

Genetic resistance of the silkworm, *Bombyx mori* to viral diseases

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The silkworm, *Bombyx mori* is infected by a number of viruses: *Bombyx mori* nuclear polyhedrosis virus (BmNPV), cytoplasmic polyhedrosis virus (CPV), infectious flacherie virus (IFV) and denonucleosis virus (DNV). Except DNV, resistance of silkworms to the virus is controlled by polygenes. Serologically, two DNV types, DNV-1 and DNV-2 are identified. The non-susceptibility to DNV types is conferred by two recessive genes, *nsd-1* and *nsd-2* and one dominant gene, *Nid-1*. Various selection and hybridization schemes to develop silkworm strains and hybrids resistant to viral diseases are discussed.

SERICULTURE has been one of the main branches of agriculture in Asiatic countries for hundreds of years. Most of the damage to sericulture can be attributed directly to silkworm diseases, rather than to unfavourable weather conditions that lead to a poor harvest of mulberry leaves. Therefore, prevention of silkworm diseases and breeding of a silkworm variety with high productivity are important problems in the commercial aspects of sericulture.

Although the disinfection of silkworm-rearing rooms and instruments by spraying with formalin or slaked lime water is generally done before each rearing season, it is not necessarily adequate to prevent the occurrence of silkworm diseases. Therefore, along with disinfection, the use of silkworm varieties which are resistant to diseases is necessary for more effective disease prevention. Of the silkworm diseases which cause economic damage, virus diseases are the most important.

Four virus diseases of the silkworm are known: nuclear polyhedrosis, cytoplasmic polyhedrosis, infectious flacherie, and denonucleosis¹.

The nuclear polyhedrosis virus (NPV) infects various tissues, and multiplies in the nucleus forming inclusion bodies called polyhedra, which occlude virus particles. The virus is rod in shape (330 × 45 nm) and contains double-stranded DNA.

The cytoplasmic polyhedrosis virus (CPV) infects the midgut epithelium and multiplies in the cytoplasm of columnar cell forming inclusion bodies which occlude virus particles. The virus is an icosahedral particle 60 nm in diameter and contains double-stranded RNA.

The infectious flacherie virus (IFV) infects the midgut epithelium and multiplies in the cytoplasm of goblet

cell without forming inclusion bodies. The virus is spherical, 28 nm in diameter, and contains single-stranded RNA.

The denonucleosis virus (DNV) infects the midgut epithelium and multiplies in the nucleus of columnar cell. The virus is a spherical particle, 22–24 nm in diameter and contains single-stranded DNA. Two strains of *Bombyx* DNV are detected serologically: DNV type-1 (DNV-1) and DNV type-2 (DNV-2). The DNV-1 is more pathogenic to the silkworm than DNV-2 (ref. 2).

The present article treats characteristics of genetic resistance of the silkworm, *Bombyx mori*, to virus diseases and also breeding of the resistant strain.

Mode of inheritance

Genetic analysis of resistance

There is a need to compare the dosage-infection responses of resistant and susceptible parent strains and their F₁ and F₂ offspring for a better understanding of the mode of inheritance of susceptibility to virus³. As shown in Figure 1, if an insect population is almost homozygous

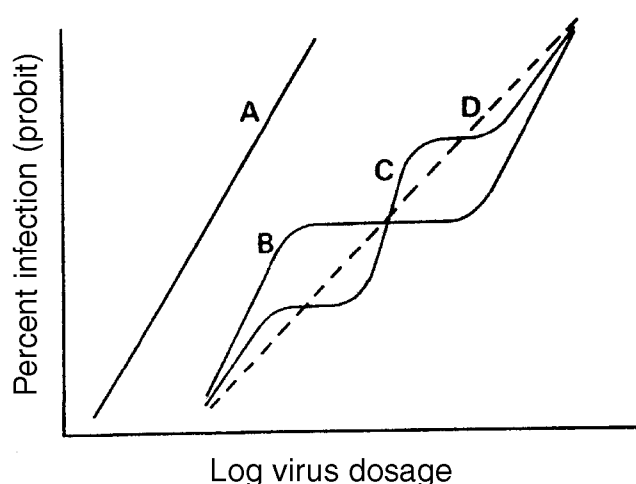


Figure 1. Dosage-infection regression lines of silkworm populations with members responding differently to virus infection: (A) Homozygous, susceptible population; (B) Population with two members having distinctly different susceptibilities; (C) Populations with three members having distinctly different susceptibilities; and (D) Population with many members with minor differences in susceptibility.

in response to the infection of a virus, the regression of the log-dosage-probit infection forms a straightline as in A. The slope of the line indicates the degree of homogeneity: that is, the steeper the slope the more homogeneous is the response. On the other hand, if individuals with distinctly different responses to infection are mixed in one population, the dosage-infection regression is not a straightline, but a curve with plateaus in certain parts of the curve. For example, when individuals with two distinctly different responses are involved in a population, the regression line forms a curve with one plateau as in B. When three members with distinctly different responses are involved in a population, the regression line forms a curve with two plateaus as in C. The more different minor responses in a population, the more the regression curve becomes linear as in D with a low slope.

