Defects, Structure and Breakage of Translocated Chromosome in the Sex-Limited Yellow Cocoon Strain of the Silkworm, *Bombyx mori*.

By TAKAO NIINO

Division of Silkworm Breeding, Sericultural Experiment Station
(Shinjo, Yamagata, 996 Japan)

Introduction

In Japan in conventional silkworm rearing to harvest cocoons for reeling, F_1 hybrids, which produce cocoon materials with good quality and are healthy and strong, are used. To produce the F_1 hybrids, it is essential to separate the male from the female before the copulation, in order to secure mating with the registered variety. The sex can be discriminated in each stage by the differences between the male and the female in pupal weight, pupal body width, gonad primordia in the larva or pupa and growth which are determined by the sex-linked *os* gene.

Currently, the observation of the gonad primordia is carried out in pupa taken out of the cocoon. The discrimation requires a high degree of expertise and recently the number of experienced researchers has markedly decreased. Therefore it becomes necessary to develop another method of sex discrimination, based on the utilization of the gene controlling the marking of the W chromosome, which determines the female traits. There are several sex-limited silkworm strains: strains with sex-limited marking[4,7,8] (marking: female, plain: male), sex-limited black egg[9] (black egg: female, white egg: male), sex-limited yellow cocoon[2] (yellow cocoon: female, white cocoon: male) (Plate 1).

The race, N131 × C131, which is the first one in which sex-limited marking is present in both parents, was registered in 1967[4]. This race does not have any physiological defects associated with the translocation of the chromosomes and is used as breeding stock and material to breed new sex-limited marking races. The efficiency of sex discrimination by this method is three times higher than that by the observation of the gonad primordia in the pupa. But this method is associated with several problems, since the discrimination must be performed manually and accurately from the 4th to the 5th instar, which coincides with the busy period of rearing. Moreover the area required for silkworm rearing and mounting is twice as large.

However, in the sex-limited yellow cocoon strain, the efficiency of sex discrimination is 16 times higher than that of the observation of the gonad primordia in the pupa, and the discrimination can be performed automatically by mechanical differentiation of the color of the cocoon[3].

Original strain

The sex-limited yellow cocoon strain was developed by Kimura et al. (1971)[2] using a female individual induced by the translocation of the yellow blood (*Y*) gene to the W chromosome by γ-ray treatment (6000R). The expected translocated type was obtained in one batch out of the 4502 batches tested. This batch showed sex-limited inheritance for the cocoon color; white in the male and yellow in the female due to the coexistence of the cocoon color gene *C* without any partial detachment or separation of the translocated 2nd chromosome to the W chromosome. At

Plate 1. Sex-limited yellow cocoon strain
Yellow cocoon: Female, ZT (W ; 2) *Y*
White cocoon: Male

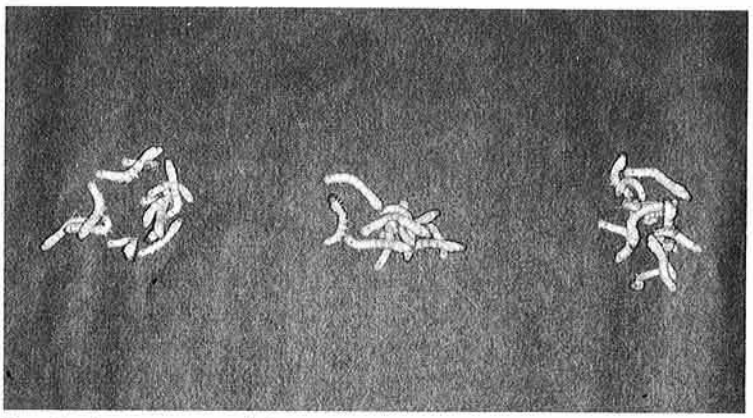

Plate 2. Breakage of $+^{i\text{-}lem}$ locus on the translocated 2nd chromosome

	Larval skin color	Genotype
Left:	Dilute lemon	ZT (W ; 2) *Y* ; *i-lem/i-lem* ; *lem/lem*
Center:	Deep lemon	ZT (W ; 2) $Y+^{i\text{-}lem}$; *i-lem/i-lem* ; *lem/lem*
Right:	Normal	ZW ; *i-lem/i-lem* ; *lem/lem*

that time, it was considered that the females of this strain showed fewer physiological defects associated with the extra chromosome than those of the other sex-limited strains. Since then, attempts have been made to improve the sex-limited yellow cocoon race for commercial purposes.

