Sizes of Polygonal Patterns of the Egg-shell and Facets of the Compound Eye in the Tetraploid Silkworm, *Bombyx mori*

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The size of polygonal patterns of the eggs laid by tetraploid female adults and that of facets of the compound eye of tetraploid male adults, both obtained by supercooling eggs at early developmental stage, were compared with those of the normal diploids. When the individuals which hatched from treated eggs became adults, in some of them the size of polygonal patterns in the lateral, central part of the laid eggs were extremely large as compared with that of the diploid adults. It was clarified that these adults were 4n from the egg-color segregation by mating and from the number of somatic chromosomes of embryo in laid eggs. In the same manner, the male adults in which the size of facets of the compound eye was extremely large as compared to adults of diploid male were ascertained to be 4n. These outstanding features of the size of polygonal patterns of facets could be utilized for the distinction of tetraploid, and distinction could readily and accurately be made as compared to the usual methods.

Hitherto, in order to distinguish tetraploid adults from diploid adults the followings were used as criteria; comparing tetraploid females with diploid females, it was noted that the formers oviposited eggs of large size (Tamazawa, 1977), and comparing the fertility of tetraploid males with that of diploid males, it was found that the formers showed quite a low fertility (Kawaguchi, 1936). In addition, the ratio of egg-color segregation was used as a criterion in a certain species. However, even in the above methods, there were the cases in which the distinction of polyploidy cannot be made. In this study, the author investigated the new means by which the distinction between tetraploid male and female adults was made.

**Materials and Methods**

_The silkworm strains used for the materials:_  
In the present study, 2 strains of re9 (red egg, striped silkworm: re/re, +w/+w, p/p) and Twl (white egg, plain silkworm: +r/+r, w2/w2, p/p) were used. A group of F1 eggs of the cross re9 ♀×Twl ♂ at 120 to 150 minutes after deposition were treated by low temperature (−10°C) for 24 hours, then kept at 26°C.

_Observation of egg-shells:_ The surface structure of the central part of the lateral region (the part which the “mizubiki” was formed after deposition) was observed under a differential interference microscope.

_Observation of facets:_ The heads of F1 male adults were decapitated and transferred to 95% alcohol. Then, the central part of the surface of the compound eye was excised and facets were observed under a differential interference microscope.

_Egg-color segregation of F2:_ Utilizing the χ²-test, a comparison was run between the ratio of egg-color segregation of various mating types.
and the ratio of theoretical egg-color segregation of F₂ (egg-color segregation of F₂ in the case of F₁ adults which resulted from re9 φ × Twl δ were 2n and 4n).

Number of somatic chromosomes of embryo in F₂ eggs: Embryos of 4 days after egg laying were dissected in normal saline, treated in 0.5% KCl solution for 10 minutes, and fixed in 3 : 1 methanol-acetic acid. Each of the fixed embryos was placed on a slide glass separately, and chromosome preparations were made according to Takagi (1971).

Results

Observation of egg-shell: According of Taki-zawa and Tamazawa (1968), among the treated eggs, the eggs in which serosa cells were large were tetraploids. Therefore, in this study the eggs in which serosa cells were large were selected. As a result, among the selected eggs a large number of 4n eggs were included together with 2n eggs. In the next step, these selected eggs were used for rearing, 384 male and female adults were obtained respectively, and they were mated at random. According to Ohtsuki et al. (1977), the surface structure of the central part of the lateral region of the egg-shell consisted of network patterns. A unit of the network was enclosed with the projecting boundary and took the shape of a regular or irregular polygon.

Firstly, after separation of the copulating adults, F₁ adults of male and female were checked by batch. The polygons of F₂ eggs in each batch resulting from mating of these male and female adults were observed. As a result, it was found that these egg batches could be divided into batches of eggs with small polygons and those with large polygons (Fig. 1). Therefore, the F₁ adults of the female that oviposited the former eggs by batch were designated as A, and F₁ adults of the female that oviposited the latter eggs by batch were designated as B.

Observation of facet: It was found that the F₁ adults of male could be divided into adults with small facets and those with large facets (Fig. 2), therefore, the former F₁ adults of male were designated as A', and the latter designated as B'.

Thus, mating in random type of the F₁ adults of male and female could be written as follows, A φ × A' δ, A φ × B' δ, B φ × A' δ and B φ × B' δ. The F₂ eggs in this order of their mating type were expressed as AA', AB', BA' and BB'.

Egg-color segregation of F₂: It was surmised from Table 1 that AA' eggs were 2n, AB' and that BA' eggs were 3n. Therefore, it was sur-

Fig. 1. Polygons as a unit of network in the central part of the lateral region of the egg-shell in F₂ eggs. (a) small polygons; (b) large polygons. Scale (a, b):50 μm.
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The number of somatic chromosomes of embryo in F2 eggs: Ten batches were selected from AA', AB', BA' and BB' egg groups, respectively. In addition, from the respective ten batches eggs were selected at random, and the number of somatic chromosomes of embryo was investigated. The chromosome sets taken as the subject were 70 per each egg group. The number of somatic chromosomes of AA' eggs was found to be 56. While, that of AB' and BA' eggs was from 76 to 84, and that of BB' eggs was from 110 to 112 (Fig. 3). From these results, it was clarified that AA' eggs were 2n, AB' and BA' eggs 3n, and BB' eggs 4n. Therefore, A female and A' male adults were 2n, B female and B' male adults were 4n, and thus it was clarified that the polyploidy of F1 adults as surmised from egg-color segregation of F2 was correct.

