Genetic Studies on Tropical Races of Silkworm (*Bombyx mori*) with Special Reference to Cross Breeding Strategy between Tropical and Temperate Races

1. Genetic nature of the tropical multivoltine strain Cambodge

By AKIO MURAKAMI and YOSHIKI OHTSUKI*

Department of Ontogenetics, National Institute of Genetics
(Mishima, Shizuoka, 411 Japan)
* Department of Insect Genetics and Breeding, National Institute of Sericultural and Entomological Science
(Tsukuba, Ibaraki, 305 Japan)

Introductory remarks–psychological effect of visible phenotypes on sericulturists

It is well-known that, in Japan, silkworm rearers or sericulturists, especially the elderly ones regard mauve-grey eggs and black newly hatched larvae as the typical representative eggs and newly hatched larvae of the silkworm. Zebra-marking silkworms (*Ze*), with appearance different from that of plain-marking (*p*) and normal marking (p^+) silkworms which are both reared by ordinary farmers, do not meet with public favor. As to the shape of the cocoon, too, sericulturists value the so-called "mayugata" shape which has long been regarded as the typical shape of the Japanese race. Also, as to the color of the cocoon, a cocoon having a golden-yellow or flesh color which is occasionally seen in European or Chinese races, is considered to be a strange color; Japanese sericulturists strikingly tend to regard a white cocoon as the typical cocoon color. In addition, they consider brown pupae in the cocoon as the normal color of the pupae and greyish-white adults as a typical type. Silkworms with those characters have long been valued as so-called "okaiko" or "dear silkworm" with even a sense of worship. Thus, Japanese sericulturists psychologically (rather than traditionally) have a distaste for silkworms having visible characters other than the above-mentioned typical representative characters.

The present authors have often heard that a similar situation is also prevalent among sericulturists in the Southeast Asian countries. Larvae reared in the tropics are smaller in size than those of both Japanese and Chinese races[11,12,28]. Also, the larval growth period in the tropical race is generally shorter than that in the temperate race[11]. Thus, the yield of silk is lower than that of silk from either the Japanese or Chinese race[11,12,28]. Moreover, the cocoon is spindle-shaped and the color is either yellow or green in the tropical silkworms[23]. Such characteristics would be considered to be favorable by the Southeast Asian sericulturists.

Invisible characters, such as physiological, biochemical, pathological ones, etc., do not generally attract special interest of farmers. This trend which is common to each country is thought to be emotional. Of course, the image of the typical silkworm is different de-

Table 1. Comparison of the cocoon shell layer percentage among tropical, Japanese, and Chinese races reared in Kobuchizawa

Races	1970	1974	1979	1985
Tropical				
Multivoltine	%	%	%	%
Annam	13.0(0.120/0.92)*	12.1(0.109/0.90)	11.3(0.113/0.93)	12.2(0.105/0.86)
Cambodge[a]	13.6(0.152/1.12)	—	12.8(0.122/0.95)	11.9(0.123/1.03)
Cambodge[b]	12.0(0.121/1.01)	10.3(0.093/0.90)	9.7(0.085/0.88)	9.9(0.120/1.21)
Mysore	12.6(0.151/1.21)	—	10.9(0.102/0.94)	11.0(0.102/0.94)
[Pure Mysore]	10.8(0.137/1.27)	9.7(0.116/1.20)	13.1(0.134/1.02)	13.2(0.123/0.73)
Ringetsu	10.9(0.114/1.04)	11.4(0.114/1.00)[c]	—	9.1(0.086/0.95)
Japanese				
Univoltine				
Akajuku	14.4(0.247/1.71)	—	13.1(0.174/1.33)	15.7(0.196/1.25)
Bivoltine				
Ohkusa	13.5(0.185/1.37)	—	12.8(0.153/1.20)	13.2(0.214/1.62)
Chinese				
Univoltine				
Shokei	14.2(0.198/1.39)	14.2(0.241/1.70)	13.8(0.216/1.57)	12.9(0.208/1.61)
Bivoltine				
Kosetsu	16.3(0.264/1.62)	15.2(0.210/1.38)	14.5(0.208/1.43)	14.5(0.262/1.81)

a) : Low non-diapause potential.
b) : High non-diapause potential.
c) : Data of 1973.
* The numerator and denominator in parentheses indicate the weight of a cocoon shell layer per capita (in g) and that of a cocoon of a pupa (in g), respectively.

