms of Phase Change in Locusts

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Abstract

Phase change in locusts is an ideal model for studying the genetic architectures and regulatory mechanisms associated with phenotypic plasticity. The recent development of genomic and metabolomic tools and resources has furthered our understanding of the molecular basis of phase change in locusts. Thousands of phase-related genes and metabolites have been highlighted using large-scale expressed sequence tags, microarrays, high-throughput transcriptomic sequences, or metabolomic approaches. However, only several key factors, including genes, metabolites, and pathways, have a critical role in phase transition in locusts. For example, *CSP* (chemosensory protein) and *takeout* genes, the dopamine pathway, protein kinase A, and carnitines were found to be involved in the regulation of behavioral phase change and gram-negative bacteria–binding proteins in prophylaxical disease resistance of gregarious locusts. Epigenetic mechanisms including small noncoding RNAs and DNA methylation have been implicated. We review these new advances in the molecular basis of phase change in locusts and present some challenges that need to be addressed.

INTRODUCTION

Polyphenism: the phenomenon in which two or more distinct phenotypes are produced by the same genotype

Phase change: the reversible transition process between solitarious and gregarious phases in locusts in response to changes in population density; also called phase transition

Locusts are the only grasshopper species (Orthoptera: Acrididae) that display extreme density-dependent phase polyphenisms and that can form dense migrating swarms under certain circumstances (65). Locusts have been among the most destructive agricultural pests throughout the course of human history (102). More than 100 countries, and the livelihoods of approximately 1 in 10 people, have been affected by locusts. After the 1950s, the frequency and duration of outbreaks of locust plagues have dramatically reduced with intensive worldwide monitoring, the application of pesticides, and the transformation of breeding and outbreak areas. However, eradication of locust plagues is still a major challenge. Locust outbreaks are attributed mainly to swarm formation and migration of high-density gregarious locusts. Among the locusts, two species stand out because of their historic and economic importance: *Locusta migratoria* and *Schistocerca gregaria*.

The term phase was first used by Sir Boris Petrovitch Uvarov (101) to describe the taxonomic revision of the genus *Locusta*. Locusts display density-dependent polyphenism in morphology, behavior, coloration, reproduction, development, physiology, immunity, and other aspects of ecology (67). Phase change in locusts is a continuous, cumulative, and easily reversible process and involves a suite of these behavioral and physiological traits in response to changes in population density. Because of its complexity, phase change in locusts has been regarded as an ideal model system of phenotypic plasticity, which can be broadly defined as the ability of one genotype to produce more than one phenotype when exposed to different environments (40, 88).

Early studies on phase change in locusts focused mostly on phase characteristics and extrinsically influenced factors. The emphasis then shifted toward the intrinsic factors, especially the endocrine and neuronal axes. These studies of locust phase change during this period not only shed light on the biological basis of locust outbreaks but also provided much insight into understanding other insect polyphenisms (4). Although Uvarov previously suggested that phase polymorphism might be based on differential phase-specific gene expression (100–102), research on the molecular biology of phase change in locusts was minimal until 1998 and lagged behind that of other insect groups, such as Diptera, Hymenoptera, and Lepidoptera (68). The advancements in genomic technologies are righting this imbalance. Many papers on the molecular regulation of phase change in locusts were published after the comprehensive review article by Pener & Simpson (67). Here, we highlight the recent advances in our understanding of the molecular basis behind locust phase change over the past 10 years and indicate gaps in our knowledge and unsolved questions.

PHASE POLYPHENISM AND ITS EVOLUTION IN LOCUSTS

A number of phase traits are clearly adaptive to changes in the social environment (86). Gregarious locusts exhibit more active and intensive migratory flight and lower fecundity than solitarious locusts. Other associated adaptive traits include development, longevity, lipid accumulation, and egg or offspring size. A reasonable explanation for this is that in gregarious locusts more energy is allocated to the construction of flight muscles and fuels, rather than reproductive investment. A recently reported example of an adaptive phase trait is cold hardiness of locust eggs. High cold hardiness in solitarious eggs could increase their overwintering success to maintain their population size in the coming year (106).

Behavioral and body color changes in animals are common phenomena in response to changes in population density. Recent phylogenetic analyses, theoretical models, and population genetics have advanced our understanding of evolutionary history (90–92), the possible mechanisms driving evolution (8, 31, 74, 113), and the adaptive signature of evolution (15–17, 33, 34). Phase-related characteristics are remarkably similar across different locust species within each monophyletic

group. Swarming locusts have evolved independently a number of times in a variety of different grasshopper lineages throughout the world, suggesting phase change is a convergent phenomenon (102). By examining the phylogeny of the subfamily Cyrtacanthacridinae, Song (90) suggested that individual reaction norms of locust phase polyphenism might be phylogenetically conserved and that certain phase traits have different evolutionary rates (92). A series of recent studies based on theoretical models have proposed important roles of interspecific, namely predation (74), and intraspecific, namely cannibalism (31), interactions during the evolution of density-dependent phase polyphenism. Chapuis et al. (17) compared genetic variation between historically nonoutbreaking and outbreaking populations of *L. migratoria* and found that the locusts from a historically outbreaking population expressed a greater degree of parentally inherited density-dependent phase changes and had higher gene flow. Molecular evidence from mitochondrial genomics shows that *L. migratoria* exhibits lower genetic differentiation, in which two lineages displaying phase polyphenism to a similar degree likely account for the maintenance of the south-north cleavage pattern (47). However, the genetic factors responsible for the evolution of locust phase polyphenism remain unknown. The rapid advent of genomics resources for the study of locusts will be of great value to future investigations (6, 19, 38).

