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Protein Pattern of Mosaic Eggs for Normal and Small Egg Charater in *Bombyx mori*

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Females of ***Bombyx mori*** heterozygous for the ***Rg*** deletion and the *sm* gene tend to lay either normal or small eggs, a single female rarely produces both types of eggs. This is explained as due to S-type position effect manifested through the right break of ***Rg*** deletion situated just left of the *sm* locus. Only one case of the exception was observed after the examination of more than two thousand females. One female of the genotype *Rg*/*sm* produced irregular shaped eggs of normal size and small. Protein pattern of larger eggs was similar to that of normal eggs, whereas protein constitution of smaller eggs rather resembled to *sm* eggs. It is assumed that this exceptional female may involve, in her ovaries, groups of cells functionally normal and *sm* which were differentiated through V-type position effect.

INTRODUCTION

Genetic surveys on the retarded growth mutant (Rg) of the silkworm, ***Bombyx mori,*** have shown that ***Rg*** is not a gene mutation but a chromosomal deletion in the third chromosome (Chikushi ***et al.,*** 1971). Females heterozygous for ***Rg*** and ***sm*** (small egg) tended to lay either normal or small eggs, which was explained as due to a position effect manifested through the right break of ***Rg*** was situated just left of *sm* locus. Eggs laid by a single female showed uniform size, and the segregation of the normal and the small eggs occurred between different batches.

The authors have shown that *sm* ***eggs*** contain less amounts of V-FP and some other proteins compared with normal eggs (Kawaguchi and Doira, 1973). In the absence of normal alleles of *sm* the ovaries encounter difficulties in the incorporation of FP as well as other proteins into the egg cells from haemolymph.

The present paper describes the protein pattern of eggs, normal or small sized with irregular shapes, laid by a single female heterozygous for ***Rg*** and ***sm*** as an exceptional phenomenon. It also discusses the possible mechanism through which distinct variations are resulted in the size and shape of eggs laid by a single female.

MATERIALS AND METHODS

Bombyx eggs used in this experiments were laid by a female moth hetero-

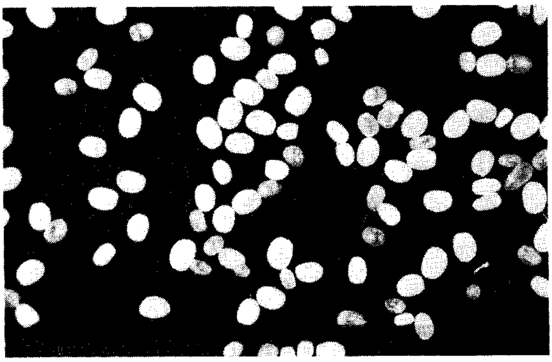

Fig. 1. Photograph of the eggs laid by a single female of the genotype *Rg* |,|*sm*, showing various sizes and irregular shapes.

zygous for *Rg* and *sm*; *Rg* is a deletion of the third chromosome segment ranging from 24.9 to 41.8 position, while the *sm* gene is recessive and inherited pseudo-maternally. The locus of *sm* is at 41.8 position on the third chromosome. More than two thousand females of *Rg*/*sm* were examined as for their egg-shape, all of them showed tendencies to produce either normal or small eggs as was reported by Chikushi *et al.* (1971). About one half of *Rg*/*sm* females produced normal eggs, whereas the other half produced small eggs. No female, but one, produced both types of the egg. An *Rg*/*sm* female laid, as an exceptional phenomenon, eggs of various sizes and irregular shapes (Fig. 1). The size of larger ones was comparable with that of normal eggs and the size of smaller ones was similar to that of *sm* eggs, though most of them showed such an irregular shape as of abnormal curvature or attenuation, truncation or notch in one end. Those irregularly shaped eggs were classified into large and small ones, and the protein patterns were separately examined by acrylamide gel disk electrophoresis. Clear haemolymph of this exceptional female was collected into glass capillary for electrophoretic analysis. After the bleeding the moth was dissected and the eggs of normal size, which were remained in ovariolcs at the vicinities of apical region of the organ, were collected. Eggs and adult's hacmolymphs of normal and *sm* genotype were also subjected to clectrophoresis. No centrifugation was attempted, instead crude homogenate of eggs in a small volume of cold saline (0.75 %) or crude haemolymph of adult female became sample solution. Running gel contained 6.5 % acrylamide. Direction of the run was toward the anode. After the migration was complete the gels were removed from the glass columns and stained for protein in a 0.5 % solution of coomasie blue for one hour, excess stain was soaked out in 10 % acetic acid.

