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

2. Multivoltine silkworm strains in Japan and their origin

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In Japan a dozen of multivoltine strains had been maintained even for a short period from the second half of the 19th century to the first stage of the next century[10]. It seems to be likely to suppose that these multivoltine strains would be derived from the tropical races. Some of these multivoltine strains had practically been cultured for the production of silk threads and/or the exportation of eggs. Since then, very little is known about the practical utilization of the multivoltine strains in our country. Nevertheless, several tropical multivoltine strains, Cambodge, Mysore, Pure Mysore, Annam and Ringetsu, have been maintained in the National Institute of Sericultural and Entomological Science, M.A.F.F. Recently, the tropical multivoltine races have been highlighted in our country by reason of physical nature (softness, fineness, etc.) of their products or silk threads. It should be noted here that the multivoltine silkworm strains raised in Japan cease embryonic development (or hibernation) during the unfavorable season from October to the coming April. This event and others are strongly suggestive that in general the tropical race is able to be cultured without difficulty in the temperate zone. The multivoltinism among the most representative characteristics of the tropical race is easily adaptable to some monovoltinisms, from the multivoltine to the tetra-, tri- and bivoltines and rarely to the univoltine, in the temperate zone. In addition, this biological event is only concerned in the phenotypic changes of the gene function controlling the multivoltinism, but that is independent from the essential changes of the gene structure.

In this part, some possible mechanisms on the adaptation of the tropical multivoltine race including the strain Cambodge to the temperate climatic zone will be discussed from a viewpoint of ecogenetics.

Multivoltine Japanese stocks and their origin

The record of non-diapause stocks in Japan dates back to the official chronicle "Shoku Nihongi" written in the 8th century[10]. In the late Edo era the term "Natsugo" (summer generation silkworms) which was coined in the documents written in those days, when artificial hatching techniques were not established yet, suggests the utilization of multivoltine stocks at that time. It is also recorded that multivoltine stocks were employed in practice on a large scale and that eggs of these stocks were exported in the Meiji era. However, these multivoltine stocks suddenly disappeared from authorized lists of practical or commercial silkworm stocks. This may be ascribed to the fact that the breeding or culture conditions for these stocks, which were not always satisfactory, resulted in a decrease of the productivity. It should be noted here that these multivoltine stocks sporadically derived from domesticated silkworms which had been introduced into Japan in ancient times. In fact, these silkworms were not directly derived from wild silkworms, *Bombyx mori mandarina* Moore, but introduced into Japan via the Korean Peninsula or directly from silkworms already domesticated in the Asian Continent, including China. Thus, it is important to clarify the introduction route of these multivoltine genes or stocks as well as of the domesticated silkworm strains in general and to analyze the expression mechanism of the multivoltine genes present in these stocks. A stock carrying a homozygous non-diapause gene factor such as the *npnd* or *pnd* gene can not survive in winter of the temperate zone such as Japan unless it becomes the dormant state[18-20]. Therefore, it is difficult to consider that such a factor is carried in the homozygous state in a population on a certain scale. In fact, the long preservation of the non-diapause genes in Japan has been involuntarily carried out. As discussed above, the presence of a facultative non-diapause type factor *npnd* seems to be suitable for the temperate climatic conditions since the factor is epistatic to *pnd*[19,20]. Accordingly, it is possible to maintain the *pnd* and *npnd* genes in a homozygous state. In short, it can be said that the non-diapause genes can be preserved and spread by a skillful natural mechanism even in the temperate region, though on a restricted scale.

Apart from the breeding standpoint, it is important to consider the origin of the Japanese multivoltine silkworms and of the *Bombyx* silkworm in general, the original place of the *Bombyx* silkworm, the speciation mechanism of several geographical races, the adaptation mechanism of diapause phenomena, etc.