Resistance controlled by polygenes

There are many silkworm varieties or strains whose genetic constitutions are almost homozygous, and they could be studied intensively to establish the basis for genetic resistance to virus diseases.

Figure 2 is an example of the dosage-infection response to a cytoplasmic polyhedrosis virus (CPV) in resistant (R) and susceptible (S) parent strains and their F_1 and F_2 offspring. The regression line of F_1 hybrid is linear and much closer to that of the resistant parent strain than that of the susceptible strain. The F_1 hybrid larvae show heterosis in their resistance to infection by CPV, i.e. they are more resistant than the larvae of their parent strains. The regression line of F_2 hybrid is also linear, but it has a fairly low slope. These results indicated that the silkworm resistance to CPV infection is controlled by a

multifactorial genetic system, that is, by polygenes. Similarly, the silkworm resistance to NPV and to IFV is controlled, in general, by polygenes^{4,5}.

Although silkworm resistance to CPV infection is generally controlled by polygenes, there is a special case in a strain called Daizo, which has a major gene for CPV resistance⁶. This was demonstrated by exposing to CPV infected larvae of F_1 , F_2 , and of backcrossed hybrids obtained from crosses between the two inbred strains, Daizo and Okusa. Okusa is one of the most highly susceptible strains to CPV. The larval resistance of F_1 hybrids and hybrids backcrossed to Daizo were nearly the same as that of Daizo, while the resistance of hybrids back crossed to Okusa was approximately intermediate between the two inbred strains. These results indicated that the resistance in the Daizo strain was inherited as a complete dominance. Furthermore, the dosage-infection regression lines of the F_1 hybrid between Daizo and Okusa, and of the hybrids backcrossed to Daizo, were quite similar to that of Daizo, while the dosage-infection regression lines of the F_2 hybrid and the hybrids back crossed to Okusa formed curves with plateaus, in part, at the 25% and 50% infection levels, respectively (Figure 3). This suggested that resistant and susceptible larvae segregated at 3:1 ratio in the F_2 hybrid and at 1:1 ratio in the hybrid backcrossed to Okusa. Thus, although the resistance to CPV infection is generally controlled by polygenes, a strain like Daizo, which is highly resistant, possesses a major dominant gene controlling CPV resistance.

Nonsusceptibility controlled by a major gene

Some of the silkworm strains are nonsusceptible to infection by DNV even after intrahemocoelic inoculation of a

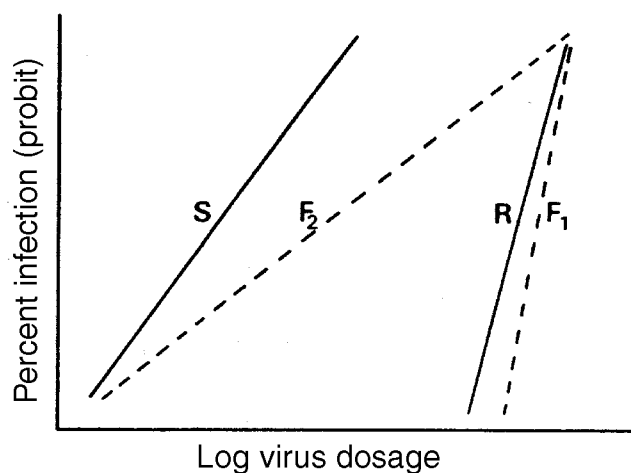


Figure 2. Concentration-infection regression lines for CPV in resistant (R) and susceptible (S) parent silkworm strains, and their F_1 and F_2 offspring.

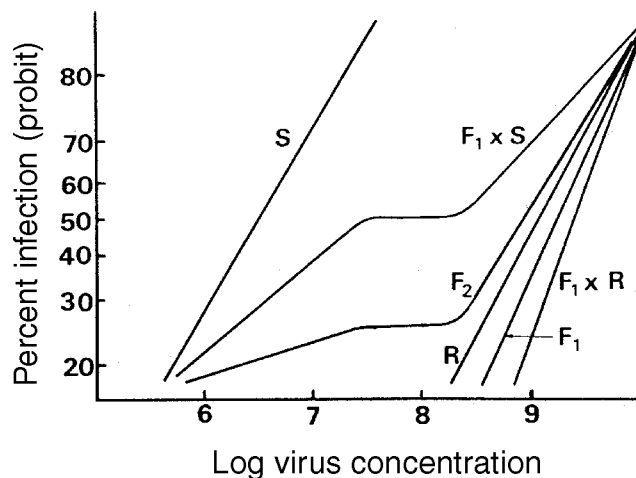


Figure 3. Concentration-infection regression lines for CPV in Daizo (R) and Okusa (S) silkworm strains, their F_1 , F_2 offspring, and hybrids backcrossed to Daizo ($F_1 \times R$) and Okusa ($F_1 \times S$).