EST: expressed sequence tag

GENETIC ANALYSES OF LOCUST PHASE CHANGE

The factors involved in the initiation of phase transition need not be the same as those controlling its subsequent maintenance, and differences exhibited between extreme phases may be the result rather than the cause of phase change. Therefore, the search for the biochemical and molecular markers of phase change in locusts is further complicated. In recent years, large-scale analyses using the techniques of functional genomics, such as microarrays, transcriptomics, metabolomics, differential displays, and proteomics, have probed the pattern of biochemical and molecular changes involved in phase change in locusts, but little information on their molecular function has been provided. These studies can provide us with insights into the genetic architectures that implement alternative phenotypes.

Genes and Transcriptomic Profiles

In one such analysis, Rahman et al. (73) investigated the phase-related gene expression by performing differential display reverse transcriptase polymerase chain reaction on the brain of *S. gregaria*. Eight differential expressed bands were found, but only one gregarious-specific band shares 80% sequence homology with the *Drosophila melanogaster* SPARC (secreted protein acidic and rich in cysteine) protein, an extracellular matrix-associated and Ca^{2+}-binding glycoprotein. Although SPARC affects mobility and morphology in nematode worms (84), any specific role in locust phase change remains to be determined.

Kang et al. (38) have contributed greatly to the exploration of the molecular genetics underlying phase transition. The authors have generated an expressed sequence tag (EST) library and a database that include 76,012 ESTs and 12,161 unigene clusters from the whole body, head, hindlegs, and midgut tissues of *L. migratoria* (49). Approximately 532 genes exhibit differentially expressed patterns between solitarious and gregarious locusts.

Most of these phase-related genes from the hindlegs and midgut were downregulated, whereas several gene classes from the head were impressively upregulated in the gregarious phase relative to solitarious insects. The upregulated genes from head tissues are involved mainly in peptidase-, receptor-, and oxygen-binding activities, as well as development, growth, external stimulus responses, and apoptosis. The JHPH protein superfamily [which consists of juvenile hormone

(JH)-binding protein, hexamerins, prophenoloxidase, and hemocyanins] was highly expressed in the heads of gregarious hoppers and in the hindlegs of solitarious hoppers. These results suggested that specific regulatory activities in nerve cells during phase change are most likely controlled through hormonal signals. Because approximately 70% of the 532 genes have no homologue in any sequenced insect genome, further studies, for example, microarray-based gene expression studies and the whole-genome sequence, should help determine those genes that initiate and/or maintain phase change and then attribute a function to those genes.

JH: juvenile hormone

Hsp: heat shock protein

The high-throughput, next-generation sequencing technology RNA-Seq has been available for de novo transcriptomes and has been widely used to explore gene structure and expression profiling of nonmodel organisms (12). To achieve high coverage of the gene content of *L. migratoria*, Chen et al. (19) generated the first de novo transcriptome (447 million reads, 21.5 Gb), which contains 72,977 sequences with a mean length of 1,170 bp. Using 12 RNA-Seq libraries of gregarious and solitarious locusts at various developmental stages, from eggs to adults, researchers investigated transcriptomic patterns and identified important genes and pathways involved in the development of and phase change in *L. migratoria*.

The number of differentially expressed genes (DEGs) between phases generally increases as locusts develop, with a sharp rise in the fourth instar. Similar differential transcriptomic profiles are observed in eggs, in the first and second instars, and between the fifth instar and the adult stage, but unique profiles are observed between the third and fourth instars. In particular, the phase change occurring in the fourth instar is very different from that in other developmental stages. This finding is consistent with the results from microarray experiments (48) and observations of the number of antennal sensilla (61), indicating that the switches in phase-related events occur during the fourth instar. Pathways involved in metabolism and biosynthesis are more active in solitarious locusts, whereas pathways associated with detecting and processing environmental information display higher activity in gregarious locusts. The most enriched pathway of the gregarious upregulated transcripts is the neural pathway involving neurotransmitter receptors, synthetases, transporters, and GPCRs. This locust transcriptome and relevant analyses provided useful resources and candidate genes to advance our understanding of locust biology, especially with regard to phase change mechanisms and pest management.

A parallel EST database from the locust *S. gregaria* was established by Badisco et al. (6). The *S. gregaria* EST database contains 34,672 ESTs assembled in 12,709 unique transcript sequences. Nearly 4,000 sequences were functionally annotated and gave many novel transcripts encoding neuronal signaling and signal transduction components. These authors also designed oligonucleotide microarrays based on the *S. gregaria* EST database and compared expression profiles in the CNS of long-term gregarious and solitarious adult desert locusts (7). A total of 214 DEGs were identified and classified under five informative GO (gene ontology) terms: multicellular organismal development, neurological system process, response to stress, generation of precursor metabolites and energy, and cellular macromolecule biosynthetic process. Solitarious locusts appear to be more strongly protected against the effects of aging by an upregulation of genes related to antioxidant systems, detoxification, and anabolic renewal. Gregarious desert locusts have a greater abundance of transcripts for proteins involved in sensory processing and nervous system development and plasticity. A comparison of these independent datasets from *S. gregaria* and *L. migratoria* shows that the major patterns of differential gene expression between solitarious and gregarious locusts are similar. This finding indicates that locust phase change has probably evolved within a framework of conserved molecular mechanisms, although locusts have undergone multiple events between different phylogenetic groups (90).

