Int. J. Indust. Entomol. Vol. 13, No. 1, 2006, pp. 7~14

International Journal of
Industrial Entomology

Introgression of Sex-Limited Larval Markings to a Productive Multivoltine Strain of Silkworm *Bombyx mori* L.

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(Received 7 December 2005; Accepted 14 February 2006)

A breeding programme was initiated during 2001 to introduce sex-limited larval markings to a productive multivoltine breed - BL67 from an inbred sex-limited line, MY1 (SL) maintained at Central Sericultural Research and Training Institute, Mysore. Introgressive hybridization, recurrent backcrossing for six generations followed by sib-mating resulted in synthesis of a new multivoltine silkworm breed BL67 (SL) with sex-limited larval markings. The new breed was studied for combining ability by crossing with eight bivoltine breeds *viz.*, NB_4D_2, CSR_2, CSR_2 (SL), CSR_2, CSR_4, CSR_8, CSR_{18} and CSR_{19}. General combining ability effects of the new breed showed its superiority over the popular Pure Mysore by expressing significant GCA effects for six out of twelve characters whereas the results are on par with the original multivoltine breed. The hybrid BL67 (SL) × CSR2 (SL) excelled in several quantitative characters such as pupation rate (90.2%), cocoon weight (1.97 g), cocoon shell weight (40 cg), cocoon shell ratio (20.3%), filament length (918 m), denier (2.96), raw silk percentage (14.96%) and neatness (90 p). Studies on cocoon size variability revealed that the cocoons of BL67 (SL) × CSR2 (SL) were found comparatively uniform showing less standard deviation of 6.55 and co-efficient of variation of 3.91%. The suitability of newly developed breed for easy grainage operation and commercial exploitation with promising hybrid have been discussed.

Key words: Larval markings, Sex-limited, General combining ability, Grainage

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Introduction

In India, sericulture development took quantum jump in mulberry silk production during recent past particularly after introduction of new bivoltine breeds during seventies. Although India stood second in world raw silk production, the quality of silk produced from cross breed between indigenous races with bivoltine races and multivoltine hybrids are poor in quality and low in yield (Datta, 1984). Several attempts have been made for the development of productive multivoltine breeds (Nagaraju *et al.*, 1987; Noamani *et al.*, 1990; Kalpana *et al.*, 1998; Raghavendra Rao *et al.*, 2002). The general practice in commercial silkworm seed production centers is mating of female parent with bivoltine male parent to prepare desired hybrid layings. Local Pure Mysore race is having the unique character of bi-modal (emergence of male moths first followed by female) pattern of moth emergence and commercial seed producers usually practice to reject male moths after emergence and by utilizing female moths required combinations will be prepared. Separation of sex by observing glands during 5^{th} instar is difficult, thus sex separation is carried out at pupal stage which requires expertise, laborious and expensive. There is a possibility of human error during pupal sex separation. To overcome all these practical problems, an attempt has been made for the introduction of sex-limited larval markings to a productive multivoltine breed, BL67 for easy grainage operation.

Materials and Methods

In the present study, a multivoltine breed with sex-limited larval markings MY_1(SL) maintained in the working germplasm of Central Sericultural Research and Training Institute, Mysore was used as donor parent and a productive multivoltine breed, BL67 was used as recurrent parent (Fig. 1). Females moths of donor parent were crossed with males of BL67. At F_1 all females carried larval markings whereas males were devoid of any markings. The selected F_1 female cocoons were back-crossed (BC) with the males of BL67. Six BC's were carried out in successive generations and selection pressure was imposed to get desired characters of recurrent parent. Afterwards, selective inbreeding was carried out for six

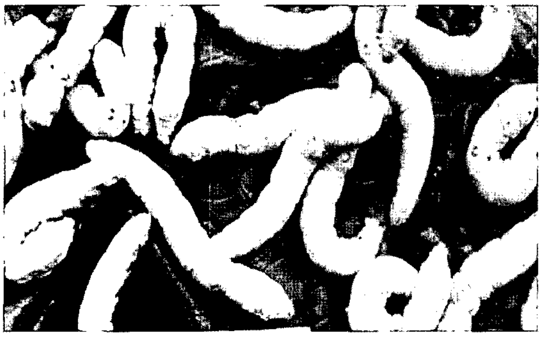

Fig. 1. Larvae of BL67 without markings.

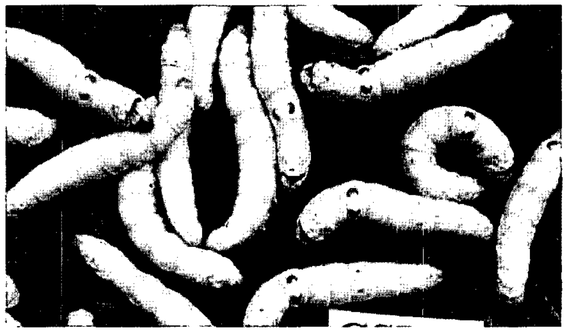

Fig. 2. BL67(SL) female larvae with markings.