1) Egg diapause as an adaptive strategy for survival in the temperate zone

Almost all the domesticated *Bombyx* silkworms reared in Japan belong to either the uni- or bivoltine type, and the embryonic developmental activity in egg-diapause state is reduced during unfavorable climatic conditions from late autumn through winter until the next early spring. During this period, mulberry leaves, on which they feed, are not available. Diapause of the mulberry silkworms is presumably triggered by the change in photoperiod[15], or daylength which is perceived by the cerebral photoreceptive organs. Photoperiodic information is transmitted to the neurosecretory system including the brain-suboesophagal ganglion (SG) system for the induction of the secretion of a diapause factor (or hormone)[6-9], which then acts on follicle epithelia. The quantitative or qualitative differences in the egg chorion secreted from the epithelia may affect the induction of the embryonic diapause state into either a non-diapause or diapause state. Initiation of the embryonic diapause of silkworms is known to resume about 24 hours after oviposition or early gastrulation[29]. Thereafter, a rapid reduction of the respiration occurs. At the same time, glycogen in the egg is converted into two kinds of polyalcohols, sorbitol and glycerol, and the glycogen content remains low[2-4]. A mixture of sorbitol and glycogen acts as an antifreeze as in the case of ethyleneglycol. Therefore, silkworm embryos can survive a very cold period in a supercooling state. When the embryos awake from the diapuase, these polyalcohols are reconverted into glycogen and become available as energy source for the late embryonic development. By such a sophisticated survival mechanism or adaptation the silkworms are able to live in the temperate zone.

It is important to determine whether the diapause phenomenon of the uni- and bivoltine races is a mechanism inherited from ancestors. It appears that these temperate races can survive in the subtropical or tropical zone like the tropical races. When the temperature exceeds ca. 35°C, the physiological activity of the silkworm larvae decreases regardless of their voltinism, and most of them die at a temperature of above ca. 40°C or more. On the basis of these facts, it is assumed that silkworms are less resistant to a high temperature than to a low temperature. Insects of a tropical origin are highly susceptible to high temperatures in general[31] as well as to low temperatures. Although cold weather seldom occurs in the tropics, at high altitudes in the tropical area these races must have acquired a resistance to cold. If this assumption is valid, it is likely that a certain ancestor of the *Bombyx* silkworm may have once lived in a cold area. In contrast, silkworms with the diapause type that were reared in the temperate zone including Japan can be reared in the tropics without much difficulty. These observations suggest that these silkworms had once been exposed to tropical or subtropical climatic conditions in the past. The diapause-nondiapause relation of the tropical race is highly reversible in general, while that of the temperate race is almost irreversible (Fig. 1). This may be ascribed to qualitative differences in the diapause mechanism between the tropical and the temperature silkworm races. Also the diapause phenomenon in the latter races may have suppressed the developmental activity at the early stage of embryonic development. Therefore, artificial hatching techniques in the silkworms were developed for the uni- and bivoltine races.

What is the original habitat of the multivoltine and uni- or bivoltine stocks? How did silkworms which inhabited subtropical or tropical zones acquire the egg diapause? Insects belonging to the *Lepidopteran* order require a moderate temperature (or effective temperature) and humidity or moisture for their growth. The forest zone, thus, is thought to be an ideal habitat. In Asia, the area which satisfies the above-mentioned environmental requirements would correspond to the subtropical zone rather than to the tropics. In such an area the minimum temperature is about 18–19°C and the average temperature about 25–27°C throughout the year. Accordingly, such a range of temperature seems to be suitable for the silkworm growth. In addition, the subtropical zones in Asia are mountainous. It is well-known that the higher the elevation the lower the temperature. It may be relatively easy for an insect population living in a flat ground to move to a mountainous region located near the flat ground and vice versa. In a highland, the daily and annual changes in the temperature are considerable. Among the insect populations which moved to a highland, i.e., a cool area, there may have been individuals which became acclimatized to the cool climate and acquired the potential to extend their niche further north by becoming resistant to a low temperature. Namely, this resistance to low temperatures may have enabled them to further move to the temperate zones. There were several glacial periods during the geological ages (*Lepidoptera* is said to have appeared 60 million years ago). The glacial periods may have exerted a selective pressure on the silkworm populations that once acquired the resistance to a low temperature. It must be emphasized that a low-temperature resistance may have been acquired in almost the same latitudinal area, and that a periodical change in the temperature under the photoperiodic conditions (change in the daylength) may have been the major environmental factor for the acquisition of the resistance. A change in the daylength is an accurate index of seasonal changes in a high latitude area. However, as far as the diapause

