Biochemical Genetics and Its Application to the Breeding of the Silkworm

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Biochemical genetics in the silkworm, *Bombyx mori*, has first been studied on the amylase in blood and digestive juice[26]. Cocoon color of the silkworm has also been studied extensively from the biochemical standpoint, and genes relating to the metabolisms of tryptophan and pteridine have further been studied[22].

According to the development of separation technique of proteins and enzymes, a lot of variants showing different mobility on gel electrophoresis have been demonstrated in some enzymes and proteins. These variants are useful for genetic mapping by determining cross-over frequencies in appropriate hybrids of genetic markers, and the localization of gene loci in some enzymes and proteins in the silkworm has been determined. Genes of biochemical interest are listed in Table 1[35]. Gene frequencies in the polymorphic loci revealed by gel electrophoresis are providing the analysis of phylogenetic differentiation of silkworm varieties. In the present paper, the author will present some results of phylogenetic analysis on the basis of gene frequencies in polymorphic loci of enzymes and proteins, the effects of these polymorphic variants on the quantitative characters of the silkworms and also on the gene loci encoding silk proteins, fibroin and sericin.

Differentiation of silkworm varieties on the basis of protein polymorphism

Geographic varieties of the silkworm which have been obtained from the world sericultural area are characterized in many genetical traits as shown in Table 2[32]. Polymorphic loci of isozymes and protein also show different gene frequencies among these geographic varieties[9,37]. Polymorphic variations in these enzymes and proteins are shown in Fig. 1. Gene frequencies in these loci observed on the geographic varieties of the silkworm reared as a stock culture in the Sericultural Experiment Station are shown in Table 3.

Genetic distances between six geographic varieties classified by geographic area and voltinism were calculated by the sum of chords of angular transformation according to the method of Cavalli-Sforza and Edwards[1]. From the genetic distances, some possible rooted trees of phylogenetic differentiation of the silkworm were proposed and length in each segment in these trees was calculated by multiregression analysis, and a phylogenetic tree was reconstructed as shown in Fig. 2. According to this tree estimated here, silkworm varieties are considered to be differentiated first into two ways and one branch further differentiated into Chinese and European univoltine varieties, while the other into two branches: Southeast Asian vs. Chinese bivoltine and Japanese varieties.

Chang[2,3], however, estimated the differentiation of silkworm varieties on the basis of historical records or archaeological evidences and considered that the domesticated silkworm was differentiated from the polyvoltine wild silkworm. Therefore, it is better to consider that the silkworm was differentiated into present uni- or bivoltine varieties from polyvoltine one.

The results of these phylogenetic analysis can be applied to the selection of F_1 hybrid

Table 1. Genes of biochemical interest in the silkworm

	Gene	Map locus	Effect
1)	Amino acid metabolism		
	rb	21–0.0	Accumulates 3-hydroxy kynurenin due to the absence of kynureninase.
	sku	22–7.1	Accumulates *iso*-valeric acid.
	w_1	10–12.7	Accumulates kynurenin in egg and eye due to the absence of kynurenin 3-hydroxylase.
	w_2	10–16.1	Accumulates 3-hydroxy kynurenin in egg and eye.
2)	Pteridins and uric acid metabolism		
	lem	3–0.0	Accumulates sepiapterin.
	og	9–7.4	Low activity of xanthine dehydrogenase.
	Sel	27–	Accumulates sepialumazine.
3)	Isozymes		
	ae	8–2.8	Amylase in digestive juice.
	be	8–4.2	Amylase in hemolymph.
	Bes	11–24.2	Esterases in hemolymph.
	Ies	Unknown	Larval integument esterases.
	Ees	Unknown	Egg esterases.
	Aph	Unknown	Alkaline phosphatase in midgut tissue.
	Bph	Unknown	Acid phosphatase in larval hemolymph.
4)	Hemolymph proteins		
	Alb	19–6.2	Small protein migrates faster towards anode on gel electrophoresis.
	Lp-s	20–6.2	Small lipoprotein with slow mobility.
	Lp-m	20–6.2	Small lipoprotein with moderate mobility.
	Lp-f	20–6.2	Small lipoprotein with fast mobility.
	Pt-3	14–	Protein migrates faster next to the Alb zone.
5)	Silk proteins		
	Fib	23–	Large subunit of fibroin.
	Src-2	11–0.0	Sericin with secondary slow mobility.
	flc	3–49.0	Low synthesis of fibroin.
	Nd	23–0.0	Absence of fibroin synthesis.
	Nd-s	14–19.2	Low synthesis of fibroin.
	$Nd\text{-}s^D$	14–19.2	Low synthesis of fibroin, induced by DES.
6)	Glutinous proteins in mucous gland		
	Ng	12–21.8	Absence of glutinous protein.
7)	Chorion proteins		
	Gr	2–6.9	Deficiency of protein components in chorion.
8)	Color of cocoon and hemolymph		
	C	12–7.2	Yellow cocoon due to the transmittance of carotenoids from hemolymph.
	F	6–13.6	Reddish yellow cocoon in the outer layer of cocoon.
	Pk	Unknown	Pink cocoon.
	Ga	Unknown	Green cocoon due to the presence of flavonoids.
	Gb	15–	Green cocoon.
	Gc	7–7.0	Green cocoon.
	Y	2–25.6	Yellow hemolymph due to the transmittance of carotenoids from midgut tissue.

