Origin and Differentiation of the Silkworm Races

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From time immemorial, the silkworm has been very closely related to everyday life of human being. No one has, however, revealed the origin of the silkworm and differentiation of the silkworm races because of a lack of more efficient technical methods for an approach to the problem.

Recently, it has become possible to study the phylogeny at the level of nucleic acid, protein and terminal product, owing to rapidly developed molecular biology. It is theoretically reasonable that the problem should be approached at the level of nucleic acid.

The efficient methods, which do not take much time in the comparison of delicate differences, have not yet been established.

Therefore, the electrophoretic or serological method has come to be accepted as a tool for detecting proteins with enzymatic activity due to their specificity and sensitivity.

It is well known that the serological method is more useful for analysis at the high level of family relation (Family and Order of insect) than for that at the low level of family relation (Species and Race of insect). The most efficient method in this investigation is found to be electrophoresis, especially at the isozyme level. From this point of view, the author analyzed the problem of racial differentiation and relatively made clear the origin of *Bombyx mori*.

Genetical analysis of various kind of isozymes in the silkworm

In general, the silkworm races can be classified into Japanese, Chinese, Tropical, European and Korean geographical races. On the other hand, they can be also classified into univoltine, bivoltine, and multivoltine from the viewpoint of voltinism. Therefore, the present investigation was undertaken on the basis of both geographical distribution and voltinism. The strains used in this experiment were Japanese univoltine, Japanese bivoltine, Chinese univoltine, Chinese bivoltine, Tropical multivoltine, European univoltine, and Korean univoltine races. Korean univoltine has trimoltings, so is called Korean trimolter.

Using many geographical races, it was ascertained that blood acid phosphatase, by means of thin layer electrophoresis with agarose, can be classified into five types viz, O, A, B, C, and D, as shown in Fig. 1-a[9]. O designates the type which is incapable of hydrolyzing naphthyl phosphate. These bands exhibited different electrophoretic mobilities but did not reveal any difference in the activity as they all reacted on the same substrate. This suggests that all the bands are isozymes.

Mating experiments revealed that the inheritance of these five bands of acid phosphatases are controlled by codominant multiple alleles, Bpy^D, Bpy^A, Bpy^B, Bpy^C and Bpy^D. In F_1 individual obtained from a mating of B and D type individuals, there existed *B* band concurrently with D band, as presented in Fig. 1-b. These three hybrid types, such as BB, BD and DD, were found to show the same enzyme activity as they were analyzed quantitatively. Namely, each band of hybrid type in F_1 individuals was found to be brought about by a half of parental activity.

As can be seen in Fig. 1-c, integument esterase can be classified into six basic types[2]. From mating experiments it was revealed that they are controlled by codominant multiple alleles as blood acid phosphatase. There was a duplicated type of which the two bands (AB and AC) were unique in integument esterase. These duplicated type bands differed from the hybrid type bands of F_1 blood acid phosphatase. The former did not segregate even if sib-mating continued several times. Consequently it is controlled by a single gene.

Relationship among geographical races from the viewpoint of isozyme types

In order to compare the frequency of isozyme types, blood acid phosphatase of about 300 races belonging to several geographical races was examined. The results as shown in Table 2 reveal that the frequency of D type follows that of C type in Japanese univoltine race (J1), Japanese bivoltine race (J2), Tropical multivoltine race (TM) or Chinese bivoltine race (C2), and conversely in Chinese univoltine race (C1), European univoltine race (E1) or Korean univoltine race (K1) the frequency of C type follows D type. The frequency of B type was only observed in