high dosage. Tests with susceptible and nonsusceptible parent strains, their reciprocal F_1 hybrids, the F_2 hybrids, and the hybrids backcrossed to either of the parents demonstrated that the nonsusceptibility to DNV-1 infection was inherited as a completely recessive character. The genetic segregation for nonsusceptibility occurred in the F_2 and in the hybrids backcrossed to the nonsusceptible parent⁷. This was confirmed by the observation that the dosage-infection regression lines of reciprocal F_1 hybrids were the same as those of the susceptible parent, while those of the F_2 hybrid and of the hybrids backcrossed to the nonsusceptible parent formed lines with plateaus at the 75% and 50% infection levels, respectively (Figure 4). These results indicated that the nonsusceptibility to DNV-1 infection was controlled by a recessive gene, and the susceptible and nonsusceptible larvae segregated in a Mendelian manner. The recessive gene (*nsd-1*) is located at position 8.3 on the 21st chromosome of the silkworm⁸.

Similarly, the dominant gene (*Nid-1*) controlling nonsusceptibility to DNV-1 infection was found⁹. It was also found that a recessive gene (*nsd-2*) (ref. 10) controls the nonsusceptibility of the larvae to DNV-2. The *nsd-2* gene is located on a different chromosome from those of *nsd-1* and *Nid-1* genes¹¹.

Mechanisms of genetic resistance

Natural infection of viruses in insects occurs perorally. Steps in the infectious process with viruses include the entering of virions into the gut lumen, the adsorption and

fusion of virus particles to the cell plasma membrane of the midgut epithelium, the penetration of virions into the midgut cell where they replicate, and the passing of the virions through the midgut epithelium into the body cavity where they eventually attack the target tissues. Accordingly, the resistance of the host to the virus might be caused by blocking any, if not all, of these steps in infection. For example, when silkworm strains that showed distinct differences in susceptibility to the peroral infection of a CPV were tested for their susceptibility to subdermal infection, the strain differences in susceptibility were quite small. The order of susceptibility of the strains to peroral infection was not correlated with that of strains exposed to subdermal infection (Figure 5)¹². The results indicated that the host resistance to peroral infection depended on inhibitory mechanisms in the gut lumen against the invasion of the virus into midgut cells, and less on the suppression of virus multiplication within the cell.

Watanabe¹³ studied differences in susceptibilities of several silkworm strains to NPV and CPV. He found that the order of susceptibility of the strains to one type of virus was significantly related to the order of susceptibilities to the second type of virus (Figure 6). The results indicated that the mechanisms of resistance of the silkworm larva to the viruses were similar and that the defense mechanisms might occur in the midgut lumen.

As mentioned previously, the resistance of the silkworm to infection by NPV, CPV and IFV is polygenic, and this is consistent with the existence of several defenses against viral infections. Antiviral substances of the silkworm gut juice, such as protease¹⁴ have been found to inactivate both NPV¹⁵ and CPV^{16,17}. Although no parallel relationship could be detected between the resistance of the strains to oral infection with viruses and the antiviral activity of the gut juice, the antiviral activity of the gut juice seemed to play some role in the mechanism of resistance of the silkworm.

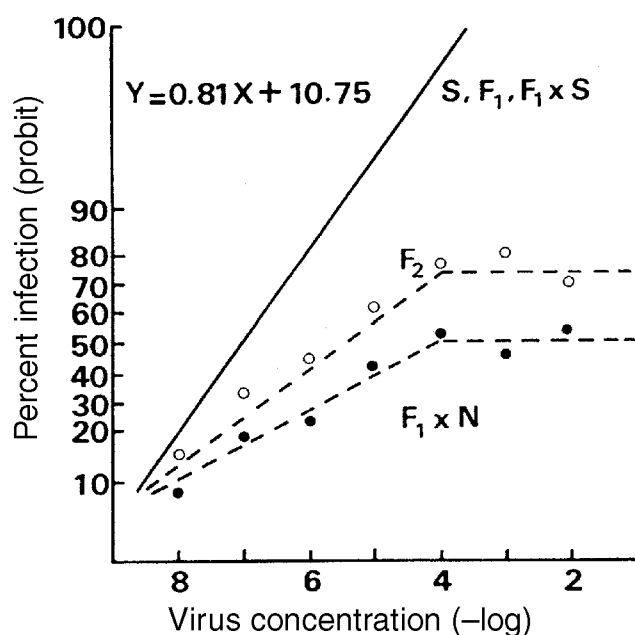


Figure 4. Concentration-infection regression lines for DNV in susceptible strains (S), F_1 and F_2 hybrids between susceptible and non-susceptible silkworm strains (N), and backcrossed hybrids ($F_1 \times S$, $F_1 \times N$).

