Morphological Features of the Mouthparts of Silkworm *Bombyx mori* L. in Relation to the Feeding Responses to Artificial Diet

-With Special Reference to Antennae and Maxillae-

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누에의 인공사료에 대한 섭식성과 구기의 형태적 특징에 대하여 - 더듬이 및 소시의 형태적 특징을 중심으로 -

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요 약

누에의 사료에 대한 섭식성 반응이 다른 2계통에 대한 두부의 형태적 차이를 전자현미경적 관찰을 하였다이들 2계통의 두부와 촉각의 형태적 차이는 변이가 심하고 다양하다. 섭식성 반응이 높은 계통에 있어서는 소시지의 돌기가 매우 잘 발달되어 있는 반면 섭식성 반응이 낮은 계통에 있어서는 형태적으로 퇴화되어 있다. 또한 촉각의 셋째마디의 유병돌기가 발달되어 있지 않으며 사료에 대한 섭식 반응이 낮고 극단적으로 퇴화되어 있는 개체는 사료를 감지하지 못하여 결국에는 굶어 죽은 것으로 사료되다.

Key words: Feeding response, Energetic feeding response, Inert feeding response, Mouthpart, Maxillary palpi, Sensilla basiconica, Trichodea sensilla,

INTRODUCTION

The first attempt for artificial diet rearing of insect was made with *calliphora vomitoria* by Bogdnov. The diet consisted of peptone, beef extract, starch and minerals.

In case of silkworm, Bombyx mori L. Fukuba

et al. reported that rearing of one generation of silkwrom was successfully grown on artificial diet. Since then, few reports were published that maxillary played an important role in feeding response as it contains contact sensilla which discriminate the taste of food. It was also reported that maxillary lobes contain sugar, minerals and water sensory hairs (Ishikawa et al., 1963, 1966;

Ito T et al., 1959; Tazima, 1982). Finally it was disclosed the sensitive organs of the feed is sensilla styloconica (Ishikawa T et al., 1966). The antenna act as feeler which is used to locate the direction. Among antennal region, middle part is more strongly stimulated (Nishino et al., 1979) and some insects have short antennal segment even feeding on the same plant species (Meyerdirk DE et al., 1987).

From the aspect of the feeding response it has been known that silkwom showed a varietal difference. Japanese variety has a better feeding response than Chinese and European varieties (Kim JU *et al.*, 1979, 1982). On the other hand Yamamoto T. has reported that feeding response was controlled by recessive gene on twenty-fifth chromosome. In the relation to feeding response, it depends on the combination of stimulant and repellent in the feed (Horie T., 1978).

It has been known that silkworm is monophagous. It prefers mulberry to other feeds. The feeding behaviour of silkworm was categorized into high, medium and low feeding response on the basis of the feeding response to aritificial diet (Hamano *et al.*, 1986). Before chewing starts smell of feeding sources arouse the feeding response and the antennae reacts with it.

On the other hand Devit BD, Smith JJB (1985) catgorized the feeding response in other insects with reference to Beck (1965) and Schoonhoven (1972) as they grouped feeding response into plant finding, sampling, active feeding and cessation feeding. Concerning the feeding response, it was resumed mandible and maxilliary palpus were primarily responsible organs. It has been known that maxilliary and antennae play key roles in the feeding response and this idea lead, to examine the infrastructural distinctions in sensory organs, using the positive feeding response of silkworm and the negative feeding response

of silkworm catergorized by a larval behaviour in the feeding reponse to artificial diet.

In these connections, the present study has made on the electron microspical observation of the morphological features of the mouthparts and some findings are reported.

MATERIALS AND METHODS

The varieties used for the experiment were previously screened on the basis of feeding response to artificial diet and selected out into two groups; energetic feeding response and inert feeding response. For the detail study of mouthparts and antennae of silkworm, *Bombyx mori*, five silkworm larva were separated to cut head part for fixation and examination of sensory organs under electron microscopes.

Head part of the silkworm larva were narcotized with insect ringer solution and cut out in cold room at 4°C, then fixed in 2% glutaraldehyde for two days, after specimens were rinsed in 0.1 mole of phosphate buffer. Post fixation was carried out with osmium tetraoxide and again rinsed in 0.1 mole of phosphate buffer and tissues were dehydrated in graded alcohole series.

Cirtical point dried and mounted on stub with double sided tape conducting silver point. They were subsequently coated with 20 nanometer clacium gold and observed under scanning eletron microscope (Hitachi S-570) at 5 to 20 Kilo volts.

RESULT AND DISCUSSION

The silkworm feeding behaviour reveals two types of feeding response; one is continuous feeding known as energetic feeding response while other is called inert feeding response. These two

new terms are used in the present study. These technical terms are quite different from Hamano K. and his associates (1986) categorized the feeding response in the silkworm into high, medium and low feeding response. Devit BD, Smith JJB (1985) categorized the feeding response in other insects with reference to Beck (1965) and Schoonhoven (1972) as they grouped feeding response into plant finding, sampling, active feeding and cessation feeding. The feeding behaviour is most important for the growth and development of the silkworm. Before feeding starts. the silkworm brings its mouthparts into a suitable position to test the diet with sensory hairs which keep contact receptors. If contact sensilla are well developed, feeding response becomes an excellence but if some detect in contact sensilla, feeding response becomes low and larva die due to starvation.