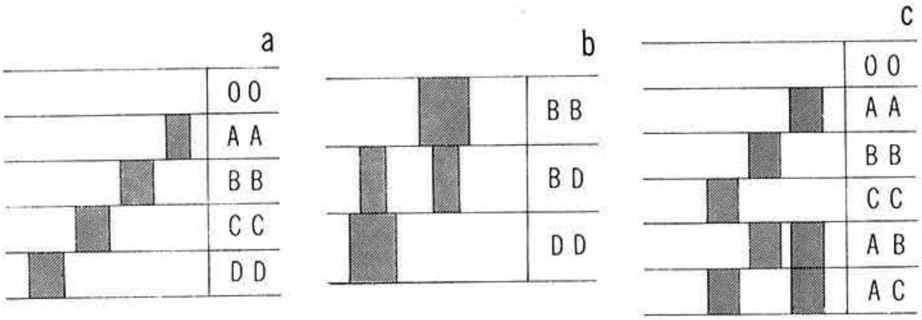

Fig. 1. Diagram showing zymogram patterns in various enzymes types of the silkworm.
a: Acid phosphatase types in the blood.
b: Acid phosphatase of the hybrid (BD) and its parental strains (BB & DD).
c: Esterase types in the integument.

Furthermore the author revealed the mode of inheritance of various kinds of enzymes by the similar method as mentioned above[1,3]. Results are summarized in Table 1.

Table 1. Various kinds of enzymes in several tissues of the silkworm

Tissues	Enzymes	Genes					
Blood	Esterase	Bes^O,	Bes^A,	Bes^B,	Bes^C		
Blood	Acid phosphatase	Bph^O,	Bph^A,	Bph^B,	Bph^C,	Bph^D	
Blood	Phenoloxidase	Phe^A,	Phe^B,	Phe^C			
Midgut	Alkaline phosphatase	Aph_1^+,	Aph_1^-				
Midgut	Alkaline phosphatase	Aph_2^+,	Aph_2^-				
Integument	Esterase	Ies^O,	Ies^A,	Ies^B,	Ies^C,	Ies^{AB},	Ies^{AC}
Silk-gland	Esterase	Ses^A,	Ses^{AA},	Ses^B,	Ses^{BB}		
Brain	Esterase	Ges^A,	Ges^B,	Ges^C			
Digestive juice	Proteinase	Dpr^I,	Dpr^{II},	Dpr^{III}			

the group of C1, E1 an K1. It was also found that C1 has more types in number than other geographical races.

Table 2. Racial differences in the frequency of the blood acid phosphatase type (*Bph*)

Geographical races	No. of strains tested	0	A	B	C	D
Japanese univoltine race	41	8.5%	1.2	0	54.7	36.6
Japanese bivoltine race	31	8.0	0	0	74.2	17.8
Tropical multivoltine race	6	33.4	8.3	0	41.6	16.7
Chinese bivoltine race	29	12.1	3.4	0	55.2	29.3
Chinese univoltine race	78	11.5	7.7	11.5	29.5	39.8
European univoltine race	38	13.2	0	13.0	32.7	41.1
Korean trimolting race	4	0	0	25.5	25.0	50.0

The values indicate the percentage of the enzyme type for each race.

The frequencies of integument esterase types are presented in Table 3. C1 had more kinds of distributing types than other geographical races and exhibited polymorphism. The frequency of *C* type was higher than that of B type in J1, J2, TM and C2, whereas the frequency of *B* type was higher than that of C type in C1, E1 and K1. It is clear that the group of J1, J2, TM, and C2 can be separated from that of C1, E1, and K1 in the case of integument esterase as well as blood acid phosphatase.

Table 3. Racial differences in the frequency of the integument esterase type (*Ies*)

Geographical races	No. of strains tested	A	B	C	AB	AC	0
Japanese univoltine race	31	0%	12.9	71.0	3.2	12.9	0
Japanese bivoltine race	53	0	18.9	64.2	1.9	15.0	0
Tropical multivoltine race	6	0	0	33.3	0	66.7	0
Chinese bivoltine race	45	0	26.6	46.6	11.1	15.7	0
Chinese univoltine race	49	4.1	45.0	26.5	14.3	6.0	4.1
European univoltine race	30	0	30.0	13.3	13.3	43.4	0
Korean trimolting race	4	0	50.0	25.0	25.0	0	0

The values indicate the percentage of the enzyme type for each race.

Schematic representation of differentiation of geographical races is shown in Fig. 2, with various kinds of enzyme analyses.[5] Generally, the genetic variation in a population is considered to increase if a species survives longer and longer in a limited area, and then a species is adapted to such a region and becomes to exhibit polymorphism. On the contrary, it is often said that the circumference of distributed area includes little variation of an animal. On considering this, the Chinese univoltine race which is the most polymorphic is supposed to be the origin of the silkworm.

