Characteristics of Se, the White-sided Egg Mutation in *Bombyx mori*

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The White-sided egg mutation (Se) of *Bombyx mori* produces eggs which are opaque with wrinkles in homozygotes, whereas they have gray bands on the anterior, posterior and ventral regions in heterozygotes. Here, the surface and internal chorion structures of these eggs were observed morphologically. No peculiarity was exhibited in the mutation with respect to apparent egg dimensions. When cross-sectional chorion specimens were observed using a scanning electron microscope, the internal features, mainly in the middle layer of the Se homozygous, were found to be deformed. Also the middle layer of eggs of the Se heterozygous was disordered in opaque areas. It can therefore be concluded that the Se alleles take part in the formation of the middle-layer architecture during choriogenesis.

Key words: *Bombyx mori*, chorion, egg character mutation, White-sided egg, Se, choriogenesis

INTRODUCTION

The domesticated silkworm, *Bombyx mori*, has many mutations that affect the egg characters related to size, shape, chorion color, chorion architecture, protein yolk spheres and follicle development, all of which are inherited in a pseudo-maternal manner (Tazima *et al*., 1975; Goldsmith, 1995). The dominant mutation named *Gre* (Green eggshell) tinges the eggs a with yellowish-green color depending on the dyes from the haemolymph and has been mapped to the position 46.4 cM on the Z chromosome (1-46.4) (Aruga, 1943). There are mutations of the chorion architecture, i.e., the Gray egg alleles (*Gr*, 2-6.9) (Toyama, 1910; Tanaka, 1919), the White-sided egg (*Se*, 15-16.9) (Kei, 1943; Chikushi and Nagai, 1975), the mottled gray egg (*mgr*, 6-30.0) (Sado, 1957; Doira *et al*., 1984; Kawaguchi *et al*., 1999) and the low temperature sensitive gray egg (*tsg*, 5-%) (Kawaguchi *et al*., 1998, 2000). These are generically called the “gray-egg” mutations, since their eggshells are wholly or partly gray. In the *Gr*, *mgr* and *tsg* mutations, the gray regions have been confirmed to be caused by the irregularity in the internal structure of chorion layer (Sakaguchi *et al*., 1973; Kawaguchi *et al*., 1999, 2000), which makes the eggshell opaque or less transparent and scarcely reveals the darkly pigmented serosal membrane, as the highly ordered, transparent chorion region does (this makes the normal appearance of chorion dark brown). *Se* is a spontaneous mutation discovered by Kei E.S. in 1943. Each of the *Se* eggs shows a gray band at the anterior, ventral and posterior areas in heterozygotes, and is wholly gray and lethal in homozygotes (Kei, 1943). In this article, the *Se* chorion morphology is observed by scanning electron (SE) microscopy. We found that the lamellate morphology at the middle layer of the opaque region of chorion in the homo- and heterozygous *Se* eggs was deformed, and, in addition, the surface structures of the chorion in the homozygous *Se* eggs were abnormal.

MATERIALS AND METHODS

Insects

The silkworm strain Yo591, bred as an experimental strain by Kawaguchi *et al.* (1997) and maintained in the Faculty of Agriculture, Kyushu University Graduate School, was used. This strain possesses the *Se* gene and, as a marker, the recessive gene *bl* (blind) belonging to the same (15th) linkage group as *Se*. The mating between + Se/bl + (females) and + *bl/* + *bl* (males) produces, in the same batch, the offspring which comprises a mixture of *bl*-heterozygotes having normal eyespots on the second thoracic segment and *bl*-homozygotes having solid black eyespots on the same segment. The female moths with normal eyespots produce *Se*-heterozygous eggs (referred to as *Se* hetero-eggs), and those with the *bl* eyespots produce normal eggs. *Se*-homozygous eggs (*Se* homo-eggs) were obtained from the sib-mating of + *Se/bl* +. Animals were raised on mulberry leaves at 25°C during the larval instars.

Sample preparation and morphological observations

Eggs were collected when the serosal membrane colored black (on days 3 to 5 after oviposition). They were washed thoroughly with distilled water using a sonicator, blotted and air dried, and then were used for experiments.
Whole eggs were fixed in Carnoy’s fluid for 4 to 5 days at 0°C and inspected for overall features under a Nikon SMZ-10 binocular. Alternatively, whole eggs were dehydrated in a graded ethanol series, freeze dried with tert-butyl alcohol, mounted on a stub with double-stick carbon tape, sputter coated with gold and observed the surface morphology by SE microscopy (JSM 5200) at 15 kV. Also eggshells were ripped with a watchmaker’s forceps in distilled water and processed as above for the observation of the inner surface and cross-sectional structures by SE microscopy.