Physiological defects

The physiological defects in the females of the sex-limited yellow cocoon strains were investigated[5]. Comparison of the weight of the 5th instar larvae between females and males indicated that in the normal and original strains the weight of the female larvae was heavier than that of the males. However in the strains bred from the sex-limited yellow cocoon strain obtained originally, there were no differences between the weight of female and male larvae. Survival rates of pupae of these females were lower than those of the males in every rearing season. A large number of proctocele-larvae appeared in the females of these sex-limited strains in the early and the late autumn rearing seasons (Table 1). Pupal weight ratios between females and males in these strains were lower than those of the normal ones. The weight of the cocoon shell of the females of the parental strains, and hybrids between Japanese and Chinese strains, was lighter than that of the males, although in the normal strains the weight of the cocoon shell of the females was heavier than that of the males (Fig. 1). The percentage of normally oviposited batches of sex-limited yellow cocoon strains was lower than that of the normal strains. The reelability percentage of the cocoon of these females tended to be lower than that of the males.

These physiological defects were similar to those reported in individuals with the trans-

Table 1. Appearance of proctocele-larvae in the sex-limited yellow cocoon strains

Season	Spring (May–Jun.)		Early autumn (Jul.–Aug.)		Late autumn (Aug.–Sep.)	
Strain	♀	♂	♀	♂	♀	♂
	%	%	%	%	%	%
Original strain	—	—	0	0	—	—
Japanese strain						
HNSY 2	0	0	—	—	1.6	0
HNSY 5	0	0	0.8	0	—	—
HNSY 20	0	0	0	0	0.7	0
HNSY 21	0	0	1.2	0	2.4	0
FNSY 17	0	0	1.4	0	0.8	0
Chinese strain						
HCSY 3	0	0	9.2	0	47.2	0
HCSY 4	0	0	0.8	0	—	—
HCSY 6	0	0	—	—	13.3	0
HCSY 10	0	0	0.8	0	6.8	0
HCSY 20	0.1	0	0.8	0	32.0	0
HCSY 21	0	0	5.2	0	—	—
FCSY 17	0	0	0	0	—	—
HCSY 22	—	—	0	0	0	0
HCSY 23	—	—	0.8	0	0	0
Hybrids						
HNSY×HCSY*	0	0	0.8	0	1.4	0
HCSY×HNSY**	0	0	0.2	0	0.4	0

Each value is the average from 1–7 batches of each strain.
*, ** Each value is the average from several cross combinations.

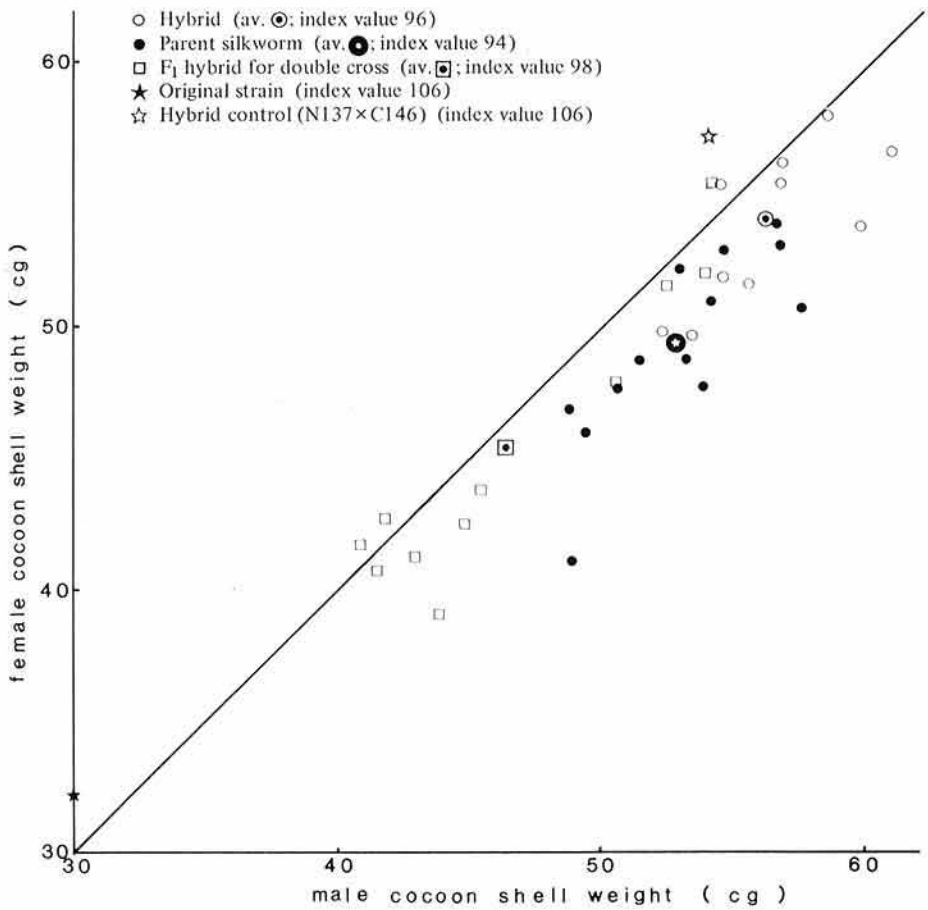

Fig. 1. Comparison of cocoon shell weight between females and males in the sex-limited yellow cocoon strains
Rearing season: Early autumn (Jul.–Aug.).
Each index value is calculated by (♀/♂)×100.

location of the autosome to the W chromosome[1,7-9], confirming the role of the translocation in the appearance of the physiological defects.

