REVIEW ARTICLE

Physiological mechanism of social insects behavior: nerve conduction, endocrine regulation, and genetic basis

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Abstract The complex behavior of social insects has been one of the focus in the field of animal behavior research. By means of caste differentiation and division of labor, social insects have occupied a favorable position in the evolution of nature. Meanwhile, the characteristics of the short reproductive cycle, high production rate, and relative simple physiological mechanisms compare to vertebrate make them a model for studying social behaviors of animal. However, the physiological and genetic mechanisms of social behavior have not been fully elucidated. The illumination of mechanisms underneath social behavior would not only improve the efficiency of human society to use the beneficial insects for the development of economic, society and the health of ecology, but also provide basic theory for the exploition of newly behavioral regulator for the management of forestry pests such as *Odontotermes formosanus*, *Coptotermes formosanus* and *Solenopsis invicta*, etc. Therefore, this article summarizes the behaviors of social insects, their nerve conduction, endocrine regulation, and genetic basis, with an aim of providing reference for related research.

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Introduction

Social insects have three remarkable characteristics including cooperation with brood care, non-reproductive individuals serving for queens and overlapped generations that children can help their parents to feed offsprings (Wilson 1971). Eusocial insects refer to the insects that contain all the three features of social insect, including all the species of Formicidae, a part species of Apoidea, and all species of Isoptera. Therefore, the social insect in this paper refers to the Eusocial insect.

Social behavior refers to a series of behaviors that individuals and their partners interact with each other for their own survival and reproduction, including foraging, migrating, gathering, mating, attacking, defending natural enemies, tending of offspring, etc. (Robinson et al. 2005). Social Insects have been valuable models to understand the behaviors of complex animal societies, principally from ethological and ecological perspectives (Hualong 2016). Although there are many kinds of social behaviors, the ecological meaning of these behaviors is to achieve the purpose of surviving and reproducing offspring (Robinson et al. 2008). All systems of social behavior share the following features:

1) They are acutely sensitive and responsive to social and environmental information; 2) The information is transduced within individual by primary sensory system; 3) The transduced neural signals are processed and integrated in specific circuits of the brain via conserved signal transduction and neuromodulatory systems; and 4) The resulting internal state of the animal ultimately controls behavioral activity. But the physiological and molecular mechanisms of these social behaviors have not been fully elucidated. With the progress of neurobiology technology (such as electrophysiological record, optical imaging, and etc.) (Mysore et al. 2009 and Mizunami et al. 2010), and the high-speed development of genomic sequencing, comparative genomics and epigenetics, as well as the maturation of RNAi and CRISPR-Cas9(Chen et al. 2016), it provides a good technical support for studying the internal mechanism of the complex social behaviors of social insects. Therefore, this paper summarizes the latest research progress of the behavioral patterns of social insects and the physiological and biochemical mechanisms of social behavior. The purpose is to provide a basis for further analysis of the mechanism of social behavior in social insects, and to design new behavioral regulation management strategies of forest pests such as *Odontotermes formosanus, Coptotermes formosanus* and *Solenopsis invicta*.

The individual development and life history of social insects

Social insects live in the community, the nest is highly structured, the division of individuals is clear and detailed, and the diversity of this behavior phenotype is non-genetic, that is, the individuals with the same genetic background are affected by different environmental signals during the development process leading to the final formation of different castes (like queen and workers). The extreme reproductive division of labor characterizes the social insects. The queen specializes in reproduction while the workers participate in co-operative tasks such as building the nest, collecting food, rearing the young and defending the colony (Wilson 1971). Social insects are haploidiploidy reproduction: the male from the unfertilized eggs developed, are haploid; the female, including the queen and worker, from the zygote developed, are diploid (Wilson 1971). The behavior of grown workers is age-oriented, which means the age polyethism. For example, the workers, emergency after 2-3 weeks, are mainly as a nurse to take care and feeding the generations and the queen. In the next 1-3 weeks, most of the workers generally become the foragers, engaged in foraging, and defending the nest (Fig. 1) (Qiu 2016; LeBoeuf et al. 2016; Robinson and Vargo 1997).

