Unique chorion structure of the mottled gray (*mgr*) egg, an egg-character mutation of *Bombyx mori* (Lepidoptera: Bombycidae)

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Abstract

The surface and internal chorion structures of the mottled gray egg (*mgr*) of *Bombyx mori*, a mutation which exhibits patched areas of gray or opaque regions, were observed in comparison with normal eggs using a scanning electron microscope. No peculiarity was exhibited in the mutant with respect to whole egg size, surface structures at the micropyle region and basic patterns as well as the average area of the network imprints (polygons) on the lateral flat region. When cross-sections of the chorions were observed, the middle layer of the *mgr* chorion corresponding to the gray areas was found to be deformed without a lamellar structure. Thus it can be concluded that the *mgr* alleles take part in the formation of the middle layer architecture during choriogenesis.

Key words: *Bombyx mori*, mottled gray egg, chorion mutation, chorion morphology

INTRODUCTION

The eggshell (chorion) of insects mechanically protects the ooplasm and embryo against water loss and pathogens, while permitting sperm entry and gas exchange through micropyles and aeropyles, respectively (Hinton, 1969). These complicated roles of the chorion are supported by the architectures constituted by scores of proteins secreted from the follicular epithelial cells (Margaritis, 1985). Morphological features of chorions in Lepidoptera have been reported for *Antheraea polyphemus* (Mazur et al., 1980; Regier and Kafatos, 1980), *Bombyx mori* (Omura and Kataoka, 1943; Akai, 1957, 1976; Sakaguchi et al., 1973; Kanda et al., 1974; Ohtsuki et al., 1977a, b; Ohtsuki and Kanda, 1978; Katsuno, 1984a, b; Kawaguchi et al., 1993) and *Bombyx mandarina* (Sakaguchi et al., 1990, 1998; Kawaguchi et al., 1996a, b).

B. mori has a number of egg-trait mutations with respect to the inner and surface structures of the chorion as well as its size, shape and color (Doira et al., 1992). In a stock strain (t15) maintained at Kyushu University, a spontaneous mutation was discovered wherein the chorion com-

prises transparent and opaque (milky-white) areas making patched patterns; this trait was inherited in a pseudo-maternal manner and named the mottled gray (*mgr*) egg (Sado, 1957). Later, it was demonstrated that *mgr* is linked with the *E^{Kp}* gene and is located at the position 30.0 cM on the 6th linkage group (Doira et al., 1984). In this study, the *mgr* chorion was observed for surface and cross-section architectures by scanning electron microscope. We found that the lamellate structure of the middle layer of the chorion was deformed in the *mgr* mutation.

MATERIALS AND METHODS

Insects. The silkworm stock strains used were d18 and p22, maintained at the Institute of Genetic Resources, Faculty of Agriculture, Kyushu University. The former contains the *mgr* gene and homozygous female adults produce mottled gray eggs. The latter is a standard strain which possesses the +*mgr* gene and deposits normal eggs. Larvae were reared on mulberry leaves at 25°C. Eggs were collected after oviposition, washed with distilled water and processed as described below.

Morphological observation of eggshells. To

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observe the surface structure, whole eggs were fixed in 2% glutaraldehyde dissolved in 0.1 M sodium cacodylate, pH 7.6, for up to 4 h at room temperature. To observe the cross-sectional structure, eggshells were torn by a watchmaker's forceps in distilled water. All samples were dehydrated in a graded series of ethanol, freeze-dried with *tert*-butyl alcohol, mounted on aluminum stubs with a double-stick carbon tape, sputter coated with gold and subjected to observation by a JSM 5200 scanning electron microscope at 15 kV.

Measurements of egg size and polygonal areas.

The average dimensions of eggs and the average areas of the polygons, i.e., the network patterns imprinted by the follicular epithelial cells from which the chorion proteins are secreted, were measured using photographs as described previously (Kawamura and Nakada, 1981; Kawaguchi et al., 1996a). Each value represents the average of 50 eggs from egg batches deposited by 5 moths (10 eggs per batch).

RESULTS

Apparent traits and dimensions of *mgr* eggs

The laterally flattened ellipsoidal shape of the *mgr* eggs was similar to that of the normal eggs (Fig. 1, top left and top right, respectively; see also the insert for normal). The lengths of the major and minor axes were 1.303 ± 0.019 mm and 1.080 ± 0.032 mm, respectively, in the mutant, while these values were 1.338 ± 0.012 mm and 1.089 ± 0.035 mm, respectively, in the normal. The lateral region of the mutant (1.037 ± 0.032 mm²) was slightly smaller than the normal (1.055 ± 0.061 mm²), although the difference was statistically insignificant. Thus, the mutant egg was almost normal in shape and size. However, the *mgr* chorion comprised patches of transparent areas and opaque/gray areas; the former appeared to be dark due to the serosal pigments whereas the latter was milky white due to the lower transparency of the chorion (Fig. 1, top left). This was in contrast to the normal egg, whose chorion was transparent throughout and appeared to be dark due to serosal pigments (Fig. 1, top right and insert). It is noteworthy that the *mgr* mutation showed various degrees of gray areas within one batch. These were roughly classified into A1, A2, B1, B2, C1, C2, D1 and D2, as

shown in Fig. 1 (bottom); A1 has faint and tiny gray areas, D2 has the largest gray areas and the other areas are intermediates whose mottledness varies from egg to egg.