Heat shock proteins (Hsps) are a family of molecules produced by organisms in response to various types of stress such as extreme temperatures, starvation, and diseases. Wang et al. (107)

compared the expression profiles of Hsp genes in the two phases of *L. migratoria* throughout embryogenesis and nymph development and found that the transcript levels of all Hsp gene families were higher in gregarious nymphs than in solitarious nymphs. During the early stages of phase change, isolation of gregarious nymphs (for 32 h) resulted in pronounced reduction in *Hsp* expression more in line with solitarious values, whereas crowding of solitarious nymphs resulted in elevated expression of three Hsp genes, *Hsp20.5*, *Hsp20.6*, and *Hsp70*. In the Australian plague locust (*Chortoicetes terminifera*), crowding led to a two- to threefold increase in the expression of only two Hsp genes, *Hsp20.5* and *Hsp20.7*, unique to members of the Orthoptera (18). The protective role of Hsp genes may actually facilitate phenotypic plasticity by helping maintain the expression of an alternative phenotype associated with stress in insects (112).

HPLC: high-performance liquid chromatography

Proteins and Peptidomes

A few protein and peptidome studies have focused on locust phase change. Using 2D gel electrophoresis, Wedekind-Hirschberger et al. (109) generated hemolymph polypeptide maps from mature adult males of *S. gregaria*. Of 238 identified polypeptide spots, 20 differential spots between solitarious and gregarious locusts were found. Three spots were solitary specific, whereas 17 were crowd specific. Field catches of solitarious and gregarious *S. gregaria* showed the same phase-specific expression for these 20 polypeptide spots. Nine of the 17 gregarious-specific polypeptide spots were repressed 15 days after treatment with the JH analogue. However, a rough estimate of the molecular weight and the isoelectric point is the only data available for these proteins; the true identity remains unknown (109).

Clynen et al. (21) presented a novel approach by combining high-performance liquid chromatography (HPLC) techniques with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to determine phase-related differential peptidomes. Using this approach, Rahman et al. (71) carried out a peptidomic analysis of the hemolymph of solitarious and gregarious phases of *S. gregaria*. Two main proteins, a 6-kDa peptide and a serine protease inhibitor (SGPI-2) with differential expression between the two phases, were identified. The 6-kDa peptide, provisionally named PRP (phase-related peptide), is abundant in the hemolymph of crowd-reared adults and its concentration is 0.1 mM. Upon solitarization of gregarious locusts, its concentration decreases progressively from generation to generation. Despite subsequent intensive studies, no clear function for PRP has been found (67). When tested in a variety of assays, PRP does not act as a protease inhibitor, as an antibacterial agent, or as an antifungal agent. It has no effect on Yellow protein expression, coloration of the cuticle, or production of the pheromonal compound phenylacetonitrile (72). PRP is taken up by the eggs; the concentration of PRP is higher in gregarious females than in eggs from solitarious *S. gregaria* (70). Using immunocytochemistry and mass spectrometry approaches, Rahman et al. (72) further found that the strongest positive immunostaining was located in the follicle cells of the ovary and in the seminal vesicle tubes of the male accessory gland complex in *S. gregaria*. These results imply that PRP may somehow play a role as a maternal factor in the determination of the phase state of the offspring; however, more experiments are required to establish its function.

Although many proteins and peptides involved in locust phase change have been identified, the studies based on the proteomic approach largely suffer from the limited genome sequence information that is available and thus are slowly moving forward. Upon the release of the whole-genome sequence of *L. migratoria*, the proteomic approach will be a more powerful tool for studying locust phase change.

Metabolites and Metabolomics

omics: a field of study in genomics, proteomics, or metabolomics

In addition to transcriptomic and proteomic approaches, metabolomics is a powerful post-omics approach for studying in vivo metabolic profiles, which provide valuable information on biological processes and gene function (11). The main analytical tools used for metabolic profiles are nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), gas chromatography/mass spectrometry (GC/MS), HPLC, and optical spectroscopic techniques (58).

Lenz et al. (44) presented a metabolic profile in the hemolymph of gregarious and solitarious *S. gregaria* using high-resolution NMR spectroscopy. Over 20 endogenous compounds were qualified and comprise trehalose, a series of amino acids, organic acids, lipids, ethanol, and the polyamine putrescine. The amounts of putrescine, trehalose, and lipids are higher in solitarious nymphs, whereas those of acetate and ethanol are higher in gregarious nymphs.

Using HPLC and GC/MS, Wu et al. (118) performed a metabolomics analysis of the hemolymph of solitarious and gregarious fourth instar nymphs of *L. migratoria* and provided distinct phase-related metabolic profiles. A total of 319 metabolites, including multiple lipids, carbohydrates, amino acids, free carnitine, and their derivatives, display a significant difference in concentration between the two phases. Most of the differential amino acids, carbohydrates, carnitines, and several lipids [e.g., lysophosphatidylcholines and diacylglycerols (18:2)] are present in higher amounts in gregarious locusts, whereas diacylglycerols (18:3) and phosphatidylethanolamines are more abundant in solitarious locusts. These patterns might reflect a real difference in the metabolism of gregarious and solitarious locusts.

MOLECULAR REGULATION OF PHASE CHANGE IN LOCUSTS

Large-scale -omics studies have highlighted thousands of candidate genes or metabolites that may have functional roles in locust phase change. What are the key regulators? How do they contribute to the functioning of molecular pathways and how do they interact with other regulatory factors in this process? In recent years, accumulating evidence has provided detailed information on the regulatory mechanisms underlying major phase traits, including behavior, color, and disease resistance (**Figure 1**).

Regulation of Behavioral Phase Change

The behavior of solitarious and gregarious locusts differs substantially in order to adapt to changes in their social environment. Major phase-related behaviors include locomotor activity, aggregation, flight, and feeding (102). A behavioral assay developed by Roessingh et al. (77) to quantify the behavioral phase state has facilitated investigations into the stimuli and neurophysiological, ecological, and molecular mechanisms underlying locust phase change (87).