Structure of translocated chromosome

It is considered that in the physiological defects associated with the translocation of the 2nd chromosome to the W chromosome in the sex-limited strain the larger the fragment of the translocated chromosome, the more pronounced the physiological defects[7]. Therefore the structure of the translocated chromosome in the sex-limited yellow cocoon strains was investigated[5].

In order to confirm the presence of the *i-lem* locus on the translocated 2nd chromosome, the F_1 hybrid between the female of the sex-limited yellow cocoon strain (ZT(W; 2)*Y*;+/+; +/+) and the male of the *i-lem* strain (ZZ; *i-lem*/*i-lem*; *lem*/*lem*) was backcrossed to the male of the *i-lem* strain. The *i-lem* strain, of which the genotype is *i-lem*/*i-lem*; *lem*/*lem*, inhibits the manifestation of the *lem* gene. When the $+^{i\text{-}lem}$ gene is present, color of the skin of the larva in the progeny is deep lemon and normal in the ratio of 1 : 1 in the female and dilute lemon, deep lemon,

Table 2. Segregation of larval skin color in the cross between ZW̆II ; +/*i-lem* ; +/*lem* female and ZZ ; *i-lem*/*i-lem* ; *lem*/*lem* male

Mating type	ZW̆II ; +/*i-lem* ; +/*lem*			×	ZZ ; *i-lem*/*i-lem* ; *lem*/*lem*		
	♀				♂		
Skin color	Dilute lemon	Deep lemon	Normal		Dilute lemon	Deep lemon	Normal
Segregation	0	246	277		138	143	263

Skin color of segregated larvae was observed at the 3rd instar.

Table 3. Segregation of moths which laid collapsing eggs in the cross between ZW̆II ; +/Gr^{col} female and ZZ ; Gr^{col}/Gr^{col} male

Mating type	ZW̆II ; +/Gr^{col}	×	ZZ ; Gr^{col}/Gr^{col}
	No. of moths laying normal eggs		No. of moths laying collapsing eggs
Segregation	139		0

and normal in the ratio of 1 : 1 : 2 in the male. When the $+^{i\text{-}lem}$ gene is absent, color of the skin of the larva in the progeny is dilute lemon, deep lemon and normal in the ratio of 1 : 1 : 2 in both sexes. The results obtained are in agreement with the hypothesis presented previously, suggesting that the $+^{i\text{-}lem}$ gene is present on the translocated 2nd chromosome (Table 2).

To determine whether the *Gr* locus was present on the translocated 2nd chromosome, backcross experiment was performed. If the $+^{Gr}$ gene is absent, the female progenies of the cross between the female of the sex-limited yellow cocoon strain (ZT(W; 2)*Y*; $Gr^{col}/+$) and the male of the strain collapsing eggs (ZZ; Gr^{col}/Gr^{col}) should produce collapsing eggs and normal eggs in the ratio of 1 : 1. But the results obtained showed that all the female progenies laid normal eggs (Table 3), indicating that the $+^{Gr}$ gene was present on the translocated 2nd chromosome.

On the basis of these results, it was concluded that the fragment of the 2nd chromosome translocated to the W chromosome of the sex-limited yellow cocoon strain contained the loci of *Gr*, *Y* and *i-lem*, which have been mapped at the 6.9, 25.6 and 29.5 loci of the 2nd chromosome, respectively.

Breakage by γ-rays of the $+^{i\text{-}lem}$ locus on the translocated 2nd chromosome

To alleviate the physiological defects, γ-ray treatment was applied to cut off the fragment of the translocated 2nd chromosome between the *Y* and $+^{i\text{-}lem}$ loci[6]. To identify the breakage of the translocated 2nd chromosome, the *i-lem* locus was used as a marker gene. The *i-lem* strain was backcrossed to the female of the sex-limited yellow cocoon strain several times and the sex-limited yellow cocoon strain carrying the *i-lem* gene was used for the irradiation experiment. The genotype of this strain is ZT(W; 2)$Y+^{i\text{-}lem}$; *i-lem*/*i-lem*; *lem*/*lem* in the female and ZZ; *i-lem*/*i-lem*; *lem*/*lem* in the male, and the phenotypic expression of the larval skin color in the 3–4 instars is deep lemon and dilute lemon, respectively. A dose of 3,000R of ^{60}Co γ-rays was applied at a rate of 527R/hr (Experiment 1) and 507R/hr (Exp. 2). Three to 4-day-old pupae were irradiated.

