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A homeotic mutation influences the wing vibration patterns during mating in males of the silkworm moth *Bombyx mori*

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ABSTRACT

An abnormality in the wing vibration pattern in males of the E^{Nc} homeotic mutant of *Bombyx mori* was investigated. The wild-type (+/+) males show a switching of the rhythmic wing vibrations from a sequential pattern to an intermittent pattern during mating, whereas the E^{Nc} mutants show a sequential pattern both before and during mating. Wing motions in +/+ males became small during mating, but those in +/E^{Nc} males did not. Ablation of the head ganglia of +/+ and +/E^{Nc} males during mating caused no change in the motor patterns of wing vibrations. Ablation or cooling of the posterior abdomen in the +/+ males during mating caused sequential wing vibrations, suggesting that the change in wing vibrations is induced by signals from the posterior abdomen. The pterothoracic ganglion in the +/E^{Nc} males is separated into two ganglia, in contrast to the complete ganglionic fusion in the +/+ males. The neurons in the pterothoracic ganglion stained from abdominal nerve cords are homologus in +/+ and +/E^{Nc} males, but many of these in +/E^{Nc} males are elongated along the anteroposterior axis. These results suggest that the wing vibration pattern is restricted by genetic factors through reconstruction of the thoracic nervous system during metamorphosis.

1. Introduction

Wing vibrations in insects have evolved as parts of diverse behaviors involved in flight, courtship, sound generation, and ventilation. Once triggered by certain releaser stimuli, the motor patterns of these wing vibrations are rhythmic. These rhythmic motor patterns of wing vibrations are thought to be generated by neuronal networks called "central pattern generators" (CPGs) (Delcomyn, 1980; Harris-Warrick and Marder, 1991; Pearson, 1993) and modified by external inputs, including sensory signals, which are essential for the correct shaping of the final motor pattern (Harris-Warrick and Marder, 1991; Burrows, 1996). Genetic factors influencing the wing vibration pattern have been reported in the flight-mutant fly (Levine and Wyman, 1973; Barnes et al., 1998) and in song production during courtship in hybrid male crickets (Bentley and Hoy, 1972; Hoy et al., 1977). These examples suggest that genetic factors influence the formation of the neuronal networks responsible for pattern generation and change the motor outputs of wing vibrations. Genetic factors may also influence the switching of one rhythmic motor pattern of wing vibrations to another pattern caused by the interaction between CPGs, or CPGs and external signals. Studies of mutations in a particular wing vibration pattern are important in clarifying the genetic basis of behavioral switching and the divergence of behaviors involving wing vibrations.

The adult male of the silkworm moth *Bombyx mori* performs a mating dance in response to the female sex pheromone. The major behavioral components of the dance are wing vibrations, walking, and occasional abdominal curvature. The mating dance plays an essential role in locating the calling female (Obara, 1979; Kanzaki, 1998). The wing vibrations of males during the mating dance are in a sequential and rhythmic motor pattern at 30–40 Hz (Kanzaki, 1998). The motor pattern during the mating dance is triggered by a command element in the head ganglia and is generated by the CPGs in the thoracic ganglia (Kondo and Obara, 1982, 1984). The motor pattern is similar to the general flight motor patterns in other moth species (Kammer, 1968; Ariyoshi and Kanzaki, 1996). When the male mates with a female, the male stops walking and vibrating his wings for several seconds and then begins another series of rhythmic intermittent wing vibrations. The rhythmic intermittent wing vibrations usually continue for several minutes during mating.

Homeotic mutations that change the identities of body segments in insects influence their basic neuronal design and behaviors. In *B. mori*, a homeotic gene complex (the *E* complex) is a cluster of homeotic genes that play important roles in the specification to the larval body segments (Ueno et al., 1992;

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Yasukochi et al., 2004; Xiang et al., 2008). In some of the *E* mutations including E^{N} and E^{Ca}, the homeodomein regions of the *Bm Ubx* and/or *Bm abd-A* genes are deleted from the *E* chromosome, which may cause depression of the *Bm Antp* gene and transformation of the abdominal segments to the other types of segments (Ueno et al., 1992, 1996). Some of the *E* mutations cause a particular directional shift in the segment, anterior to posterior or vice versa, and cause dramatic phenotypic transformations in embryos, such as from an abdominal segment to a thoracic segment with thoracic legs (Itikawa, 1952; Ueno et al., 1992) or another abdominal segment with abdominal legs (prolegs) (Sasaki, 1940; Itikawa, 1952; Shimada et al., 1986; Xiang et al., 2008). Homozygotes and heterozygotes for the E^{Nc} mutation express a pair of prolegs in the second abdominal segment that is normally without prolegs in the larval stages (Sasaki, 1940; Itikawa, 1952). The additional prolegs are as functional as normal prolegs, with mechanosensilla, muscles, and the nerve innervation required for walking, suggesting a dramatic transformation from an abdominal second ganglion to another abdominal ganglion that involves the movement of the prolegs (Sasaki et al., in preparation). The mutant larvae can develop into pupae with reorganization of the central nervous system and finally become adults. Both the mutant male and female adults are fertile. However, abnormalities in their behavior and neuronal morphology in the adult stages have not been investigated. How the abnormal larval abdominal ganglia are reconstructed to perform adult behaviors during metamorphosis and whether the mutant adults can perform normal reproductive behaviors remain unknown.

