The detection of mosaics and polyploids in a hereditary mosaic strain of the silk moth, *Bombyx mori*, using egg colour mutants

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Summary

To analyze abnormal fertilization in a hereditary mosaic strain (mo/mo) of *Bombyx mori*, the percentages of diploidy mosaic, polyploidy mosaic and polyploid eggs in a batch were estimated by using egg colour mutants (pe re). Among 48 890 eggs from crosses of pe $+/+$ re, mo/mo females with pe re/pe re males, 9409 abnormal eggs were obtained; 4472 of them were diploidy mosaics (red-white eggs), 4038 were polyploids (black eggs) and 899 were polyploidy mosaics (566 black-white, 256 black-red and 77 black-white-red eggs). The total number of diploidy mosaic eggs was approximately equal to that of polyploid eggs. A significant correlation was detected between the diploidy mosaic and polyploid egg ratios within a batch. This suggests that diploidy mosaics are produced by double fertilization in which two genetically non-identical egg nuclei are fertilized in turn by a sperm, and polyploids are formed by the fertilization of a diploid, non-disjunctive egg nucleus gamete by a single sperm. Our results also indicated the presence of common factors modifying both mosaic and polyploid frequency. The concordance of the observed ratio of polyploidy mosaic eggs (1·84%) with the expected value (diploidy mosaic ratio × polyploidy ratio × 2 = 1·83%) suggests that the formation of mosaics occurs independently of the formation of polyploids in this abnormal fertilization process. We point out that it is necessary to modify Goldschmidt & Katsuki's general model to explain abnormal fertilization, and we propose several possible models.

1. Introduction

The female fertilization mutant gene *mo* in *Bombyx mori* provides us with valuable material for the study of mechanisms of female maturation division and fertilization. Katsuki & Akiyama (1926) reported the appearance of several mosaic larvae in a hereditary mosaic strain homozygous for the gene *mo*. In their genetical and cytological study, Goldschmidt & Katsuki (1927, 1928a, b, 1931) proposed that by suppression of polar body elimination, independent fertilization of each of the egg and second polar body nuclei gives rise to different parts of the mosaic embryo (Fig. 1, Tables 1 and 2). Almost all silkworm geneticists have accepted their double fertilization hypothesis as a general model to explain the formation of mosaics in this hereditary mosaic strain (Tazima, 1964).

After these studies, the appearance of polyploids (Hasimoto, 1934) and polyploidy mosaics consisting of both diploidy and polyploidy cells (Kobayashi *et al.* 1983) was reported in the mosaic strain in addition to diploidy mosaics consisting of diploidy cells. In
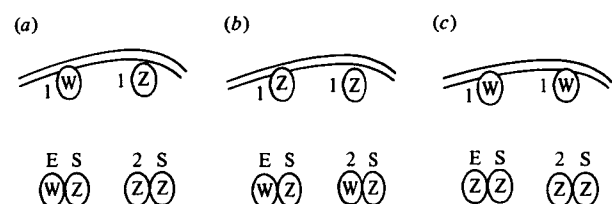
F: W/Z × M: Z/Z

Fig. 1. Goldschmidt & Katsuki's model proposed to explain the mechanism of abnormal fertilization in a hereditary mosaic strain of *B. mori*. F, Female; M, male; W, Z, sex chromosomes; E, egg nucleus; 1, first polar body nuclei; 2, second polar body nucleus; S, sperm pronucleus; a, gynandromorph; b, mosaic female; c, mosaic male.

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Table 1. *The models proposed to explain mosaic and polyploid formation*

(*a*) Meiotic division
    (1) Mixed-reduction
    (2) Post-reduction
    (3) Pre-reduction
(*b*) Female nuclei take part in double fertilization
    (1) Egg and second polar body nuclei
    (2) Two randomly selected nuclei
(*c*) Polyploid formation
    (1) Fusion of two fertilized nuclei
    (2) Fusion of one sperm with two or three egg nuclei
    (3) Fusion of one sperm with a non-disjunctional egg nucleus
        in second meiotic division
(*d*) Phenotypes of mosaics and polyploids (Table 2)
    (1) Segregational types made by two genetically identical egg nuclei
    (2) Non-segregational types (combined sex and somatic mosaic)
        made by two genetically non-identical egg nuclei
    (3) Recombinant type (sex or somatic mosaic) which is
        segregational type for one allele but also non-segregational
        type for the other allele