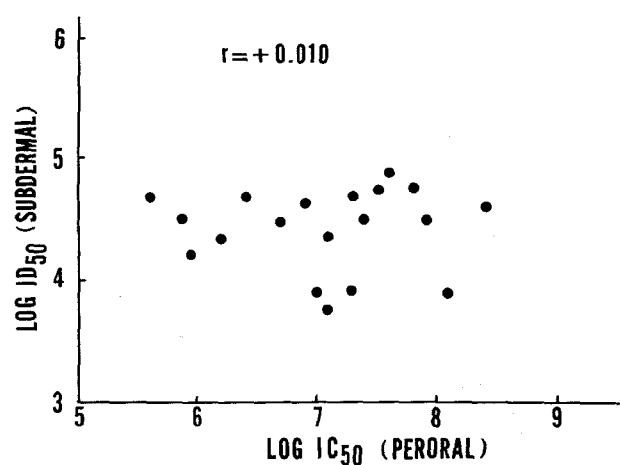


Figure 5. Relationship between susceptibilities of silkworm strains to peroral infection with CPV and those to subdermal infection, showing no correlation between susceptibilities to the both types of infections.

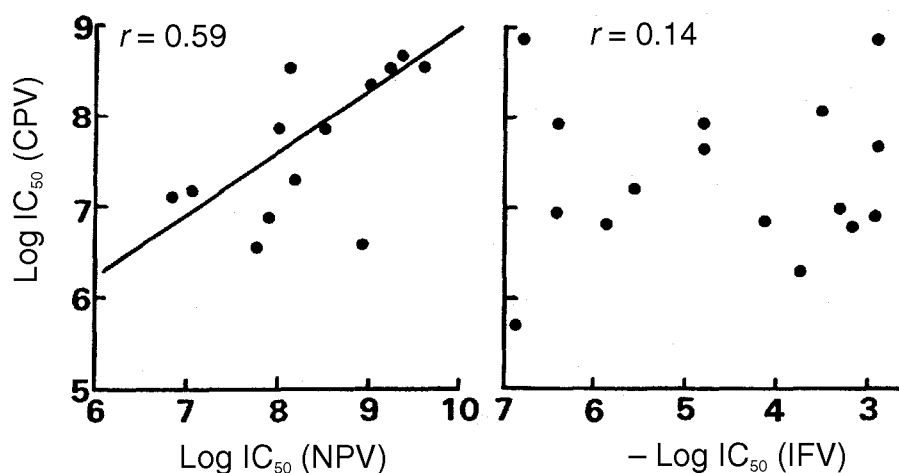


Figure 6. Relationship among various susceptible silkworm strains to CPV, NPV and IFV, showing correlation in susceptibility between CPV and NPV but not between CPV and IFV.

There are some reports that the peritrophic membrane may be one of the major barriers preventing virus adsorption to the midgut epithelium. The peritrophic membrane is supposed to play a role similar to that of the mucus membrane in the vertebrate gut and protect the midgut cells from mechanical damage caused by abrasive food particles¹⁸. In Lepidoptera, this sheath is formed along the length of the midgut and is presumed to be a secretory product of the epithelial cells. It is renewed at each molt. Biochemically, the peritrophic membrane is composed of chitin, protein, and different mucopolysaccharide and hyaluronic acid-like compounds. The virus receptor of a susceptible animal cell is lipoprotein or glycoprotein, which is also plentiful in the peritrophic membrane. The peritrophic membrane of the silkworm larva is able to absorb CPV *in vitro*¹⁹.

Accordingly, the chemical and physical properties of the peritrophic membrane appear responsible for its action as a barrier in filtering or preventing most of the ingested viruses from reaching the midgut epithelium.

In the case of CPV, old midgut columnar cells infected with the virus are discharged into the gut lumen and new cells develop to take their place²⁰. The degree of this epithelial regeneration varies with the silkworm strain, and this may be one of the reasons for interstrain differences in tolerance to disease development. The IFV multiplies in the midgut epithelium to the extent in both susceptible and resistant larvae. But in the resistant strain, infected goblet cells are discharged into the gut lumen at each molt, and regenerative cells rapidly develop into new goblet cells, prolonging the larval period of lethal infection more than that of susceptible strain²¹. Although this mechanism for IFV is similar in action to that for CPV, it seems to have a different genetic basis. Watanabe *et al.*²² have shown that there is no significant

genetic correlation between interstrain differences in the susceptibility to IFV and CPV (Figure 6).