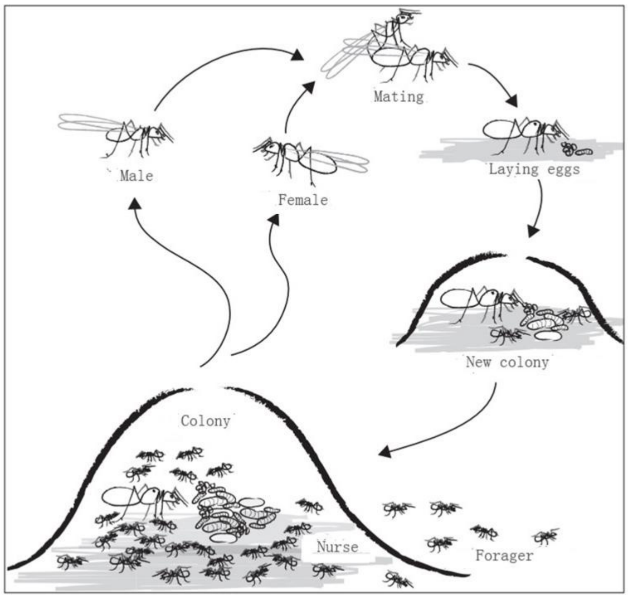

Fig. 1 The life cycle of *Solenopsis invicta* (LeBoeuf et al. 2016)

Pheromone communication mechanism of social insects

Social insects live in groups, with extremely tight organization. A set of all individuals within the same nest is called “superorganism”, where the individual is mainly communicated by a volatile or semi-volatile pheromone (Karlson 1959). Pheromone, also named ectohormone, is a kind of chemical substances secreted by an animal to the same species, e.g., a release of certain behavior or a determination of physiologic development (Karlson 1959). Analogous to the importance of hormones in controlling organism homoeostasis, pheromone play a major role of coordinating the association of unitary organisms into a coherent social unit or so-called superorganism (Alaux et al. 2010). Pheromones are usually divided into two categories of releaser pheromones and primer pheromone. Releaser pheromones have the releaser effect that causes the nestmates or the opposite sex to make an immediate behavior change. For example, the alarm pheromone releases an alarm behavior when the colony is attacked, inducing the nestmates to carry on the collective defense. The trail pheromone can mark their own route and food places, guiding the nestmates to find prey in time. The funeral pheromone on the surface of corpse induces the nestmates to carry the corpse outside the nest. The brood pheromone induces the nurse workers to feed the larvae in the colony. The cuticular hydrocarbons are used to identify nestmate.

The primer pheromone has the primer effect that gradually change the physical development of the receivers and result in the alteration of behavior in a long-term. For example, queen's mandibular gland pheromone (QMP) can inhibit the worker's ovarian development to prevent nestmate from oviposition (Wilson and Bossert 1963). Pheromones is usually secreted by the exocrine glands of insects, and the distribution of exocrine glands in social insects is shown in Fig. 2. Most of the social information exchanged among individuals in the nest of social insects is produced by exocrine glands, which can induce the behavioral response or physiological changes of the nestmates through individual contact or diffusion of airflow (Billen and Morgan 1998).

