カイコ光沢小眼突然変異体における幼虫側単眼の発育成長と形態

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Features and growth of the stemmata in the varnished eye mutant of *Bombyx mori*

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The varnished eye mutant (*ve*) of the silkworm, *Bombyx mori* exhibited undeveloped ommatidia in the adult compound eyes. However, the morphology and growth rate of the stemmata, a larval optic organ located on both sides of the head capsule in 6 pairs, were completely normal in developing *ve* larvae. Also the sectional structures of the stemmata observed under a light microscope did not show any abnormality in the mutant. These results suggest that the formation and growth of larval stemmata are not affected by the *ve* mutant gene.

Key words: *Bombyx mori,* optic organ, stemmata, varnished eye mutant

The larval optic apparatus termed lateral ocelli or stemmata is located on the head capsule in 6 pairs in *Bombyx mori* and during metamorphosis the organ is replaced by a pair of the adult compound eyes. The morphological details of optic organogenesis in *B. mori* were studied from the larval to the adult stages (KOYAMA, 1954; KOYAMA and TANAKA 1954; 1956; YAGI and KOYAMA, 1963). *B. mori* has 2 adult optic apparatus mutants, lustrous (*lu*; UDA, 1930; HASIMOTO, 1965) and varnished eye (*ve*; DOIRA, 1975), both due to spontaneous mutation (DOIRA *et al.*, 1992). The *ve* mutant was of special interest; the compound eyes in adults is small in size having characteristically glossy surface in contrast to the dull reflection of the normal eyes. The *ve* gene belongs to the 6th linkage group at the 11.1 position (DOIRA *et al.*, 1992). This mutant is thought to affect the organogenesis of optic systems but the problem as to whether the *ve* gene is expressed during development of the larval visual apparatus was uncertain. In this study we observed the *ve* mutant larvae for the morphology and growth rate of the stemmata by scanning electron microscopy and light microscopy. There was no difference between *ve* and normal larvae, suggesting that the mutant gene does work during the adult compound eye differentiation.

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Materials and Methods

Insects: The experimental silkworm strain u711 (a stock of the Institute of Genetic Resources, Faculty of Agriculture, Kyushu University), which contains the *ve* mutant gene, was used. This strain includes the E^{El} gene as a maker and has been maintained by the cross E^{El} +/+ *ve* (females) × + *ve* /+ *ve* (males). The E^{El} heterozygotes with extra-crescents and extra-legs and the + homozygotes with normal marking and normal legs were segregated in the ratio of 1 : 1 within the same batch; the former will be designated the normal type while the latter the *ve* mutant.

Scanning electron and light microscopy: Heads were cut from newly hatched and newly ecdysed larvae after each molt as well as from mature larvae. To obtain surface views of larval optic apparatus, whole heads were fixed in Carnoy's fluid for 2 or 3 days at 0 ℃. After fixation, the samples were dehydrated in a graded series of ethanol, freeze dried in the presence of *tert*-butylalcohol, mounted on aluminum stubs with a double-stick carbon tape followed by the sputter coating with gold and subjected to scanning electron microscopy (JSM 5200) at 15 kV. To obtain specimens for sectional structures of stemmata, whole heads from the mature larvae were fixed as above, embedded in Technovit resin 7100 (Heraeus Kulzer) and cut into 3 μm sections by a microtome. The sections were stained with hematoxylin-eosin and mounted in eukitt for light microscopical observation (Zeiss-Axioplan). The average diameters of stemmata were calculated from the enlarged photographs from the scanning electron microscopy.

Results and Discussion

The *ve* mutant was found when adult traits were investigated. When the surface structures of the compound eyes were observed under a scanning electron microscope, the normal eyes were confirmed to be hemispherical with numbers of regularly arranged ommatidia (Fig. 1A), whereas the *ve* eyes were smaller in size and the surface was smooth with incomplete ommatidia (Fig. 1B). This observation was in agreement with a previous report (DOIRA, 1975). Then we observed the larval heads by scanning electron microscopy for the optic apparatus, stemmata. In the normal larvae (shortly after hatching), the 6 stemmata S1 to S6 were apparent on the lateral side of the head, with S1 occupying the nearest position to the antenna (Fig. 2A and B). Each