showing the highest heterotic effect. In Japan, usual F_1 hybrids for sericulture are produced by the crossing between Japanese and Chinese inbred strains. From the result of this experiment, Chinese univoltine strain may be better to be used for one of the parent strains of hybridization than Chinese bivoltine one. Hirobe[18] described that European univoltine strains have the highest general combining ability in some quantitative characters of cocoons. Hirata et al.[17], however, observed that survival rate of silkworm larvae is the

Table 2. Difference in main characters among geographical races of silkworms

	Japanese	Chinese	European	Tropical
Egg	Serosa color, greyish purple. Chorion color, white. Occasionally colored non-hibernating egg, dead egg after body pigmentation stage and white rot egg.	Serosa color, palely greyish purple. Chorion color, light yellow. Scarcely white rot egg.	Large egg. Hatching unsynchronous.	Light yellow color egg. Weak in wintering-over.
Larva	Normal marking. Many redripening and yellowish molters. Rather slow growth. Susceptible to viruses, N[1] and F[2]. Insensitive to mulberry leaf quality.	Plain marking. Many yellow-ripening silkworm and whitish molters. Comparatively fast growth. Susceptible to muscardine. Insensitive to temperature.	Body shape, slender. Many red-ripening and yellowish molters. Very slow growth. Susceptible to pebrine and virus C[3]. Sensitive to unfavorable condition.	Plain marking. Body, small and slender. Very rapid growth. Susceptible to muscardine. Very strong against diseases.
Cocoon	Bale shape. White. Occasionally double cocoon. Thick fiber in size and short in length.	Elliptical or spherical shape. White or yellow. Thin fiber in size and long in length.	Long elliptical shape. White or flesh color. Abundant in sericin. Heavy cocoon shell weight. Thick fiber in size.	Spindle shape. White. Light green. Great quantities of floss. Light cocoon shell weight. Thin fiber in size. No double cocoon.
Voltinism	Univoltine Bivoltine	Univoltine Bivoltine	Univoltine	Polyvoltine

1) N: Nuclear polyhedrosis, 2) F: Flacherie, 3) C: Cytoplasmic polyhedrosis

highest in the crossing between Japanese and Chinese bivoltine strains. Therefore, the results of phylogenetic analysis can not be applied directly to the selection of F_1 hybrids showing the highest combining ability, and further study is necessary.

Effects of genotypes in polymorphic loci on quantitative characters

Effects of the genotypes in biochemical loci on the quantitative characters of the silkworm were examined on blood protein (*Alb*), digestive amylase (*ae*) and two isozymes.

1) Albumin protein in larval haemolymph

F- and S-individuals segregated at F_2 generation of crossing between two local strains possessing F and S proteins were isolated after testing the *Alb*-phenotype displayed by gel electrophoresis, and several quantitative characters were observed by the rearing of larvae in their offspring[6].

Significant effects of *Alb*-genotypes were observed in two quantitative characters, cocoon weight and cocoon shell weight (Tables 4 and 5), although there were no effects observed on the other characters. Values of the both characters were highest in the group of *F/F* genotype, which was followed by *F/S* or *S/F* and *S/S*. Amounts of F- and S-proteins in albumin zone on polyacrylamide gel were determined by densitometric scanning of gels during the larval and pupal stages. Two proteins, F and S, were existent in almost constant concentration during the whole developmental stages observed. Physiological role of this protein remains unclear. However, proteins migrated with the same mobility on electrophoretic gels as the albumin protein are

