Int. J. Indust. Entomol. Vol. 9, No. 1, 2004, pp. 41~46

International Journal of
Industrial Entomology

Development of a Polyvoltine Breed - BL_{67} (Pg) of the Silkworm, *Bombyx mori* L. with Parthenogenetic Origin

Ravindra Singh*, D. Raghavendra Rao, Debnirmalya Gangopadhyay, Nazia Choudhary, B. K. Kariappa and S. B. Dandin

Central Sericultural Research and Training Institute, Mysore - 570 008, Karnataka, India.

(Received 14 April 2004; Accepted 22 June 2004)

A breeding programme was initiated by utilizing a robust bivoltine breed CSR_{18} and a polyvoltine breed Cambodge with the main objective of developing robust polyvoltine silkworm breeds/hybrids. At F_1 and F_2, parthenogenetic development was induced following warm water treatment of eggs at 46°C for 18 min followed by two backcrosses with Bl_{67}, an evolved polyvoltine breed. The newly developed breed was subjected for hybrid study using eight hybrid combinations in the laboratory at F_8 generation. F_1 hybrids between newly developed breed Bl_{67} (Pg) and promising bivoltine breeds exhibited their superiority by expressing significant hybrid vigour for several economic characters like cocoon yield/10,000 larvae, cocoon weight, cocoon shell weight, cocoon shell ratio and denier. Study on cocoon shape variability revealed that cocoons of all the F_1 hybrids except BL_{67} (Pg) × NB_4D_2 were found comparatively uniform in shape.

Key words: *Bombyx mori* L., Breeding, Hybrid vigour, Cocoon shape variability, Parthenogenetic development

Introduction

Hybrid vigour in F_1 hybrids is expressed to greater extent in hybrids involving homozygous lines (Nacheva *et al.*, 1999). Application of parthenogenesis in silkworm is of special advantage in silkworm breeding in order to develop homozygous silkworm breeds with less phenotypic variability, high hybrid vigour, viability and combining ability (Strunnikov *et al.*, 1982; Strunnikov, 1986; Takei *et al.*, 1990). Origin and mechanism of hybrid vigour through artificial parthenogenesis have been studied (Ohkuma, 1971; Strunnikov, 1983). Hirokawa (1990, 1995) has studied the practical utilization of F_1 hybrids of the silkworm through selection of high parthenogenetic lines by hot water treatment. Attempts have been made to develop silkworm breeds by means of androgenesis (Xu *et al.*, 1997; Nacheva *et al.*, 1999). The present study was undertaken to explore the possibility of application of parthenogenesis in silkworm breeding for the development of superior polyvoltine breed of the silkworm, *Bombyx mori* L.

Materials and Methods

The study was conducted at Silkworm Breeding laboratory of Central Sericultural Research and Training Institute, Mysore during the period of 2002 – 2004. A bivoltine × polyvoltine hybrid CSR_{18} × Cambodge was utilized as breeding resource material. The breeding plan of new polyvoltine silkworm breed has been given in Fig. 1. The F_1 was reared in mass during September October, 2002. From F_2 onwards, cellular rearing was resorted to. Females were separated on the basis of larval markings. After emergence, eggs were obtained by squeezing out the ovarioles. Exactly after 12 hrs, eggs were treated in warm water at 46°C for 18 min as per Astaurov (1967). Eggs were dried and incubated at 15°C for 72 hrs. With the appearance of light brownish serosa colour, eggs were treated with hot hydrochloric acid at 46°C for 5 min. Then eggs were incubated at 25°C till hatching. Rearing of larvae hatched from parthenogenetic eggs was carried out. Females were backcrossed with males of BL_{67}. Hybrid evaluation was carried out at F_8 by utilizing seven CSR

*To whom correspondence should be addressed.
Central Sericultural Research and Training Institute, Mysore-570 008, India. Tel: 091-0821-362406; Fax: 091-0821-362845; E-mail:kalarsingh@rediffmail.com