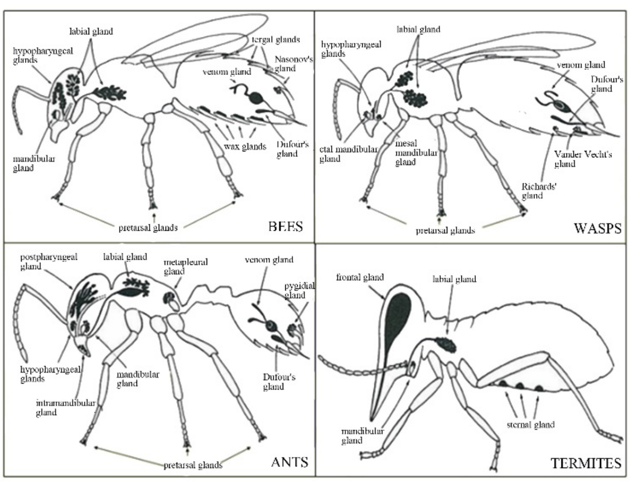

Fig.2 Schematical profile drawings showing the commonly found exocrine glands in wasps, bees, ants, and termites. Glands with a pheromonal function are indicated with capital lettering (Billen and Morgan 1998).

Neuro-regulation of insect's social behavior

Social insects' high degree of structuring relies heavily on the precise chemical information communication system. Only external semi-chemicals are converted into electrophysiological signals is it able to cause individuals' specific behaviors (Mizunami et al. 2010). Social insects' pheromones are conducted to the brain via olfactory organs and are deployed by the brain. This process is as follows: odorant molecules enter the olfactory receptor on the tentacle, and odorant binding proteins (OBPs) in the lymph of the olfactory receptor convey the odor to olfactory receptor neurons (ORNs) which project the odor to the primary nerve fiber web-olfactory lob (AL) in the insect's brain. Insects' AL is like the olfactory bulb of vertebrates and they process odor in a similar way. Dendrites of ORNs of the same type gather in some single glomeruli in the AL. Glomeruli is the functional unit of the AL and is projected to higher-level brain center which is the mushroom body in the forebrain and the lateral horn(LH) in the lateral forebrain via axons of projection neurons at the periphery of the AL.

Formic acid and n-undecane are alarm pheromones of *Camponotus obscuripes*. Mizunami et al. (2010) studied the signal transmission path of formic acid and n-undecane in the brain. First, odorant molecules reach the AL via ORNs on tentacles, signals are then projected to the mushroom body in the forebrain and the lateral horn via projection neurons. It's found that projection neurons sensitive to alarm pheromones are intensive in certain areas of the lateral horn, while other neurons processing ordinary odors are not, which indicates that these neurons are related to alarm information. And neurons at the lateral horn exist only in the pre-motor area, which may be related to aggressive behaviors.

Social insects center on nests and can have accurate navigation between nests and food place. Brockmann et al. (2007) studied the neural projection path of information conveyed by bees' waggle dance, he found that sensory neurons in bees' ommatidium region can sense the polarized light information in the ultraviolet region, and project the information to the medulla at the end of the brain's back. Worker bees in the honeycomb can convert sun compass information into gravity sensing information through mechanical sensory neurons of the nuchal hair plate and project the information into the suboesophageal ganglion, sense the direction of and distance from food with sensory hairs at tentacle nodes and according to the direction to which dancing bees swing their abdomens and the frequency of fluttering wings, convert them into electrophysiological signals, and project the signals to the notopodium of the midbrain, the suboesophageal ganglion and the forebrain.

The endocrine regulation of social behavior

Social behaviors are regulated by endocrine hormones that widely distributed in the lymph (Sasaki et al. 2012). Hormones are chemical messengers of multi-cellular organisms, which can transport to different organ via hemolymph circulation to facilitate communication among various cells. Juvenile hormones and molting hormones are endocrine hormones regulating insects' behavior and ovary development (Doleazl et al. 2012). In social insects, juvenile hormones are deemed to relate to behaviors of worker ants or worker bees, while molting hormones are deemed to relate to ovary development (Dong et al. 2009). Well-developed ovaries are the main source of *Bombus terrestris*' molting hormones (Bloch et al. 2000). Juvenile hormones participate in the division of labor of *A. mellifera* and worker bees of *B. terrestris* (Robinson and Vargo 1997; Bloch et al. 2000) and the division of labor behavior of worker ants of *Streblognathus peetersi* (Brent et al.2006). In bees, field bees have more juvenile hormones than nurse bees in the honeycomb. After nurse bees are processed with juvenile hormones, they gather nectar early (Amdam et al. 2006). This phenomenon is also found in ants (Aonuma and Watanabe 2012). The content of molting hormones in nurse ants is high; nurse ants have well-developed ovaries and can produce alimentary eggs. The content of molting hormones in foraging ants is low; their ovaries degenerate and cannot lay eggs (Robinson and Vargo 1997).