In the present paper, we describe the abnormal switching in the wing vibration patterns of the adult males of the E^{Nc} homeotic mutant. We also show the abnormal morphology of the thoracic ganglia in the adult mutant and discuss how the abnormal reorganization of the central nervous system during metamorphosis influences the switching of the rhythmic wing motor patterns.

2. Materials and methods

2.1. Animals

Adults of both sexes of the homeotic mutant strain E^{Nc} of the silkworm moth, *B. mori*, were used for behavioral, electrophysiological, and morphological experiments. The E^{Nc} mutation is a spontaneous mutation first reported by Sasaki (1940). Selection of larvae for strong expression of the E^{Nc} trait, coupled to inbreeding for over 60 years, has resulted in a stock that shows constant expression of a pair of supernumerary prolegs on the second abdominal segment. The E^{Nc} strain was obtained from the Genebank at the National Institute of Agrobiological Sciences in Japan. Because E^{Nc} is an autosomal dominant mutation, heterozygote mutants (+/E^{Nc}) and wild-type (+/+) larvae were separated from the crosses between heterozygotes and the wild-type. Larvae were reared on mulberry leaves (*Morus alba* L.) throughout all stages. All larvae were reared at 28 °C during the first and second instars and at 25 °C during the third, fourth, and fifth instars, with a photoperiod of 12 light:12 dark.

2.2. Analyses of wing motions in fore- and hindwings

To determine wing motion patterns and coordination between fore- and hindwings in males, video images with a high-speed camera were analyzed. Images of the wing motions in both +/+ and +/E^{Nc} males were captured with a high-speed camera (Model 10000 Mono, Red Lake Imaging, Morgan Hill, CA, USA) and associated computer at 1000 frames per second and with an exposure time of 1 ms during wing vibration before and during mating. Selected images were stored as computer files for later analysis with computer software (Motionscope, Red Lake Imaging). Movements of the tips of fore- and hindwings were traced from these images.

2.3. Recording flight muscle activities

Electromyograms were recorded from one of the indirect flight muscles, the right first dorsal longitudinal muscle (DLM1) (Kondo and Obara, 1982) by inserting a fine silver wire electrode (diameter: 75 μm), insulated to its tip, into the muscle through a hole in the dorsal mesothorax. A reference electrode was inserted into the posterodorsal thoracic cavity. Both electrodes were fixed with wax and were guided by the movement of the male moth during the recording. After the recordings were made, the recording sites were confirmed by dissecting the thorax of the moth. It has been reported that the potential of the DLM1 recorded by electromyograms corresponds to the downstroke of the wing vibration in *B. mori* (Ariyoshi and Kanzaki, 1996). This was true in our preliminary experiments when simultaneous recordings of electromyograms and high-speed camera images were made. The muscle potentials were amplified with an extracellular amplifier (MEG2100, Nihon-Kohden, Tokyo, Japan), recorded on a computer through an A/D converter (PowerLab, ADInstrument, Castle Hill, NSW, Australia), and analyzed with computer software (Chart 5, ADInstrument). The recordings from the DLM1 were filtered with a digital high-pass filter (5 Hz), because the baseline of the recordings usually fluctuated with the movement of the males.

2.4. Ablation of head ganglia or posterior abdomens of males during copulation

To determine whether signals from the brain or the posterior abdominal nervous system are involved in the generation and modification of the wing vibration patterns in males during copulation, the head ganglia of +/+ and +/E^{Nc} males or posterior abdomens of +/+ males were ablated. Ablation of the whole head from the body was performed with fine scissors during mating. As a control, some individuals were injured on the dorsal head with scissors, but the brain was not injured, and the head remained attached to the body. The posterior abdomen was ablated between the sixth and seventh abdominal segments during intermittent wing vibrations, resulting in the ablation of the nerve connection between the third and fourth (terminal) abdominal ganglia or between the fourth abdominal ganglion and the nerve terminal at the genitalia. In either case, the pairs of testis, seminal ducts, and seminal glands remained intact in the abdomen after ablation. Control individuals were injured dorsally between the sixth and seventh abdominal segments, but not at the ventral nerve cord. The wing vibration patterns of males were recorded from the DLM1 as electromyograms during the experiments. On the electromyograms, the number of spikes occurring between 1 and 4 s before the end of the mating dance (“before mating”), between 1 and 4 s before the ablation treatment (“during mating but before treatment”), and between 1 and 4 s after the onset of wing vibrations (“during mating but after treatment”) were counted and compared.

2.5. Cooling the posterior abdomens of males during copulation

Because the ablation of the posterior abdomen could have strong effects on neuronal activities during wing vibrations, we inactivated the abdominal nerve cord by cooling the posterior abdomens of +/+ males. During the intermittent wing vibrations, the posterior abdomen around the genitalia was cooled with a cold spray gun (Spot freeze, Fine Chemical Japan Ltd., Osaka, Japan) with a fine nozzle tip, for less than 1 s. The wing vibration patterns of the