Surface structures at the anterior pole and lateral flat regions

The anterior pole region of the eggshell had a micropyle with a single external opening surrounded by comparatively uniform petal-shaped patterns making a double-petaled rosette (Fig. 2, A and C), which, like the polygons, was imprinted by follicular epithelial cells. The size of the rosette was a little smaller in *mgr* (C) than the normal (A), although the overall features were similar to each other. The surface structure of the lateral flat region was a network of polygons (Fig. 2, B and D), the imprints of follicular epithelial cells. The boundaries between the polygons had ridges which reflect the cell junctions. The size of the polygon was 514.7 ± 29.6 μ m² in the *mgr* egg and 524.7 ± 18.9 μ m² in the normal egg. Namely, the shape and size of polygons did not differ between the *mgr* (D) and normal (B).

Internal structure of the *mgr* chorion

Figure 3A shows a cross-sectional structure at the lateral flat regions of the normal egg, composed of the three layers, outer, middle and inner. The outer layer (O) revealed a lamellate and smooth crust which was compactly integrated with a thinner strata. The middle layer (M), having regular and parallel lamellae, was the thickest and occupied most of the chorion layer. The thinnest inner layer (I) consisted of small columns oriented perpendicularly to the oocyte surface. Also lateral flat regions of the *mgr* chorions were observed. The translucent areas of the mutant, observed using group A1 eggs (see Fig. 1), had three layers (Fig. 3F) which resembled those of normal eggs. However, the opaque area of the mutant was found to be abnormal at the middle layer, which appeared to be crushed and had quite different features compared to normal chorion with regular and parallel lamellate architectures. Figure 3B shows an example with the most extensive mutant character (group D2). On the other hand, the *mgr* eggs belonging to groups B1 to C2 were normal at the upper

Fig. 1. Apparent features of mottled gray eggs deposited by the d18 strain and normal eggs deposited by the p22 strain. A (1 and 2), B (1 and 2), C (1 and 2) and D (1 and 2) denote the mutant eggs, roughly classified into 8 groups by the degree of the mutant gene expression. Insert, a magnified normal egg. Scale bar, 1 mm.

Fig. 2. Surface structures of anterior and lateral flat regions of the chorions observed by scanning electron microscope. A and B, normal egg (p22); C and D, *mgr* eggs (d18). A and C, micropyles at the anterior region; B and D, polygons at the lateral flat regions. Scale bars, 10 μ m (short) and 50 μ m (long).

parts of the middle layer with regular lamellae, but abnormal at the lower part (Fig. 3, C and D). Using group A2 to B2 chorions, the middle layer structure at the boundaries between the transparent and opaque areas was observed; expectedly, anomalous and regular structures coexisted (Fig. 3E).

DISCUSSION

The surface of the chorion exhibits spatially differentiated areas, i.e., lateral, ventral, dorsal, anterior and posterior, with the micropyle area and polygons (cf. refs. cited in Introduction). The present observations confirmed that the *mgr* mutant had no differences in surface structures. These facts indicate that the mottled gray character consisting of patches of transparent and milky-white areas is ascribed to the internal mor-

phology of the chorion of the three layers; in fact the cross sections of the mutant eggshell exhibited specific features at the middle layer, which had disordered and irregular forms without the lamellate structure at the opaque area. No specific traits were found at the outer and inner layers. It can therefore be concluded that the morphological traits of this mutation are due to a structural abnormality at the middle layer of the chorion. The mechanisms responsible for the occurrence of the various degrees of mottled areas remain unclear.

The highly specialized chorion proteins synthesized in and secreted from the differentiated follicular epithelial cells which surround the oocyte are coded by the multigene family (Matsuzaki, 1968; Kafatos et al., 1977; Mazur et al., 1980; Regier and Kafatos, 1980; Bock et al.,

Fig. 3. Cross-sectional structures at the lateral flat region of the chorion observed by a scanning electron microscope. A, normal egg (p22); B, C, D, E and F, *mgr* mutation egg (d18) belonging to the A1 to D2 groups as seen in Fig. 1 (bottom). B, opaque area of D2 egg; C and D, opaque areas of B1 and C2 eggs, respectively; E, transparent plus opaque areas of A2 egg; F, translucent area of A1 egg. All patterns were reproducible even when different groups of mutant eggs were used. O, M and I denote the outer, middle and inner layers, respectively. Scale bar, 8 μ m.

1986). These were identified to be *Ch-1*, *Ch-2* and *Ch-3*, which have been localized to three linked clusters belonging to the 2nd linkage group (Goldsmith and Basehoar, 1978; Goldsmith and Clermont-Rattner, 1979). The current finding confirmed that the *mgr* alleles also have a role in choriogenesis in terms of the formation of the stratified structure of the middle layer. Further study of this mutation from the viewpoints of protein and genetic constitutions will be of special importance in understanding gene expression in relation to choriogenesis.

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