pending on the tradition established throughout the long history of each people or race. It is of interest to note that unlike the case of *Drosophila melanogaster*, there is no genetical definition of "wild" type for the mulberry silkworm (*Bombyx mori* L.) and there is only a geographical classification for convenience sake regarding the respective standard type for the "European", "Chinese" and "tropical" races. Moreover, the main criteria for such a practical classification are morphological (or visible) characters observed by the people engaged in the rearing of silkworms in each country and region. On the other hand, voltinism, which is an invisible character, can be regarded as one of the important criteria for scientific classification.

The silkworms reared in the tropical and temperate zones show a marked difference in voltinism. Japan, Korea, China, European countries and other countries in the temperate zone are usually rearing either the uni- or bivoltine race which undergoes one or two generations per year, while the Southeast Asian countries in the tropical zone are rearing only multivoltine races which undergo several generations per year.

As can be seen in Table 1, the ratio of cocoon shell layer weight to the total cocoon weight in a pupa of several tropical races tends to be lower than that of typical Japanese and Chinese races. Is this situation attributable to the low productivity a character peculiar to the tropical race? Or can the two conditions be separated from each other? If the characters can be separated, it may be possible to increase the productivity, while maintaining the visible phenotypes characteristic of the tropical race by improving and replacing only the factor(s) responsible for the low productivity. However, hybrid silkworms between the uni- or bivoltine and tropical multivoltine races have often been used as materials for various biological studies since the tropical race is generally sturdy or robust during the summer generation and has a short generation time to some extent. These hybrids show typical tropical characteristics in terms of the shape and color of the cocoon, and the number of generation times per annum. Consequently, it appears that the gene factors controlling these characters did not split into separate factors. These phenomena may be explained as follows: various phenotypic factors or genes which characterize the tropical race are linked to each other on a narrow region of the same chromosome or a dominant character.

It is well-known, that the tropical race is remarkably vigorous as compared with the other geographical races[11,14]. However, no attempt has been made so far to analyze scientifically the mechanism, which is involved in this biological phenomenon. As stated elsewhere, the tropical multivoltine race produces moths which lay only non-diapausing eggs in the tropics, while they produce diapausing egg batches in addition to the non-diapausing ones in Japan (Murakami, 1988)*. This phenomenon strongly suggests that the tropical race is endowed with an adaptive ability to the change of environmental conditions (Murakami, 1988)*. Therefore, the introduction of the sturdiness character of the tropical race into the temperate race offers promising breeding materials for improving the vigor of the Japanese or Chinese stock.

In this paper the results on extensive studies and frequent discussions of the present authors and others concerning the characterization of the genetic characteristics of the Cambodge** stock as a typical tropical multivoltine race are presented. In the paper, attempts were also made to outline ecogenetical nature of the multivoltine gene peculiar to tropical races.

* Presented at the International Meeting of Tropical Sericultural Practices held at Bangalore, India in Feb., 1988.

** Cambodge is French for Cambodia.

Genetical characterization of some typical visible phenotypes in the Cambodge stock

1) Non-diapausing eggs

The Cambodge stock, a tropical multivoltine race, which is reared in Indonesia undergoes generation changes without any diapause[13]. The same situation is generally observed in other tropical races[23]. On the other hand, when the stock was introduced into Japan the life cycle changed with the seasonality (Murakami, 1988)* : (1) almost all the eggs laid by the spring generation silkworms were of the non-diapause type, (2) some eggs laid by the summer generation entered the diapause, (3) almost all the eggs laid by the late autumn generation entered the diapause and (4) non-diapausing eggs were seldom laid throughout the year. It is obvious that the climatic conditions in the temperate zone are attributable to the phenomenon observed in Japan[19]. The induction or acquisition of diapause phenomena seems to be an adaptation of the tropical multivoltine race for survival under unfavorable environmental conditions.

