SHORT COMMUNICATION

LINKAGE ANALYSIS OF THE FIBROIN GENE IN THE SILKWORM, *BOMBYX MORI*

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The occurrence of three fibroin phenotypes in the silkworm, *Bombyx mori*, has been demonstrated by comparing the electrophoretic mobility of fibroin large subunit on SDS-polyacrylamide gel (Hyodo, A. and Shimura, K. (1980) Japan. J. Genetics, **55**: 203-209). In the present paper, linkage analysis of the fibroin gene was performed by observing the fibroin phenotype, using J-131 silkworm strain and twenty-four silkworm stocks carrying genetic markers on each chromosome. The fibroin gene was found to be linked with the *Nd* gene locating on the 23rd chromosome.

In the previous paper (Hyodo *et al.* 1980), we reported that silk fibroin phenotype can be classified into at least three groups (F, M, and S phenotypes) by analyzing the mobility of fibroin large subunit on SDS-polyacrylamide gel electrophoresis. The symbols F, M, and S were used for fibroin phenotypes showing fast, moderate, and slow moving profiles of fibroin large subunit on the gel, respectively. Using this technique, we have carried out a linkage analysis of the fibroin gene in the chromosomes of *Bombyx mori*. In this paper, the structural gene of fibroin is given the symbol *Fib*.

All the silkworm strains used here were obtained from the Sericultural Experimental Station, the Ministry of Agriculture, Forestry and Fishery. As the materials carrying genetic markers on each chromosome, twenty-four genetic stocks possessing M or S fibroin phenotype were subjected to the genetic analysis. J-131 strain was used as a material of F variant with fast moving profile of fibroin large subunit.

Linkage analyses of the *Fib* gene were carried out by observing the fibroin phenotype in the F_2 generation or in the back cross of moth possessing recessive gene markers to the F_1 obtained from the crosses between moths of J-131 (this is referred to as normal silkworm) and each genetic stock. Silk fibroin was prepared mostly from the posterior silk glands of silkworm larvae at the 5th day in the 5th instar. In the case where *mln* or *so* mutant strains were used, its fibroin was prepared from cocoon.

Table 1. Results of linkage test of the *Fib* gene in the silkworm

Gene and linkage group	Mating		Segregation of characters					Remarks
	Female	Male	F	M	S	FM	FS	MS
Sex (1)	(M♀ × F) F ₁					♀ 10 ♂ 10		Independent
<i>p</i> (2)	(p S × +M) × +M			<i>p</i> 8 + 5				<i>p</i> 2 + 4 ibid
<i>Ze</i> (3)	(Ze M × +F) × +F		<i>Ze</i> 7 + 5			<i>Ze</i> 3 + 5		ibid
<i>L</i> (4)	(L M × +F) × +F		<i>L</i> 5 + 7			<i>L</i> 5 + 3		ibid
<i>oc</i> (5)	(oc M × +F) × oc M			<i>oc</i> 11 + 10		<i>oc</i> 13 + 14		ibid
<i>E</i> (6)	(E M × +F) × +F		<i>E</i> 3 + 5			<i>E</i> 7 + 5		ibid
<i>st</i> (8)	(st M × +F) <i>inter se</i>		<i>st</i> 1 + 4	<i>st</i> 3 + 4		<i>st</i> 8 + 13		ibid
<i>I-a</i> (9)	(I-a M × +F) × +F		<i>I-a</i> 11 + 9			<i>I-a</i> 8 + 11		ibid
<i>w</i> ₁ (10)	(w ₁ M × +F) × w ₁ M			<i>w</i> ₁ 6 + 3		<i>w</i> ₁ 4 + 7		ibid
<i>K</i> (11)	(K M × +F) × +F		<i>K</i> 10 + 10			<i>K</i> 10 + 9		ibid
<i>es</i> (12)	(es M × +F) × es M			<i>es</i> 11 + 6		<i>es</i> 12 + 5		ibid
<i>ch</i> (13)	(ch M × +F) × ch M			<i>ch</i> 12 + 9		<i>ch</i> 12 + 15		ibid
<i>U</i> (14)	(U S × +F) × +F		<i>U</i> 4 + 5				<i>U</i> 8 + 8	ibid
<i>Nd-s</i> (14)	(Nd-s S × +F) × +F		<i>Nd-s</i> 5 + 4				<i>Nd-s</i> 9 + 9	ibid
<i>bl</i> (15)	(bl M × +F) × bl M			<i>bl</i> 3 + 4		<i>bl</i> 7 + 6		ibid
<i>cts</i> (16)	(cts M × +F) × cts M			<i>cts</i> 5 + 6		<i>cts</i> 4 + 7		ibid
<i>bts</i> (17)	(bts S × +F) × bts S				<i>bts</i> 6 + 8		<i>bts</i> 4 + 4	ibid
<i>mln</i> (18)	(mln S × +F) × mln S				<i>mln</i> 1 + 5		<i>mln</i> 5 + 5	ibid
<i>nb</i> (19)	(nb S × +F) × nb S				<i>nb</i> 1 + 9		<i>nb</i> 11 + 11	ibid
<i>oh</i> (20)	(oh S × +F) × oh S				<i>oh</i> 2 + 3		<i>oh</i> 0 + 0	ibid
<i>rb</i> (21)	(rb M × +F) × rb M			<i>rb</i> 8 + 5		<i>rb</i> 2 + 5		ibid
<i>or</i> (22)	(or S × +F) × or S				<i>or</i> 10 + 10		<i>or</i> 10 + 10	ibid
<i>Nd</i> (23)	(Nd M × +F) × +F		<i>Nd</i> 0 + 23			<i>Nd</i> 8 + 0		Linked
<i>so</i> (26)	(so S × +F) × os S				<i>so</i> 8 + ND*		<i>so</i> 2 + ND*	Independent

