カイコにおける血液エステラーゼ遺伝子の連関分析

誌名	The Japanese journal of genetics
ISSN	0021504X
巻/号	532
掲載ページ	p. 129-131
発行年月	1978年4月

JAPAN. J. GENETICS Vol. 53, No. 2: 129-131 (1978)

SHORT COMMUNICATION

CHROMOSOME MAPPING OF THE BLOOD ESTERASE GENE IN THE SILKWORM, *BOMBYX MORI*

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Received December 9, 1977

In non-specific esterase appeared in the hemolymph of the silkworm, *Bombyx mori*, four fundamental types, A, B, C and O, have been demonstrated by agar gel electrophoresis and their expression are controlled by codominant allelic genes, Bes^A, Bes^B, Bes^C and Bes^O (Eguchi *et al.* 1965). However, the location of this gene on chromosome still remains obscure. In the present investigation, the author examined the linkage relation of the *Bes* gene and determined its location on the chromosome.

The *Nd-s* strain (No. 803) was used as a material of null allele of blood esterase, Bes^O. As the materials carrying genetic markers on the chromosome, three genetic stocks, No. 919 (p^M *nb or*), No. 941 (*UK*) and No. 943 (p^M *bp mp*), possessing the Bes^A allele were used. All the silkworm stocks have been cultured in the Sericultural Experiment Station (cf. Chikushi 1972). For examining the recombination value between the *Bes* and the *mp* genes, the Bes^O *mp* homozygote strain was used in crossing after its establishment by selection.

The linkage analysis of the *Bes* gene was carried out by observing the esterase phenotype in hemolymph in the F_2 generation or in the back cross of the moth of null allele to the F_1 obtained from the crosses between moths of null and normal esterase activity which has the genetic markers as described above.

The detection of esterase activity was carried out on a filter paper (3MM; Whatman). Three μl of hemolymph was spotted on a filter paper with a micro pipet (Microdispenser; Drummond Sci. Comp., Pa.) and the paper was incubated in 0.2 M Tris-citrate buffer (pH 6.8) containing 0.05% α-naphthyl acetate and 0.125% naphthanil diazo blur B for 10 min at 37°C. The detection of enzyme activity on the filter paper is simple and is capable of analysing the linkage of enzyme loci genetically in a large number of individuals. Larval hemolymph was mainly used for the esterase assay, but pupal hemolymph was also used for the recombination experiment with the *mp* (micropterous wing) gene because there were no differences in the zymogram of esterase between larval and pupal hemolymph by polyacrylamide gel electrophoresis, as shown in Fig. 1.

As the result of linkage analysis of the *Bes* gene with marker genes located in five different linkage groups, the *Bes* gene was found to be linked with the *K* (knobbed) and the *mp* genes belonging to the 11th linkage group of the silkworm, as shown in Table 1.

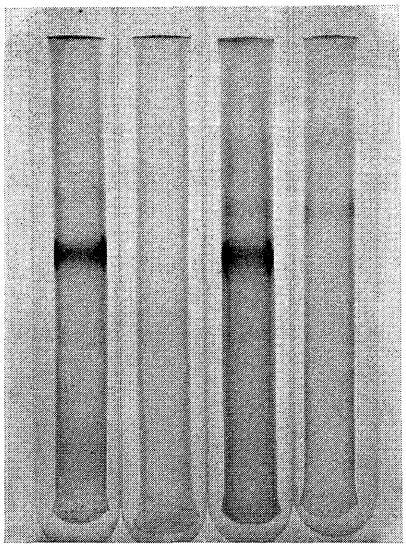

Fig. 1. Zymograms of non-specific esterase in larval and pupal hemolymph of the silkworm displayed by disc electrophoresis on 5% polyacrylamide gel (Davis 1964).
L) 4-day-old 5th instar larvae, P) 3-day-old pupae,
A) Bes^A, O) Bes^O.

Table 1. Linkage test of the *Bes* gene

Linkage group	Mating	Segregation of phenotype								Remarks	Recombination value (%)
		A				*O*					
2	$p^M/+$, *A/O inter se*	p^M	5	+	3	p^M	10	+	2	independent	—
11	*K A/+O inter se*	*K*	263	+	5	*K*	6	+	80	linked	6.22
11	*mp O/mp O*×*mp O/+A*	+	286	*mp*	76	+	69	*mp*	259	linked	21.02
14	*U/+*, *A/O inter se*	*U*	40	+	5	*U*	13	+	2	independent	—
19	*nb/+*, *A/O inter se*	*nb*	2	+	6	*nb*	3	+	9	ibid	—
22	*or/+*, *A/O inter se*	*or*	1	+	7	*or*	4	+	8	ibid	—

Recombination values for *Bes-K* and *Bes-mp* were observed to be 6.2 and 21.0%, respectively. Since the distance between the *K* and the *mp* loci has been given as 24.0 (Hirobe *et al.* 1947; Tazima *et al.* 1975), it can be considered from these recombination value that the *Bes* locus is located on the plus side of the *K* gene (0.0).

Further, a three-point experiment for the recombination among the *Bes*, *K* and *mp* genes clearly demonstrated that these three loci were arranged in the order of *K-Bes-mp* on the 11th chromosome, and the recombination values were calculated to be 11.06 and 18.49% for *K-Bes* and *Bes-mp*, respectively (Table 2). Recombination value between the *K* and the *mp* genes obtained from this experiment was higher than the established one, 24.0%. Similarly high value of recombination between the *K* and the *mp* genes was also obtained in our another recombination experiment for the *pnd* (pigmented

Table 2. Determination of recombination values among the *Bes*, *K* and *mp* genes by three-point experiment in the cross of $(+\ O\ mp/+\ O\ mp)\times(+\ O\ mp/K\ A\ +)$

Character / Batch	Non-crossovers		*K-Bes* crossovers		*Bes-mp* crossovers		Double crossovers		Total
	KA+	*+Omp*	*KOmp*	*+A+*	*KAmp*	*+O+*	*KO+*	*+Amp*	
1	145	159	22	20	50	34	0	0	430
2	98	83	12	21	24	18	0	1	257
Total	243	242	34	41	74	52	0	1	687

Recombination values calculated are as follows, *K-Bes*: 11.06%, *Bes-mp*: 18.49%, *K-mp*: 29.26%.

non-diapausing) gene on the 11th chromosome, and the distance between these two gene loci will be revised as 28.6% (Yamamoto, Gamo and Hirobe, in press in Japan. J. Sericult. Sci. 47(3)). Therefore, the value between the *K* and the *Bes* genes should be adjusted to give 28.6 for *K-mp*, and then the value was determined to be 10.7%.

It can be concluded, from the present investigation, that the esterase locus in the silkworm hemolymph is located on the 11th chromosome in the order of *K*(0.0)-*Bes*(10.7)-*mp*(28.6).

The author wishes to express his sincerest gratitude to Dr. H. Doira in Kyushu University for his valuable advices of this investigation, and also to Dr. Y. Kuroda in the National Institute of Genetics for his kindness in reading and correcting the manuscript.

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