In the Cambodge stock, a recessive pigmented but non-diapausing egg (*pnd*) gene is known to be present in the 11th linkage group[30]. If the eggs are homozygous for the *pnd* gene, they undergo a non-diapause cycle regardless of the environmental conditions. This fact indicates that the non-diapause character is an obligatory type and that it is indifferent to environmental conditions. It was also revealed that the *pnd* gene is different from the non-diapause gene of the facultative type detected in the Cambodge stock, which is strongly dependent on the environmental conditions[20]. Therefore it appears that the non-diapause factor, whose function is different from that of the *pnd* gene, is also present in the Cambodge stock as well as other tropical stocks. For the sake of convenience, the factor was designated as the non-pigmented and non-diapause egg, *npnd*[20], because the egg is characterized by the absence of pigmentation of the serosa membrane cells. Genetical characteristics of the *pnd* gene enable to determine whether the gene is present in the Cambodge stocks, which have long been preserved in Japan as well as in Indonesia, by simple mating between females homozygous for *pnd* and the Cambodge males. The results of the mating experiments indicated that some silkworms presented at the National Institute of Genetics (Mishima) harbors the *pnd* gene in homozygous or heterozygous conditions[20]. However, most of them were found to lack the *pnd* gene. As the *npnd* gene shows a maternal inheritance[18,20], no mating experiments between the Cambodge females and *pnd* males were performed.

The results were also roughly in agreement with the frequency of appearance of the non-diapausing and diapausing egg batches in the Cambodge stock, as described elsewhere by Murakami[19]. That is, the non-diapausing egg batches may correspond to a highly multivoltine Cambodge line which harbors both the *npnd* and *pnd* genes, while the diapausing batches may correspond to a low multivoltine line which harbors only the *npnd* gene or is heterozygous for the *pnd* gene. This interpretation is naturally supported by Katsumata[13] who reported the presence of the *pnd* gene in the Cambodge stock in Indonesia and inferred that the gene had originally been maintained in that line. In such a case, the genetic constitution for the non-diapause characters of the Indonesia (or neighboring countries) native Cambodge stock can be represented as *npnd*/Y : *pnd*/*pnd*. The *npnd* gene would be functioning as a non-diapausing factor under the tropical climatic condition of Indonesia. On the other hand, the function of *npnd* in the temperate region including Japan is considered to be low due to the adaptation to the change of the environmental conditions. It is important to recognize that since the *npnd* gene exerts maternal effects on the phenotypic expression, the genetic behavior is epistatic to the *pnd* gene even when the *pnd* gene is present in a homozygotic condition. This special feature of the *npnd* gene may account for the maintenance of *pnd* in both tropical and temperate stocks. In other words, it can be said that the *pnd* gene can not operate in the temperate zone without the presence of the *npnd* gene.

2) *Egg-color in diapausing eggs*

In the preceding section, the genetic factors controlling the non-diapause in the Cambodge stock as a model were discussed. As a result, it became clear that there are at least two genetic factors responsible for the non-diapause phenomenon, *npnd* and *pnd*, which are located on the X and 11th chromosomes, respectively.

Diapausing eggs laid by the Cambodge stock are characterized by a yellow ochre color similar to that of gardenia fruit. In order to analyse the genetic characteristics of this egg-color (*gar*, gardenia), a mating experiment was carried out between Cambodge females and J106 males and vice versa. Segregation ratios of the egg-color in the subsequent F_1 and F_2 generations were analyzed (Table 2). Most of the F_1 hybrid eggs obtained by a mating cross between Cambodge females and J106 males were non-pigmented and did not enter the diapause, while other eggs were yellow ochre in color. However, the F_2 off-

Table 2. Segregation of egg-color in F_2 from a cross between Cambodge females and J 106 males

Egg-color		Total
Black	Yellow ochre	
437	154	591
357	138	495
452	150	602
475	147	622
428	166	594
424	150	574
2,573	905	3,478
74%	26%	
3 :	1	

Color of diapausing eggs in the J 106 stock is black, but that in the Cambodge stock is yellow ochre.

F_1 : Almost all non-diapausing eggs.

F_2 : All diapausing eggs.

spring obtained by a sibcross among the F_1 hybrids gave eggs with a normal (or grayish lavender) (+) and yellow ochre (*gar*) color in the ratio of 3:1. In the case of reciprocal crossing, all the F_1 eggs showed a normal color and the F_2 offspring from sibmating among the F_1 hybrids gave a ratio of 3:1 with a normal and yellow ochre color. These findings clearly suggest that the yellow ochre egg is under the control of a single recessive gene located on an autosome. It was also revealed that the *npnd* gene is located on the X chromosome, while the *gar* gene is located on an autosome, suggesting that these two genes behave independently each other.

