Int. J. Indust. Entomol. Vol. 7, No. 1, 2003, pp. 5~10

[Review]

International Journal of *Industrial Entomology*

Genetic Basis of Screening of Molecular Markers for Nuclear Polyhedrosis Virus Resistance in *Bombyx mori* L.

Keping Chen*, Qin Yao, Yong Wang and Jialin Cheng[1]

Institute of Life Sciences, Jiangsu University, 301 Xuefu Road, Zhenjiang-212013, P. R. China.
[1]Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang-212018, P. R. China.

(Received 14 April 2003; Accepted 10 June 2003)

The nuclear polyhedrosis virus (NPV) resistance of silkworm is controlled by a pair of dominant genes on autosome and micro-effect modificator genes on sex chromosome Z and has the phenomenon of patroclinal inheritance. Based on its hereditary characteristics, methods of preparing near isogenic lines and their F_2 populations for screening molecular markers were designed.

Key words: Genetic basis, Molecular markers, NPV resistance, *Bombyx mori*

Introduction

China has a history of over 5,000 years in raising silkworms (*Bombyx mori* L.). At present, over 30 million farmer households are involved in sericultural production in over 10 China provinces. Its output of silkworm cocoons, silk and silk fabrics account for over 70% of the worlds total. Apart from China, sericulture is also practiced in Japan, the former Soviet Union and Brazil etc. Silkworm viral diseases are major diseases causing great loss in sericulture, among which the disease caused by nuclear polyhedrosis virus (NPV) is the most disastrous.

The worlds silkworm raising countries all expect to develop new silkworm strains resistant to NPV disease for commercial uses. Silkworms resistance to NPV disease varies greatly among various species (Zhang *et al.*, 1982; Chen *et al.*, 1991). This is the basis for breeding disease resistant strains. Regarding the inheritance of NPV disease resistance, many scholars hold fairly different views. Yet most of them incline to assume that it is mainly controlled by a pair of dominant genes located on an autosome, that it has the phenomenon of patroclinal inheritance, that it has micro-effect modificator genes on sex chromosome Z (Meng, 1982; Chen *et al.*, 1996), and that it is one of the qualitative-quantitative characters (Xu *et al.*, 2000). By employing traditional hybridization method, strains with enhanced resistance to NPV disease can be obtained through selection among progeny of crosses between resistant species and susceptible species. However, the traditional method has a long selection cycle and a relatively low accuracy, resulting in low breeding efficiency. Therefore, we have firstly attempted to develop molecular markers and use them to establish molecular marker-assisted selection system for developing new silkworm strains resistant to NPV disease. This article introduces methods of preparing the near isogenic lines and their F_2 populations for screening molecular markers linked to NPV disease resistance. Meanwhile, the heredity of NPV resistance is discussed.

*To whom correspondence should be addressed.
Institute of Life Sciences, Jiangsu University, 301# Xuefu Road, Zhenjiang-212013, Jiangsu, P. R. China.
Tel: 86-511-8791923; E-mail: kpchen@ujs.edu.cn

Basic assumptions and their verification

Silkworm sex chromosomes are of ZW type, *i.e.*, its females have W/Z chromosomes and its males have Z/Z ones. Thus, the homozygous genotypes of silkworm species resistant to NPV disease can be expressed as *NN*-W/Z^m (♀) and *NN*-Z^m/Z^m (♂), among which *NN* denotes major dominant genes on autosome and Z^m denotes micro-effect modificator genes on sex chromosome Z. And those of susceptible species can be expressed as *nn*-W/Z (♀) and *nn*Z/Z (♂) among which *nn* denotes recessive genes on autosome and Z denotes that there is no micro-effect modificator gene on sex chromosome Z.

The baseline value of silkworms resistance to NPV

In 1991, we conducted a thorough survey on silkworm's

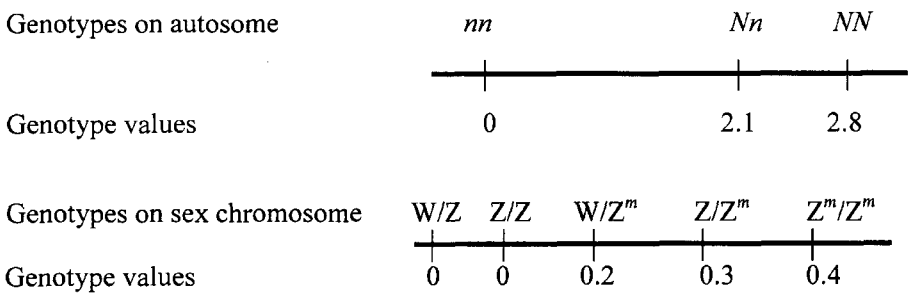

Fig. 1. Assumed values of genotypes related to NPV resistance in silkworms.