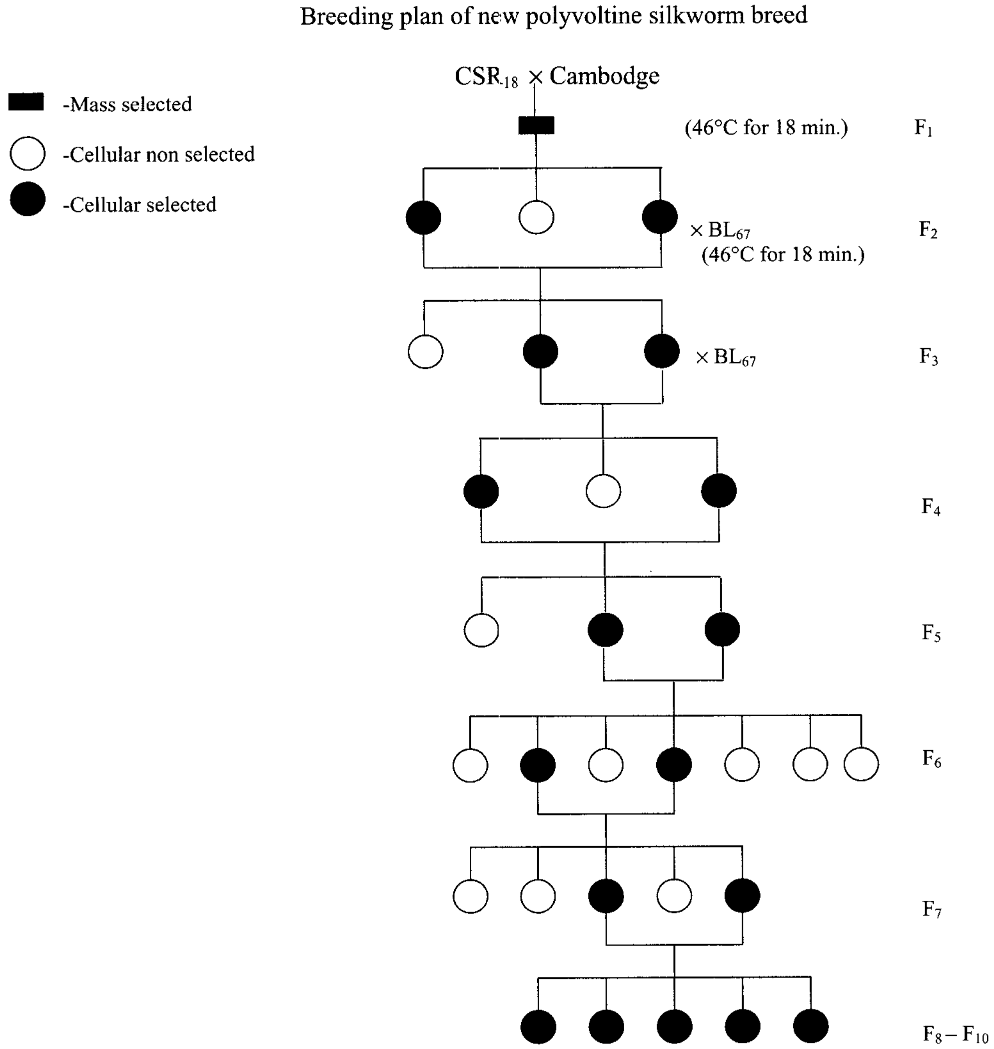

Fig. 1. Breeding process of new polyvoltine silkworm breed.

breeds *viz.*, CSR_2, CSR_2 (SL), CSR_3, CSR_4, CSR_8, CSR_{18} and CSR_{19} along with NB_4D_2. PM × NB_4D_2 was used as control. Cocoon shape measurement was studied in F_1 hybrids between newly evolved polyvoltine breed and bivoltine breeds. During breeding process, the batch showing better performance for viability, cocoon yield, cocoon shape, cocoon colour and cocoon characters were selected for further continuation. The post cocoon parameters such as filament length, denier, raw silk %, neatness and reelability were considered in each generation.

Results

At F_1, 3092 eggs of CSR_{18} × Cambodge were treated towards parthenogenetic development and 1763 eggs showed reddish brown pigmentation. Eighty eight larvae hatched and 43 reached up to cocoon stage. All the parthenotes were females. Females were backcrossed to males of BL_{67}. At F_2, same procedure was repeated. Out of 4123 eggs treated, 2517 eggs showed pigmentation. 128 larvae hatched and 63 larvae reached up to cocoon stage. Females were backcrossed with males of BL_{67}.