Goldschmidt & Katsuki's model: a-1, b-1, d-1-2-3
new models:
    (1) a-1, b-2, c-2, d-1-2-3
    (2) a-2, b-1, c-2, d-2
    (3) a-2, b-2, c-2, d-1-2
    (4) a-3, b-2, c-2, d-1-2

Table 2. *Chromosome constitution of egg, first and second polar body nuclei in the mixed-, pre- and post-reductional meiotic division*
Mating scheme: F: W/Z, $oc/+$ × M: Z/Z, oc/oc.

Egg*	Second polar body*	First polar body (1)	(2)
Pre-reduction (no mosaic*: segregational type)			
s 5	s 5	s 5	s 5
Post-reduction			
(sex and somatic mosaic*: non-segregational type)			
s 5	s 5	s 5	s 5
Mixed-reduction (no mosaic*: segregational type)			
s 5	s 5	s 5	s 5
(Sex and somatic mosaic*: non-segregational type)			
s 5	s 5	s 5	s 5
(Sex or somatic mosaic*: recombinant type)			
s 5	s 5	s 5	s 5
s 5	s 5	s 5	s 5

* Phenotypes of mosaics which are made by the fusion of each of the egg and second polar body nuclei with a sperm (Table 1). F, Female; M, male. Single and double lines indicate alternative homologues for (s) sex chromosome (W: single, Z: double; or W: double, Z: single) and (5) fifth chromosome (*oc*: single, +: double; or *oc*: double, +: single).

Goldschmidt & Katsuki's estimation of the frequencies of the three kinds of mosaics (sex mosaics: gynandromorph but not somatic character mosaic, somatic mosaics: somatic character mosaic but not gynandromorph, and combined sex and somatic mosaics) that appeared in the crosses, we (Ebinuma and Kobayashi 1986) pointed out an observational error since it was difficult to distinguish 'sex and somatic mosaics' from 'sex mosaics' or 'somatic mosaics' because there was no coincidence in time and place of expression of the markers they used to identify mosaic individuals (sex character: internal and external sex organs of the adult moth; somatic character: larval skin colour) (Ebinuma and Kobayashi, 1986). Moreover, a study which attempted to modify the frequency of mosaics by artificial selection (Ebinuma *et al.* 1983) indicated the presence of genetic factors modifying the action of the *mo* gene. Therefore, to investigate the mechanism of abnormal fertilization and the nature of modifiers present in this hereditary mosaic strain, we studied the percentages of diploidy mosaic, polyploidy mosaic and polyploid eggs in a given batch by using egg colour mutants (*w2*, *pe* and *re*).

2. Materials and Methods

These studies were conducted with the hereditary mosaic strain which had been maintained at The Sericultural Experiment Station, Tsukuba. We had selected this strain for high percentage of mosaic eggs in a batch (eggs from a single pair mating) by using the egg colour mutation *w2*. We then used this strain to make two new strains homozygous for *mo*: one homozygous for the egg colour mutations *w2* and *re*

and the other heterozygous for *pe* and *re*. The mutations used were: serosa colours: +*pe*, +*re*, +*w2* (normal, 5–0·0, 5–31·7, 10–16·1 dark brown in egg colour), *pe* (pinked-eyed white egg, 5–0·0, light orange in egg colour), *re* (red egg, 5–31·7, reddish brown in egg colour) and *w2* (white egg, 10–16·1, yellowish white in egg colour). As the *pe* and *w2* genes are epistatic to the *re* gene, the egg colours of *pe re* and *re*; *w2* are the same as those of *pe* and *w2*, respectively.

3. Results

Egg color mosaics

The serosa cells, a single-layer membrane lying underneath the chorion, are derived from cleavage nuclei and, unlike the chorion and yolk, their genotype is that of the embryo. Since non-diffusible substances are responsible for the serosa colour characters used in our experiments, we were able to detect a single mosaic egg whose serosa cells differ from normal with respect to their phenotype (Fig. 2). Therefore, we could more accurately estimate the percentages of exceptional phenotypic individuals appearing in the mosaic strain at the egg stage than in larval stages because there was no reduction in the frequency of certain phenotypes caused by differences in viability.