The nonsusceptibility of the silkworm to a DNV is controlled by a single recessive gene⁷. The gene may cause a deficiency of an enzyme involved in virus multiplication or in the receptor synthesis of the midgut cell.

The larva of the genetic mosaic strain has a midgut epithelium consisting of a mosaic of genetically nonsusceptible (*Nid-1/+*) and susceptible (*+/+*) areas. In the area infected by DNV-1, the degraded columnar cells accumulate and attach to the increased number of intact goblet cells to form a multiple-layered epithelium. In the uninfected areas, the single-layered epithelium has a large number of tightly arranged columnar cells with a few goblet cells among them. The increased number of columnar cells in the uninfected area may compensate for the deficient function of the degraded cells in the infected area. The overall effect is prolongation of the lethal infection with DNV-1 in the larva with midgut mosaicism²³. When DNV-2 is fed to the genetic mosaic larvae with susceptible (*nsd-2/+*) and nonsusceptible (*nsd-2/nsd-2*) areas in the midgut, DNV-2 infection only occurs in the midgut area of susceptible cells (*nsd-2/+*), causing non-lethal infection to the host^{24,25}.

External factors affecting susceptibility

It is well known that the virus multiplication in the silkworm or the degree of resistance/susceptibility to virus infection is changed by internal factors such as larval age, molt, metamorphosis and diapause²⁶. The susceptibility, especially when it is controlled by a polygenic system, may be readily modified by external factors, such as route of infection, temperature, chemicals and food, that are applied naturally or artificially to the silkworm.

Route of infection

There is, so far, no evidence of natural infection with viruses through the integument or spiracle; most virus infections occur perorally and some through the egg. However, insects in general are highly susceptible to viruses injected into their hemocoels. Watanabe¹² found 100 to 10,000 lower LD₅₀ values when silkworm larvae were given subcutaneous injections of CPV than when given perorally. This result suggested that much of the ingested virus did not enter the susceptible epithelial cells of the midgut, and the resistance might depend more on inhibitory mechanisms in the gut than on the suppression and virus multiplication within cell.

Food

There is some evidence that food quality is important in the susceptibility of insects to viral infections. Certain varieties of mulberry increase the incidence of silkworm mortality from viral diseases²⁷. The prevalence of most viral diseases in the silkworm is low in spring but increases in autumn. The high incidence of viral diseases in autumn seems to be associated with the quality of the mulberry leaves. For example, when silkworm larvae are reared on an artificial diet containing powdered mulberry leaves obtained from different seasons of the year, those fed on artificial diet containing autumn-harvested leaves are more susceptible to viral infections than the ones reared on artificial diet with spring-harvested leaves²⁸.

However, mulberry leaf alone affects susceptibility since silkworm larvae reared on an artificial diet without mulberry leaf are more susceptible to infection by polyhedrosis viruses than those reared on a diet with mulberry leaves²⁹. Silkworm larvae fed on artificial diets low in protein, low in sucrose, or high in cellulose contents tend to increase in susceptibility to viral infections³⁰. When the larvae are fed with an artificial diet low in protein, a protease in the gut juice is reduced, resulting in the low antiviral activity³¹.

Temperature

Temperature is the most important external physical factor for both silkworm susceptibility and multiplication of viruses in the host. Most silkworm varieties have been adapted to rearing at 25°C, which is the most suitable for their development. Accordingly, temperatures much higher or lower than 25°C tend to act as a stress and increase the larval susceptibility to viral infections³². Exposure of silkworm larvae to low temperatures (5°C for several hours) before peroral infection with CPV³², NPV³³, or an IFV³⁴, enhanced susceptibility to each virus. On the other hand, high temperatures are known to increase resistance or cause the disappearance of viral

infections in plants and higher animals. This is also true in the silkworm. The virus-infected silkworm larvae, when reared at an elevated temperature (36–37°C), were able to survive virus infection^{35,36}. Inoue³⁷ demonstrated thermal therapy in the silkworm infected with IFV. Larvae that had been given a lethal dose of IFV just after hatching did not succumb to the disease but made cocoons when they were repeatedly exposed at each larval ecdysis to a high temperature (37°C) for 24 h.

In the case of DNV, viral multiplication was reduced when infected silkworm larvae reared at 25 to 28°C were subsequently reared at 37°C (ref. 38). Autoradiographic results with ³H-thymidine and ³H-tyrosine revealed that the synthesis of both virus and protein was greatly reduced in infected larvae maintained at 37°C. Fluorescent antibody studies also confirmed that the synthesis of DNV-antigen in the larvae was inhibited at 37°C. These results indicated that high temperatures (37°C) apparently reduced the activity of enzymes concerned with viral DNA and protein syntheses.