**RESULTS**

**Apparent characters and dimensions of Se eggs**

The Se hetero- and Se homo-eggs each exhibited a similar ellipsoidal shape of the lateral flat side in comparison with the normal (Fig. 1A, B and C). Many wrinkles were found in the Se homo-eggs (C). There were no significant differences in size among the normal, Se hetero- and Se homo-eggs: the lengths of the major/minor axes were 1.26 ± 0.02/0.92 ± 0.03, 1.21 ± 0.04/0.98 ± 0.04 and 1.28 ± 0.03/0.95 ± 0.02 mm, respectively, and the areas of the lateral flat region were 0.95 ± 0.04, 0.95 ± 0.06 and 0.95 ± 0.03 mm², respectively. It was confirmed that the Se homo-eggs were wholly gray (C), whereas the Se hetero-eggs had gray areas in the anterior pole, posterior and ventral regions (Fig. 1B, red arrows; Fig. 2A, B and C) but not in the dorsal region (Fig. 2D).

**Surface structures at the anterior pole and lateral flat regions of Se chorion**

The surface structure of the chorion at the anterior pole region was observed by SE microscopy (Fig. 3A to D). There is a micropyle with a single external orifice, which is surrounded by petals known to be formed as imprints of chorion-secreting follicular epithelial cells. In the present case, the number of petals was from 11 to 13, changing from individual to individual (A and C); a difference in number due to the mutation was scarcely detected. The surface of the micropyle and margin areas at the anterior pole region was smooth in the normal (A) and Se hetero-eggs (not illustrated since the micropyle located in the gray region was indistinguishable from the normal), but rough in the Se homo-eggs (C). The micropylar channels on the inside surface at the anterior pole region, previously confirmed to be present in eggs of plural *B. mori* strains (Kawaguchi *et al.*, 2002), were again validated in the present strain (B and D), and the number of channels was mainly three or four, corresponding to the types II and III, respectively (Kawaguchi *et al.*, 2002), both in the normal and the mutation. The inner surface was again smooth in the normal, but rough in the Se homo-eggs (B and D, respectively). Also, the inner surface was smooth in the Se hetero-eggs (not shown).

The surface structures at the lateral flat side were observed by SE (Fig. 3E to H). The polygonal network patterns, i.e., the imprints of follicular epithelial cells, were clearly observed and the surface was smooth in the normal eggs (E and F) and in the Se hetero-eggs (patterns omitted), but highly abnormal in the Se homo-eggs (G and H). The aeropyles, seen at three-cell junctions in the normal eggs (arrows in F) and also in the Se hetero-eggs (not shown), were not found in the Se homo-eggs, but instead many holes and slits of various sizes were present.
in these eggs (arrows in H). This feature was observed in whole surface regions of an Se homo-egg.

**Internal morphology at anterior and posterior regions of Se chorion**

Fig. 4 shows SE micrographs of cross-sectional structures at the anterior (A, C and E) and posterior regions (B, D and F), both of which in the Se hetero-eggs are gray (cf. Fig. 2A and C). The outer layer (o), which is a lamellate and smooth crust integrated compactly with thin strata, was thinner in the mutant eggs (Fig. 4C to F) than in the normal eggs (A and B). The Se homo-eggs revealed roughness in the crust, which was loosely integrated with the thin strata (F). The thickest middle layer (m), having regular and parallel lamellae in the normal (A and B), was highly irregular in the Se hetero-eggs (C and D), which exhibited loosely integrated vertical lamellas, making the chorion layer very interstitial. This tendency was more marked in the Se homo-eggs (E and F) compared to the Se hetero-eggs. The thinnest inner layer (i), consisted of small columns oriented perpendicularly to the oocyte surface (A and B), seemed to be normal in the Se hetero-eggs (C and D), but the small columns were slender in the Se homo-eggs (E and F).

**Internal structures at dorsal, ventral and lateral regions of Se chorion**

Next, the internal structures were observed by SE at the dorsal and ventral regions of the normal and mutant chorions (Fig. 5A to F). Overall results were similar to those of the above-illustrated anterior and posterior regions, except that the Se hetero-eggs showed almost normal features of the middle layer not only at the normal part (D, dorsal region, cf. Fig. 2D) but also at the gray part (C, ventral region, cf. Fig. 2B), although the Se hetero-eggs often showed partial aberration in the upper-middle layer (e.g. shown by asterisks in D). In the Se homo-chorion, the morphology of the middle layer was consistently abnormal (E and F), making the whole structures highly porous.