Fertilization

We crossed +*re*/+ +, *w2*/*w2*, *mo*/*mo* females to *pe re*/*pe re* males in order to confirm the fact that the formation of mosaics in this hereditary mosaic strain was due to double fertilization of two female pronuclei, as first noted by Goldschmidt and Katsuki (1927). The failure to detect black-white and red-white mosaic eggs which contained in their white areas cells repressing the chromosome set of the male or female gamete only indicated that there was neither androgenesis nor gynogenesis in the formation of mosaics (total number of eggs: 7334; number of mosaic eggs: 747 black-red, 0 black-white and 0 red-white) (Table 3).

Correlation

We detected diploidy mosaics and polyploids as non-disjunctional phenotypes of heterozygous marker alleles appearing in the cross *pe* +/+ *re*, *mo*/*mo* × *pe re*/*pe re* as follows. In Table 4, we show the genotypic models of abnormal eggs appearing in our hereditary mosaic strain. Because there is no crossing over in *Bombyx mori* females, polyploids may be detected as black eggs in which non-disjunction of heterozygous marker alleles (*pe* +/+ *re*) has occurred. Diploidy

Table 3. *Mating scheme to detect androgenesis and gynogenesis in abnormal fertilization*

Mating scheme:

$$ F: \frac{+\ \ re}{+\ \ +}, \frac{w2}{w2} \times M: \frac{pe\ \ re}{pe\ \ re}, \frac{+}{+} $$

Normal fertilization:

(a) Red egg
$$ \frac{+\ \ re}{pe\ \ re}, \frac{w2}{+} $$

(b) Black egg
$$ \frac{+\ \ +}{pe\ \ re}, \frac{w2}{+} $$

Abnormal fertilization*

(a) Black-red egg (diploidy mosaic)

(Black region)
$$ \frac{+\ \ +}{pe\ \ re}, \frac{w2}{+} $$

(Red region)
$$ \frac{+\ \ re}{pe\ \ re}, \frac{w2}{+} $$

(b) White egg

(Androgenesis)
$$ \frac{pe\ \ re}{pe\ \ pe}, \frac{+}{+} $$

(Gynogenesis)
$$ \frac{+\ \ re}{+\ \ re}, \frac{w2}{w2} \ \ \text{or} \ \ \frac{+\ \ +}{+\ \ +}, \frac{w2}{w2} \ \ \text{or} \ \ \frac{+\ \ re}{+\ \ +}, \frac{w2}{w2} $$

* Genotypes of polyploid and polyploidy mosaic were neglected; F, female; M, male.

Table 4. *Genotype model of mosaic and polyploid eggs in pe +/+ re,
mo/mo × pe re/pe cross*

Mating scheme: F: $\dfrac{pe\ +}{+\ re}$ × M: $\dfrac{pe\ re}{pe\ re}$

Normal fertilization:

(a) White egg
$\dfrac{pe\ +}{pe\ re}$

(b) Red egg
$\dfrac{+\ re}{pe\ re}$

Abnormal fertilization:

(a) Diploidy mosaic: red-white egg

(Red region) $\dfrac{+\ re}{pe\ re}$ (White region) $\dfrac{pe\ +}{pe\ re}$

(b) Polyploid: black egg

(Triploid)
$\dfrac{pe\ +}{+\ re}\ \dfrac{pe\ re}{}$

(Tetraploid)
$\dfrac{pe\ +}{+\ re}\ \dfrac{pe\ +}{pe\ re}$ or $\dfrac{pe\ +}{+\ re}\ \dfrac{+\ re}{pe\ re}$

(c) Polyploidy mosaic:

Black-white egg

(Black region) $\dfrac{pe\ +}{+\ re}\ \dfrac{pe\ re}{}$ or $\dfrac{pe\ +}{+\ re}\ \dfrac{+\ re}{pe\ re}$ (White region) $\dfrac{pe\ +}{pe\ re}$

