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Morphological and electrophysiological differences in tarsal chemosensilla between the wild silkmoth *Bombyx mandarina* and the domesticated species *Bombyx mori*

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ABSTRACT

Gustatory and olfactory senses of phytophagous insects play important roles in the recognition of host plants. In the domestic silkmoth *Bombyx mori* and its wild species *Bombyx mandarina*, the morphologies and responses of adult olfactory organs (antennae) have been intensely investigated. However, little is known about these features of adult gustatory organs and the influence of domestication on the gustatory sense. Here we revealed that both species have two types of sensilla (thick [T] and slim [S] types) on the fifth tarsomeres of the adult legs. In both species, females have 3.6–6.9 times more T-sensilla than males. Therefore, T-sensilla seem to play more important roles in females than in males. Moreover, gustatory cells of T-sensilla of *B. mandarina* females responded intensely to mulberry leaf extract in electrophysiological experiments, while T-sensilla of *B. mori* females (N4 strain) hardly responded to mulberry leaf extract. These results suggest that T-sensilla of *B. mandarina* females are involved in the recognition of oviposition sites. We also observed that, in three *B. mori* strains (N4, p50T, and Kinshu × Showa), the densities of sensilla on the fifth tarsomeres were much lower than in *B. mandarina*. These results indicate that domestication has influenced the tarsal gustatory system of *B. mori*.

1. Introduction

Domesticated animals typically differ from their wild progenitors regarding appearance, behavior, and physiological properties (Diamond, 2002). About 5000–10,000 years ago, humans discovered the ability of moth larvae to spin silk threads and began to breed these moths to optimize silk production (Goldsmith et al., 2005). This artificial selection led to the evolution of the domesticated silkmoth, *Bombyx mori*. The putative ancestor of *B. mori* is *Bombyx mandarina,* an extant wild silkmoth of East Asia (Sun et al., 2012). *B. mandarina* is commonly found in mulberry fields in Japan, Korea, and China. In the laboratory, *B. mandarina* and *B. mori* readily mate (Kuwahara et al., 1983), and their hybrids are fertile and develop normally. In the process of domestication, several phenotypic characteristics including body color, growth rate, cocoon color, cocoon size, and flying ability have changed (Guo et al., 2011). Moreover, the oviposition behaviors of the two species are very different.

Generally, *B. mori* and *B. mandarina* are considered to be monophagous or oligophagous insects that feed only on *Morus* species and *Maclura tricuspidata* belonging to family Moraceae. The adult females of *B. mori* do not show any preference regarding their oviposition site. They lay eggs on paper for egg production regardless of whether mulberry is present or absent. On the other hand, Ômura (1950) reported that *B. mandarina* females lay eggs predominantly on the bark and leaves of mulberry trees in mulberry fields though they occasionally lay eggs erroneously on non-host woody plants near mulberry trees, suggesting that they somehow recognize mulberry for oviposition.

Host plant-specific oviposition is crucial for monophagous and oligophagous insects, because it promises the survival of the next generation, particularly during larval development. It is known that host plant recognition in butterflies depends on several factors including leaf color, leaf shape, and phytochemical compounds

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(Rausher, 1978; Honda, 1995; Kelber, 1999). Extensive work on chemical mediators in the oviposition of lepidopteran insects has demonstrated clearly that chemical attributes of plants serve as the crucial cues that enable the adult females to discriminate and recognize their hosts (Renwick and Chew, 1994; Honda, 1995; Honda et al., 2010). Several butterfly species in the families Pieridae (Ma and Schoonhoven, 1973; Du et al., 1995; Städler et al., 1995), Nymphalidae (Baur et al., 1998), and Papilionidae (Roessingh et al., 1991) recognize their host plant components using tarsal gustatory senses to decide oviposition sites. We expected that tarsal gustatory senses of *B. mandarina* females play some roles in the recognition of their oviposition sites.

Gustatory and olfactory senses play important roles for phytophagous insects to recognize the chemical attributes of plants. Bisch-Knaden et al. (2014) reported that the perception of plant odorants in both sexes of *B. mori* has deteriorated as compared with the wild species *B. mandarina*. In females, this physiological impairment was found to be reflected in a clear reduction in the numbers of olfactory sensilla (Bisch-Knaden et al., 2014). However, the influence of domestication on the gustatory system in *B. mori* has not yet been clarified. Moreover, the physiological and ecological functions of the gustatory senses of the adult legs of *B. mandarina* and *B. mori* have not been reported. In this study, we report (1) morphological features of tarsal sensilla of two species by electron microscopic observation and (2) response patterns of the taste cells of tarsal sensilla by electrophysiological techniques. Here, we show the differences in anatomical and functional properties between the two species and suggest that these differences are derived from the change of selection pressure during domestication.