Chemicals

Some chemical insecticides and adjuvants increase insect susceptibility to pathogens, resulting in a synergistic enhancement of infection³⁹. Sublethal dosages of certain insecticides enhanced the susceptibility of the silkworm to viral infection⁴⁰. When sublethal doses of DDT and Sumithion, an organophosphorous insecticide, were applied topically to larvae, no signs of intoxication such as paralysis, vomiting, reduction of feeding, or growth inhibition appeared. However, larvae treated with Sumithion were more susceptible to peroral infection with NPV or CPV than larvae not treated with the insecticide, and larvae treated with DDT showed an increased susceptibility to NPV.

Other pathogens

When a mixture of pathogens is inoculated into an insect, the pathogens may coexist, react synergistically, or interfere with one another during infection. In some cases, one of the ingested pathogens or its infection acts as a biological stressor and increases the susceptibility of an insect to another pathogen. For example, Ishikawa and Miyajima⁴¹ have reported that silkworm larvae that have been exposed to bacteria show an increase in susceptibility to viral infection. Watanabe and Shimizu⁴² reported synergism between an IFV and a DNV in the silkworm, but the mechanism was unknown. The interaction took place in a silkworm population susceptible to DNV and probably resulted in a severe epizootic of both viruses. On the other hand, an interference phenomenon was reported to occur between two strains of CPV in the silkworm⁴³.

Breeding of resistant strains

Selection

One of the procedures of breeding for silkworm strain with polygenetically inherited characters is selection for the desired characters. Polygenic resistance of silkworm to viral diseases such as nuclear polyhedrosis, cytoplasmic polyhedrosis, or infectious flacherie will develop after a prolonged selection, under exposure to the virus. The process of selection changes the frequency of genes for viral resistance in a population and causes it to increase. The failure to select for viral resistance in certain population does not necessarily mean that such selection is impossible. It may be the result of the absence of a gene or genes for resistance, a small population size, a population with limited genetic variability or the use of an inappropriate agent to select for the genetic trait.

There are two practical methods for selection of resistant strain; so-called batch selection and individual selection. In batch selection, sample larvae from each batch are tested for susceptibility to a virus. As a result, the batch showing the highest resistance to the virus is selected for further breeding. The batch test for susceptibility to the virus is continued in the subsequent generations. There is no risk of losing the strain in breeding due to the viral infection, but the selection effect comes out slowly. In individual selection, the larvae of mixed batches are fed with a virus and the offspring of the surviving individuals are further exposed to the virus. The exposure to the virus is continued in the subsequent generations. In this method, the selection effect comes out fast, but the concentration of virus suitable for selection pressure (70–80% mortality is preferable) is difficult to determine. There is also risk of losing the strain in breeding by a highly lethal viral infection due to the strong selection pressure applied.

Uzizawa and Aruga⁴⁴ and Funada⁴⁵ attempted the selection of silkworms resistant to IFV infection by repeated exposure of the virus, and they succeeded in obtaining a resistant strain after several generations. Aratake⁴ also succeeded in breeding of a strain resistant to IFV by means of the batch selection.

Watanabe⁴⁶ attempted to select a silkworm strain resistant to CPV. The larvae were fed with a diet containing CPV, and the offspring of the surviving individuals were exposed to the virus. The exposure to CPV was continued for eight generations. There was no increase in resistance to CPV up to the fourth generation, but resistance increased suddenly in the fifth generation (Figure 7). The lack of resistance in early generations may be partly caused by the application of a low dosage of virus which caused a low per cent mortality. The study suggested that greater than 60% mortality was required as a selection pressure in order to induce and retain resistance to CPV infection. Despite continued rigorous selection, there was

no further increase in resistance after a certain plateau of about 10 to 16-fold of that of the unselected strain.

When two selected strains were crossed, the hybrid was more resistant than the hybrid from crosses of unselected strains (Figure 8). However, when the two hybrids were compared on the basis of heterosis in resistance, several different features were observed. The resistance of control hybrids appeared greater than that of the unselected parent strains, and this indicated the existence of marked heterosis. On the other hand, none of the hybrids from crosses of selected strains were more likely to develop marked heterosis in resistance, while in the selected strains resistant genes of similar genetic constitution became integrated during the course of selection, and the genetic combination of the two selected strains tended to show poor heterosis.