3) Color and shape of cocoons

Although the color of cocoons is not highly appraised from the viewpoint of breeding, this phenotype may be of interest. As stated in an earlier section, the cocoon color is one of the important criteria for the differentiation of races or strains. This applies also to the shape of cocoons but that is related to the productivity of silk to some extent. The cocoon color of the wild silkworm (*Bombyx mori mandarina* Moore) is biologically important in terms of the protective function against various agents and sunlight in nature.

When Cambodge females with bright reddish yellow cocoon which is peculiar to the stock are crossed with J106 males with white cocoon, the F_1s display only bright reddish yellow cocoon and the F_2s segregate into a typical monogenic ratio or three bright reddish yellow and one white color cocoons. In the reciprocal crossing of J106 females with Cambodge males, similar results were obtained. Accodringly, it appears that the C^* gene located on the 12th linkage chromosome (0.0 units) which infers the bright reddish yellow color of the cocoon is a simple dominant gene to the gene inferring the white color. It is difficult to interpret the delicate color tone of bright reddish yellow cocoons which is similar to gamboge as seen in the surplice of high rank buddhist bonzes.

When the Cambodge strain is crossed with the Daizo strain with the green color (which is similar to bamboo-grass) of cocoons, only bright reddish yellow cocoons are produced in the F_1 and three bright reddish yellow and one green cocoons in the F_2, indicating that the gene responsible for the green cocoons is a simple recessive gene to that for the bright reddish yellow cocoons. The gene for the white cocoons of the J106 strain is dominant over the gene for the green cocoons of the Daizo strain (Table 3). In crossing the mutant strain *od* with white cocoons with the Daizo strain characterized by green cocoons, cream color cocoons appear in the F_1. When the cream cocoon F_1s are sibcrossed, the F_2 ratio is roughly one white, two cream, and one

Table 3a. Dominant-recessive relationship in the color and shape of cocoons between Cambodge, Daizo and J106 stocks

Cross		Cocoon	
Female	male	Color	Shape
Cambodge ×	Cambodge	Bright reddish yellow	Spindle
Daizo ×	Daizo	Light green	Irregular or semilunar
J 106 ×	J 106	White	Standard shape of the Japanese type
Cambodge ×	Daizo	Bright reddish yellow	Spindle
Daizo ×	Cambodge	Bright reddish yellow	Spindle
Cambodge ×	J 106	Bright reddish yellow	Close to Japanese standard type
J 106 ×	Cambodge	Bright reddish yellow	Close to Japanese standard type
Daizo ×	J 106	Cream	Close to Japanese standard type
J 106 ×	Daizo	Cream	Close to Japanese standard type

The Daizo and J 106 stocks are typical Chinese and Japanese bivoltine races respectively.

* Outer-layer yellow cocoon

Table 3b. Segregation of the color and shape of cocoons observed in the F_1 backcross (Daizo females × Cambodge males) females × Daizo males

	Spindle	Irreg.	Total
Bright reddish yellow	227	264	491
Light green	248	277	525
Total	475	541	1,016

The Daizo stock has a light green cocoon with an irregular or semilunar shape peculiar to this stock. The color of cocoons in the Cambodge stock is bright reddish yellow (or gambodge). The cocoon in this stock is a spindle shape. The bright reddish yellow color of the cocoon in the Cambodge stock is dominant over the white, and light green color of the cocoon is an incompletely dominant character.

green cocoons (Murakami, unpublished observations). This finding accounts for the fact that cream is an intermediate color between white and green cocoons and the dominance to the latter is incomplete. In summary, the relation of the dominance for the cocoon color is generally in the order of bright reddish yellow, green, and white.