Breeding Line

Generation-wise performance of the new polyvoltine breeding line for twelve economic characters is presented in Table 1. Maximum pupation rate (96.5%) was recorded at F_3 followed by 93.5% and 93.3% at F_1 and F_8 respectively. Maximum cocoon yield/10,000 larvae was recorded at F_3 (15.4 kg) followed by 14.5 and 14.3 kg during F_6 and F_7 respectively. Cocoon shell weight was maximum (29.4 cg) at F_7 whereas cocoon shell ratio was highest 20.6% at F_2. Maximum raw silk of 15.8% was observed at F_2. It ranged from 12.3 – 15.8%. The filament length ranged

Table 1. Performance of parthenogenetic line BL_{67} (Pg) during breeding process

Generation	Fecundity (no)	Hatching %	Pupation rate (%)	Yield/ 10,000 larvae by wt. (kg)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Raw silk (%)	Filament length (m)	Denier (d)	Reel-ability (%)	Neatness point (p)
F_1	489	96	93.5	14.0	1.59	28.3	17.8	13.61	789	2.35	82	89
F_2	487	97	91.5	12.8	1.40	28.9	20.6	15.75	753	2.29	84	88
F_3	463	97	96.5	15.4	1.56	28.6	18.3	14.22	783	2.33	81	89
F_4	476	96	87.6	11.6	1.34	23.7	17.5	13.27	729	2.26	80	89
F_5	515	95	83.6	10.5	1.27	21.0	16.5	12.39	659	2.21	82	88
F_6	517	97	92.1	14.5	1.47	26.1	17.8	13.69	769	2.24	83	87
F_7	522	98	87. 9	14.3	1.64	29.4	18.0	14.96	731	3.01	84	88
F_8	505	96	93.3	11.8	1.27	21.5	17.0	12.31	661	2.23	83	89
Mean	497	97	90.7	13.1	1.44	25.9	17.9	13.77	734	2.36	82	88
SD	20	0.83	3.84	1.6	0.134	3.2	1.13	1.11	47	0.247	1.4	0.81

Table 2. Performance of polyvoltine × bivoltine hybrids

Sl. no.	Hybrid	Pupation rate (%)	Yield/ 10,000 larvae by wt. (kg)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Raw silk (%)	Reelability (%)	Neatness (p)	Denier (d)
1.	BL_{67} (Pg) × CSR_2	95.0	20.7	2.11	41.7	19.8	871	15.5	85	88	3.1
2.	BL_{67} (pg) × CSR_3	92.7	19.7	2.09	41.7	19.9	861	13.6	84	87	2.9
3.	BL_{67} (pg) × CSR_4	97.0	20.4	2.08	44.0	21.1	754	14.6	83	87	3.2
4.	BL_{67} (pg) × CSR_{18}	91.3	19.3	2.15	44.0	20.4	777	13.7	83	87	3.2
5.	BL_{67} (pg) × CSR_{19}	96.5	20.3	2.10	41.5	19.8	727	13.3	86	86	2.9
6.	BL_{67} (pg) × CSR_2(sl)	89.5	18.7	2.05	40.6	19.8	757	13.9	82	86	3.0
7.	BL_{67} (pg) × NB_4D_2	89.7	19.0	2.06	38.9	18.9	750	12.4	86	86	2.9
8.	BL_{67} (pg) × CSR_8	89.0	18.0	2.01	38.2	19.0	878	14.7	85	88	2.9
9.	CV at 5%	3.02	0.9	-	1.6	0.97	100.3	0.7	-	1.0	-

from 659 m at F_5 to 789 m at F_1. The reelability ranged from 80.0 – 84.5%. Maximum neatness of 89.3 p was recorded at F_4 followed by 89.1 p at F_1.