RESULTS

Protein patterns of eggs and haemolymph obtained from female moths of the genotype +/*sm* and *sm*/*sm* are shown in Fig. 2. The former produced normal eggs and the latter small eggs, respectively. Gel-l represents that of normal

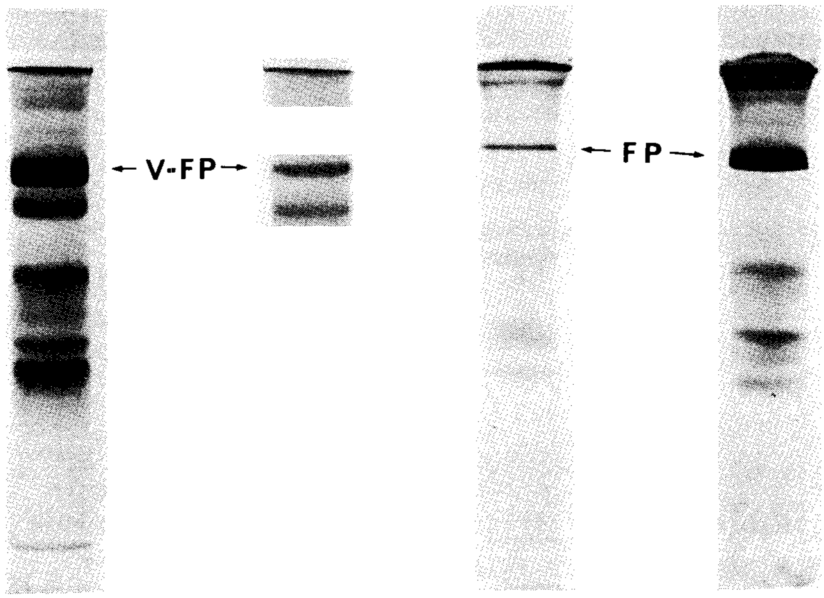

Fig. 2. Protein pattern of egg and adult's haemolymph obtained from females of normal and sm genotype. Samples were applied on the top of the gel and migrated towards the anode. (1) Normal egg. (2) *sm egg.* (3) Normal haemolymph. (4) *sm* haemolymph. V-FP : Vitelline female protein ; FP: Pupal female protein, sex-limited and vitellogenic protein of the pupal haemolymph.

eggs, vitelline-female protein (V-FP) which was incorporated from pupal haemolymph into the oocytes is clearly shown as the major component. Eggs of *sm* contained traces of V-FP and less amounts of moderately migrating proteins as compared with normal pattern (gel-2). Haemolymph of normal adults contained only traces of pupal female protein (FP) and some other proteins which migrated moderately (gel-3), whereas large amounts of such proteins as well as FP were accumulated in adult's hacmolymph of *sm* (gel-4).

Fig. 3 shows the protein pattern of eggs of large group (gel-l), small group (gel-2) and those collected from ovarioles (gel-3), respectively, all of which were produced by the single *Rg/sm* female as an exceptional phenomenon. Protein pattern of adult's haemolymph of the same animal is also demonstrated (gel-4). As is evident from the figures, eggs of normal sized groups, laid or remained in ovarioles, contained fairly large amounts of V-FP and H-protein, whereas only traces of V-FP and H could be detected in eggs of small size. Vitellogcnic FP was accumulated enormously in haemolymph of the animal even after the emergence rather than undergoing a normal decrease. H-protein could also be detected in the haemolymph of the adult.

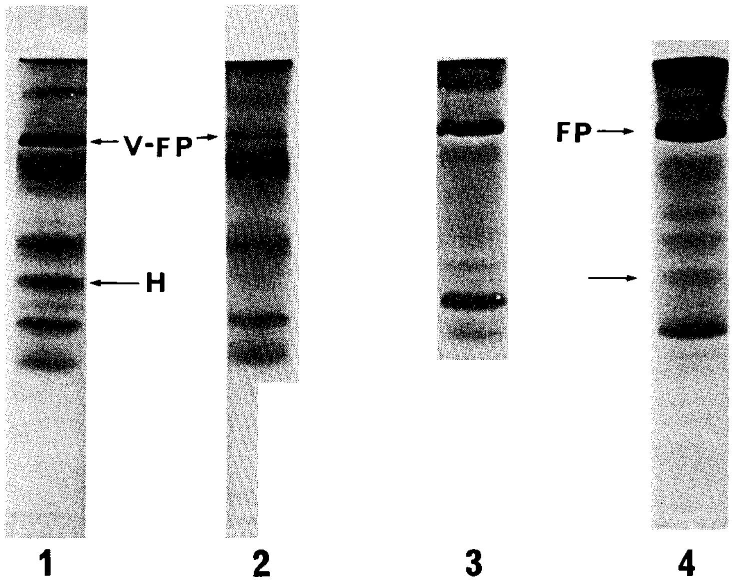

Fig. 3. Protein pattern of egg and adult's haemolymph obtained from the female of *Rg+/+sm* which produced various size and shape of the eggs shown in Fig. 1. (1) Eggs of larger group, normal size. (2) Eggs of smaller group, size comparable to *sm*-egg. (3) Normal-sized eggs, remained in the ovarioles. (4) Adult's haemolymph. V-FP : Vitelline female protein ; H : Non-sex limited protein of the pupal haemolymph, transferred to the oocytes or maintained in the haemolymph after the emergence.