Table 1. Genotypes and their values

♂	*NN*-Z^m/Z^m	*NN*-Z/Z^m	*Nn*-Z^m/Z^m	*Nn*-Z/Z^m	*NN*-Z/Z	*Nn*-Z/Z	*nn*-Z^m/Z^m	*nn*-Z^m/Z	*nn*-Z/Z
	8.20	8.10	7.50	7.40	7.80	7.10	5.40	5.30	5.00
♀		*NN*-W/Z^m		*Nn*-W/Z^m	*NN*-W/Z	*Nn*-W/Z		*nn*-W/Z^m	*nn*-W/Z
		8.00		7.30	7.80	7.10		5.20	5.00

resistance to NPV disease. According to the results obtained, we divided all the tested silkworm species into 4 groups, namely the susceptible, fairly susceptible, fairly resistant and resistant groups. The susceptible group had an average lethal concentration 50 (LC_{50}) value of 4.95 and the resistant group had an average LC_{50} value of 8.25 (Chen *et al.*, 1996). On the basis of our results, we assumed 5.0 as the baseline value of LC_{50} for NPV resistance and assumed *NN* and Z^m/Z^m genotype values of 2.8 and 0.4 beyond the baseline value, respectively. Thus genotype *NN*-Z^m/Z^m had a value of 8.2 (2.8 + 0.4 + 5.0 = 8.2). Finally, *Nn* and Z/Z^m was assumed genotype values of 2.1 and 0.3 beyond the baseline value, respectively, according to the principle of incomplete dominance of resistance versus susceptibility (Fig. 1). According the above assumptions, all genotypes and their values of both female and male individuals are calculated as follows (Table 1).

Theoretical verification of the assumptions

Fourteen combinations of R (resistant), S (susceptible), F_1, F_2 and backcrosses etc. were prepared with resistant breed (R) and susceptible breed (S). Their LC_{50} values were calculated and compared with the practical values (Table 2).

χ^2 test was conducted to data in Table 2. The resulting χ^2 was 0.0135. Meanwhile, $\chi^2_{0.05,\,13} = 22.36$ and $\chi^2_{0.01,\,13} = 27.69$. This showed that LC_{50} values of these 14 combinations were in accordance with the assumed theoretical values very well, implying that our assumptions are rational.

Preparation methods of the near isogenic lines and F_2 populations

Based on the above assumptions and verification, the following methods were designed to prepare near isogenic lines and F_2 populations for screening the molecular markers linked to NPV resistance.

Table 2. Comparison of practical and theoretical LC_{50} values of various combinations on NPV disease resistance

Combinations	Theoretical LC_{50} values	Practical LC_{50} values	Combinations	Theoretical LC_{50} values	Practical LC_{50} values
R	8.25	8.20	(R×S)×R	7.68	7.70
S	4.95	5.00	R×(R×S)	7.32	7.68
R×S	7.23	7.30	(S×R)×R	7.59	7.75
S×R	7.30	7.35	R×(S×R)	7.27	7.68
(R×S)F_2	6.57	6.88	(S×R)×S	5.86	6.20
(S×R)F_2	6.75	6.98	S×(S×R)	6.15	6.20
(R×S)×S	5.78	6.05			
S×(R×S)	6.10	6.05			

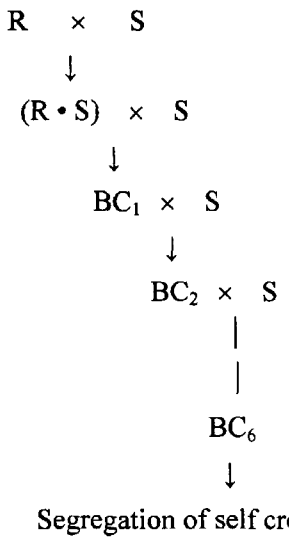

Fig. 2. Establishment of near isogenic lines with major genes *NN*. BC_1 and BC_2 stand for the first and second backcrosses and so forth. The same below.

