Breeding of the silkworm race “Taisei” non-susceptible to a densonucleosis virus type 1

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The No.908 strain of the silkworm, Bombyx mori, has a dominant gene, Nid-1, in homozygous, which controls the non-susceptibility of the silkworm to densonucleosis virus type 1 (DNV-1). To establish a silkworm race, non-susceptible to DNV-1, the Nid-1 gene was introduced to a practical race, N 150; the F1 hybrid of N 150 x No.908 was recurrently backcrossed with N 150 for several generations and individuals having heterozygous Nid-1 (Nid-1/+) were ascertained in every generation as survivors of the oral inoculation of DNV-1. In the 6th generation of backcrossing, Nid-1/+ individuals were sib mated to establish a strain having homozygous Nid-1. In the subsequent generation, the progeny was exposed to DNV-1, and the survivors, whose genotype was supposed to be Nid-1/Nid-1 or Nid-1/+, were further sib mated. Some males which had been used in the sib mating were also mated with female of the susceptible race to test whether the genotype of the male is homozygous Nid-1 or not. In the 2nd generation of the sib mating with males which had been ascertainment to have homozygous Nid-1, a progeny line produced all of batches containing only non-susceptible larvae was determined to be the strain with homozygous Nid-1. After improvement in economic characters for several generations, the strain with homozygous Nid-1 was established as the practical race, N 203, non-susceptible to DNV-1. The cross, N 203 x C 150, was authorized by the Minister of Agriculture, Forestry and Fisheries in March 1996, as the commercial silkworm race named “Taisei” for spring rearing.

Key words: silkworm breeding, Bombyx densovirus type 1, non-susceptible to DNV-1.

Introduction
Most of the damage to sericulture can be attributed directly to silkworm diseases, rather than to unfavorable weather conditions that lead to a poor harvest of mulberry leaves. Therefore, the prevention of silkworm diseases is one of the most important problems in the commercial aspects of sericulture. Although the disinfection of rearing rooms and instruments by spraying disinfectants is generally done before each rearing season, it is not necessarily adequate to prevent the occurrence of silkworm diseases. Along with disinfection, the use of silkworm races which are resistant to diseases is necessary for more effective disease prevention. Of the silkworm diseases which cause economic damage, virus diseases are very dangerous.

Resistance of the silkworm to nuclear- and cytoplasmic-polyhedrosis virus (NPV, CPV) and infectious flacherie virus (IFV) are controlled by...
polygenes (WATANABE, 1991). Accordingly, breeding of resistant strain to these viruses is possible by selection of survivors after virus exposure (WATANABE, 1967; ARATAKE, 1973a, b).

On the other hand, the silkworm non-susceptible to densonucleosis virus type 1 (DNV-1) was ascertained to have a homozygous recessive gene (nsd-1) (WATANABE and MAEDA, 1978, 1981). The nsd-1 gene is located at position 8.3 on the 21st chromosome of the silkworm (EGUCHI et al., 1991). Similarly, the dominant gene (Nid-1) controlling non-susceptibility to DNV-1 infection was found (EGUCHI et al., 1986). These genes can be introduced to an existing commercial race by crossing. The use of a non-susceptible to DNV-1 race thus bred is attractive, because no action is needed for the virus prevention during sericulture.

We introduced Nid-1 gene into an existing practical race by backcrossing and further established the commercial race named “Taisei” that is non-susceptible to DNV-1. We describe in the present report the breeding process of “Taisei.”

Materials and Methods

Materials for breeding: A silkworm strain, No.908, having Nid-1 (No infection with DNV-1) gene in homozygous was used as a paternal strain for the original crossing. The No.908 strain stocked in our institute also has mutant genes such as pW (Moricaud), Ng (No glue), Bm (Black moth), and I* (sooty plain yellow inhibitor). A commercial race, N 150, susceptible to DNV-1, was used as the race to which Nid-1 gene would be introduced. The race is one of the parents of the hybrid race “Owashi,” N 150 x C 150, bred in our institute (EGUCHI et al., 1995).

Virus and virus exposure: DNV-1 (densonucleosis virus type 1) provided by Dr. Y. FURUTA was fed to the silkworm larvae. Ten grams of the diseased larvae were triturated with 90 ml of distilled water in a mortar, and the homogenate was centrifuged at 10,000 rpm for 30 min. The supernatant diluted 10 times with distilled water was used as the virus inoculum. For screening of non-susceptible larva with Nid-1 gene, mulberry leaves which had been smeared with the virus solution were fed to the newly hatched larvae for 24 hr. Afterwards, they were reared on uncontaminated leaves and survivors, 7-12 days after the virus inoculation, were determined to be non-susceptible larvae.