A rough framework of behavioral phase change at ecological and physiological levels based mainly on these studies on *S. gregaria* has been recently proposed (67). Once solitarious locusts are forced into a crowd, their behaviors rapidly switch to that of gregarious locusts (gregarization). The change is triggered by two distinct sensory pathways: (*a*) the combined sight and smell of other locusts and (*b*) direct contact with other locusts through repeated stimulation of specific hindleg mechanoreceptors (79, 85). Moreover, the time-course patterns of behavioral phase change vary among different locust species. *S. gregaria* exhibits rapid gregarization and slow solitarization (76); the time-courses of two processes in *C. terminifera* are similar (27), whereas in *L. migratoria* gregarization is remarkably slower than solitarization (29). Furthermore, the exact route map of phase change from the studies on *S. gregaria* cannot be applied to other locust species. For

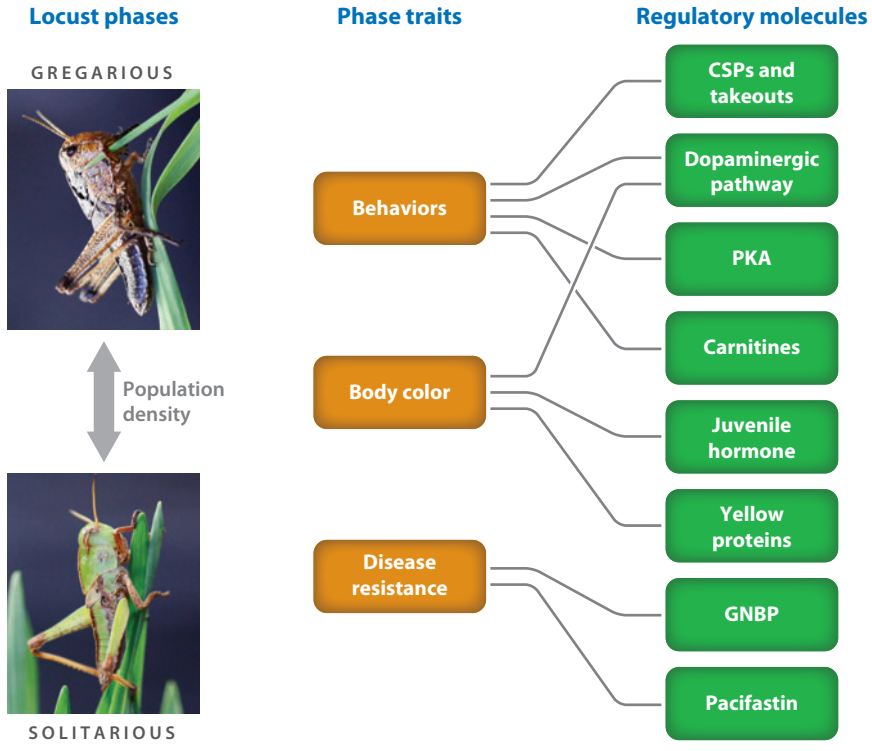

Figure 1

Overview of major uncovered molecular regulatory mechanisms underlying phase change in locusts. Locust phase change involves a series of life traits. Several genes or metabolites had been identified to have important roles in the differences of behavior, coloration, and immunity between solitarious and gregarious locusts. See text for details. Abbreviations: CSPs, chemosensory proteins; GNBP, gram-negative bacteria-binding protein; PKA, protein kinase A.

example, in *C. terminifera*, touching of the antennae rather than the hindlegs induces behavioral gregarization (50).

Extensive alterations of structures, circuits, and physiology of the central and peripheral nervous systems can shape the course of behavioral phase change. Various ultrastructure experiments have demonstrated that solitarious locusts have a generally higher number of sensilla on the antennae, frons (25), and outer side of the hind femora (79) than gregarious locusts do. Accordingly, solitarious locusts display more sensitive olfactory responses to aggregation pheromones and higher sensitivity to touch stimuli than gregarious locusts do (60, 79). Compared with the solitarious locust brain, the brain of *S. gregaria* gregarious locusts are 30% larger and have a larger central complex, yet they have smaller primary visual and olfactory neuropils (62). The properties and actions of individual neurons responsible for phase-related behavioral responses also differ remarkably between the two phases. For example, descending contralateral movement detectors (DCMDs) (51, 78), tritocerebral commissure giants (TCGs) (25), and slow extensor tibiae (SETi) (13) exhibit many phase-dependent differences. The change in the number of several potential neurotransmitters and/or neuromodulators in the CNS, such as octopamine, serotonin, dopamine, GABA, glutamate, acetylcholine, tyramine, and citrulline, may play an important role

in remodeling the CNS during phase change (26, 56, 80). In addition, hormones, such as JH, are involved in mediating phase-related behavior and relevant neuron actions (3, 35, 111).

CSP: chemosensory protein

The shift in aggregation behavior, e.g., attraction/repulsion behavior, between gregarious and solitarious locusts in response to changes in population density is crucial for the formation of large swarms (67). Using a high-density oligonucleotide array with 9,154 locust unigenes, Guo et al. (29) and Ma et al. (48) performed comparative gene expression profiling on head tissues of fourth instar gregarious and solitarious nymphs of *L. migratoria* that underwent the initiation stage of population density changes (within 64 h). They found that locusts exhibit rapid and complicated transcriptomic changes [1,444 differentially expressed genes (DEGs), 15% of total detected genes]. Gene enrichment analysis during both time-course processes highlighted the insect pheromone-binding family A10/OS-D. These genes have antenna-rich expression levels and differential expression between solitarious and gregarious fourth instar nymphs. The gene expression levels also exhibit a strong correlation with the time-course of attraction/repulsion behavioral changes in response to changes in population density.