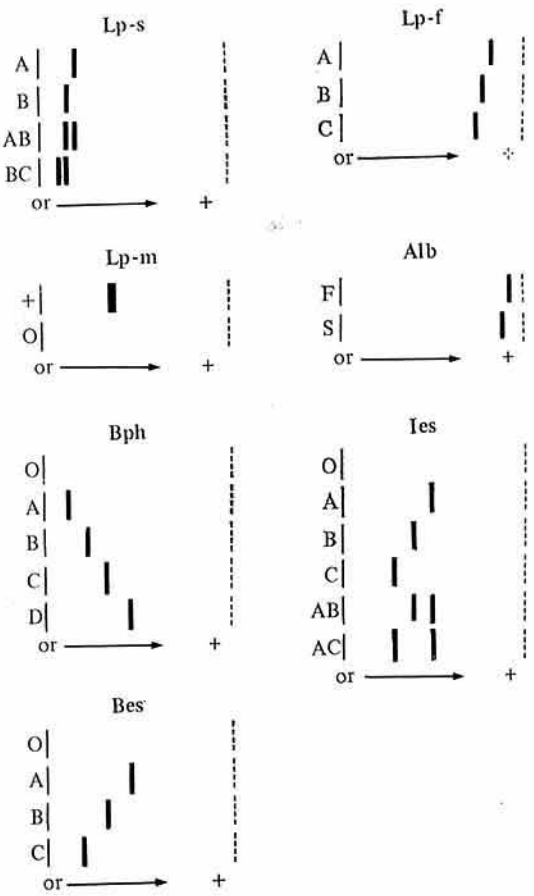

Fig. 1. Diagrammatic representation of polymorphic variations in the loci of four blood proteins and three isozymes in the silkworm. (cf. Table 1 for gene symbol)

known to have some effects on the haemomuscular reaction of silkworm larvae[36]. Therefore, it is better to consider that this protein has some effects on the quantitative characters of the silkworm.

2) Digestive amylase

In the locus of digestive amylase of the silkworm, it is known that two allelic genes exist showing null (*ae*) and high (*Ae*) activities[26]. The *Ae* gene was further classified into some allozymes with different mobilities by gel electrophoresis[13]. The null mutant gene, *ae*, is distributed in many varieties of the silkworm randomly[14,27]. Therefore, digestive amylase can be considered to have no effects on the growth and survival rates of the silkworm.

As the result of analysis of relationship between amylase activity in digestive juice and quantitative characters[15], silkworms with low amylase activity (*ae*) usually showed high cocoon and cocoon shell weights than those with high activity (*Ae*) when the larvae were reared on usual mulberry leaves (Table 6). However, *Ae* larvae showed high values in the both characters, when the larvae were reared on hardened leaves in the late autumn. Digestivity in *Ae* larvae also showed high values than in *ae* ones (Table 7). Most of the recent improved silkworm strains in Japan have low amylase activity in digestive juice and it is considered that they are carrying the *ae* gene. However, two types, low and middle, of amylase activity probably caused by allozymic effect are segregating in these improved inbred strains, and the larvae showing the middle activity cause high values in quantitative characters such as pupation rate, cocoon and cocoon shell weights[16].

These results give the conclusion that amylase in digestive juice has the beneficial effects on some quantitative characters, especially its high activity is effective on the survival rate when the larvae are reared on hardened leaves. Recombination of the *Ae* allele into improved commercial races will give the improvement of dietary efficiency in the silkworm.

3) Proteinase in digestive juice

Proteinase in digestive juice is an essential enzyme for the proteolytic digestion of mulberry leaves, and larvae in all silkworm races show high activity. Racial variation in proteolytic activity in digestive juice is small and there is no null mutant in this enzyme[5,28]. Only allozymic variations by gel electrophoresis have been demonstrated[4].

A selection experiment on the proteinase activity in digestive juice has been carried out[5]. Response to its selection was low, although the activity changed gradually as the selection proceeded, and it can be considered that the proteinase activity in digestive juice

Table 3. Gene frequencies in several loci of blood proteins and isozymes characterizing six geographical varieties

Alleles		Geographic varieties					
		J_1	J_2	C_1	C_2	E	SEA
*Lp-s**	*A*	.029	0	.413	.069	.191	.138
	B	.029	0	.067	.167	0	.333
	AB	.942	1.00	.520	.712	.809	.528
	BC	0	0	0	.052	0	0
*Lp-m**	+	.965	.940	.909	.975	.825	.885
	O	.035	.060	.091	.025	.175	.115
*Lp-f**	*A*	.441	.240	.382	.371	.682	0
	B	.401	.665	.580	.333	.318	.666
	C	.158	.095	.038	.296	0	.334
*Alb**	*F*	.515	.623	.242	.526	.161	.442
	S	.485	.377	.758	.474	.839	.558
*Bph***	*O*	.085	.080	.109	.121	.334	.132
	A	.012	0	.073	.034	.083	0
	B	0	0	.122	0	0	.130
	C	.547	.742	.293	.552	.416	.327
	D	.366	.178	.403	.293	.167	.411
*Bes***	*O*	.303	.240	.133	.172	.172	.333
	A	.562	.630	.769	.760	.760	.667
	B	.025	.023	.049	.017	.017	0
	C	.110	.107	.049	.051	.051	0
*Ies***	*O*	0	0	.038	0	0	0
	A	0	0	.038	0	0	0
	B	.129	.189	.453	.266	0	.133
	C	.710	.642	.264	.466	.333	.300
	AB	.032	.019	.150	.111	0	.133
	AC	.129	.150	.057	.157	.667	.434