Biogenic amines are a class of nonpeptide neurohormones present in the nervous system of the invertebrates' brain, including histamine, serotonin, octopamine, tyramine and dopamine, which function as neurotransmitters or neuromodulators to regulate insect energy metabolism, learning and memory, muscle contraction and other physiological or behavioral processes (Aonuma and Watanabe 2012; Baumann et al. 2009). Dopamine promotes mating behavior of social insects and the development of reproductive organs (Sasaki and Nagao 2001; Sasaki et al. 2007). Octopamine plays an important role in recognition of companions among bees and ants (Robinson et al. 1999 and Vandermeer et al.2008), and participates in the attacking behavior of *Camponotus japonicus* (Aonuma and Watanabe 2012). Octopamine in bees' brain stimulates secretion of juvenile hormone by the pharyngeal body (Kaatz et al. 1994). The content of juvenile hormone in the lymph of the larvae is low, but gradually increases after emergence. Treating the male bees with methamphetamine, an analogue of juvenile hormone, leads to an increased level of dopamine in the brain, as well as increased expression of dopamine receptor (Sasaki et al.2012). Virgin ant creates a new nest after mating, when the contents of serotonin, octopamine, and dopamine in the brain significantly decrease compared to that before mating, while tyramine level significantly increases. Such changes in biogenic amines may be related to the oviposition behavior of queen (Aonuma and Watanabe 2012). However, the specific mechanisms how biogenic amines within the brain affect endocrine of social insects and how they regulate the insects' social behavior remain unclear.

The genetic basis of social behavior

Genetic factors play an important role in the behavior of social insects (Fitzpatrick and Shahar 2005). The global invasive species Solenopsis invicta has two forms of social organization: monogyne and polygyne. (Keller and Ross 1998). The genotype of the odor-binding protein Gp-9 of *Solenopsis invicta* determines the number of queens in the nest. The genotype of the worker ants in the monogyne colony is Gp-9BB, which allows only one queen of the same genotype; while the genotype of most workers in the polygyne colony is Gp-9Bb, which allows several external queens of the same genotype (Lucas et al. 2015; Valles and Porter 2003). Bonasio et al. (2010) studied the degree of DNA methylation in ants of different labor divisions in the colonies of *Camponotus floridanus* and *Harpegnathos saltator*. And it was found that phenotype of *Camponotus floridanus* significantly differed between different labor divisions and was relatively fixed, once differentiated, there's no conversion between classes. On the contrary, there are insignificant differences in the form and lifespan of queen ants and worker ants of *Harpegnathos saltator*; the behavior is flexible; when the ant nest loses queen ants, worker ants can also produce eggs. It's found there are significant differences in the degree of methylation among different grades of the two ants species (P<0.05), and most of the highly methylated genes were "housekeeping genes", whose products were present in all cells of the body (Bonasio et al. 2010). The methylation of these genes is likely to participate in the morphological differentiation between queen bees and worker bees and between queen ants and worker ants and the social division of labor between worker bees or worker ants (Chittka et al. 2012).