After *CSP* (chemosensory protein) gene expression was silenced by RNAi, gregarious nymphs lost their attraction behavior and exhibited repellent behavior in response to other individuals. For *takeout* genes, knockdown of gene expression induced a decrease of repellent behavior in solitarious locusts. However, RNAi treatments of these genes did not affect other behavioral parameters relating to locomotor activity. Therefore, the results indicated that *CSP* and *takeout* genes initiate the phase change in the locusts in the short term. The influence of these genes on aggregation behavior might exert their effect through mediating changes in olfactory sensitivity. In addition to *CSP* and *takeout* genes, several other gene families, for example, odorant-binding proteins and olfactory receptors, are involved in insect chemoreception in peripheral olfactory organs (43); however, their functional roles in locust behavioral phase change remain unknown. Further, little is known about how neuronal plasticity in the CNS contributes to shifts in aggregation behavior and how peripheral systems interact with the CNS.

In the CNS, the dopamine pathway is involved in behavioral phase change in *L. migratoria* (48). Ma et al. (48) performed comparative gene expression profiling on head tissues of gregarious and solitarious nymphs at each stadium (1–5 instar) of *L. migratoria*. As the nymphs develop, the number of DEGs increases from the first to the fourth stadium but decreases significantly during the fifth stadium. This development-dependent pattern implies that the fourth stadium is a key developmental stage, when the largest number of DEGs exists between the two phases. DEGs in the fourth stadium are classified into 21 functional categories involved mainly in general metabolism, molecular transport, production of cuticular protein, and chemosensory transduction. Using GO enrichment analysis, these authors found many genes associated with the catecholamine metabolic pathway.

In the dopaminergic pathway, these pivotal genes, including phenylalanine hydroxylase (*henna*), tyrosine hydroxylase (*pale*), *ebony*, *RD1*, and vesicular amine transporter 1 (*vat1*), have higher expression levels in gregarious locusts and exhibit significant differential expression during the time-course of phase change, consistent with changes in dopamine concentration. The knockdown of these genes by RNAi induced in gregarious locusts a behavioral shift toward the solitarious phase. The application of an antagonist of *pale*, α-methyl-DL-tyrosine methyl ester hydrochloride (AMPT), to third instar gregarious nymphs also induced the progress of gregarization, whereas the direct injection of dopamine or its receptor agonist, apomorphine, into solitarious nymphs induced gregarious behavior. These results provided clear evidence that dopamine acts as a key neurotransmitter in the regulation of behavioral phase change in *L. migratoria* (48). Many studies have suggested an important relationship between dopamine and behavior in various animal species (83).

In the locust *S. gregaria*, the amount of serotonin in the thoracic ganglia was positively correlated with the extent of gregarious behavior induced by different periods of crowding. A series of pharmacological and behavioral experiments demonstrated that serotonin plays a key role in inducing initial behavioral gregarization (2, 80). However, serotonin is not responsible for maintaining gregarious behavior because its amount in long-term gregarious locusts is less than half that in long-term solitarious locusts (80). In *L. migratoria*, the injection of serotonin can also slightly initiate gregarious behavior, but serotonin when accompanying crowding treatment induced more solitarious-like behavior than did serotonin injection alone (48). Significant differences in serotonin levels were not found in brain tissues between the two phases of *L. migratoria*. A recent report by Tanaka & Nishide (97) measured attraction/avoidance behavior in *S. gregaria* after single and multiple injections of serotonin at different concentrations. Serotonin had only a short-term effect on the level of some locomotor activities and was not involved in the control of gregarious behavior (97). In addition, it is not clear how the neurotransmitter influences this unique behavior, because a binary logistic regression model used in these studies for the behavioral assay focused mostly on only one behavioral parameter representing an overall phase state. Obviously, behavioral phase change might involve alternative regulatory mechanisms in different locust species. Therefore, these studies demonstrate that CNS regulatory mechanisms governing initiation and maintenance of phase change are species specific and involve the interactions between these neurotransmitters.

Aminergic: activated by, characteristic of, or secreting one of the biogenic amines

Given the key roles of aminergic signaling, what are the downstream pathways involved in the establishment of long-term memory? Ott et al. (63) investigated the role of two protein kinases in the phase change in *S. gregaria*: the *foraging* gene product, which is a cGMP-dependent protein kinase (PKG), and cAMP-dependent protein kinase A (PKA). Through use of pharmacological and RNAi intervention, these authors have demonstrated that PKA, not PKG, has a critical role in modulating the propensity of locusts to acquire and express gregarious behavior. When injected with the PKA inhibitor KT5720 or double-stranded RNAs of the PKA catalytic subunit C1 gene, solitarious locusts behaved less gregariously after 1 h of forced crowding, whereas RNAi against the inhibitory R1 subunit promoted more extensive gregarization. Adenylyl cyclase/PKA signaling has been proposed to have a central role in diverse forms of plasticity, including reflex sensitization, contextual fear conditioning, appetitive and aversive conditioning, and addiction (1, 22, 53, 57, 89). Unfortunately, although a correlation between serotonin and PKA was hypothesized, direct evidence was not provided. Additionally, although no role for PKG in mediating short-term locust phase change has been demonstrated, its activity is higher in the brain of gregarious desert locusts, (45). This finding suggests that PKG plays possible roles in other phase traits such as foraging and nutritional regulation.