2. Material and methods

2.1. Insects and plants

Mulberry trees (*Morus alba* L. cv. Shin-Ichinose) grown in the experimental field in the Yayoi Campus of the University of Tokyo (35°42′N, 139°45′E) were used throughout this study. Larvae of *B. mandarina* were collected in a mulberry farm (35°74′N, 139°54′E) at Nishi-Tokyo, Japan and maintained in our laboratory. We focused on the relationships between the adult sizes and the tarsal morphologies (i.e. sensillum numbers) among *B. mori* strains. Therefore, we used three *B. mori* strains (N4, p50T, and Kinshu × Showa). N4 and p50T were maintained at the University of Tokyo, and their adult body sizes are similar to that of *B. mandarina.* The body size of Kinshu × Showa is larger than those of the two other *B. mori* strains and *B. mandarina*. Eggs of F1 hybrid, Kinshu × Showa, were purchased from Ueda Sanshu Ltd (Ueda, Japan). Larvae of *B. mandarina* and *B. mori* were reared on mulberry leaves at 25 °C ± 1 °C under a 12L/12D photoperiod. Pupae were sexed, and males and females were put into separate cages before eclosion. The adult females (within 48 h after eclosion) were used for electrophysiological experiments.

2.2. Scanning electron microscopy

Tarsi of fore-, mid-, and hindlegs of living moths were excised using scissors and were air dried at room temperature. To obtain a better view of the sensilla, we gently removed the dust on the tarsal samples using an air pump. The tarsal samples were fixed on a metal stage with dielectric double-sided tape (Okenshoji Co. Ltd., Tokyo, Japan) and electroconductive paste (Dotite; Fujikura Co. Ltd., Tokyo, Japan) and sputter coated with platinum–palladium. The leg surface and tarsi were observed under a scanning electron microscope (SEM), TM-1000 (Hitachi Ltd., Tokyo, Japan), and the hair structures were observed under an SEM S-4800 (Hitachi Ltd., Tokyo, Japan).

2.3. Measurements of numbers and densities of sensilla

Kachikachi-counter software ver. 2.71 (http://www.geocities.jp/gen_0715/softs/katikati/index.html) was used to count the numbers of sensilla. ImageJ (National Institutes of Health, Maryland, USA) was used to analyze the area possessing thick-type sensilla (T-sensilla) on the fifth tarsomeres of the forelegs. The procedures were as follows: SEM views of the ventral sides of the fifth tarsomeres were used for ImageJ analysis. For each of the anterior and posterior areas of the ventral side, the sensilla located on the outside in a cluster of T-sensilla were connected by a straight line, and its regions were measured and regarded as areas possessing T-sensilla.

2.4. Preparation of mulberry leaf extracts

(1) Methanol extract: Methanol-soluble substances were extracted from mulberry leaves at room temperature as follows. A total of 1 g of young mulberry leaves (*M. alba* L. cv. Shin-Ichinose) was placed in a vial containing 10 ml of methanol. After 4 days, mulberry leaves were removed from the methanol. A portion (100 μl) of the methanol extract was dried using an air pump. The residue was dissolved in 100 μl of ethanol (99.5%) and stored at −30 °C until use. Just before an electrophysiological experiment, the ethanol solution was mixed with 900 μl of 11 mM NaCl using a vortex mixer. This solution included approximately 10% ethanol and 10 mM NaCl and was used as 0.01 × methanol extract.

(2) Water-soluble extract: A total of 0.5 g of young mulberry leaves (*M. alba* L., Shin-ichinose) was homogenized with 19.5 ml of 10.3 mM NaCl using a pestle, mortar, and sea sand (300–600 μm; Wako) on ice. The homogenate was centrifuged at 14,000 rpm and 4 °C for 20 min. The supernatant was stored at −30 °C until use. Just before an electrophysiological experiment, a portion of the supernatant (100 μl) was mixed with 900 μl of 10 mM NaCl. This solution was used as 0.0025 × water-soluble extract.

2.5. Electrophysiological experiments

The responses of the sensory neurons of *B. mandarina* and *B. mori* hair sensilla were recorded using a typical tip-recording method (Hodgson et al., 1955). The foreleg or midleg of the moth was cut at the proximal region of the tibia and was inserted into a glass capillary (Drummond Scientific Co., USA) filled with Ringer's solution (147.2 mM NaCl, 4.0 mM KCl, and 2.2 mM $CaCl_2 \cdot 2H_2O$) to prevent drying of the sample. A platinum wire (indifferent electrode) was inserted in the other side of the glass capillary. Under a stereomicroscope (MZ 125; Leica Microsystems Ltd., Switzerland), the glass capillary was fixed on a stage, but the tarsus was attached to expose its ventral surface so that a stimulating/recording electrode could be placed on the sensilla. A capillary puller (P-97, Sutter Instrument Co., USA) was used to make a fine-tipped glass capillary with a tip diameter of approximately 10 μm. This fine-tipped glass capillary containing a stimulus solution (leaf extract or control solution) was used as the recording electrode. The stimulation of sensillum was conducted by capping the tip of a sensillum with the glass capillary (recording electrode). The responses (electrical impulses) of sensory neurons were obtained immediately after mounting the glass capillary on a sensillum. A platinum wire inserted into the recording glass-capillary electrode was connected

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