Preparation of near isogenic lines for the major gene

Near isogenic lines with major genes on autosome were established as follows (Fig. 2). Because there were only two genotypes (*Nn*-W/Z and *nn*-W/Z) in female individuals of (R · S)×S and each of them accounted for 50%, the progeny of selected *Nn*-W/Z individuals from NPV infection crossing with cyclical backcross parent also had 50% female individuals of *Nn*-W/Z genotype.

After backcrossing reached the sixth generation, theoretically its major characters had already been the same with the cyclical backcross parent only except the resistance. And a further self cross would be able to reach the homozygous individuals of genotypes *NN*-W/Z and *NN*-Z/Z (Table 3).

Preparation of near isogenic lines for both major gene and modificator genes

Near isogenic lines with major genes on autosome and modificator genes on sex chromosome Z (*NN*-Z^m/Z^m) were established as follows (Fig. 3). The major difference between this mating pattern and the above method is that the cyclical backcross parent could only be the maternal parent, the backcross progeny could only be the paternal parent, and male individuals with genotype of *Nn*-Z/Z^m were selected. This mating pattern led to no alternation in

Table 3. Genotypes and their values of self crosses

♀	♂	
	n-Z	N-Z
N-Z	Nn-Z/Z	NN-Z/Z
Genotype value	7.10	7.80
n-Z	nn-Z/Z	Nn-Z/Z
Genotype value	5.00	7.10
N-W	Nn-W/Z	NN-W/Z
Genotype value	7.10	7.80
n-W	nn-W/Z	Nn-W/Z
Genotype value	5.00	7.10

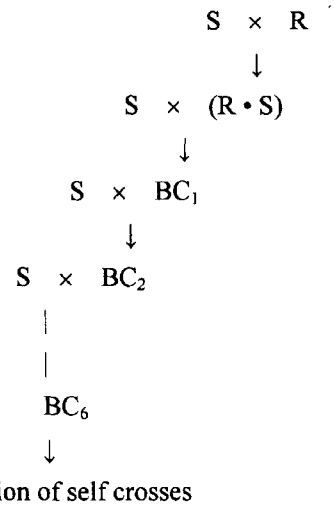

Fig. 3. Establishment of near isogenic lines with *NN*-Z^m/Z^m.

Table 4. Genotypes and their values in self crosses

♀	♂			
	n-Z	*n*-Z^m	*N*-Z	*N*-Z^m
N-Z^m	*Nn*-Z/Z^m	*Nn*-Z^m/Z^m	*NN*-Z/Z^m	*NN*-Z^m/Z^m
Genotype value	7.40	7.50	8.10	8.20
n-Z^m	*nn*-Z/Z^m	*nn*-Z^m/Z^m	*Nn*-Z/Z^m	*Nn*-Z^m/Z^m
Genotype value	5.30	5.40	7.40	7.50
N-W	*Nn*-W/Z	*Nn*-W/Z^m	*NN*-W/Z	*NN*-W/Z^m
Genotype value	7.10	7.30	7.80	8.00
n-W	*nn*-W/Z	*nn*-W/Z^m	*Nn*-W/Z	*Nn*-W/Z^m
Genotype value	5.00	5.20	7.10	7.30

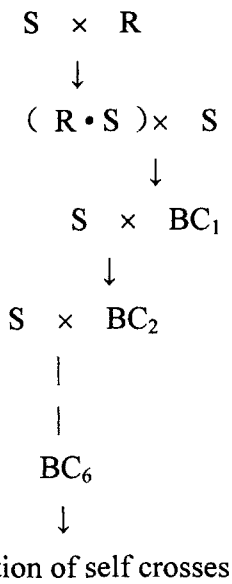

Fig. 4. Establishment of near isogenic lines with *nn*-Z^mZ^m and *nn*-W/Z^m.

the distribution probability of female and male genotypes in backcross populations. After backcrossing reached the sixth generation, self crossing with *Nn*-Z/Z^m and *Nn*-W/Z^m which were selected from NPV infection yielded near isogenic lines with *NN*-Z^mZ^m and *NN*-W/Z^m (Table 4).