Performance of F_1 hybrids

The performance of polyvoltine × bivoltine hybrids is presented in Table 2. The hybrids BL_{67} (Pg) × CSR_{19} and BL_{67} (Pg) × CSR_2 recorded an average pupation rate of 96.5 and 95.0%. The cocoon yield of 20.7 and 20.4 kg/10,000 larvae was recorded in BL_{67} (Pg) × CSR_2 and BL_{67} (Pg) × CSR_4 respectively. Maximum cocoon weight of 2.15 g and 2.11 g was recorded in BL_{67} (Pg) × CSR_{18} and BL_{67} (Pg) × CSR_2. Maximum cocoon shell weight (44.0 cg) was recorded in BL_{67} (Pg) × CSR_4 and BL_{67} (Pg) × CSR_{18} followed by BL_{67} (Pg) × CSR_2 and BL_{67} (Pg) × CSR_3 where it was 41.7 cg. Maximum cocoon shell ratio of 21.1% was recorded by BL_{67} (Pg) × CSR_4 followed by BL_{67} (Pg) × CSR_{18} (20.4%). Longest filament length of 878 m was recorded in BL_{67} (Pg) × CSR_8 followed by BL_{67} (Pg) × CSR_2 (871 m) and BL_{67} (Pg) × CSR_3 (861 m) respectively. The raw silk percentage was 15.5 and 14.7% in BL_{67} (Pg) × CSR_2 and BL_{67} (Pg) × CSR_8 respectively. Maximum reelability of 86.0% was recorded in BL_{67} (Pg) × NB_4D_2 and BL_{67} (Pg) × CSR_{19}. Two hybrids BL_{67} (Pg) × CSR_2 and BL_{67} (Pg) × CSR_8 recorded neatness of 88.0 p. Filament size recorded for BL_{67} (Pg) × CSR_4 and BL_{67} (Pg) × CSR_{19} was 3.2 d followed by BL_{67} (Pg) × CSR_2 (3.1 d) respectively.

Hybrid vigour studies

Heterosis over mid parent and better parent value for five economic characters in F_1 hybrids have been shown in Table 3. BL_{67} (Pg) × CSR_4 showed its superiority by exhibiting maximum significant hybrid vigour over mid parent and better parent values for three characters whereas BL_{67} (Pg) × CSR_{18} showed significant heterosis over mid parent value for 2 characters. It was interesting to note that all the F_1 hybrids except BL_{67} (Pg) × CSR_8 exhibited significant hybrid vigour for three economic characters over better parent value. Significant hybrid vigour for pupation rate was recorded in BL_{67} (Pg) × CSR_4. Highest significant heterosis for cocoon yield over better parent value (46.56%) was exhibited by BL_{67} (Pg) × CSR_2. Highest significant hybrid vigour for cocoon weight (31.56% was recorded in BL_{67} (Pg) × CSR_{18} over better parent value. Highest and highly significant hybrid vigour for cocoon shell weight was also exhibited by BL_{67} (Pg) × CSR_{18} over mid parent (52.01%) and better parent value (49.60%) respectively. Maximum significant heterosis for cocoon shell ratio over mid parent value (6.06%) was recorded in BL_{67} (Pg) × CSR_4 followed by BL_{67} (Pg) × CSR_{18} (5.03%).

Studies on cocoon shape measurement

Cocoon shape variability in polyvoltine × bivoltine F_1 hybrids is presented in Table 4. Majority of F_1 hybrids expressed uniform cocoon shape by exhibiting their Standard Deviation (SD) less than 8. Among 9 polyvoltine × bivoltine hybrids BL_{67} (Pg) × CSR_3 exhibited more cocoon shape uniformity with 5.73 standard deviation and 3.58 coefficient of variation (CV%) followed by BL_{67} (Pg) × CSR_2 and BL_{67} (Pg) × CSR_{18} with 6.16 and 6.66 SD and 3.85 and 4.03 CV%. Two hybrids viz. BL_{67} (Pg) × CSR_4 and BL_{67} (Pg) × CSR_{19} were found relatively uniform in cocoon shape with CV% of 3.93 and 3.81 respectively.

Discussion

Artificial parthenogenesis has been applied in silkworm breeding to get marked stability of characters like viability, cocoon weight and cocoon shell weight, amount of silk per cocoon and raw silk (Dznealaidze and Tabliashvili, 1990), evolution of homozygous silkworm breeds and improvement of selection efficiency (Strunnikov, 1983; Takei *et al.*, 1990), determination of perspective hybrids (Plugaru *et al.*, 1993) and to get low phenotypic variability in F_1 hybrids (Strunnikov *et al.*, 1982; Strunnikov, 1986). Silkworm breeds with high pupation rate, cocoon weight, cocoon shell weight and cocoon shell ratio are very important for improvement of cocoon yield (Kang *et al.*, 2001, 2002).

In the present study, marked significant hybrid vigour was observed for various quantitative characters like cocoon yield/10000 larvae, cocoon weight, cocoon shell weight and cocoon shell ratio in F_1 hybrids between BL_{67} (Pg) females and males of bivoltine breeds. Origin and mechanism of hybrid vigour through artificial parthenogenesis have been studied (Ohkuma, 1971; Strunnikov, 1983). F_1 hybrids between parthenogenetic female and normal males have exhibited increase in hybrid vigour (Strunnikov, 1986; Takei *et al.*, 1990; Ravindra Singh *et al.*, 1994). Strunnikov (1974, 1986) has observed 15% more hybrid vigour in hybrids between parthenoclone and normal lines. Attempts have been made to examine practical utilization of F_1 hybrids through application of artificial parthenogenesis (Sarkisyan, 1977; Hirokawa, 1990; 1995).