The spindle shape of the cocoons in the Cambodge stock is also a characteristic feature. In crosses with the J106 strain, cocoons with a typical Japanese shape were observed in the F_1 and three Japanese (or normal) and one spindle types in the F_2. In crossing the bivoltine C108 strain with a Chinese race, cocoons with a shape peculiar to the Chinese race were obtained in the F_1 and the color of the cocoons was bright reddish yellow. In the F_2 segregation for the cocoon shape, a ratio with three Chinese and one spindle types was observed. In addition, segregations in a monogenic ratio with three spindle and one crescent or croissant-shaped cocoons were obtained in the F_2 of the reciprocal cross between the Cambodge and Daizo* (a bivoltine Chinese race) strains. These findings indicate that the spindle-shaped cocoon derived from the Cambodge stock dominates the crescent cocoon from the Daizo stock. It is difficult to determine whether the spindle-shaped cocoon is epistatic to the irregular crescent one. These two phenotypes are common in the tropical race as a rule and are usually associated with a floosy character, giving a cotton-like appearance. In any case, the dominance relation for the shape of cocoons is in the order of typical representative Japanese or Chinese, spindle and irregular crescent type. Some typical visible genetic phenotypes in the Cambodge stock are listed in Table 4.

* In India, the Daizo stock is considered to be a multivoltine race.

Attempt to detect some useful but not yet established quantitative characters in the tropical race

As mentioned in the preceding section, there are several visible traits characteristic of the tropical multivoltine Cambodge stock, such as the embryonic non-diapause, diapausing egg-color, cocoon color and shape which are associated with different linkage groups. From a breeding standpoint, a genetical analysis of economic characters, such as growth rate, weight of cocoon shell layer, vigors, etc. in the tropical race is important. The analysis of the quantitative characters mentioned above is however difficult compared with that of the other visible characters outlined. However, this approach would contribute significantly to breeding practices as well as to a better understanding of the biology of *Bombyx* in general. It is important to identify and analyze the genetic factors controlling either the growth rate or growth period for each stock or race. As pointed out already, the growth rate (or period) in the tropical race tends to be more rapid (or shorter) than that of the temperate race (under the relatively high ranges of the rearing temperature) in general. Accordingly, the replacement of the genetic factor responsible for the growth rate (or period) in the tropical race with the factor for late growth rate (or long growth period) in the temperate

Table 4. List of typical visible genetic traits in the Cambodge stock

Character (or gene)	Linkage	Reference
Voltinism & egg-color		
Non-diapause		
a) Pigmented but non-diapausing egg (*pnd*)	11	Indifferent to environmental conditions
b) Non-pigmented and non-diapausing egg (*npnd*)	X(1)	Sensitive to photoperiod or daylength and highly adaptable to changes of environmental conditions
Diapause		
a) Pigmented eggs with ochre yellow similar to the outside color of fruits of gardenia trees (*gar*)	A*	This trait often appears in the tropical multivoltine race reared in the temperate zone.
Growth rate		
Short generation or life span (short larval duration, early adult emergence, etc.) (*pre*: premature)	X(1)	This trait usually reduces the whole cocoon weight or cocoon shell weight.
Cocoon color & shape		
Color		
Bright reddish yellow both 12? outside and inside the cocoon similar to gamboge or color of pigment in fruits of gardenia trees (*Gam*).		The trait seems to be analogous to the gene *C*.
Shape		
Spindle (*sp*)	A*	This trait is often accompanied with flossiness, giving a cotton-like appearance.
Sturdiness		
Resistant to both high temperature and relative humidity	?	This physiological trait seems to be a very common feature to all the tropical multivoltine races including the Daizo stock, a Chinese bivoltine race.

* The abbreviated word "A" indicates a certain autosome.

race would be useful for the improvement of the productivity of the former race. Conversely, the introduction of the vigor factor of the tropical race into the temperate race, while maintaining the high productivity of the race may further increase the productivity.

The cocoon shape may also be related to the productivity to some extent. Spindle-shaped cocoons, one of the characteristic traits of the tropical variety, are under the control of a single recessive character for which the phenotype is not expressed in the F_1 hybrids of a cross between the temperate race with a normal cocoon shape and a tropical race with a spindle-shaped cocoon. It is well known that the elimination of genes from the gene pool of hybrids is difficult. However, such elimination may be advantageous since the recessive gene is usually covered with its dominant gene in the process of hybridization to develop commercial strains. The factor responsible for the shape of cocoons is related to the nervous system controlling the gooseneck movement of mature larvae for the spinning of cocoons. Therefore, this character is an important subject in neurobiology or ethology of the silkworm.

Undoubtedly, it may be difficult to analyze genetically the nature of a quantitative trait and/or to isolate the genes responsible for the trait.