Cerapachys biroi is a parthenogenetic ant, and the behavioral of individuals in the nest can be divided into a queen ant stage (oviposition) and a worker ant stage (foraging) (Libbrecht et al. 2016). Libbrecht et al. (2016) compared the DNA methylation of *Cerapachys biroi*'s brain in the queen ant stage and worker ant stage and analyzed its potential regulatory effect of DNA methylation on the behavior of *Cerapachys biroi*, but no significant difference in DNA methylation of *Cerapachys biroi* in the queen ant stage and worker ant stage. These studies show that DNA methylation has different regulating effects on different social insect species and plays a different role in different social insects.

Alaux et al. (2012) injected lipopolysaccharide (LPS: a non-pathogenic immunogenic factor derived from the outer membrane of gram-negative bacteria) into the Italian *Apis mellifera* Ligustica after emergence, and found that the expression of foraging genes in the brain significantly increased and the nurse bees went to foraging earlier. Lucas et al. (2009) found that the foraging(*p-pfor*) genes of *Pheidole pallidula* encodes the cyclic AMP dependent kinase (cGMP-KPG). The kinase is expressed in five cells in the mushroom body of the soldier ants' brain but it is not expressed in the mushroom of foraging ants. Expression of foraging genes decreased when the soldier ant was stimulated with food (*Tenebrio molitor*), but significantly increased when the nest needed protection. The above studies indicate that foraging genes control foraging behavior in both ants and bees, but the expression of foraging genes has a diametrically opposite effect on the foraging behaviors of ants and bees, indicating that the expression of foraging genes plays an important role in the differentiation of non-genetic behavior polyphenisms of social insects.

Conclusion and perspective

Compared with mammals and human beings, the nervous structures of social insects are simple and relatively easy to operate. The highly evolved social behaviors and other characteristics provide a good mode for the study on physiological regulation of social behavior of mammals and even human. For example, social insects usually launch violent attacks to defend their nest upon intrusions, to understand the physiological mechanism of these attacks would help us understand the underlying mechanism of attacking behaviors of other insects and animals. With the development of electrophysiological technologies such as voltage clamp, patch clamp, electroantennogram, single-cell recording, calcium imaging and laser confocal microscopy, the complete nerve conduction pathway of the social insects' complex social behaviors – from signal reception to responsive behavior – will be presented to us clearly soon.

Social insects and individuals within the same nest have the same genome, but show phenotypic and behavioral polymorphisms. Studying the role of hormones in the process of differentiation and formation of polymorphism would help us understand the role of hormones in controlling behaviors.

Although studies on the role of genes in the regulation of insects' social behavior have made great progresses, many problems still require further investigation. For example, whether their social behavior is determined by genetic or acquired environmental factors, which remains controversial (Robinson 2004). Beside genes and behaviors, there are many factors influencing the performance of behavior, such as changes in neuro and endocrine; transcriptional, translational, post-translational changes; and changes in brain metabolism and nerve regulation, etc., each may lead to changes in the final behavior. Despite these challenges, advances in genetic and molecular biological technologies have provided a bright future for studies on the relationship between genes and social behaviors. Transgenic animals' experiments, RNAi technology and CRISPR/Cas9 gene editing technology make it possible to study the function of specific genes (Chen et al.2016; Helbing and Lattorff 2016; Qiu and Cheng 2017). With the development of technologies, neurotransmission electrical signals, hormone secretion, and changes in whole genome transcription and protein translation of tissues and organs can be monitored in real time as specific behavior occurs, and it is expected that more detailed physiological and molecular mechanism of the complex behavior of social insects would be revealed. *Odontotermes formosanus*, *Coptotermes formosanus* and the global invasive insect *Solenopsis invicta* are major forestry pests that cause serious losses to China's agricultural & forestry production and social economy. These insects have strong reproductive capacity, live in hidden lair, and show other characteristics that make chemical control very difficult. Through the advanced physiological & biochemical technology, gene sequencing & synthesis, gene editing and other tools, the social insect's behavior and the underlying mechanism shall be revealed. And these regulation agents should be used to against these pests to interfere with their social behavior and disrupt the normal order of the nest, the nest would fell apart and the purpose of biological control can be achieved.

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