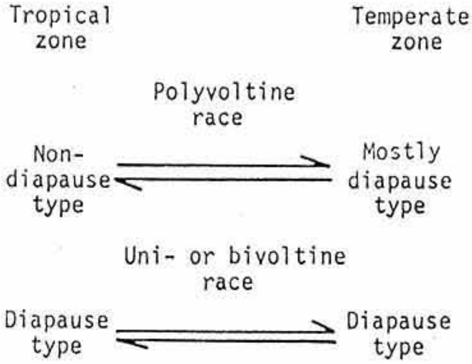

Fig. 1. Transformation of voltinism between the uni-, bi- and multivoltine silkworm races in their respective habitats

is concerned, the atmospheric temperature is apparently the primary factor. The difference in the number of generations per annum or voltinim is considered to be due to differences in the environmental conditions which the silkworms encountered during the course of expansion of their habitat in each climatic zone after the acquisition of the embryonic diapause.

Since it appears that a diapause population can be separated from a non-diapause population in a relatively narrow area, crossing between the two populations may occur frequently. As a result, the genes which control the egg-diapause can be maintained in either homozygotic or heterozygotic conditions in the country of origin of the non-diapause silkworms. Populations which moved to the northern temperate zones are considered to be well adapted to the environment because they harbor the diapause genes in a heterozygotic (most of the population) or homozygotic (some of the population) condition. It should be noted that the silkworms did not require other adaptive characteristics after they moved to northern regions since they had already acquired the diapause gene in the country of origin.

Native *Morus* mulberry trees are widely distributed between latitude 10°N and 45°N, but the number of species is larger in the subtropical zone than in the temperate zone. It is suggestive that in *Moraceae* it includes bread fruit tree, which grows in the tropical and subtropical zones of Asia[16]. In these areas, there is a large number of species in the family *Bombycidae* as well[1,24]. Therefore it is conceivable that a certain origin of non-diapause type silkworms is located in the subtropical zone.

Diapause phenomena appear to be the driving force which enabled silkworms, originating from the subtropical zone, to diffuse and distribute in the general concept that various kinds of insects which originated in the subtropics or tropics became distributed into the temperate zone as a result of the acquisition of the diapause strategy for survival. This hypothesis can account for the fact that the multivoltine forms of *Bombyx* silkworms sometimes appeared and were utilized in Japan. Some ancestor of the commercial silkworm population (or stock) introduced into Japan, through trade or cultural exchange between Japan and China or other countries, is considered to have harbored heterozygotic combinations for the non-diapause and diapause genes.

2) *Attempt to estimate the genetical nature of the Japanese multivoltine stocks*

In *Bombyx* silkworm, there are several genes responsible for the embryonic diapause. One is the *pnd* gene (pigmented but non-diapause egg) which is located on the 11th linkage group[30]. This *pnd* gene is indifferent to environmental conditions[13] but is not related to the diapause hormone[32]. The *pnd*-2 gene, which is considered to operate like the *pnd* gene, has a locus on the 12th linkage group[5]. The third one is the so-called V gene which is related to voltinism and located on the 6th linkage (21.5 units). The fourth one is the *npnd* gene (non-pigmented and non-diapause egg) which is a sex-linked recessive type[18]. Also, the gene exerts a maternal effect on the phenotypic expression. This gene is related to the hormonal switching mechanism from the non-diapause to the diapause state in anticipation of unfavorable environmental conditions. In addition to these, there are other gene factors related to the diapause phenomenon in silkworms.

Most of the temperate races with either the uni- or bivoltine form are considered to be homozygous for the *pnd* gene, while multivoltine silkworms such as the Cambodge stock in Indonesia are homozygous for the *pnd* gene. It is assumed, however, that the *pnd* allele was separated and fixed long time ago since this allele is indifferent to the changes of the environmental conditions unlike the *npnd* one. On the other hand, the *npnd* character in the Cambodge stock is considered to be relatively new compared with the *pnd* one. It is possible that the *npnd* gene allows a subtropical population to expand into both tropical and temperate populations. In fact, as already mentioned, the *npnd* gene confers a high degree of adaptation to the change of the environmental conditions in the new niches. Also, it is likely that the multivoltine Japanese stocks which appeared in the past may have been derived from the populations haboring a *npnd*-like gene in the heterozygous state rather than a *pnd*-like one.