* ND: Not determined.

Fig. 1. Electrophoretic patterns of fibroin extracted from the posterior gland (1, 6, 7, 8), the middle gland (3, 4, 5) and cocoons (2), showing the linkage between the fibroin, *Fib*, and the *Nd* genes on the chromosome 23 in the silkworm. 1, J-131 strain; 2, *Nd* strain; 3-5, *Nd* larvae in BF_1 ; 6-8, Normal larvae in BF_1 .

Fibroin of *Nd* mutant (Rayo) was obtained from the posterior part of the middle silk gland.

The results of linkage analysis of the *Fib* gene are shown in Table 1. In all cases except for the crossing with the naked pupa (*Nd*), two protein bands derived from both parental strains appeared without linking to the marker gene on each chromosome. On the contrary F and FM type appeared only in + and *Nd* larvae, respectively, and no recombinated phenotypes, *Nd* F and + FM, were observed in the BF_1 generation of crossing, (*Nd* M $\times$ +F) $\times$ +F, because there is no crossing over in the female of the silkworm (Tanaka 1914). Fig. 1 shows the fibroin phenotypes displayed by SDS-PAGE in this BF_1 generation. In the gels of the *Nd* gland, contamination of many sericin fractions was observed because the proteins were extracted from the middle silk gland. However, M type appeared in *Nd* strain (Fig. 1 lane 2) and FM type in the *Nd* larvae segregated (Fig. 1 lane 3-5), although normal larvae possessed

only F type (Fig. 1 lane 6-8). These segregation in the BF_1 generation clearly demonstrated the linkage between the *Fib* and the *Nd*, and thus the structural gene of fibroin can be said to be located on chromosome 23. In the present investigation, we did not examine the determination of recombination value to find the exact region of the *Fib* gene on chromosome, because no other marker gene has been found in chromosome 23 (Tazima *et al.* 1974).

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LITERATURE CITED

- Hyodo, A., and K. Shimura, 1980 The occurrence of hereditary variant of fibroin large subunit in the silkworm, *Bombyx mori*. Japan. J. Genetics **55**: 203-209.
- Tanaka, Y., 1914 Sexual dimorphism of gametic series in the reduplication. Trans. Sapporo Nat. Hist. Soc. **5**: 61-64.
- Tazima, Y., H. Doira, and H. Akai, 1974 The domesticated silkmoth, *Bombyx mori*. In "Handbook of Genetics" (R.C. King, ed.) Vol. 3, pp. 63-124. Plenum Publishing Corp., New York.