In parallel, other downstream signaling pathways involving amino neurotransimitters may also contribute to locust phase change. For example, two octopamine receptors, sgOctαR and sgOctβR, have higher transcript levels in the CNS of long-term gregarious desert locusts, and *sgOctβR* expression levels increase during the first four hours of gregarization (105). Multiple GPCR genes involved in signal transduction cascades also are differentially expressed between solitarious and gregarious phases of *L. migratoria* (19).

Metabolomics is a branch of systems biology in which metabolites reflect the phenotype directly and are biologically informative, compared with genomics and proteomics. Metabolomics analysis of the hemolymph from solitarious and gregarious locusts treated by time-course crowding and isolation has highlighted the importance of lipid metabolism in locust phase change (118). This study identified carnitine and its acyl derivatives, which are involved in the lipid β-oxidation process, 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 corresponding changes of metabolic profiles. In contrast, solitarious locusts injected with exogenous acetylcarnitine displayed gregarious behavior during gregarization. These results suggest that carnitines mediate locust phase transition possibly by modulating lipid metabolism and influencing the nervous system of the locusts.

DNA methylation: a biochemical process involving the addition of a methyl group to the cytosine or adenine DNA nucleotides

Some signaling molecules associated with energy metabolism, including hormones, neuropeptides, and intermetabolites, are also part of regulatory networks controlling locust behavioral phase change. The high locomotor activity and intensive flight capacity of gregarious locusts require a higher metabolic rate and more lipid storage compared with solitarious locusts (4). Adipokinetic hormone (AKH) is produced in the intrinsic cells of the glandular lobes of the central complex of locusts and induces hyperlipemia, as lipids are the main fuel for flight (103). The adipokinetic response is remarkably higher in gregarious locusts than in solitarious locusts (5). Octopamine, JH, and insulin-like peptides are involved in the AKH pathway and can induce an adipokinetic response (9, 67). Recent work has suggested that carnitines play a direct role in behavioral phase change as signaling molecules, acting as intermetabolites in cell energy metabolism (118). Acetylcarnitine can promote the biosynthesis and release of several neurotransmitters, such as acetylcholine, GABA, melatonin, and dopamine (82), or influence phenotypic changes through epigenetic modulation, such as histone acetylation and DNA methylation (64).

Molecular Regulation of Body Coloration

The change in body color during locust phase transition is remarkable. Gregarious locusts display a contrasting pattern of black and orange, with little to no variation in pattern among individuals in the same crowd. Solitarious locusts are cryptic and range from green to brown depending on external environmental factors such as humidity and temperature (65, 95, 102). The mechanism(s) by which endocrine controls phase color polyphenism in *L. migratoria* and *S. gregaria* has been studied in great detail. JH is a key regulator of the induction of green body color (3). Implantation of extra corpora allata, the glands that produce JH, or injection of synthetic JH or JH analogs, stimulated gregarious *L. migratoria* nymphs to turn green. However, green solitarious nymphs lost their green color after being allatectomized with precocene III but did not develop the body coloration of gregarious nymphs (66). By establishing an albino strain of *L. migratoria*, Tawfik et al. (98) identified a dark-color-inducing neuropeptide, [His^7]-corazonin, which consists of 11 amino acids, from the corp cardiaca of *S. gregaria* and *L. migratoria*. However, [His^7]-corazonin does not induce the bright yellow background body color characteristic of last instar gregarious nymphs of *S. gregaria* (94). In addition to the interaction between JH and [His^7]-corazonin, the control factors involved in the regulation of yellow coloration are still unknown.

Ma et al. (48) have proposed that the dopamine pathway might be involved in the regulation of color phase polyphenism. The silencing of *pale* gene expression led to a lightening of the pronotum of gregarious nymphs, whereas for solitarious nymphs the pronotum darkened (48). Melanin deposition induced by *pale* and *ebony* has been demonstrated in other insect species (117) and occurs by mechanisms different from those of corazonin. Future studies are needed to determine how the interaction between these factors relates to color polyphenism.

The cuticle of gregarious adult males of *S. gregaria* turns bright yellow around day 10, coinciding with full sexual maturity (59). The beta-carotene-binding Yellow protein, produced by the epidermal cells integrated into the cuticle, is responsible for this coloration (119). It is 250 amino acids long and its chromophore-free molecular mass is 25,682 Da. The protein is devoid of cysteine and has low levels of methionine and tryptophan. In crowd-reared adult males the transcription of Yellow protein gene began on day 5 and reached a maximum on day 12. JH

and insulin have positive effects on inducing mRNA transcription of Yellow protein, whereas corazonin, ecdysone, and 20-hydroxyecdysone do not (10).

Molecular Regulation of Disease Resistance

DDP: density-dependent prophylaxis hypothesis

GNBP: gram-negative bacteria-binding protein

High-density populations have been linked to increased rates of parasitism and disease (41). As a result, organisms are predicted to increase their investment in disease resistance mechanisms (immunological, behavioral, chemical, and/or physical) as population density increases, and this might lead to a positive relationship between population density and per capita parasite and pathogen resistance. This phenomenon can be explained by the density-dependent prophylaxis (DDP) hypothesis (114, 115). It relies on three important assumptions: (*a*) Parasite transmission is generally positively density dependent, (*b*) potential hosts can alter their phenotype in response to cues associated with population density, and (*c*) parasite defense is costly. DDP is likely to be particularly prevalent in species exhibiting density-dependent phase polyphenism. Gregarious adult *S. gregaria* or *L. migratoria* survive topical application of spores of the fungal pathogen *Metarhizium anisopliae* var. *acridum* better than solitarious adults do (107a, 116). Gregarious locusts had higher antibacterial activity and somewhat higher hemocyte counts, but there was no difference between the phases in phenoloxidase activity, encapsulation, or behavioral fever responses (54, 116). However, a recent field study reported a negative correlation between total hemocyte counts and population densities for *C. terminifera* (55).