Preparation of near isogenic lines for modificator genes

Near isogenic lines with only modificator genes on sex chromosome Z ($Z^mZ/^m$) were established as follows (Fig. 4). In fact, this mating pattern only changed to use F_1 female individuals (genotype *nn*-W/Z^m) for crossing with the cyclical backcross parent at F_1 stage. After the BC_1 was obtained, the following mating was the same. But this pattern led to a higher selection probability of the target female and male individuals. Self cross between *nn*-Z/Z^m and *nn*-W/Z^m individuals selected from BC_6 yielded the near isogenic lines of *nn*-Z^mZ^m and *nn*-W/Z^m (Table 5).

Table 5. Genotypes of self crosses

♀	♂	
	n-Z	*n*-Z^m
n-Z^m	*nn*-Z/Z^m	*nn*-Z^mZ^m
Genotype value	5.30	5.40
n-W	*nn*-W/Z	*nn*-W/Z^m
Genotype value	5.00	5.20

Preparation of F_2 populations for screening molecular markers

Segregation pattern of cross R×S

F_2 segregation pattern of cross R×S was obtained by crossing resistant individuals (*NN*-W/Z^m) with susceptible ones (*nn*-Z/Z) (Table 6). There were *NN*-W/Z^m and *NN*-Z/Z^m individuals but no *NN*-Z^mZ^m ones among these populations. Therefore, this mating pattern could only be used to screen molecular markers linked to major gene (*NN*) of NPV resistance on autosome. The modificator genes on sex chromosome Z did not appear.

Segregation pattern of reverse cross S×R

F_2 segregation pattern of reverse cross S×R was obtained by crossing the female susceptible individuals (*nn*-W/Z) with resistant male individuals (*NN*-Z^mZ^m). Its segregation populations were different with those of the above (Table 7). There were individuals of both *NN*-W/Z^m and *NN*-Z^mZ^m genotypes among the segregation populations. They can be used for screening molecular markers through NPV infection tests.

Table 6. Genotypes and their values of F_2 population of R×S

♀	♂			
	n-Z	*n*-Z^m	*N*-Z	*N*-Z^m
N-Z	*Nn*-Z/Z	*Nn*-Z/Z^m	*NN*-Z/Z	*NN*-Z/Z^m
Genotype value	7.10	7.40	7.80	8.10
n-Z	*nn*-Z/Z	*nn*-Z/Z^m	*Nn*-Z/Z	*Nn*-Z/Z^m
Genotype value	5.00	5.30	7.10	7.40
N-W	*Nn*-W/Z	*Nn*-W/Z^m	*NN*-W/Z	*NN*-W/Z^m
Genotype value	7.10	7.30	7.80	8.00
n-W	*nn*-W/Z	*nn*-W/Z^m	*Nn*-W/Z	*Nn*-W/Z^m
Genotype value	5.00	5.20	7.10	7.30

Table 7. Genotypes and their values of F_2 populations of S×R

♀	♂			
	n-Z	*n*-Z^m	*N*-Z	*N*-Z^m
N-Z^m	*Nn*-Z/Z^m	*Nn*-Z^m/Z^m	*NN*-Z/Z^m	***NN***-Z^m/Z^m
Genotype value	7.40	7.50	8.10	8.20
n-Z^m	*nn*-Z/Z^m	*nn*-Z^m/Z^m	*Nn*-Z/Z^m	*Nn*-Z^m/Z^m
Genotype value	5.30	5.40	7.40	7.50
N-W	*Nn*-W/Z	*Nn*-W/Z^m	*NN*-W/Z	***NN***-W/Z^m
Genotype value	7.10	7.30	7.80	8.00
n-W	*nn*-W/Z	*nn*-W/Z^m	*Nn*-W/Z	*Nn*-W/Z^m
Genotype value	5.00	5.20	7.10	7.30

Conclusion

Silkworm's resistance to NPV disease is controlled by both dominant major gene on autosome and modificator genes on sex chromosome Z, belonging to qualitative-quantitative categories. Its heredity is quite complicated. Traditional breeding can only rely on screening with NPV infection. Its progress is quite slow. Nevertheless, it is also influenced by a number of environmental factors. Selection based on molecular markers can accurately trace fragments linked to the resistance. It not only avoids trouble of doing NPV infection but also eliminates artifacts resulted from environmental factors.