Black-red egg

(Black region) $\dfrac{pe\ +}{+\ re}\ \dfrac{pe\ re}{}$ or $\dfrac{pe\ +}{+\ re}\ \dfrac{+\ re}{pe\ re}$ (Red region) $\dfrac{+\ re}{pe\ re}$

Black-white-red egg

(Black region) $\dfrac{pe\ +}{+\ re}\ \dfrac{pe\ re}{}$ (White region) $\dfrac{pe\ +}{pe\ re}$ (Red region) $\dfrac{+\ re}{pe\ re}$

F, female; M, Male; We neglect the genotypes of segregational types of mosaics and polyploids which are made by two genetically identical egg nuclei because we cannot detect them in this cross.

mosaics may be observed as red-white eggs in which white regions are *pe +/pe re*, red parts + *re/pe re*. The marker characters Goldschmidt & Katsuki used to distinguish mosaic individuals were expressed at a different time and place (sex character: internal and external sex organs of the adult moth; somatic character: larval skin colour). However, since the time and place of expression of the *pe* mutation is the same as that of *re* (egg colour), we can estimate the number of mosaic and polyploid eggs without having to correct for observational error caused by a lack of coincidence in the time and place of expression of these markers. Nevertheless, we obtained a poor fit to the expected 1:1 ratio of black-red and black-white egg mosaics ($\chi^2 = 156{\cdot}1$, d.f. $= 46$, $P < 0.1\%$). This is a different kind of observational error, arising because
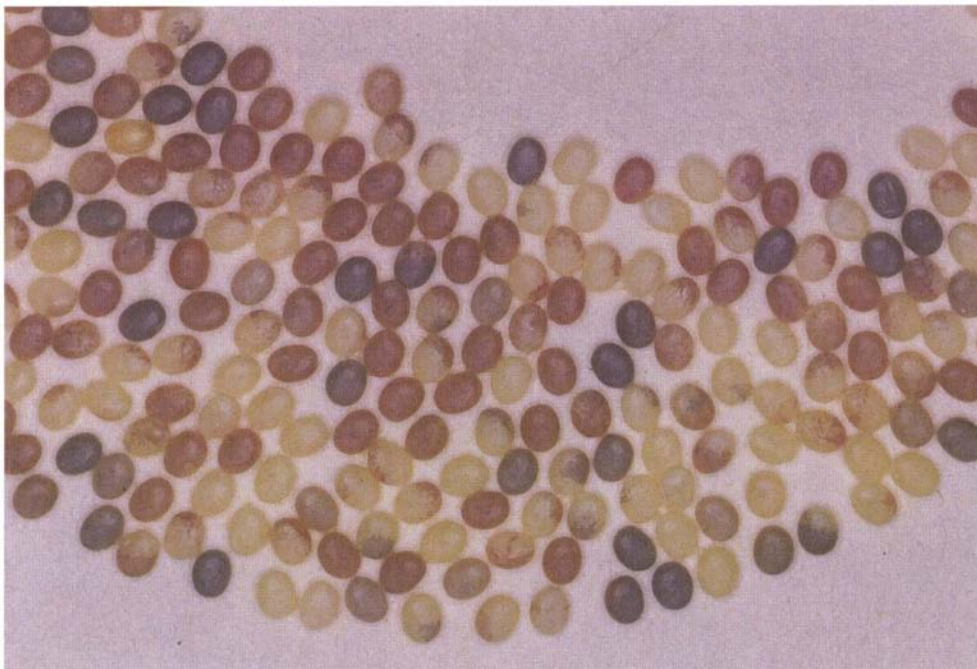
FIGURE 2. Mosaic and polyploid eggs in *pe +/+ re, mo/mo × pe re/pe re* cross. Red-white are diploidy mosaic eggs; black are polyploid eggs; and black-white, black-red and black-white-red are polyploidy mosaic eggs.