Concluding remarks

Based on a general view of the Cambodge stock, a tropical race, several unsuspected but useful genetic characters were revealed which could be used for the breeding of *Bombyx* mulberry silkworms. On the other hand, various unfavorable characters are maintained. It should be noted that characteristics of the tropical race are complementary to those in the temperate race: the former race growing more rapidly in the larval stages than the latter and the former producing much more silk materials per capita than the latter, for example. Based on the genetic analysis of the Cambodge stock it was shown that some of the characters distinctive features of the tropical race are inherited in a single recessive (or dominant) type, while those in the Japanese or Chinese race are inherited in a dominant (or recessive) one. For the voltinism, the Cambodge stock harbors, at least, two different recessive genes, *npnd* and *pnd*, controlling the non-diapause state, while the temperate race, Japanese or Chinese one, harbors dominant alleles, $npnd^{+}$ and pnd^{+}, which are responsible for the diapause state. The non-diapause trait of the *npnd* gene confers a high adaptability to the change of the environmental conditions including photoperiodism, whereas that of the *pnd* is entirely indifferent to the environmental conditions. Moreover, the former seems to be related to the endocrinological system which affects the diapause state, while the *pnd* gene does not participate in such a system[32].

The recessive gene *gar* detected in the Cambodge stock, which is responsible for the yellow ochre pigmentation of the diapausing eggs and is similar to the color of fruits of gardenia trees, is located on a certain autosome. This trait is considered to be common to the tropical race. The cocoon of the Cambodge stock shows a bright reddish yellow color similar to that of gambodge prepared from fruits of gardenia trees which is under the control of a single dominant gene *Gam* located on a given autosome, too. A spindle shaped cocoon is characteristic of the tropical race in general. This trait is under the control of a single recessive gene located on a certain autosome as well, while both Japanese and Chinese races have a dominant gene factor responsible for the so-called well-shaped cocoon. Prematurity is a common trait in the tropical race and the character is under the control of a single recessive sex-linked gene *pre* (premature), while the dominant factor is maintained in the temperate race. The reason why the tropical race displays the sturdiness trait which is the most important economic character in contrast to the temperate race has not been elucidated.

In this paper, a breeding strategy was proposed to further improve the productivity of a certain race which preserves its superior and visible characters, for which the latter has long been valued by local sericulturists, by introducing some superior characters from another race which eliminating unfavorable ones. It is well known that in the tropical race some quantitative characters such as the cocoon shell weight ratio, flossiness, cocoon shape, etc., are inferior to those in the temperate race, while the sturdiness is a favorable character. The growth rate of the tropical race tends to be earlier than that of the temperate race under the relatively high temperature ranges prevailing in the tropics. Accordingly, the introduction of the sturdiness into the summer generation strains of the temperate race would contribute to the improvement of the productivity by reducing the loss of larvae. This strategy can also be applied to the other generations. As the insects are vigorous rearing would become easy and time could be saved. In the tropical race some of the unfavorable characters listed above should be replaced with those of the temperate race, while preserving the sturdiness.

The genetic analysis of the Cambodge stock revealed that some of the characters are located on different linkage groups, while others are located on the same linkage group, which would allow recombination. Although linkage or gene map analysis takes time, it is essential to undertake a genetic analysis of the tropical race to promote DNA recombinant studies in the silkworm.

It should be emphasized that the tropical race is able to adapt to the climatic conditions of Japan, as evidenced by the transformation of the non-diapausing egg form to the diapausing form under unfavorable climatic conditions for growth and that eggs from the multivoltine stock have been exported on a large scale to other countries in the past.

The breeding strategy should emphasize major genes rather than polygenes. In the current breeding methodology of the commercial strains, hybrids between the Japanese and Chinese races are observed in Japan, while those between the tropical and temperate races are very rare. In India, however, the commercial strain, Mysore Princess which is highly productive is derived from a hybrid cross between a Japanese bivoltine and an Indian multivoltine race. The tropical race shows various characteristics related to quality of products such as softness of thread (M. Miki, personal communications), high temperature resistance, etc., which have not been thoroughly studied frequently. Accordingly, the introduction of unknown superior quantitative as well as qualitative traits in the tropical race could be realized in a short period of time compared with polygenic selective breeding. It must also be emphasized that environmental conditions such as photoperiodism, temperature, daily digestive rhythm or effective time zone, time interval between feeding, etc., affect the expression of several genetic characters. Biological response to different environmental variables is different in each geographical race. Therefore, it is important to coordinate several research fields of sericultural science including breeding and rearing methods and to adapt an integrated approach to sericulture.

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