Note on the formation and disintegration of yolk cell in the silkworm, *Bombyx mori* (Lepidoptera)

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In the eggs of *Bombyx mori*, the yolk undergoes a secondary yolk cleavage into large spherical masses at the stage of germ band formation, as in some of other lepidopterans (Kobayashi and Ando, 1988). Each spherule (here called yolk cell) contains one yolk nucleus within. During diapause, yolk cells tightly encapsulate the embryo, and yolk cells are speculated to function as a protection of the embryo in diapause against the coldness in winter. On the breaking of diapause, the yolk cells recede from the embryo, then the morphogenesis is re-activated with supply of nutritious substance from the yolk cells. Prior to hatching, yolk cells are disintegrated into or absorbed by the embyro (Toyama, 1902; Iwasaki, 1931).

The yolk cell is considered to be formed by the interaction of the yolk material produced by female and yolk nucleus originated from zygote nucleus. In this report, we compared the sizes of yolk cells in the eggs different in ploidy and/or size, to give some materials for the discussion on the mechanism concering yolk cell formation.

Materials and Methods

Two types of polyploid eggs were used in this study. One of the triploids was induced by hot water treatment (46℃, 18 min) of eggs 0-10 min after oviposition according to Yokoyama *et al.* (1990). In this triploid eggs, egg materials were produced by the diploid female, whereas the embryo and yolk nucleus were triploid. Another triploid eggs were obtained as follows. The tetraploid female was induced by refrigeration treatment (-10℃, 24 h) of eggs 2-3 h after oviposition (Tamazawa and Takizawa, 1977). Then, this tetraploid female was mated with the diploid male, and the triploid eggs were produced. In this triploid eggs, the egg materials were produced by the tetraploid female, whereas the embryo and yolk nucleus were triploid. The eggs were anatomized in a physiological saline, and yolk cells were measured. The size of embryos was estimated from the weight of newly hatched larva, since it is difficult to measure.

Results and Discussion

The egg size of the giant eggs and triploid eggs derived from 4n female were 10-15% larger than normal eggs (2n). On the other hand, the size of triploid eggs derived from 2n female was the same as normal eggs (Table 1). The newly hatched larvae from the giant eggs (*Ge*) and triploid eggs from 4n female moths were clearly heavier than the larvae of the control (2n), whereas the larvae hatched from the triploid eggs from 2n female moths was the same as control (2n).

The size of yolk cells in the triploid eggs derived from 2n female moths and the giant eggs (*Ge*) was almost the same as the control. On the other hand, the yolk cells of the triploid eggs from tetraploid moths were larger than those of the control (Table 2). Thus, we may conclude that the size of yolk cells formed during the germ band formation was determined by polyploidy of the mother moths, regardless of the size

Table 1 Egg size of polyploid and giant egg strain.

Crossing type ♀ × ♂	No. of egg examined	Length (mm)			Width (mm)		
		$\bar{X} \pm SD$	Max	Min	$\bar{X} \pm SD$	Max	Min
4n × 2n[1]	22	1.47 ± 0.04	1.53	1.39	1.12 ± 0.05	1.22	1.04
2n × 2n: hot water treatment[2]	25	1.25 ± 0.02	1.28	1.22	1.01 ± 0.01	1.02	0.99
2n × 2n: giant egg strain[3]	20	1.41 ± 0.02	1.44	1.37	1.16 ± 0.04	1.22	1.09
2n × 2n: control	20	1.28 ± 0.02	1.31	1.26	1.02 ± 0.01	1.05	1.00

[1]Triploid eggs were produced by tetraploid female induced by low temperature treatment (-10℃, 24 h) of the eggs 2-3h after oviposition.
[2]Triploid eggs were induced by hot water treatment (46℃, 18 min) of the eggs 0-10 min after oviposition.
[3]Eggs were obtained from the giant egg strain (*Ge*).

Table 2 Size of yolk cell in polyploid and giant egg strain.

Crossing type ♀ × ♂	No. of egg examined	Diameter (μm)		
		$\bar{X} \pm SD$	Max	Min
4n × 2n	550	90.5 ± 11.8	130.0	63.9
2n × 2n: hot water treatment	590	78.1 ± 11.4	117.0	52.5
2n × 2n: giant egg strain	600	76.7 ± 11.3	121.0	49.8
2n × 2n: control	400	77.3 ± 9.8	111.6	50.3

Table 3 Change in size of yolk cell in normal eggs.

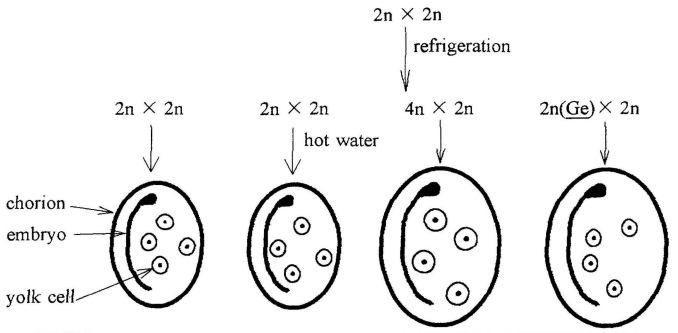
Days after breaking of diapause	Developmental stage	Diameter of yolk cell (μm)		
		$\bar{X} \pm SD$	Max	Min
1	Abdominal outgrowth appearance	78.6 ± 9.66	104.0	56.5
2	Shortening	75.9 ± 9.77	116.0	50.3
3	Head and thorax differentiation	74.1 ± 8.74	96.0	53.8
4	Revolution	65.1 ± 9.24	90.7	47.0
5	Completion of revolution	immeasurable		

by measurement of about 200 yolk cells.

of egg and embryo and the polyploidy of yolk cells (Fig. 1).

No significant changes were observed in the yolk cells until the breaking of diapause, and the disintegration of yolk cells commenced at the beginning of abdominal outgrowth. The yolk cells started to decrease in size, easily to break down after revolution (Table 3). The transformation of yolk nuclei which were adjacent to the embryo was especially remarkable at the stage of revolution (Fig. 2).

In conclusion, the formation of the yolk cells of the silkworm, *Bombyx mori*, may be controlled by the genotype of the mother moths, regardless of the genotype of embryos.



Egg size	normal	normal	large	large
Embryo size	normal	normal	large	large
Polyploidy of embryo and1 yolk cell	2n	3n	3n	2n
Size of yolk cell	normal	normal	large	normal

Fig. 1 Size of yolk cells in the polyploid eggs and the giant egg (*Ge*).

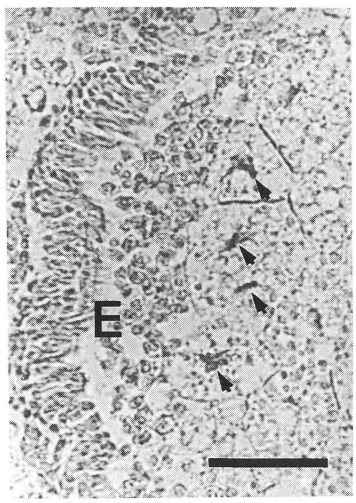


Fig. 2 Longitudinal section of embryo at the head-thorax differentiation. Arrowheads: yolk nuclei, E: embryo. Bar = 50 μm.

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