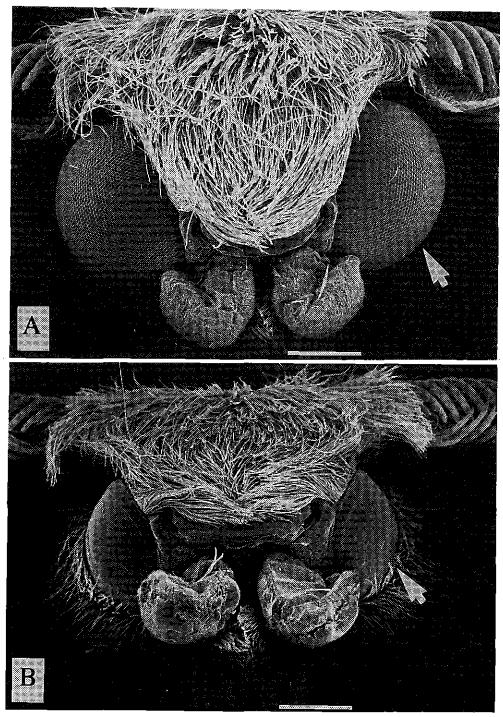

Fig. 1. Scanning electron micrographs of the adult optic organs. A, normal; B, *ve* mutant. Arrow indicates the compound eyes. Scale bar, 500 μm.

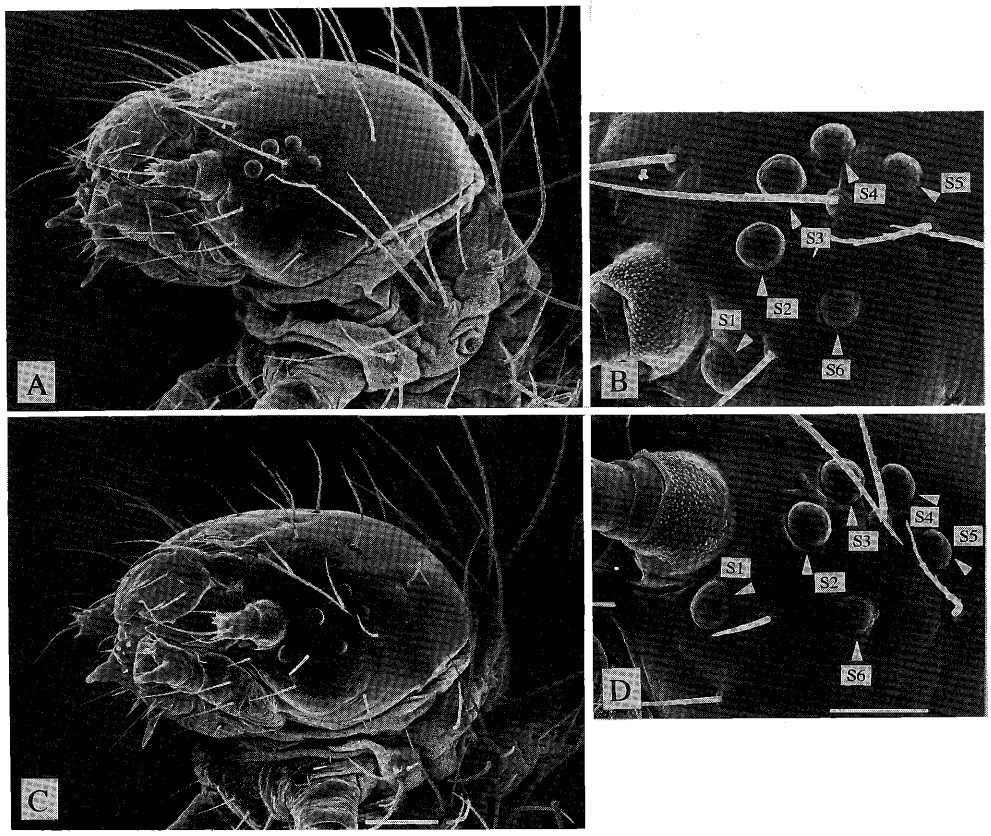

Fig. 2 Scanning electron micrographs of the head capsule of the newly hatched larvae and the optic apparatus (stemma). A and B, normal; C and D, *ve* mutant. S1 to S6, the 1st to the 6th stemmata. Short scale bar (A and C), 100 μm; long scale bar (B and D), 50 μm.

had a hemispherical shape, but S6 exhibited irregularity. These features of morphology and distribution of stemmata were as reported previously (AKAI, 1976). In the *ve* mutant, the features of stemmata of newly hatched larvae were found to be completely normal as seen in Fig. 2C and D.