Along with combined application of different breeding strategies like androgenesis and cloning, artificial parthenogenesis would be beneficial in the development of silkworm hybrids with less phenotypic variability and to obtain genetically identical copies of silkworm (Strunnikov *et al.*, 1982; Strunnikov, 1986). In the present study, majority of the F_1 hybrids between BL_{67} (Pg) and bivol-

Table 3. Heterosis over mid parent (MPV) and better parent (BPV) values in hybrids between polyvoltine and bivoltine silkworm breeds

Sl. no.	Hybrid	Pupation rate	Cocoon yield/ 10,000 larvae	Cocoon weight	Shell weight	Shell ratio	Denier
	BL_{67} (Pg) × CSR_2						
1.	MPV	6.5	47.3	32.5	31.3	-0.95**	9.8
	BPV	4.9	46.56**	28.89**	22.41**	-10.18**	24.32**
	BL_{67} (Pg) × CSR_3						
2.	MPV	5.3	40.1	28.1	24.2	-3.38**	2.07**
	BPV	5.1	39.24**	27.61**	10.50**	-14.53**	17.12**
	BL_{67} (Pg) × CSR_4						
3.	MPV	9.72*	47.6	31.3	39.68**	6.06**	13.0
	BPV	9.1	45.48**	27.22**	31.08**	-3.74**	28.9
	BL_{67} (Pg) × CSR_{18}						
4.	MPV	3.1	46.8	43.9	52.01**	5.03**	22.2
	BPV	2.2	37.50**	31.56**	49.60**	-2.74**	29.6
	BL_{67} (Pg) × CSR_{19}						
5.	MPV	7.1	56.7	41.2	39.73**	-1.74**	11.9
	BPV	4.6	44.99**	28.32**	38.49**	-11.49**	20.11**
	BL_{67} (Pg) × CSR_2 (SL)						
6.	MPV	-0.9	35.8	32.4	35.2	1.98**	18.5
	BPV	-3.6	33.61**	25.47**	32.72**	-5.27**	23.23**
	BL_{67} (Pg) × NB_4D_2						
7.	MPV	0.0	31.7	25.9	21.5	-3.16**	5.7
	BPV	-1.9	28.16**	25.66**	12.44**	-10.57**	17.39**
	BL_{67} (Pg) × CSR_8						
8.	MPV	5.6	50.7	39.7	37.45**	-2.90**	5.7
	BPV	1.3	28.57**	23.0	29.67**	-10.39**	16.58**
	PM × NB_4D_2 (Control)						
9.	MPV	9.4	58.7	48.2	36.7	-4.8	4.9
	BPV	5.6	27.5	23.3	1.7	-17.4	28.1

* and ** denote Significant at 1% and 5% level respectively.

Table 4. Cocoon size variability in polyvoltine × bivoltine F_1 hybrids

Sl. no.	Hybrid	Cocoon length (mm)	Cocoon width (mm)	Cocoon length/ Cocoon width ratio	Coefficient of variation
1.	BL_{67} (Pg) × CSR_2	33.94 ± 1.34	21.24 ± 0.69	159.93 ± 6.16	3.85
2.	BL_{67} (Pg) × CSR_2 (SL)	34.48 ± 1.25	20.86 ± 0.84	165.61 ± 7.24	4.37
3.	BL_{67} (Pg) × CSR_3	35.34 ± 1.10	20.10 ± 0.77	159.98 ± 5.73	3.58
4.	BL_{67} (Pg) × CSR_4	36.36 ± 1.15	18.49 ± 0.85	197.04 ± 7.75	3.93
5.	BL_{67} (Pg) × CSR_8	35.10 ± 1.44	18.19 ± 0.57	193.17 ± 8.33	4.31
6.	BL_{67} (Pg) × CSR_{18}	34.91 ± 1.09	21.12 ± 0.77	165.38 ± 6.66	4.03
7.	BL_{67} (Pg) × CSR_{19}	34.96 ± 1.03	18.31 ± 0.60	191.04 ± 7.29	3.81
8.	BL_{67} (Pg) × NB_4D_2	34.72 ± 1.56	18.68 ± 1.19	186.82 ± 11.98	6.41
9.	PM × NB_4D_2	37.09 ± 1.27	18.64 ± 0.84	199.73 ± 10.56	5.29

Data mean ± SD of 100 cocoons. Cocoon width was measured in the central region of cocoons.

tine breeds exhibited comparatively uniform cocoon shape. Less phenotypic variability may be attributed to homozygous nature of the individuals utilized for production of F_1 hybrids (Ravindra Singh *et al.*, 1997).