Using near isogenic lines has been proved very effective in screening molecular markers for plant characters (Luo *et al.*, 2001; Wang *et al.*, 2001a,b). Thanks to its short life cycle and high reproduction rate, silkworm is very suitable for screening molecular markers by employing near isogenic line technique. In order to find molecular markers for resistance to NPV disease, theoretically three near isogenic lines that contain genotypes of *NN*, Z^m/Z^m and *NN*-Z^m/Z^m respectively need to be established. If RAPD can find specific fragments of each of the *NN* and Z^m/Z^m genotypes, there should be the corresponding fragments in genotype *NN*-Z^m/Z^m. The reciprocal verification from three sides should lead to the appearance of molecular markers of both major gene and micro-effect genes on sex chromosome Z theoretically. In accordance with this inference, we had prepared the relevant experimental lines and found a 700 bp specific band in both near isogenic line *NN*-Z^m/Z^m (resistant) and parental line *NN* (resistant). There was no this band in the susceptible parental line and its near isogenic lines. Meanwhile, an accurate verification was also performed in their F_2 populations (Yao *et al.*, 2003). The same methods were also proved effective in screening molecular markers linked to major genes controlling silkworms endurance to fluoride toxicity (Chen *et al.*, 2001).

One thing to be noted is that, while considering the size of populations for study, *NN*-Z^m/Z^m individuals can only be found in F_2 populations derived from S×R segregation pattern and the rate of its appearance is 1/16. Thus, when F_2 populations are used to screen molecular markers, there must be a certain number of individuals being mixed so as to eliminate the difference of their hereditary background.

Acknowledgments

This project was supported by the Jiangsu Agricultural High-Tech Research Fund (No. BG2001317).

References

Chen, K. P. and C. Q. Lin (1991) Resistance of preserved silkworm races to nuclear polyhedrosis virus disease. *Acta Sericologica Sinica* **17**, 45-46.

Chen, K. P., C. Lu, Z. H. Xiang, Q. Yao and C. Q. Lin (2001) Cloning and sequencing the RAPD markers of enduring fluoride in silkworm (*Bombyx mori*). *J. Agric. Biotech.* **9**, 136-138.

Chen, K. P., C. Q. Lin and Q. Yao (1996) Studies on the resistance and heredity of silkworms to nuclear polyhedrosis virus disease. *Acta Sericologica Sinica* **22**, 160-164.

Luo, M., M. Y. Li, L. Hou, Y. H. Xiao and Z. S. Zhang (2001) An AFLP marker related to fibrogenesis in upland cotton (*Gossypium hirsuturm* L.). *Acta Genet. Sinica* **28**, 677-682.

Meng, Z. Q. (1982) Inheritance of resistance to nuclear polyhedrosis by peroral inoculation in the silkworm (*Bombyx mori* L.). *Acta Sericologica Sinica* **8**, 133-138.

Wang, X. Y., P. D. Chen and S. Z. Zhang (2001a) Pyramiding and marker assisted selection for powdery mildew resistance genes in common wheat. *Acta Genet. Sinica* **28**, 640-646.

Wang, Z. L., L. X. Wang, J. R. DAI, B. Wang and X. Z. Li (2001b) Molecular identification and mapping of a maize gene (Rf3) in S-type CMS using AFLP, RFLP and SCAR

techniques. *Acta Genet. Sinica* **28**, 465-470.

Xu, C. W., H. D. Mo and Y. Ao (2000) Maximum likelihood method for the qualitative-quantitative inheritance of endosperm traits and its application in the genetic analysis for amylose content in Indica rice. *Sci. Agric. Sinica* **33**, 23-29.

Yao, Q., M. W. Li, Y. Wang, J. Lu, Y. Dong and K. P. Chen (2003) Screening of molecular markers for NPV resistance in *Bombyx mori* L. *J. Appl. Entomol.* **127**, 134-136.

Zhang, Y. N., S. X. Liu, Y. M. Huo and S. Y. Ou (1982) Identification of the resistance of certain silkworm races to six types of silkworm disease. *Acta Sericologica Sinica* **8**, 94-97.