Introduction of Nid-1 gene: First, N 150 females were crossed with No.908 males, and secondly females of the F₁, were backcrossed with N 150 males. Thus backcrossing with N 150 females was further continued for 6 generations. In every generation, non-susceptible larvae Nid-1/+ were selected as survivors after the virus exposure and their female moths were backcrossed with N 150 males. In the 6th generation of backcrossing non-susceptible individuals were sib mated and the non-susceptible progeny was selected by virus exposure. Their sib mated progeny batches were reared separately for further two generations, and the non-susceptible strain, Nid-1 homozygote, who produced all of batches containing only non-susceptible larvae, was established.

Improvement in economic characters and breeding of commercial race: The breeding line (HN92) with Nid-1 was further improved in economic characters such as cocoon quality, reelability, number of eggs laid, etc., by intra- and inter-batch selections. During the process, a test of combining ability of HN92 to an existing race, C 150, was carried out for establishment of a commercial hybrid race. The method was the same as that conducted in the breeding of commercial races, “Shoho” and “Owashi” (EGUCHI et al., 1995).

Results and Discussion

Establishment of a breeding line with Nid-1 gene in homozygous: In the recurrently backcrossing for 6 generations, most of the chromosomes, except which Nid-1 is located on, are theoretically supposed to be similar at 99.3% to that of the parental race used recurrently for the backcross (TAZIMA et al., 1955).

In every generation, larvae were exposed to DNV-1 and survivors, Nid-1/+, were used for the next backcrossing with N 150. In the virus
Exposure conducted in every generation of backcrossing, the survival larvae were obtained at about 50% level as shown in Table 1. In the 6th generation of backcrossing the survivors of the virus exposure were sib mated to obtain Nid-1 homozygotes. Theoretically, in the progeny from the sib mating, Nid-1/Nid-1, Nid-1/+ and +/+ larvae are segregated in a ratio of 1:2:1. As the result of virus exposure, about 75% of the treated larvae, whose genotype was supposed to be Nid-1/Nid-1 or Nid-1/+ survived.

At the next step, the survivors were sib mated and, at the same time, the males which had been used in the sib mating were also doubly mated with females of the susceptible race to test genotype of the male. If the batch from the test mating contains only survivors of the virus exposure, genotype of the male is ascertained to be Nid-1/Nid-1. Forty six males were used in the test of genotype, 5 males were ascertained to be Nid-1/Nid-1. Seven females were mated with those 5 males with Nid-1/Nid-1, and 7 batches (lines) were obtained. These 7 lines were supposed to contain only Nid-1/Nid-1 individuals or both Nid-1/Nid-1 and Nid-1/+ individuals at a 1:1 ratio. Furthermore, 13-18 individuals in each line were mated with the adults (race, N124) susceptible to DNV-1. In the subsequent generation, 114 batches were tested for the susceptibility to DNV-1 by the virus exposure. As a result, in 3 lines out of 7, all of their progeny batches containing only non-susceptible larvae, indicating these 3 lines were Nid-1/Nid-1. In the following progeny of these lines, all of their batches were also ascertained to contain only non-susceptible larvae. From these 3 lines we established the non-susceptible breeding line (HN92) to DNV-1 by introduction of Nid-1 to N 150.

Breeding process for economic characters: In earlier steps of breeding of HN92 line by backcrossing, individuals showing above average value for cocoon characters, such as cocoon weight, cocoon shell weight, etc., were selected, and the females were backcrossed with N 150 males. Their 6-8 egg batches were mixed and reared to avoid severe selection and extreme inbreeding.
Although cocoons of No.908 strain and N 150 race were white, their F1 hybrids made yellow cocoons. This might be concerned with that No. 908 strain has a $I^S$ gene, an inhibitor of yellow blood. Along with the yellow color of cocoon, other mutant characters such as “Moriaud ($p^m$)” and “No glue ($Ng$)” originated from No.908 strain were eliminated in earlier steps of breeding. However, “Black moth ($Bm$)” character derived from No.908 strain was not eliminated but remained as a marker character for the breeding line.

As for the breeding process for economic characters, heterosis markedly took place in the F1 hybrid of No.908 and N 150, resulted in a large cocoon weight and pupal weight, but also in a small percentage of cocoon shell weight. However, in the 3rd generation of recurrent backcrossing, most of the economic characters except percentage of cocoon shell weight were showed similar level to those of N 150, the male parental race used in recurrent backcrossing. In the 3rd sib mating generation after recurrent backcrossing, when Nid-1 gene was homozygoously introduced into the breeding race, HN92, values of economic characters except reelability were much the same as those of N 150 race (Table 1).

In spring 1992, a test of F1 hybrid was conducted at Tsukuba and Matsumoto. The test results indicated that cross of HN92 x C 150 was superior in most of economic characters to N 137 x C 146, which was used as a control race of the test, but showed some defects that larval duration was a little longer and reelability of cocoon was lower than that of the control race. Accordingly, to improve cocoon reelability of HN92 strain, 35 and 31 batches were reared in early autumn and late autumn 1992 respectively, and batches showing high reelability were selected. As a result, cocoon reelabilities of 6 batches selected in spring 1993, were 85-100%, suggesting the reelability was much improved as compared with the value in three generations before, when average reelability of 3 batches was 73%.