Combining ability test

Usually, practical silkworm variety consists of F₁ hybrids of two strains or double crossed hybrids of four strains

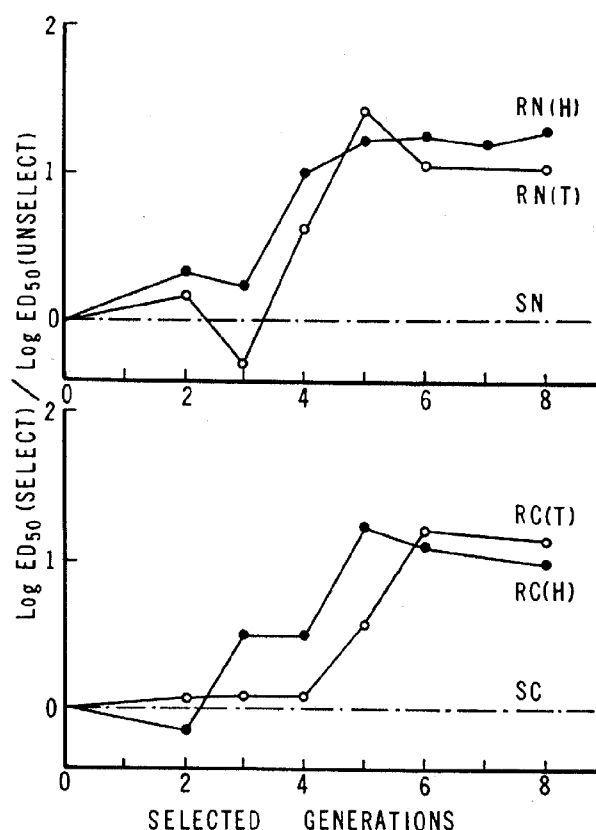


Figure 7. Development of resistance in the 4th-instar larvae to CPV infection in successive generations of the silkworms selected with the virus. RN(H) and RN(H) are selected strains with HC virus forming hexagonal polyhedra and TC virus forming tetragonal polyhedra, respectively, from SN control Japanese strain. RC(H) and RC(T) are selected strains with HC virus and TC virus, respectively, from SC control Chinese strain. The degree of resistance is presented as a logarithmic ratio obtained by dividing the log ED₅₀ of the selected strain by the log ED₅₀ of the unselected strain.

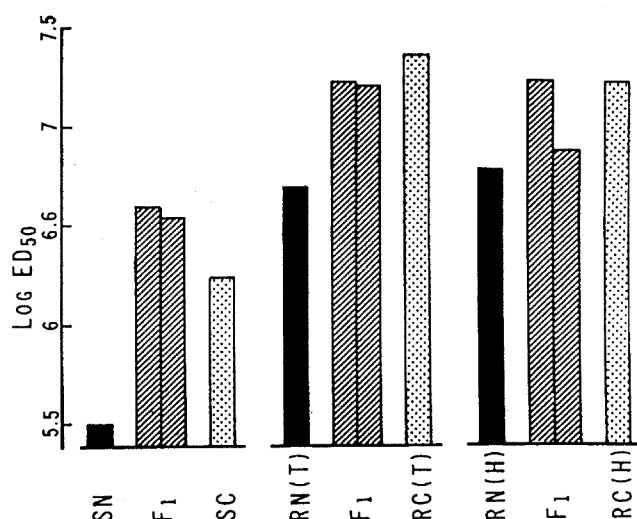


Figure 8. Comparative resistance in the 4th-instar larvae to CPV among selected (RN, RC), unselected (SN, SC) strains and their hybrids.

(tetra-parental hybrids). As mentioned previously, F₁ hybrid shows heterosis in polygenic resistance to virus: F₁ hybrid is more resistant than the parental strains. Therefore, on the basis of the combining ability test of existing strains, it is possible to establish a silkworm variety, or cross combination of strains, which shows high heterosis in resistance to virus.

The expression of polygenic traits, such as cocoon weight, resistance to disease, etc., is easily affected by external factors. The polygenic expression in double-crossed hybrids is more stable than single-crossed hybrids in unfavourable environment because of their flexibility in gene constitution within the population. Accordingly, under unfavourable rearing conditions it is reasonable to use double-crossed hybrids for preventing diseases and getting stable cocoon production.

Introduction of major genes

The use of nonsusceptible or refractile varieties to diseases is attractive from the managerial and economic standpoints because it requires no action for the disease prevention. Breeding for a nonsusceptible variety is much easier if the mechanism of nonsusceptibility is controlled by a single major gene. The gene can be introduced into a breeding program, or transferred to an existing suitable variety by backcrossing.

Recessive major gene, such as *nsd-1* or *nsd-2*, controlling nonsusceptibility to DNV infection can be introduced easily into a strain in an early stage of the breeding program for productive characters. As shown in Figure 9, if a strain A (+/+) in an early stage of breeding does not possess gene(s) for resistance, it should be crossed with a strain B (*r/r*) which is homozygous for the genes for resistance.