The detailed results of the breakage test are presented in Table 4. Nine female individuals, which had the expected yellow blood and dilute lemon phenotype for the larval skin color, were identified out of 10,400 female

Table 4. Breakage of $+^{i\text{-}lem}$ locus on the translocated 2nd chromosome by treatment with γ-rays

	No. of pupae treated	No. of moths laying fertilized eggs	Hatchability (%)	No. of fertilized eggs used	No. of larvae observed				Total
					Female		Male		
					Yellow blood		White blood		
					Deep lemon	Dilute lemon	Deep lemon	Dilute lemon	
Cont.	60	54	88.9	11,880	5,502	0	0	5,784	11,286
Exp. 1	200	163	39.9	16,193	7,085	6	0	7,483	14,574
Exp. 2	80	57	56.2	7,292	3,306	3	0	3,400	6,709

Rearing experiments were performed for half a batch.

Table 5. Segregation of progeny of cross between ZT (W; 2) *Y*; *i-lem/i-lem*; *lem/lem* and ZZ; *i-lem/i-lem*; *lem/lem*

	Segregation			
	Female		Male	
	Yellow blood		White blood	
No.	Deep lemon	Dilute lemon	Deep lemon	Dilute lemon
1	0	51	0	60
2	0	26	0	55
3	0	51	0	60
4	0	31	0	32
5	0	15	0	62
6	0	89	0	99
7	0	51	0	62
8		Non-fertilized eggs		
9		Non-fertilized eggs		
Cont.	277	0	0	278

larvae observed. All the female larvae except these had the yellow blood and deep lemon phenotype for the larval skin color. In the non-irradiated controls, all the female larvae obsreved had the yellow blood and deep lemon phenotype. In the males, all the larvae observed had the white blood and dilute lemon phenotype. From these results, it appears that the 9 female individuals (new line) did not harbor the $+^{i\text{-}lem}$ gene on the translocated 2nd chromosome due to the breakage of the fragment carrying the $+^{i\text{-}lem}$ gene locus or due to DNA damage of the $+^{i\text{-}lem}$ gene locus. The disappearance of the $+^{i\text{-}lem}$ gene resulted in the change of the larval skin color in females from deep lemon to dilute lemon. The rates of detection were 8.5×10^{-4} (Exp. 1) and 9.1×10^{-4} (Exp. 2). These values were compatible with the mutation rate of the female young pupae, which was about 4.6×10^{-7} R/locus/basis[10].

Attempts were made to determine whether the new line produced females with the yellow blood and dilute lemon phenotype (Table 5 and Plate 2). In the female progeny of a cross between the female of this new line and the male of the *i-lem* strain with the ZZ; *i-lem/i-lem*; *lem/lem* genotype, there were individuals with the yellow blood and dilute lemon phenotype and the ZT(W; 2)*Y*; *i-lem/i-lem*; *lem/lem* genotype, while in the male progeny, there were individuals with the white blood and dilute lemon phenotype and the ZZ; *i-lem/i-lem*; *lem/lem* genotype. However, in the female progeny resulting from sib-mating of control animals, there were individuals with the deep lemon phenotype and the ZT(W; 2) $Y+^{i\text{-}lem}$; *i-lem/i-lem*; *lem/lem* genotype. These results indicated that the new line did not harbor the $+^{i\text{-}lem}$ gene on the translocated 2nd

chromosome.

Based on these results, it was concluded that the induction of the breakage of the $+^{i\text{-}lem}$ locus on the fragment of the translocated 2nd chromosome attached to the W chromosome had been successfully achieved and that the fragment was presumably attached to the W chromosome from the side of the *p* locus.

Future orientation of research

It is important to develop a commercial silkworm race of the sex-limited yellow cocoon as early as possible. Presently experiments on the rearing and silk reeling characteristic of the cross combinations between the sex-limited yellow cocoon strains are being conducted in the laboratory where the strains were bred and in the laboratories of the Sericultural Experiment Station. Among the crosses, some yielded a higher amount of cocoon crop than the control race and they displayed a good quality. However, these crosses are characterized by the lower survival rate of the pupae of the females and by that the weight of the female's cocoon shell is similar to that of the male's. These strains are improved to eliminate the defects by applying two methods. In the first method strains with few defects are used, that is, the values of the ratio of the weight of the cocoon shell between females and males are identical with that of the standard race and the survival rate of the pupae is higher. The second method consists of the breakage of the fragment of the translocated 2nd chromosome remaining on the *Y* gene, and shortening of the fragments to alleviate the defects. The new strains obtained in the breakage experiments of the $+^{i\text{-}lem}$ locus were backcrossed to the registered silkworm races, and the resultant new strains will be used as breeding materials.

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