Table 5. *Correlation between mosaic and polyploid frequencies*
Mating scheme: *pe* +/+ *re, mo/mo* × *pe re/pe re* number of matings: 111, total number of eggs laid: 48,890 (Mean*: 440·45; M.S.: 58·33)

Penotype of eggs	Number of eggs			Percentages			
	Mean*	M.S.	Total	Mean	M.S.	(Min.,	Max.)
Diploidy mosaic (red-white)	40·29	24·26	4472	9·31	5·85	0·26	24·42
Polyploid (black)	36·38	16·36	4038	8·40	4·06	2·23	23·51
Polyploidy mosaic	8·10	6·21	899	1·84	1·42	0.00	6·68
(Black-white)	5·10	4·00	566	1·16	0·92	0.00	4·22
(Black-red)	2·31	2·32	256	0·52	0·50	0·00	2·29
(Black-white-red)	0·69	1·10	77	0·16	0·26	0·00	1·06
(Expected)				1·83	1·76	0·06	9·57
Total	84·77	39·56	9409	19·55	9·61	4·80	46·73

* All the eggs from a single pair mating (one batch); M.S.: mean square; expected: diploidy mosaic ratio × polyploid ratio × 2.

it is difficult to distinguish correctly small-red-spot black mosaic eggs from normal black eggs or small-black-spot red mosaic eggs from normal red eggs (Fig. 2, Table 5).

The percentages of mosaic and polyploid eggs varied from a few fractions to several per cent of the total number of eggs laid in a batch (Table 5). The total number of diploidy mosaic eggs was approximately equal to that of polyploid eggs and a significant correlation was detected between the mosaic and polyploid egg ratios in a batch (Fig. 3). These results indicated the presence of common factors modifying both diploidy mosaic and polyploid frequencies.

4. Discussion

In normal strains, only one sperm and one egg nucleus can take part in fertilization. In this mosaic strain, however, two kinds of abnormal fertilization appear to occur as follows. One is diploidy mosaic formation in which each sperm fuses with a single egg nucleus but two sperm and two egg nuclei take part in the fertilization of a given egg. The other is polyploid formation in which only one sperm takes part in fertilization events but it fuses with two or three egg nuclei. Diploidy mosaic formation would occur independently from polyploid formation in this abnormal fertilization process because the frequency of polyploidy mosaics (1·84%) which were formed when two such abnormal fertilization events occurred at the same time was be in accordance with the expected value (diploidy mosaic ratio × polyploidy ratio × 2 = 1·83%, $F = 0·005$) (Table 5). So these results suggest that in our strain selected for high mosaic frequency the factors modifying *mo* gene activity do not have specific effects on dispermic
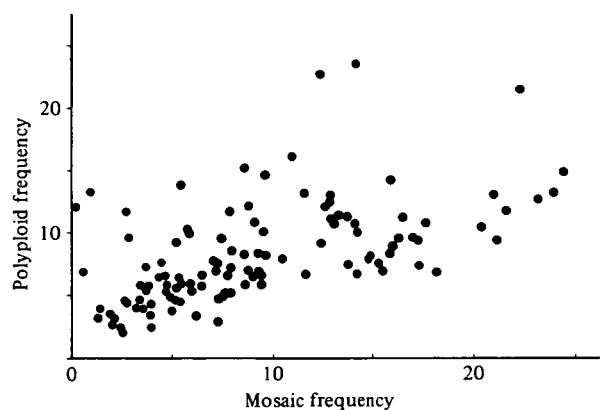

Fig. 3. Scatter diagram of transformed mosaic and polyploid egg ratios of a batch in the *pe* +/+ *re, mo/mo* × *pe re/pe re* cross. number of matings $(n) = 111$; coefficient of correlation $(r) = 0·555$***.

fertilization during mosaic formation. Rather, they are more likely to have general effects on the abnormal fertilization process before the determination of either diploidy or polyploid mosaic formation.

Goldschmidt & Katsuki (1928b) proposed that double fertilization in the mosaic strain was brought about by the fusion of each of the egg and second polar body nuclei with a sperm. In the case of post-reductional meiotic division, the first division is always equational with respect to the disjunction of the heterozygous marker alleles, since there is no crossing-over between the centromere and the marker gene loci in the female of *B. mori*. One of the egg and second polar body nuclei contains a Z-chromosome, the other a W-chromosome because of the female heterogametry. So fertilization of each of the nuclei by a sperm containing one Z-chromosome always results in a combined somatic and sex mosaic in the +/*oc*, *mo/mo* × *oc/oc* cross, used by Goldschmidt & Katsudi