The structures of sensilla and other structures of functional organs in mouthparts and antennae in the energetic and inert larva were examined under scanning electron microscope. There is a pair of antennae composed of five jointed segments and used as sensory organs. The antennal loabes contain contact sensilla and act as a feeler. There are some differences in a morphological differences between the energetic and the inert larva; that is, the energetic larva showed a symetrical feature of antennae on both sides, while the inert larva fomed assymetrical features of antennae, showing paticuarly a different number of sensilla basiconicum.

In Fig. 1 the right and the left sensory hairs of antennal organs in the energetic larvae has shown. There are four sensilla trichodea on the second antennal segment and are fully grown. On the third segment, four sensilla basiconica are well developed, when larva move in search of food, these contact sensilla play an inportant

role to locate the food and direction and then maxillary sensory hairs perform the function of food selection as well sa discriminate the taste. The sensory hairs of the inert larvae have shown. On the left side, the four sensillum trichodea on the second antennal segment are well developed. Similarly, four sensilla basiconica which are located on the third segment are fully grown. When these sensilla contact an organism or food, they feel its presence or absence as well as direction. On the right side, the four sensillum trichodea on the second antennal segment are developed but on the third segment only two quite deformed sensilla basiconica are present. These deformed structures can not feel the present of food or locate the direction. That is why the larva has strong feeling or desire to locate the direction and check the food but because of deformed sensilla becomes sluggish, do not grow well and finally dies to starvation. The antenna are primary site of the olfactory organs (Ishikawa et al., 1964). Structure act as chemosensilla which are two types i.e., thick and thin type of chemosensilla.

The thick wall chemosensilla take the form of hairs as sensilla basiconica and sensitive to storage of feelings. Thin wall chemosensilla are the outer and inner hair of the second segment known as sensiluim trichodium which are sensitive to stimuli.

Morphologically more than half of the trichodea sensilla in the adult male antenna appear in the adult stage in the animal (Schafer and Sunchez; 1973) as reported by Nishino C *et al.* (1979). Schaller (1978) classified sensilla of american cockroach antenna into three categories i. e., Wp (wall pore), Np (nonpole), and Tp (terminal pole) sensilla on the basis of his transmission electron microscope and scanning electron microscope observations.

In Fig. 2 the sensilla of the energetic larva are shown with maxillary palpus and maxillary lobes. There are eight maxillary palpi grown at the top of the maxillary palpus known as sensilla basiconica which differentiate the taste of food. The energetic larvae revealed the right and the left maxillary lobes with sensilla Styloconica (Ss). These are three in number i.e., Ss 2 and Ss 3 which are associated with contact chemoreceptors. Ss 1 is sugar sensory hair and stimulated by sucrose, inositol and glucose, while Ss 2 is sensory hair which responsed to stimulate water and Ss 3 (B) is little degveloped and functions are unknown (Ishikawa and coworkers; 1963). The other three sensory hairs are contact sensilla, two are large and called sensilla trichodea and small one is known as sensilla chaeticum. They are well developed and fully grown at the top of the maxillary plapus. Contrarily the inert larva has abnornal shape (dull) of the sensilla basconicum and they show inactive response to artificial diet with a sluggish movement against food.

In Fig. 3 the left and the right maxillary palpus with sensilla basiconica are shown in the inert larvae. The ultrastructures of sensilla basiconica on the left side are fully developed and eight are in number but on the right side these structures are deformed and can not perform its fully function.

Therefore, when larvae has desired to select the food, they try but fail to discriminate the taste of food as organs deformed. In the energetic and the inert larvae comparison of sensilla basiconica is displayed with close magnification. These morphological observations reveal that maxillary sensory hairs are fully developed. And they act as contact chemoreceptors, easily discriminate the food and move in search for diet but if sensilla are deformed or sbsent. The

larva showing a dull or no response to diet move with sluggish movement and grow poorly and finally die of starvation.

SUMMARY

The silkworm feeding behaviour reveals two types of feeding response i.e., energetic and inert feeding response to diet. These two new technical terms are being introduced in the present study.

The structures of functional organs of mouthpart and antenna of energetic and inert feeding response to diet were examined by scanning electron microscope. There is a big variations in the ultrastructures of different sensilla. The maxillary palpi or sensilla basiconica at the top of maxillary palpus of maxilla are well developed in the energetic larva while in the inert larva, these organs on the right side are extremely deformed. Similarly, these sensilla on the third segment of antenna are well developed in the larva energetic to diet has only two deformed sensilla basiconica, that is why silkworm can not show a proper form. This is related to abnormality and it is due to the fact that behaviour is too strong to select the food but fails and finally dies of the starvation.

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FIGURE LEGENDS

- **Fig. 1.** Scanning electron micrograph of antennae in the silkworm, *Bombyx mori* L. In the normal antennae of silkworm(energetic, A), four sensilla basiconica are available but the inert larva (B) form two sensilla basiconica. ×2000
- Fig. 2. Scanning electron micrograph of maxilla with sensory hairs in the silkworm, *Bombyx mori* L. M1: Maxillary lobe, Mp: Maxillary palpus, Sb: Sensilla basiconica, Sc: Sensillum chaeticum, Ss 1,2,3: Sensilla styloconica 1, 2, 3, St: Sensilla tricodea. Sensory hairs on Maxillary lobes of larva in the silkworm, *Bombyx mori* with the energetic response (A) and the inert response to diet (B). ×1500
- Fig. 3. Scanning electron micrograph of sensory hairs on the left and the right maxillary palpus of larva in the silkworm, *Bombyx mori*, with energertic (A) and inert response to diet (B).

 Mp: Maxillary palpus, Sb: Sensilla basiconica, ×800