Finally, the layer structures at the lateral flat region in the normal eggs and the Se homo-eggs were inspected by

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**Fig. 2.** Binocular views of the normal eggs and Se hetero-eggs featuring the anterior pole region (A), ventral region (B), posterior pole region (C) and dorsal region (D). In each panel, the top line shows the normal eggs and the bottom line indicates the Se hetero-eggs. Gray areas are highlighted by red arrows. Scale bar, 1 mm
SE (Fig. 6A and B). These were very similar to those of other regions (cf. Figs. 4 and 5, A/B and E/F); again the Se homo-chorion was remarkably porous with vertical lamellae. The Se hetero-eggs gave results resembling those of the normal, and thus illustrations were not shown.

**Fig. 3.** SE micrographs at the anterior pole (A to D) and lateral flat (E to H) regions of the normal eggs (A, B, E and F) and Se homo-eggs (C, D, G and H). A and C, micropyle on the outer surface; B and D, micropylar channels on the inside surface; E and G, polygonal network patterns; F and H, enlarged network patterns. Arrows in F and H indicate the aeropyles and various openings, respectively (see text for details). Scale bar, 10 μm.
The distinctness in manifestation of the White-sided egg (Se) gene between the heterozygotes and homozygotes was confirmed in terms of the surface and internal chorion morphology. As to the surface structures, the Se heterozygous phenotype showed no difference from the normal, but the homozygotes were abnormal in that the eggs had large wrinkles, numerous openings and lacked a smooth surface. The cross sectional structures of the Se chorion exhibited specific features mainly at the middle layer, particularly in the homozygotes. Such mutant micrographic traits were seen at the gray (opaque) regions, and rarely detected at the normal (transparent) areas of the mutant chorion (although Se hetero-eggs often showed half-abnormal lamellar structures at the middle layer as seen in Fig. 5D). Thus it can be concluded that the morphological traits of this mutation are due to structural abnormality of the surface and cross-sectional layers of the chorion. Embryos of Se homo-eggs are lethal and cannot hatch. Possibly, the porousness of the mutant chorion (see Figs. 3H and 6B etc.) causes the eggs to dehydrate. This inference is supported by our unpublished observation that the Se/Se chorion formed wrinkles, like those seen in Fig. 1C, two or three days after oviposition.

The best known, representative gray eggs are the Gr egg mutations, which include five spontaneous (Gr, Gr^p, Gr^col, Gr^im, Gr^K) and three X-ray induced (Gr^{X−1}, Gr^{X−2}, Gr^{X−i}) alleles (Fujii et al., 1998). The cross sectional structures, mainly at the middle layer, of the Gr^col (col-
lapsing egg) chorion exhibited many thin lamellae, which are irregularly arranged with strikingly cracked features (Sakaguchi et al., 1973). The middle layer of the Gr16 (European-16 gray) chorion consisted of oblique or disorganized lamellae and vertical lamellae (Gautreau et al., 1993). The internal morphology of the Se homozygous and/or heterozygous chorion looked like those of the Gr1 col and Gr16 mutations in many aspects; however the Se gene is not a member of Gr multiple alleles (Kei, 1943; Chikushi and Nagai, 1975).

The programmed formation of chorion architecture in B. mori has most extensively been investigated through the molecular and expression analyses of the multigene families localized to the Gr locus (e.g., Sakaguchi et al., 1973; Goldsmith and Basehoar, 1978; Goldsmith and Clermont-Rattner, 1979; Goldsmith and Kafatos, 1984; Lecanidou et al., 1986). Our previous (Kawaguchi et al., 1999, 2000) and present morphological studies confirmed that the wild type alleles of mgr, tsg and Se genes, whose loci are independent of the Gr gene members, are also needed for the completeness of lamellar structures. The establishment of an internal chorion layer during development may thus be highly sophisticated, polygenic processes, which should additionally be studied from the viewpoints of phenogenetics, molecular genetics and morphology.

![Fig. 5. SE micrographs of the cross-sectional structure at the dorsal (A, C and E) and ventral (B, D and F) regions of the normal eggs (A and B), Se hetero-eggs (C and D) and Se homo-eggs (E and F). o, m and i, the outer, middle and inner layer, respectively. Asterisks indicate the areas of disordered lamellae at the middle layer of the Se hetero-egg. Scale bar, 5 µm.](image-url)
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Fig. 6. SE micrographs of the cross-sectional structure at the lateral flat regions of the normal egg (A) and Se homo-egg (B). For other details, see the legend to Fig. 5. Scale bar, 5 µm.

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