Afterwards, variation in economic characters among batches was small, indicating a practical race was finally established by breeding of HN92 breeding line. We named the practical race as “N 203” and all of the economic characters were retained by minor selection.

Establishment of commercial race “Taisei”: For establishment of a commercial race which is non-susceptible to densonucleosis virus type 1, we carried out rearing tests of N 203 x C 150, and the results were compared with those of N 150 x C 150 and N 137 x C 146 reared as a control race. Results of the rearing tests made in the spring of 1992 and 1993, indicated most of the economic characters were superior to those of the control races, but inferior in cocoon reelability of cocoon. In further rearing tests conducted in late autumn 1994, however, N 203 x C 150, whose maternal race had been improved in reelability, showed 90% reelability, whereas control race showed 93%. Thus it had been cleared that most of the economic characters of N 203 x C 150 were superior to those of the standard variety and the reelability was improved to the standard level.

Accordingly, we made an application to the Ministry of Agriculture, Forestry and Fisheries for registration of N 203 x C 150 as a new silkworm race. And in spring 1995, rearing tests of N 203 x C 150 was conducted in 13 places of experiment stations and institutes distributed in Japan. The summarized results of tests are shown in Table 2.

The results indicated that although reelability of N 203 x C 150 was a little less than that of the control race, N 137 x C 146, most of the economic characters were considerably superior to that of the control. And also the cocoon of N 203 x C 150 retained favorable characters those derived from the original race, N 150 x C 150, such as long filament, fine (middle) size of filament, etc. Because of these good results, the Minister of Agriculture, Forestry and Fisheries accepted to authorize N 203 x C 150 as a new silkworm race named “Taisei” in March 1996.

Acknowledgments

We thank Dr. H. INOUE, in our institute and
Table 2. Characteristics of N203 × C150. The average of 13 laboratories in the examination directed by the Ministry of Agriculture, Forestry and Fisheries in the spring rearing season, 1995.

<table>
<thead>
<tr>
<th>Race</th>
<th>Duration of feeding period (day)</th>
<th>Percentage of pupation of the 3rd eclosed larvae (%)</th>
<th>Amount of cocoons produced per 3rd eclosed larvae (kg)</th>
<th>Cocoons shell weight (g)</th>
<th>Percentage of cocoons shell weight (%)</th>
<th>Raw silk filament weight (mg)</th>
<th>Length of cocoons filament (mm)</th>
<th>Neocapsid defect per day of 5th instar (%)</th>
<th>Point (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, N203 × C150 (TAISEI)</td>
<td>24.79</td>
<td>95.8</td>
<td>2.34</td>
<td>58.7</td>
<td>25.1</td>
<td>21.19</td>
<td>1,627</td>
<td>1,334</td>
<td>2.74</td>
</tr>
<tr>
<td>N137 × C146 (Control)</td>
<td>24.17</td>
<td>96.3</td>
<td>2.26</td>
<td>53.7</td>
<td>23.7</td>
<td>20.28</td>
<td>1,333</td>
<td>1,125</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Index(A/B × 100) 103 99 104 104 109 106 104 123 119 88 96 100 113

Figure 1. Cocoons shape of "Taisei", non-susceptible to DNV-1 and the parent races No.908, N150, C150 and N203. No.908 strain has Nid-1 in homozygote. N150 race is susceptible to DNV-1. N203 has Nid-1 in homozygote. C150 has not Nid-1. Taisei (N203 × C150) is newly authorized silkworm race of non-susceptibility to DNV-1. N137 × C146 hybrid is general control of the commercial silkworm.

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References


江口良橘・原和二郎・崎崎 旭・広田勝美・一場もとえ・蜷木 理・永易健一：濃核病ウイルス1型非感受性蚕種「大成」の育成

保存系統 No.908 は濃核病ウイルス（DNV-1）に対し非感受性の優性遺伝子（*Nid-1*）を持ちつつある。日 150 号と No.908 を交雑したのち、日 150 号を反復親とした戻し交雑法により、*Nid-1* 遺伝子の実用品種（日 150 号）への導入を行った。毎世代孵化幼虫にウイルスを添食し、生存蛾を *Nid-1* 遺伝子をもつ個体として選抜した。戻し交雑を 6 世代繰り返したのち、同雌区内交配を 2 回繰り返し、ウイルス添食試験を併せて *Nid-1* 遺伝子ホモの系統を選抜した。さらにこの系統の実用形質の改善と向上を図り、濃核病ウイルスに非感受性の実用品種「日 203 号」を確立した。この日 203 号と中 150 号の交雑組合せは、1996 年に春蚕用新品種「大成」として農林水産大臣の指定を受けた。