The stemmata of newly ecdysed normal 5th instar larvae showed the same arrangement as those of newly hatched larvae, although the size differed among them; S3 was the largest. The surface structure of stemmata was very smooth. We occasionally observed coronary loops on the surface (Fig. 3A) but these may be artifacts brought about during fixation, since the surface is fragile. The size, number, shape, surface structure and mode of distribution of stemmata in the *ve* mutant were similar to those of normal larvae (Fig. 3B).

During the larval growth, the stemmata did not change their number and location but enlarged. Fig. 4 shows the changes in

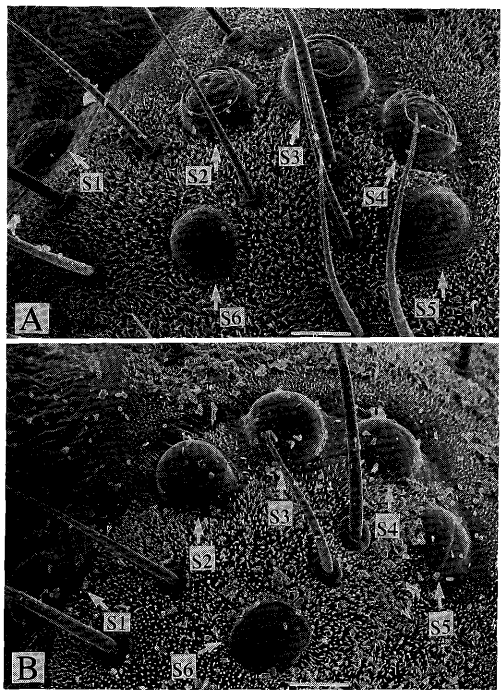

Fig. 3. Scanning electron micrographs of the stemmata in newly ecdysed 5th instar larvae. A, normal; B, *ve* mutant. For abbreviation see Fig. 2. Scale bar, 100 μm.

diameter of each stemma from the 1st to the 5th instars. Again no marked difference was seen between the normal and the mutant animals, although the average diameters at the 5th instar were about 5.5 and 5.1-folds compared to those at the 1st instar in the normal and the mutant, respectively, but the difference was insignificant.

Light microscopical observation of stemma sections revealed that the organ in the normal larva consists of cornea, crystalline body, distal and proximal sense cells and rhabdom, as well as the hypodermis around the stemma (Fig. 5A shows those in a mature larva). All features in the *ve* mutant were the same as those in the normal animal (Fig. 5B).

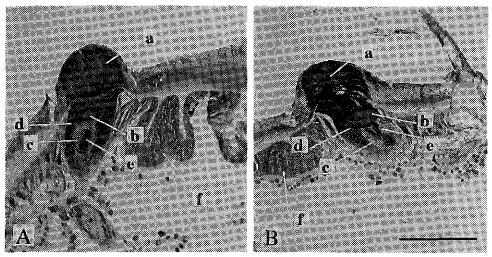

Fig. 5. Light micrographs of the stemmata in the mature larvae. A, normal; B, *ve* mutant. a, cornea; b, crystalline body; c, distal sense cell; d, proximal sense cell; e, rhabdom; f, hypodermis. Scale bar, 100 μm.

As a whole we conclude that the *ve* mutant gene scarcely affects the larval stemmata formation, functioning only in the process of forming the adult compound eyes. Further morphological analysis of the pupal and adult compound eyes in this mutant is in progress in our laboratories.