Using RNA-Seq, Wang et al. (107a) found that *M. anisopliae* induced the expression of at least twice as many genes in the solitarious phase than in the gregarious phase of *L. migratoria* and that the transcription of immune molecules such as pattern recognition proteins, protease inhibitors, and antioxidation proteins was increased in prophylactic immunity of gregarious locusts. Three gram-negative bacteria-binding protein (GNBP) orthologous members (i.e., GNBP1, GNBP2, and GNBP3) were identified from a de novo transcriptome database of *L. migratoria*. GNBP3 was susceptible to proteolysis, whereas GNBP1, induced by *M. anisopliae* infection, resisted proteolysis. Silencing of *gnbp3* by RNAi significantly shortened the life span of gregarious locusts but not solitarious locusts. By contrast, *gnbp1* silencing did not affect the life span of both gregarious and solitarious locusts after *M. anisopliae* infection. In insects, four pathways [Toll, immunodeficiency (IMD), c-Jun N-terminal kinase (JNK), and Janus kinase/signal transducers and activators of transcription (JAK/STAT)] are involved in the recognition of an invasive microbe that leads to signal production (93). GNBPs are upstream of the Toll pathway and might be responsible for the prophylactic immunity of crowded animals. These results suggest that the activation of upstream rather than downstream modulators of immune cascades is involved in the phenotypic resistance of gregarious locusts to fungal infection, preferring to quarantine rather than eliminate pathogens to conserve energy.

The pacifastins, a family of serine protease inhibitors found in the hemolymph and CNS of arthropods, might contribute to the prophylactic immunity of gregarious locusts. Pacifastins play a role in the innate immune system, inhibiting the prophenoloxidase (PO)-activating system or preventing fungal penetration (24, 39). To date, eight pacifastin-like precursors encoding 22 different peptides have been identified in locusts (104). Rahman et al. (71) reported that levels of the pacifastin SGPI-2 in the hemolymph were higher in solitary-reared than in crowd-reared *S. gregaria* adults, with concentrations rising across four progressive generations of solitary-reared locusts. When the *L. migratoria* EST database was searched, *L. migratoria* pacifastin-like precursor (LMPP) mRNAs were found to be widespread, occurring in the midgut, hindleg, and head tissues (20, 38). Kang et al. (38) found from their EST library that the unigene cluster coding for LMPP-2 was expressed at higher levels in solitarious than in gregarious *L. migratoria* nymphs. Franssens

et al. (24) explored the effect of SGPIs on PO activation in hemolymph of 10-day-old crowd-reared adult *S. gregaria*. Neither SGPI-1 nor SGPI-2 inhibited the induction of PO activity in response to challenge by the immune elicitor laminarin (a glucan from brown algae). However, increased levels of transcripts of two pacifastin-like peptide precursors (SGPP-1 and SGPP-2), which encode SGPI-1, SGPI-2, and SGPI-3, were found in the fat body of locusts that were injected 20 h previously with laminarin.

Epigenetics: the study of changes in gene expression or cellular phenotype, caused by mechanisms other than changes in the underlying DNA sequence

In addition to broadening the taxonomic scope of these studies, the mechanisms underpinning DDP need to be examined at a much finer scale. There is some evidence relating to the regulation of immunological mechanisms in response to changes in population density. However, so far these investigations have been done at a fairly crude level, and there is a need for refinement and examination of the DDP responses at the cellular and molecular genetic levels. There is also a need to determine precisely how gross changes trigger immunological and other resistance mechanisms.

EPIGENETICS OF PHASE CHANGE

An intriguing phenomenon in locust phase change is transgenerational accumulation of a phase state (36, 52, 65, 67, 81, 96, 102). A number of phase traits (e.g., morphometry, mass and body size, hatchling behavior, coloration, ovariole number, development time, disease resistance, and cold hardiness) not only change within an individual's lifetime but also are cumulative across several generations through a maternal effect (28, 54, 67, 106). In addition, the eggs produced by solitarious and gregarious locusts display different gene expression profiles and cold hardiness, and genes involved in the dopamine pathway were upregulated in the eggs of gregarious locusts (106). Clearly, this phenomenon belongs to the field of epigenetics, and it is very challenging for us to uncover the mechanisms underlying transgenerational accumulation of these phase traits. Although some controversies in this field still exist between different laboratories (67, 96), epigenetic mechanisms, including noncoding RNA, DNA methylation, and histone acetylation, among others, have been proposed to determine the dramatically developmental flexibility controlled by population density without standard genetic changes (69).

Small noncoding RNAs play an important role in posttranscriptional gene expression regulation during development and other biological processes. Small RNAs include several kinds of short noncoding RNAs such as microRNA (miRNA), small interfering RNA (siRNA), and Piwi-associated RNA (piRNA). Wei et al. (110) used high-throughput sequencing to characterize the small RNA transcriptome and compare small RNA expression patterns of the two phases of *L. migratoria*. Fifty conserved miRNA families and 185 potentially locust-specific miRNA families were identified. Longer small RNAs including endo-siRNA and piRNA-like small RNA were found to be more abundant in the solitarious phase (108).