DISCUSSION

As is well known, the egg shell and yolk are produced in the maternal body during pupal development, and therefore belong to a different generation from the embryo contained in the same egg. Eventually the size and the shape of eggs that are exclusively dependent on the shell tend to show a uniformity among those laid by a single female. Segregation of the traits of eggs, of which shells are concerned, occurs between different batches and not in a single batch, that is the phenomenon of "pseudo-maternal inheritance." Small egg character shows a typical pattern of this mode of inheritance.

Examination of two thousand females has shown that *Rg/sm* females tend to produce either normal or small eggs. Because there existed no difference between the normal egg forming females and the small egg forming females as for their genic constitutions, and because the right break of ***Rg*** deletion in the chromosome situated just left of the *sm* locus, observed segregation of the egg-type of the female moths is explained as due to a position effect belonging to the category of the S-type. S-type position effect comes into expression among individuals of the same genic constitution associated with a chromosomal break near a specific gene locus. Some individuals manifest normal phenotype, the rest manifest mutant phenotype of the concerned gene according to the case may be.

It has been reported that a mixoploid female consisted of 2n and 4n cells produces both normal eggs and large ones showing a wide range of egg-size with bi-modal distribution. Normal sized eggs are differentiated from 2n cells and large sized ones are from 4n cells. Ploidy of an oocyte was found to be closely correspondent with that of the surrounding follicle cells. Such mixoploid females were obtained by artificial parthenogenesis through heat treatment of unfertilized eggs (Hasimoto, 1953).

Present case of the exceptional *Rg/sm* female resembles to this type of mixoploid in the sense that a single female produces various sized eggs. It seems, however, that she was not a mixoploid one as the egg-size of larger group (LE in the following) was comparable to normal eggs derived from 2n cells and the size of smaller eggs (SE) was similar to those of *sm/sm* female. All the eggs laid were unfertilized, being impossible to perform progeny test.

Comparative analyses of the protein patterns of eggs or adult's haemolymph of the present exceptional female with those of normal and sm have provided an evidence to get insights as to the mechanism leading a *Rg/sm* female to produce irregularly shaped eggs of various size. Protein patterns of LE, especially that of laid ones, resemble much to the pattern of normal eggs, showing large amounts of V-FP, H and moderately moving bands. Whereas in SE only traces of V-FP and H were detected showing considerable similarities to the pattern of ***sm eggs*** rather than to that of normal eggs. H-protein was reported to be transferred from haemolymph to the egg yolk during pupal development similarly as in the case of V-FP (Kawaguchi and Doira, 1974). Large amounts of FP were detected in the haemolymph of the adult exceptional *Rg/sm* female. Further, H and some moderately moving proteins made an appearance in her haemolymph in higher concentrations when compared with normal egg forming females. To maintain proteins in high concentrations in adult's haemolymph is a characteristics for the ***sm*** females.

Protein patterns of normal or ***sm eggs*** are determined by the genotype of the ovaries (Kawaguchi and Doira, 1973). That indicates that cellular function of the ovaries determines the protein composition of the eggs as well as the egg-shape or egg-size.

We may safely infer, thus, that the present exceptional female involves two functionally differentiated groups of cells in her ovarian tissues through a certain mechanism similar to V-type position effect which cause variegated expression of normal and mutant phenotypes in a single individual accompanied by a chromosomal break. One group is affected by the ***sm*** allele and the other in which being manifested the action of normal allele of ***sm.*** Co-existence of these two types of cells in each group of follicle cells, which is responsible for the determination of the size or the shape of a single egg, might cause variance and irregularity of egg shape. When functionally normal cells were predominating in the follicles larger eggs would be resulted, whereas smaller eggs would be produced in the predominance of follicle cells affected by the *sm* gene. Functional differentiation of the oocytes into normal or sm might also be involved there. Irregularity of egg-shape may be caused by the patch of functionally normal cells on the background of cells functionally ***sm,*** or ***vice versa.***

Further studies of the ***Rg*** deletion and the ***sm*** gene are in progress, and may provide information as for the mechanism of position effect based on some striking evidences.

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