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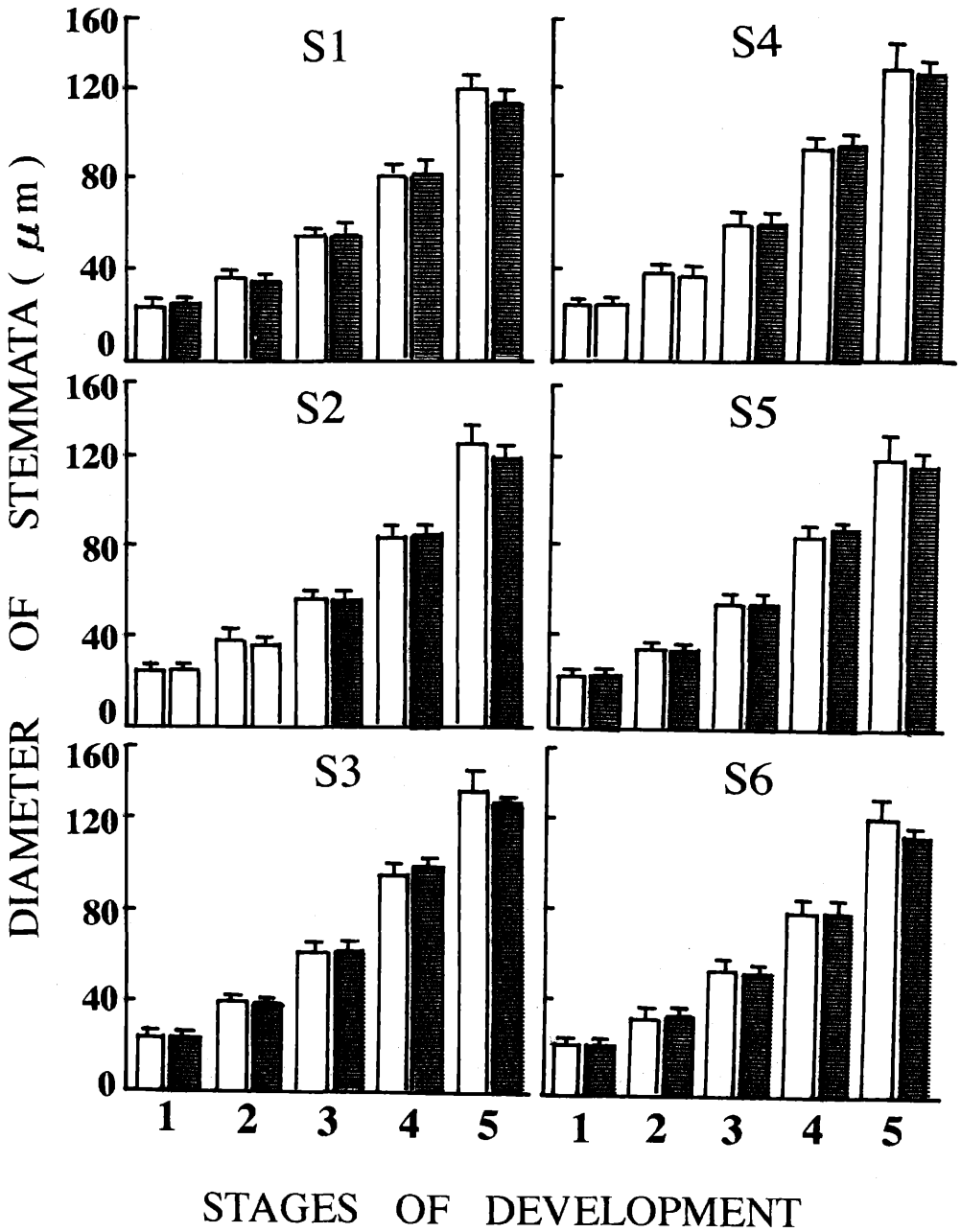

Fig. 4 Changes in size of the stemmata during the larval development. 1 to 5, the 1st instar to the 5th instars. Open column, normal; shadowed column, *ve* mutant. For abbreviation see Fig. 2.

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河口　豊・炭田恵子・伴野　豊・古賀克己・土井良　宏：カイコ光沢小眼突然変異体における幼虫側単眼の発育成長と形態

カイコ光沢小眼変異体（*ve*）の幼虫視覚器官である側単眼の外部と内部の形態および幼虫発育に伴う成長について正常との比較観察を行った。*ve* 幼虫の側単眼は頭部側面に6対存在，それぞれの配置および形態は正常幼虫のそれらと全く同じであった。さらに正常と *ve* との間において，6個の各側単眼に対応するそれぞれのサイズはもとより幼虫発育に伴う6個の側単眼の発育程度においても有意な差異は認められなかった。一方，熟蚕の側単眼内部構造にも正常との間に相違は観察されなかった。これらの結果から，*ve* 遺伝子は幼虫側単眼の外部および内部形態の形成と発育成長には影響を及さないものと推察した。