From the above results, it was clarified that the female adults that oviposited eggs with small polygon were 2n and those that oviposited eggs with large polygons were 4n, and at the same time that the male adults with small facets were 2n and those with large facets were 4n.

Discussion

The size of polygons: According to Tamazawa (1977), the size of the eggs deposited by adults of 4n female was larger than that of adults of 2n female. Omura and Kataoka (1943) reported that the size of polygons was large in the following order of 4n, 3n and 2n, especially the size of polygons in 3n and 4n being markedly larger than 2n. The present report revealed that the female adults that oviposited eggs with large size of polygons were 4n, and that in contrast the female adults that oviposited eggs with small size of polygons were 2n. Moreover, since in the present study any female adults that oviposited eggs with small size of polygons were 2n.
Fig. 2. Facets in the central part of the compound eye in F1 adults of male. (a) small facets; (b) large facets. Scale (a, b) : 50µm.

Fig. 3. Somatic chromosomes of embryo in F2 eggs. (a) AA' egg (2n=56); (b) AB' egg (3n=84); (c) BA' egg (3n=84); (d) BB' egg (4n=112). Scale : 10µm.
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Posited eggs with an intermediated size of polygons was not found, it was entirely possible to distinguish accurately the polyploidy of female adults by observing the size of the polygons of the laid eggs. Further, even if the number of the laid eggs was only one per single adult, or even when the laid eggs were either fertilized or not, the polyploidy of the female adults could be distinguished by the size of the polygons of the laid eggs. The size of the polygons of the laid eggs can be judged readily and accurately under a dissecting microscope.

The size of the facets: Koyama (1954) reported the number and size of the facets which covered the outer surface of the compound eye of the silkworm. However, there is so far no report on the facets of polyploids. From the present study, it was clarified that male adults with large sized facets were 4n, and male adults with small sized facets were 2n. Furthermore, because no adults with intermediate sized facets were found, the polyploidy of male adults could be accurately distinguished by the size of the facets. In order to check the size of facets the observation must be done under a differential interference microscope or an ordinary light microscope, even though slightly more difficult than observing the size of the polygons of the laid eggs. Further, the polyploidy of female adults could be distinguished by the size of the facets as in the case of male adults.

Egg-color segregation of F2: The eggs of F2 are segregated into black, red and white in color. However, it was impossible to surmise the polyploidy of the eggs of each batch by the ratio of egg-color segregation, because the ratio of egg-color segregation of one batch did not often coincide with the ratio of theoretical egg-color segregation, and because the fertility of the adults of 4n male was low, and thus the female adults mated with the adults of 4n male oviposited only a limited number of eggs, and in addition many of the unfertilized eggs were formed. For this reason, the ratios of egg-color segregation of many of egg batches must be examined in order to get a standerd value for the distinction of polyploidy of F2 eggs. Further, it was noted that the ratio of egg-color segregation of BB' in Table 1 did not coincide with the ratio of theoretical egg-color segregation. This seemed to be due to the fact that distinction of fertilized and dead eggs was not clear.

The number of somatic chromosomes of the embryo in F2 eggs: The polyploidy of the F2 eggs could be distinguished by counting the number of somatic chromosomes of a small number of embryo in each egg batch. In addition, by checking the size of the polygons of F2 eggs, the polyploidy of the F1 adults of female which were used for mating and the polyploidy of the F1 adults of male used for mating could be distinguished. Regarding the distinction of the polyploidy of F2 eggs, this method was found to be easier than counting the number of somatic chromosomes of the embryo. The number of laid eggs by female adults mated with the adults of 4n male was usually quite low, and the polygon observation has an advantage of not sacrificing the eggs.

Applications of the distinction method: The method of distinction described here could be used not only when the polyploidy of male and female adults used in the mating was unknown, but also when the polyploidy of either male or female adult was unknown. Especially, in in-breeding when the ratio of egg-color segregation could not be used for distinguishing the polyploidy of the male and female adults, the present method would effectively be used for this purpose.

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References


勝野貞哉：家蚕4倍体の卵紋ならびに複眼小眼面の大きさについて

発生初期卵の過冷却処理によって得られた4倍体雄成虫の産下卵における側面中央部の卵紋。ならびに4倍体雄成虫の複眼小眼面について、それぞれの大きさを2倍体のそれと比較した。過冷却処理した卵から孵化した個体のかなには、成虫となって産下する卵の側面中央部の卵紋が、無処理の2倍体のものに比べて著しく大きいものがあった。この成虫は交配による卵色分離と、その卵に形成された胚子の体細胞の染色体数から4倍体であることが判った。また同様にして、雌成虫において複眼小眼面が2倍体より著しく大きいものは、4倍体であることを確かめた。上記のように、卵紋あるいは複眼小眼面の大きさにみられる特異性は、4倍体を従来の方法よりも簡単かつ正確に判定するために利用することができる。