References

Astaurov, B. L. (1967) Experimental alterations of the developmental cytogenetic mechanisms in mulberry silkworm: Artificial parthenogenesis, polyploidy, gynogenesis and androgenesis. *Adv. Morphog.* **6**, 199-257.

Dznealaidze, A. N. and T. S. I. Tabliashvili (1990) Cloned hybrid lines of the silkworm. *Shelk* **4**, 7-8.

Hirokawa, M. (1990) Selection of the high parthenogenetic lines and examinations of their practical characters in commercial F_1 hybrid races of the silkworm, *Bombyx mori*. *Bull. Fukushima Seric. Exp. Stn.* **24**, 1-6.

Hirokawa, M. (1995) Studies on sex control in the silkworm, *Bombyx mori*. *Bull. Fukushima Seric. Exp. Stn.* **28**, 1-104.

Kang, P. D., B. H. Sohn, S. U. Lee and S. J. Hong (2002) Breeding of a new non-cocooning silkworm variety, Hachojam, suitable for autumn rearing season. *Int. J. Indust. Entomol.* **4**, 77-81.

Kang, P. D., K. M. Kim, B. H. Sohn, S. U. Lee, S. O. Woo and S. J. Hong (2001) Breeding of a new silkworm variety chumsujam, with a high yielding for spring rearing season. *Int. J. Indust. Entomol.* **2**, 65-68.

Nacheva, Y., N. Petkov, P. Tzenov, K. Maninova and Chun Gen Su (1999) Breeding of bisexual lines of the silkworm *Bombyx mori* L. with androgenetic origin. *Bull. Indian Acad. Seric.* **3**, 36-41.

Ohkuma, T. (1971) Studies on the mechanism of hybrid vigour by means of artificial parthenogenesis in the silkworm, *Bombyx mori* L. *J. Seric. Sci. Jpn* **40**, 422-430.

Plugaru, I. G., R. I. Plugaru, V. A. Golouko, M. I. Stotskii, T. I. Spiridonova and V. U. Klimenko (1993) Perspective hybrids of silkworm, *Bombyx mori* L. found during parthenogenesis. *Bull. Acad. Repub. Moldova Biol. Sci.* **3**, 12-16.

Ravindra Singh, K. P. Jayaswal and B. Saratchandra (1994) Parthenogenetic development of ovarian eggs in some breeds of silkworm, *Bombyx mori* L. *Entomon.* **19**, 57-62.

Ravindra Singh, M. M. Ahsan and R. K. Datta (1997) Artificial parthenogenesis in the silkworm *Bombyx mori* L.: A review. *Indian J. Seric.* **36**, 87-91.

Sarkisyan, S. M. (1977) Artificial parthenogenesis in the silkworm and the prospects of the practical parthenoclone use. *Vestnik-sel. Skokhozyajstvennoj-Nauki (USSR)* **10**, 54-59.

Strunnikov, V. A. (1974) Initiation of the compensating gene complex is one of the causes of heterosis. *Zh. Obshch Biol.* **35**, 666-677.

Strunnikov, V. A. (1983) Control of Silkworm Reproduction, Development and Sex. Mir Publishers, Moscow.

Strunnikov, V. A. (1986) Nature of heterosis and combining ability in the silkworm. *Theor. Appl. Genet.* **72**, 503-512.

Strunnikov, V. A., S. S. Lezhenko and N. L. Stepanova (1982) Genetically identical copies of the mulberry silkworm. *Theor. Appl. Genet.* **63**, 307-315.

Takei, R., M. Nakagaki, R. Kodaira and E. Nagashima (1990) Factor analysis on the parthenogenetic development of ovarian eggs in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). *Appl. Entomol. Zool.* **25**, 43-48.

Xu, A. Y., M. W. Li, A. Fang, M. H. Fei and J. T. Huang (1997) Isolation of a self-bred line of *Bombyx mori* L. by means of dispermic androgenesis. *Sericologia* **37**, 199-204.