As already mentioned above, there were two groups, of J1, J2, TM, C2, and C1, E1, K1. There was much closer family relation within a group than between the groups. From this result, the author concluded that E1 and K1 are differentiated directly from C1, and both TM and J2 from C2. Further it is suggested that C2 is differentiated from C1 which has been gradually adapted to the southern region through the Chinese continent.

It is interesting to note that the Japanese and Korean races are not related in the means of biological aspects although they are geographically relevant. Although it was known that Japanese sericultural technique was spread all over Japan from China through the

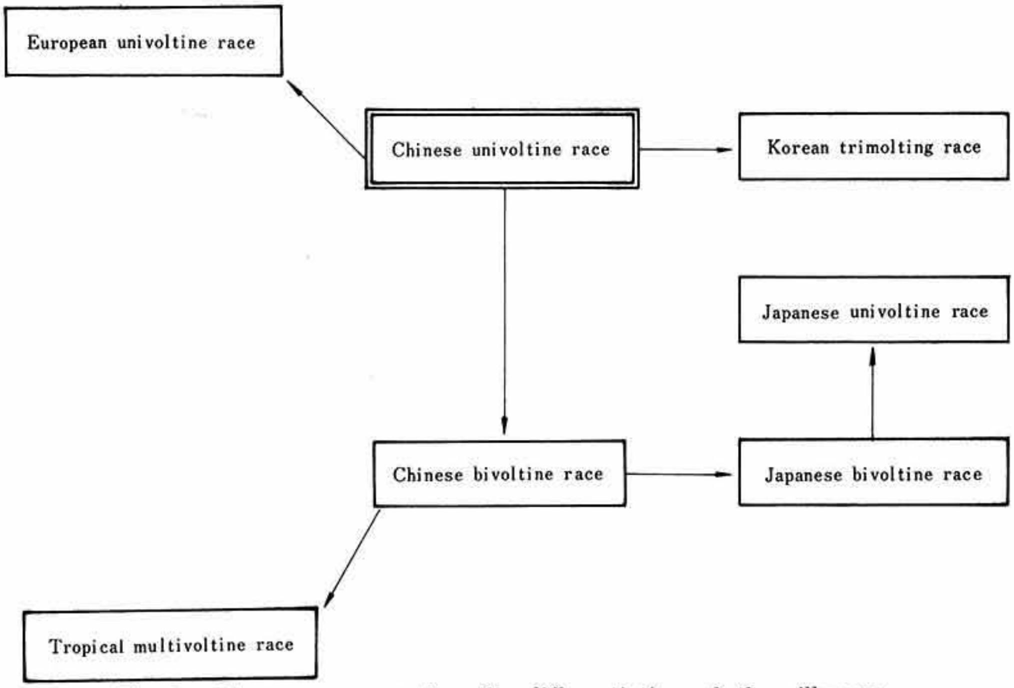

Fig. 2. Diagram representing the differentiation of the silkworm geographical races.

Korean peninsula, Japanese races had a close relation to Chinese bivoltine and Tropical multivoltine, which had been popular in the southern region. The races of northern China and Korea resemble each other in terms of isozyme.

Differentiation mechanism of *Bombyx mori* and *Theophila mandarina*

For a long time it has been accepted that *Bombyx mori* and *Theophila mandarina* are very closely related, and their ancestor is the same because they closely resemble each other in their morphology and ecology. The author compared a great number of Japanese and Korean wild silkworms (*Theophila mandarina*) with domestic silkworms (*Bombyx mori*) from the point of view of isozyme. Results for blood acid phosphatase, blood esterase, and integument esterase are summarized in Table 4.