In the mulberry silkworm, male moths tend to emerge earlier than females as a rule. However, when the F_1 males, of a cross be-

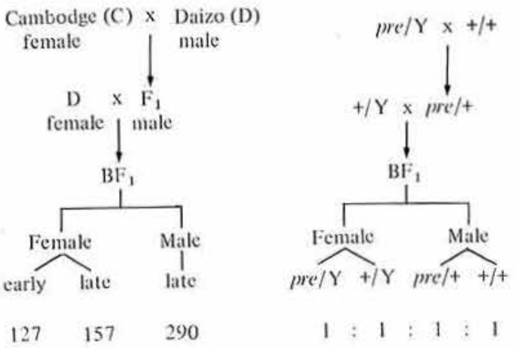

Fig. 1. Genetic analysis of the adult emergence in a cross between the Cambodge females and Daizo males

An early emergence gene is designated as *pre* (premature) and Y is the female-determining chromosome. *pre* seems to be analogous to the Lm^e gene. The Cambodge stock harbors the *pre* gene which is peculiar to tropical races.

tween Cambodge females and Daizo males, were again crossed to the Daizo females, the females in the backcross F_1 progeny tended to emerge earlier than the males as in the case of the F_1 males of a cross between the Daizo females and Cambodge males. This fact suggests that the gene, tentatively designated as *pre* (premature), is located on the X-chromosome in the Cambodge stock as in the case of the *npnd* gene, while in the Daizo and probably other temperate stocks the pre^+ gene occupies a dominant allele instead of the *pre* (Fig. 1).

When the Cambodge and Daizo stocks, all together with many other stocks were reared in the insectaria under a controlled temperature of 25°C, the Cambodge stock completed its feeding activity 19–20 days after hatching. Naturally, this stock was the first to start spinning cocoons and to emerge from the cocoons. Two or three days later, the Daizo stock had completed its feeding and two or three days later an egg-color mutant (*pe/re*) ended the feeding. Aojuku, a typical Japanese bivoltine race and C108, a Chinese bivoltine race, fed for one more day. Naturally, the Daizo stock started spinning cocoons two or three days later than the Cambodge stock. Furthermore, the Cambodge stock seems to exhibit a somewhat shorter pupal stage as compared with the other stocks. It is also clear that the Cambodge stock has a shorter adult lifetime than other stocks such as Aojuku and C108. In *Bombyx mori*, the adult lifetime of the Daizo stock is the shortest and that of the males is shorter to some extent than that of the females (Murakami, unpublished observations). In other words, these observations indicate that the Cambodge stock may harbor a gene responsible for the short lifetime, namely the short larval, pupal and adult stages. The situation seems to be a common feature for tropical races regardless of the rearing conditions in general. It is natural that in tropical races the larval body weight tends to be reduced in comparison with temperate races. Accordingly, productivity of silk materials or weight of the cocoon shell layer per capita in tropical races is inevitably lower than that in temperate ones.

As mentioned above, it became clear that the duration of the larval stage affects the body weight of larvae and/or productivity of silk materials. This character appears to be linked to the X chromosome. The present observation is in agreement with those made previously[17,21,22,25-27]. Nagatomo[21,22] designated this gene as either sex-linked early Lm^e or late maturation gene Lm^+. The saying goes that the longer the duration of the larval stage, the heavier the cocoon or its shell layer weight per capita. Hence if the gene controlling the short larval period (*pre*) in the Cambodge stock were to be replaced with the gene controlling a long larval period, productivity of the stock could be improved.

Although sturdiness is highly appreciated as a useful suprior trait in the Cambodge stock as well as in tropical races in general, the genetical nature of the trait has not been characterized and there is no information about the gene system involved in this character (either major single gene or polygene) in autosomes because no sexual difference in the character has been observed so far.

To close this part we want to thank especially Mr. Hideo Sasaki, Chief of the Kobuchizawa Office of the National Institute of Sericultural and Entomological Science, MAFF., for providing us some data of the breeding records accumulated by the staff members of the office. The senior author thanks Mr. Y. Fukase and Mrs. M. Suzuki for their assistance in performing the genetic experiments in Mishima.

(References cited in this report (Part 1) will be listed together with those cited in Part 2 to be published in the next issue.)

(Received for publication, June 28, 1988)