* Gamo and Otsuka (1976)
** Yoshitake (1968)
*** J_1: Japanese univoltine, J_2: Japanese bivoltine, C_1: Chinese univoltine, C_2: Chinese bivoltine, E: European, SEA: Southeast Asian (tropical)

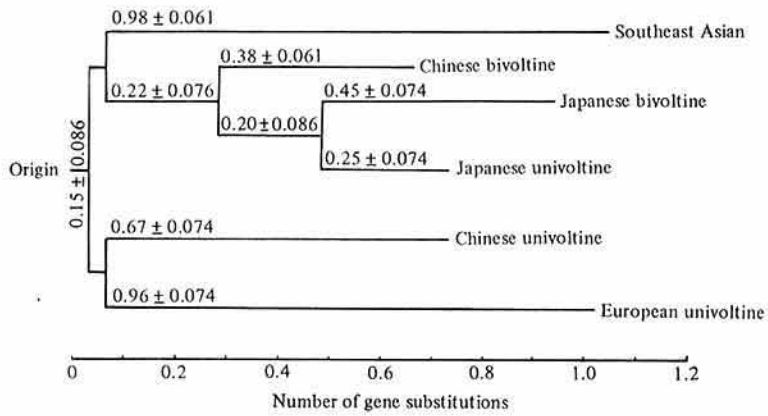

Fig. 2. Tree of silkworm evolution reconstructed from the matrix of genetic distances

Table 4. Effects of albumin genotypes upon the cocoon weight

Strain	Genotype	♀ 1	♀ 2	♂ 1	♂ 2	Mean
		g	g	g	g	g
ok	*F*/*F*	1.68	1.97	1.28	1.57	1.63
×	*F*/*S*	1.65	1.82	1.19	1.43	1.51
Kaijyo	*S*/*F*	1.56	1.76	1.19	1.34	1.47
	S/*S*	1.51	1.68	1.15	1.29	1.41
Tenryu-	*F*/*F*	1.35	1.74	1.16	1.36	1.41
seihaku	*F*/*S*	1.32	1.62	1.13	1.32	1.35
×	*S*/*F*	1.32	1.69	1.03	1.32	1.35
Kaijyo	*S*/*S*	1.25	1.69	1.03	1.31	1.32

1; Late autumn in 1968, 2; Spring in 1969
Cocoon weight in parent strains; Kaijyo (S) > ok (F) & Tenryu-seihaku (F)

Table 5. Effects of albumin genotypes upon the cocoon-shell weight

Strain	Genotype	♀ 1	♀ 2	♂ 1	♂ 2	Mean
		cg	cg	cg	cg	cg
ok	*F*/*F*	22.6	27.1	21.0	26.4	24.3
×	*F*/*S*	22.0	25.2	21.1	24.3	23.2
Kaijyo	*S*/*F*	21.0	24.8	19.7	23.0	22.2
	S/*S*	20.1	24.3	19.5	22.6	21.7
Tenryu-	*F*/*F*	17.8	24.0	19.4	23.1	21.1
seihaku	*F*/*S*	18.1	22.8	18.8	21.9	20.5
×	*S*/*F*	17.7	23.1	18.0	22.4	20.4
Kaijyo	*S*/*S*	16.7	22.6	16.8	22.0	19.6

1; Late autumn in 1968, 2; Spring in 1969
Cocoon shell weight in parent strains; Kaijyo (S) > ok (F) & Tenryu-seihaku (F)

Table 6. Effects of genotypes in digestive amylase on some quantitative characters

Leaf condition	Genotype of amylase	Pupation rate (%)	Cocoon wt (g)	Cocoon shell wt (cg)
Normal	*Ae*/*Ae*	92.0	2.04	38.5
	ae/*ae*	89.2	2.12	40.5
Hardened	*Ae*/*Ae*	63.6	1.19	17.2
	ae/*ae*	43.2	1.12	13.8

Season and year tested: late autumn in 1968
Race used: D24(+) ×WG(+), D24(−) ×WG(−)