The frequency of the C type blood acid phosphatase was high in *Bombyx mori*, but in *Theophila mandarina* the D type was

Table 4. Difference in the distribution of several genes between *Bombyx mori* and *Theophila mandarina*

		Bombyx mori	*Theophila mandarina*
Number of larvae examined		768	942
Blood acid phosphatase (*Bph*)	O	7.3%	2.2
	A	4.2	0
	B	9.4	2.2
	C	51.0	45.0
	D	28.1	50.6
Blood esterase (*Bes*)	O	28.1	17.1
	A	62.5	19.2
	B	3.1	1.9
	C	6.3	59.8
	D	0	2.0
Integument esterase (*Ies*)	A	1.5	0
	B	20.7	0
	C	48.2	7.8
	AB	7.5	0
	AC	22.1	0
	D	0	79.0
	E	0	13.2

dominant. The frequency of A type blood esterase was the highest in *Bombyx mori*, whereas that of the C type predominated in *Theophila mandarina*. The continuity of gene frequencies appeared between them even if they represented a different variation of genes.

In the case of integument esterase only the C type was found in both *Bombyx mori* and *Theophila mandarina* but in the latter the frequency of C type was 8 per cent of the total individuals examined. Most of the D and E types, which did not appear in *Bombyx mori*, were found in *Theophila mandarina*. Accordingly, it may be concluded that these two insects have no similarity, but barely keep the continuity between them as far as the gene of integument esterase is concerned.

It is obvious that in *Theophila mandarina* AB and AC duplicated types can not be detected at all, while those types can be analyzed in a high frequency in *Bombyx mori*. It has been established that except for a few minor differences observed in the frequency of other kinds of enzymes, all the enzyme systems are continuous in both of them. Hence, it is reconfirmed that both of them are closely related.

As previously mentioned, the frequency of duplicated types, AB and AC in the integument esterase of *Bombyx mori* were noted, besides the A, B, and C types. This duplicated type is controlled by a single gene. As shown in Table 5, there was a marked difference of esterase quantity between the duplicated and the single types. It was found that the activity of the duplicated type is twice as much as that of the single type.

The author presumes that the AB or AC type is controlled by Ies_A and Ies^B gene, or is a product of the action contributed by both Ies^A and Ies^C genes. This duplicated phenomenon was not only limited to the integument esterase but also noted in many other enzymes like silk-gland esterase, midgut alkaline phosphatase, and blood acid phosphatase.

Table 5. Difference of the esterase activity among strains

Esterase activity of the integument

Strain	Type of enzyme (Isozyme)	Esterase activity (μ moles/g/hr)
Bombyx mori (Shinshocho)	Ies^A	292
Bombyx mori (Mukonaka)	Ies^C	233
Bombyx mori (Koishimaru)	Ies^C	272
Bombyx mori (Sekuzato)	Ies^{AB}	501
Bombyx mori (Cambodge)	Ies^{AC}	580
Theophila mandarina	Ies^D	282
Theophila mandarina	Ies^E	267

Esterase activity of the silk-gland

Strain	Type of enzyme (Isozyme)	Esterase activity (μ moles/g/hr)
Bombyx mori (Cambodge)	Ses^{AA}	121
Bombyx mori (Heiwa)	Ses^{AA}	131
Bombyx mori (Koishimaru)	Ses^{BB}	119
Bombyx mori (Annan)	Ses^A	46.3
Theophila mandarina	Ses^A	45.3
Theophila mandarina	Ses^A	50.0

When such duplicating gene frequency was compared in *Bombyx mori* and *Theophila mandarina*, the duplicating genes could scarcely be found in *Theophila mandarina*. The evidence that the duplicating gene occurred rests on unequal crossing over. If it is clear that *Bombyx mori* and *Theophila mandarina* are originated from the same ancestor and the former gradually acclimated, it may imply that the accumulation of duplicated genes in *Bombyx mori* is promoted by artificial selection during a long period.

It is known that the agricultural plants are associated with polyploid phenomenon as most of them reveal polyploidy. In practice the polyploidy is seldom in an animal because of sterility except for a few cases. The duplication of gene induces a slight increase of genetic substances, by which the animal is influenced little in propagation, as compared with the duplication or partial duplication of chromosome. In this connection, there is the possibility which gene duplication occurs very frequently in animal. There may be an equal mechanism by which the duplication of chromosome in agricultural plants and accumulation of the duplicating gene in domesticated animals take place.

The author claims that the duplication of genes might have played an important role in the differentiation of *Bombyx mori* and *Theophila mandarina* from the same ancestor.

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