By developing a new algorithm based on a k-mer scheme, Zhang et al. (120) further predicted 87,536 locust piRNAs and found 12,386 gregarious-specific piRNAs and 69,151 solitarious-specific piRNAs. Transposable elements are proposed to be important sources of small RNAs such as piRNAs and endogenous siRNAs in both germ line and somatic cells (99). On the basis of a de novo assembly from deep-sequencing RNA-Seq data, 105 retroelements in the locust transcriptome were identified and differential expression profiles of these retroelements were determined in solitarious and gregarious locusts at the fifth instar and adult stage (37). A type of transposable element, I element, exhibits phase-related differential expression patterns in central and peripheral nervous tissues, such as the brain, antenna, and labial palps, in *L. migratoria* (30). These data supported the functional roles of small RNA in locust phase change. Further experiments are needed to elucidate the detailed underlying mechanisms.

DNA methylation has recently been linked to phenotypic plasticity in eusocial insects through the regulation of alternative splicing or gene expression (32, 42, 46). DNA methylation involves the addition of a methyl group, typically to the C_5 of a cytosine-pyrimidine ring that occurs next to a guanine nucleotide, a so-called CpG site. Unlike in plants and vertebrates, DNA methylation in insect genomes occurs at very low levels and most CpG methylation is specifically confined to gene bodies or transcriptional units rather than nongenic regions (46). During silencing of DNA methyltransferase 3 (Dnmt3) gene expression, young honey bee worker larvae that were not fed royal jelly were still able to develop into queen-like adults with developed ovaries (42). A liquid chromatography–mass spectrometry analysis showed that 1.3–1.9% of cytidines are methylated in *S. gregaria* (14). By searching locust EST databases of *S. gregaria* and *L. migratoria*, two DNA methyltransferase genes, *Dnmt1* and *Dnmt2*, but not *Dnmt3*, were identified (14, 75). Phase-specific expression of *Dnmt1* and *Dnmt2* were also found in certain tissues of *S. gregaria* (14). By using genome-scale bisulfite sequencing, Falckenhayn et al. (23) showed that overall methylation levels are higher in locusts than in other invertebrates and a significant fraction of locust transposons were methylated.

However, these conclusions, which are based on EST data rather than whole-genome information, are largely limited. For example, we have never excluded the possibility of *Dnmt3* existing in locust genomes because of their higher methylation levels. Future studies are required to characterize the functional roles of DNA methylation in locust phase change, especially with the availability of the complete locust genome sequence. In addition, whether other epigenetic mechanisms, such as histone acetylation, protein phosphorylation, and alternative splicing, are also involved in locust phase change has not yet been touched on until now.

CONCLUDING REMARKS AND PERSPECTIVES

Substantial progress has been made in better understanding the molecular basis and regulation of phase change in locusts over the past decade. A picture of how the actions of genes and some small molecules regulate phase change in response to changes in population density is beginning to emerge. Genome-wide analyses have shown that phase change in locusts is accompanied by widespread changes in gene expression and involves multiple conserved molecular pathways among different species. Some explored genes, molecules, and pathways involved in phase change, including CSP genes, *takeout*, Hsp genes, hexamerin genes, GNBP genes, the dopamine pathway, corazonin, PKA, and carnitines, have furthered our insight into the regulation of unique phase traits, such as behavior, coloration, and disease resistance, among others. Additionally, epigenetic regulatory mechanisms such as small RNAs and DNA methylation are increasingly being implicated in phase change in locusts. These findings highlight the importance of locusts as a model system for studying the comprehensive molecular mechanisms of phenotypic plasticity.

Revealing the molecular regulatory mechanisms of locust phase change presents a formidable intellectual challenge for several reasons. First, large-scale gene expression analyses have highlighted a number of candidates; however, it is a challenge to establish cause-and-effect roles for gene expression in phase-related genes. Second, the molecular mechanisms are invariably complicated. Many levels of physiological and molecular regulation lie between the genome and phase-related traits, including transcription, posttranscriptional and translational events, and epigenetic change. The interplay between these mechanisms remains to be discovered. The third challenge is determining how locust phase polyphenism evolves. Locusts do not belong to a monophyletic taxon and have evolved multiple times within subfamilies. Moreover, not all forms of phase polyphenism are alike, with some species exhibiting the full suite of morphological and physiological changes and others exhibiting just some of the differences. Whether locust phase polyphenism

evolves independently through unique molecular mechanisms or similar pathways remains ripe for investigation.

Despite these challenges, genetic and genomic approaches hold great promise for elucidating the molecular basis of locust phase change. We have a strong and growing arsenal of powerful technologies and increasingly sophisticated methods of system biology to profile changes during phase transition. The use of RNAi or transcription activator-like effector nuclease (TELEN), approaches already proving to be effective, will intensify work that establishes causal relationships between genes and phase change. When the whole genome sequence of locusts becomes available, the time to combine these tools to elucidate molecular mechanisms of phase change will be under way.

SUMMARY POINTS

1. Locusts are among the most important agricultural pests and have long served as a model for insect physiology, neuroscience, and behavior. Phase change in locusts lies at the heart of locust swarming and outbreaks.
2. Locust phase change is a reversible, cumulative, transgenerational process in response to changes in population density and involves a suite of phenotypes. Therefore, locust phase change is an extreme example of phenotypic plasticity.
3. Large-scale genomic and metabolomic analyses have demonstrated that thousands of genes or metabolites are involved in locust phase change and that there are conserved molecular pathways among different locust species.
4. Several genes or metabolites have important roles in the regulation of locust phase change. For example, CSP genes, *takeout*, the dopamine pathway, PKA, and carnitines help regulate behavioral change; dopamine and corazonin are important for coloration; and GNBPs promote phenotypic resistance of gregarious locusts to fungal infection.
5. Epigenetic mechanisms, such as DNA methylation, histone acetylation, and noncoding RNAs, have been implicated in the regulation of phase change in locusts, but their functional roles have not yet been elucidated.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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