Table 7. Effects of genotypes in digestive amylase on dietary efficiency of the silkworm

Genotype of amylase	Amount of leaf ingested (g)	Amount of leaf digested (g)	Digestivity (%)
Ae/*ae*	403.0	133.8	33.2
ae/*ae*	397.5	126.1	31.7

Races used: D24(+) ×Kenpaku, D24(−) ×Kenpaku

of the silkworm is controlled by polygenic genes. Proteinase activity has no significant effect on quantitative characters of tse silkworm. However, proteinase activity seems to show a negative correlation with the activity of digestive amylase, and the F_1 larvae produced by the crossing moths of high amylase and of high proteinase were well developed on an artificial diet[28].

Effects of proteinase activity in digestive juice on the quantitative characters of the silkworm can be said to remain unclear at the present time. However, increase of proteinase activity by its selection perhaps contributes to the improvement of dietary efficiency of silkworm larvae.

4) Isozymes in larval haemolymph

Effects of genotypes in the esterase (*Bes*) and the acid phosphatase (*Bph*) on quantitative characters of the silkworm were examined[11]. However, no relations between genotypes in two isozymes and many quantitative characters were observed. From these results, the genotypes in most isozymes can be said to have no relations with quantitative characters and selection experiments on the isozymic polymorphism will not be effective to the improvement of silkworm characters.

5) α-ketoglutaric acid in larval haemolymph

Concentration of α-ketoglutaric acid in larval haemolymph of the silkworm has been reported to have significant correlation with the quantitative characters such as cocoon and cocoon shell weights[23]. Therefore, the

colorimetric determination of this chemical in larval haemolymph at the 5th instar seems to be applicable to the improvement of silkworm races, especially for the selection of F_1 hybrids showing the highest combining ability.

Gene loci encoding fibroin and sericin proteins

Silk thread is composed of two classes of proteins, fibroin and sericin, synthesized in the different sections of silk gland[8,24]. Existence of variant polypeptides in both silk proteins has been found in some silkworm strains[7,19,33], and allows mapping of the corresponding genes.

1) Fibroin genes

Fibroin is composed of two polypeptides, one large (360K) and one small (26K), linked by disulfide bonds[29,30,34]. In the large subunit, some genetic variants encoding the different sizes of polypeptides have been demonstrated and linkage analysis was carried out using these variants.

As the results, the *Fib* gene encoding the large subunit of fibroin protein was found to be located at the adjacent region of the *Nd* (naked pupa) gene on chromosome 23[20]. On the other hand, some variants migrating with different mobility on polyacrylamide gels were found in small subunit of fibroin protein and the gene locus encoding this small polypeptide is located on the different autosomal chromosome from that of the *Fib* gene[21].

2) Sericin genes

Sericin is composed of five main polypeptides synthesized in different sections of the middle silk gland[7]. The existence of variant polypeptides in certain silkworm strains allows mapping of the corresponding genes. Using a variant polypeptides (S-2) in sericin originally found in the *Nd-s* mutant strain, linkage analysis of the *Src-2* locus encoding the S-2 sericin migrating with secondarily slow mobility by acid gel electrophoresis was carried out[11]. As the result, the *Src-2* locus was found to be located at 0.0 map unit on chromosome 11. Further, linkage analysis of other sericin genes was attempted. The gene locus of the heaviest sericin polypeptide (S-1) was also found to be located on the same chromosome, although the map unit is quite different from that of the *Src-2*[31].

Variant sericin polypeptides have some effects on quantitative characters such as sericin content and reelability of cocoons[10,12]. Variant gene caused by a deletion or duplication of DNA affects the content of sericin in cocoons, while one caused by the replacement of base sequences in DNA affects the reelability of cocoons.

Chromosomes possessing polygenes relating to the content of sericin in cocoons were also analysed using a strain of low sericin content[25]. Chromosome 2,12,15, 21 and 22 were estimated to carry their polygenes.

Conclusion remarks

Biosynthesis of fibroin and sericin in the silk gland has been studied widely. Especially, the DNAs encoding the both proteins were cloned using gene manipulation technique and some base sequences in their nucleotides were analysed. Genetic engineering technique is considered to be useful for the improvement of characters in plants and animals. This technique is also applicable to the silkworm breeding and recombination of foreign genes encoding enzymes into silkworms will improve the efficiencies in feeding of mulberry leaves and in protein synthesis of silkworm larvae. However, this technique is not applied to the silkworm breeding at once. We need to study more to find the suitable vector which makes it possible to carry appropriate DNA, and to establish the technique for the introduction of foreign DNA into insect cells.

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