The F₂ population is administered with a DNV solution of high concentration so as to select the survived larvae having nonsusceptibility gene in homozygous condition. After the F₂ generation when the introduction of resistant gene is established, the breeding of strain will be continued only for productive characters.

Figure 10 shows a schematic procedure for the introduction of a recessive nonsusceptibility gene (*r*) such as *nsd-1* or *nsd-2* to an existing superior variety A (+/+) by backcrossing at every two generations. The F₂ population from a cross between variety A and strain B (*r/r*) having recessive nonsusceptibility gene in homozygous condition is exposed to DNV. As a result, larvae surviving the infection are supposed to have nonsusceptibility gene in homozygous condition and their male moths are backcrossed to female moths from variety A, expecting crossing over on the male chromosomes.

In the first backcrossed generation, sister and brother mating is done to get the progeny. In the next generation, the population is exposed to DNV to select the survived individuals who have nonsusceptibility gene in homozygous condition. Their males are used for backcross to variety A. Thus, if the backcross is repeated six times, a variety almost similar to variety A at 98% of genetic similarity with DNV nonsusceptibility gene in homozygous condition could be established.

If the nonsusceptibility gene is dominant such as *Nid-1*, introduction of the gene by backcrossing and exposure to DNV for selection of nonsusceptible larvae are repeated every generation. After the backcross is repeated six times over, sister and brother mating is done in the population. The batch containing no susceptible larva appearing in the following few generations is supposed to

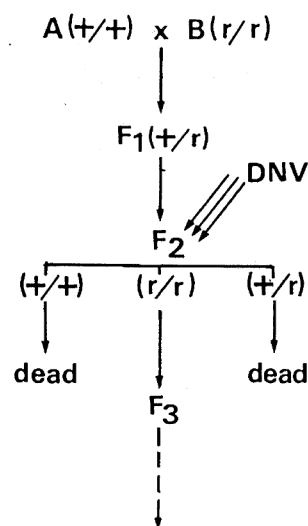


Figure 9. Schematic procedure of introduction of DNV nonsusceptibility gene to a breeding strain. A(+/+), a susceptible strain to DNV in an early stage of breeding; B(*r/r*), nonsusceptible strain with a recessive nonsusceptibility gene (*r*), such as *nsd-1* or *nsd-2*, in homozygous condition; DNV, a densovirus.

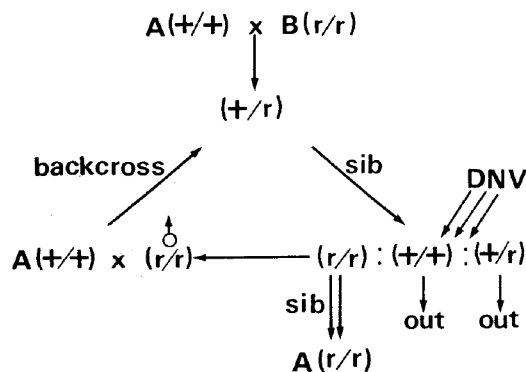


Figure 10. Schematic procedure of introduction of DNV nonsusceptibility gene to an existing variety by recurrent backcross in every other generation. A (+/+), an existing variety with excellent traits but susceptible to DNV; B (r/r), a nonsusceptible strain with a recessive nonsusceptibility gene (r), such as *nsd-1* or *nsd-2*, in homozygous condition; sib, sister and brother mating.

be the established variety introduced with dominant nonsusceptibility gene in homozygous condition.

Conclusion

Resistance of the silkworm to viral diseases such as nuclear and cytoplasmic polyhedroses and infectious flacherie is controlled by polygenes. The polygenes are supposed to be mainly concerned with defense mechanisms of midgut such as antiviral activity of gut juice, characteristics of peritrophic membrane, etc. On the other hand, nonsusceptibility to densonucleosis is controlled by recessive (*nsd-1*, *nsd-2*) or dominant (*Nid-1*) major genes. The major gene may cause a deficiency of an enzyme involved in viral multiplication or in the receptor synthesis within the midgut cell.

Polygenic resistance can be introgressed into a silkworm variety by selection in a breeding program of the silkworm variety. A hybrid of two strains usually shows high heterosis in polygenic resistance to viral diseases. Therefore, it is advisable to establish the resistant variety on the basis of the combining ability test of existing strains.

The breeding procedure for nonsusceptible variety to DNV is much easier because the mechanism of nonsusceptibility is controlled by a single major gene. The gene can be introduced into the breeding program, or transferred to an existing superior variety by backcrossing. Thus, use of nonsusceptible variety to DNV is attractive because of no action for the control of virus during sericulture.

Genetic studies on the resistance of silkworm to diseases other than viral diseases are still limited. The general methodologies for analysis of the genetic basis and mechanism of viral disease resistance described in this article would help in achieving progress in the non-viral diseases of silkworm as well.

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