Table 6. *Genotype of recombinant diploidy mosaic in mixed reductional division hypothesis and polyploidy mosaic in the* +/oc, mo/mo × oc/oc *cross.*

	Sex mosaic		Somatic mosaic	
	(Female region)	(Male region)	(+ Female region)	(oc Female region)
Diploidy mosaic	W/Z, +/oc	Z/Z, +/oc	W/Z, +/oc	W/Z, oc/oc
Polyploidy mosaic	W/Z/Z, +/oc/oc	Z/Z, +/oc	W/Z/Z, +/oc/oc	W/Z, oc/oc

(*oc*: larval skin character (chinese translucent, 5-40·8)). In a pre-reductional meiotic division, no mosaic occurs (Table 2).

To explain the appearance of three kinds of mosaics (sex mosaics, somatic mosaics, and sex and somatic mosaics) in the +/oc, mo/mo × oc/oc cross, they proposed the mixed-reductional meiotic division such that one of the sex and fifth chromosomes segregates in the post-reductional division and the other in the pre-reductional division, or alternatively, both chromosome pairs segregate in the same division (post-reduction or pre-reduction) owing to independent assortment of a heterozygous pair of marker alleles on each of the sex and fifth chromosomes at the first or second meiotic division (Table 2). We (Ebinuma and Kobayashi 1986) pointed out that the segregation ratio of three kinds of mosaics in their genetical study is not in accordance with expectation on the basis of completely independent assortment of each chromosome pair (sex mosaics:somatic mosaics:sex and somatic mosaics = 141:83:457 (Katsuki's observed data, 1927); ≠ 1:1:1 (expected ratio)) (Table 2).

In the +/oc, mo/mo × oc/oc cross, it is difficult to distinguish 'sex and somatic mosaics' from 'sex mosaics' or 'somatic mosaics' because of the markers used to discriminate mosaic individuals (sex character: internal and external sex organs of the adult moth; somatic character; larval skin colour). Also polyploidy mosaics could not be distinguished phenotypically from diploidy sex mosaics or somatic mosaics (Table 6). So we propose that if almost all diploidy 'sex mosaics' and 'somatic mosaics' which appeared in Katsuki's study (1927) were 'sex and somatic mosaics', it is possible to explain diploidy mosaic formation in the hereditary mosaic strain without the need to resort to a mixed reductional meiotic division, as follows. In the case of post-reductional meiotic division, diploidy mosaics (sex and somatic mosaics) are produced by double fertilization: the egg nucleus and the second polar body nucleus are fertilized in turn by a sperm (Table 1). In the mating scheme of our experiment, we only fail to detect the diploidy mosaics which are made by genetically nonidentical egg nuclei. The detection of tri-colour mosaics, however, indicates that first polar body nuclei also take part in fertilization in addition to egg and second polar body nuclei. This means that we cannot use only a mixed-but also post- and pre-reductional meiotic division to

explain a double fertilization in which the two egg nuclei are selected randomly from among the four egg nuclei (i.e. egg, two first polar body, and second polar body nuclei) (Table 1).

In conclusion, we discuss the methods to test the proposed hypotheses (Table 1). Randomly selected hypothesis can be tested in the cross of female homozygous and male heterozygous for a marker allele. Because we cannot detect only a mosaic (non-segregational type) which is made by genetically non-identical egg nuclei but also a mosaic (segregational type) which is made by genetically identical egg nuclei when two randomly selected egg nuclei take part in double fertilization. We can test the mixed-reductional hypothesis in the cross of female doubly heterozygous for unlinked marker alleles and male double homozygote, since we can detect a recombinant type mosaic (for one marker allele, segregational type but the other, non-segregational type) which is caused by recombination between unlinked marker alleles (Table 2). In this experiment, we cannot test three possible models proposed to explain polyploid formation because we can detect only a non-segregational type polyploid as a non-disjunctional phenotype of female's heterozygous marker alles. Hasimoto (1934) reported that tetraploids which appeared in the mosaic strain, consisted of three egg nuclei and a sperm. So the best explanation would be that one sperm fuses with two or three egg nuclei (model c-2 in Table 1).

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