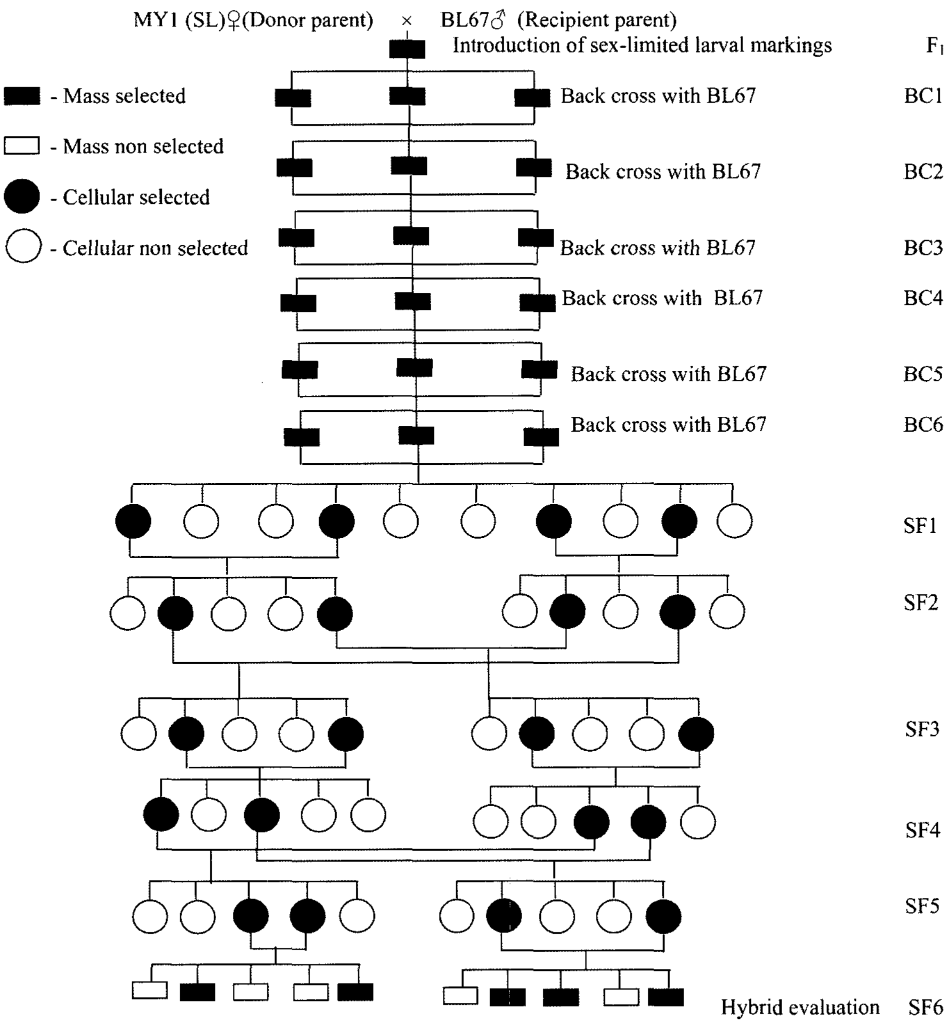

Fig. 3. Breeding plan for development of multivoltine silkworm breed with sex-limited larval markings.

more generations (Breeding process presented in Fig. 3). After six subsequent sib-matings up to SF6, hybrid evaluation was carried out with eight bivoltine breeds *viz.*, NB_4D_2, CSR_2, CSR_2 (SL), CSR_3, CSR_4, CSR_8, CSR_{18} and CSR_{19}. During breeding process and also during hybrid evaluation, important characters *viz.*, fecundity, larval span, pupation rate, cocoon yield/10,000 larvae, cocoon weight, cocoon shell weight, cocoon shell ratio, filament length, denier, raw silk percentage, reelability and neatness were recorded. Rearing was conducted by standard method of Krishnaswami (1978). General Combining Ability of newly developed breed and Specific Combining Ability, heterosis percentage over Mid Parent Value (MPV) and Better Parent Value (BPV) of hybrids were estimated by Kempthorne, (1957). Cocoon uniformity of hybrids was calculated in terms of standard deviation of the ratio of cocoon length and breadth of 100 green cocoons for each hybrid (Mano, 1994).

Results

Performance of new sex-limited line in different generations:

Newly developed multivoltine breed with sex-limited larval markings in female larvae and plain male are presented in Fig. 1. Rearing performance of newly developed multivoltine breed with sex-limited larval markings for ten characters is given in Table 1. The fecundity of the new line ranged from 422 (BC_2) to 524 (SF_3). Hatching

Table 1. Average rearing performance of sex-limited larval markings breed, BL67(SL) over the generations

Generation	Period	Fecundity (No)	Hatching %	Larval span (d : h)	Pupation rate (%)	Cocoon weight (g)	Shell weight (cg)	Shell ratio (%)	Filament length (m)	Denier	Neatness (p)
F1	Dec.2001	521	97	20:00	♂ 96.5	1.22	22.7	18.7	728	2.41	86
					♀ 90.0	1.46	22.4	15.4	653	2.66	86
BC1	Feb.2002	504	98	18:06	♂ 92.0	1.20	22.0	18.3	711	2.39	87
					♀ 86.7	1.38	21.8	15.8	633	2.56	86
BC2	Apr.2002	422	89	18:06	♂ 96.0	1.51	26.0	17.3	722	2.37	86
					♀ 93.3	1.71	25.0	14.8	616	2.63	86
BC3	Jun.2002	452	95	20:06	♂ 89.0	1.17	21.5	18.3	744	2.33	87
					♀ 82.3	1.32	20.3	15.3	621	2.58	86
BC4	Aug.2002	506	97	19:06	♂ 86.7	1.25	25.0	20.1	709	2.31	86
					♀ 62.9	1.59	28.0	17.6	601	2.52	85
BC5	Oct.2002	517	94	19:06	♂ 90.4	1.23	23.7	20.9	752	2.41	87
					♀ 83.5	1.52	26.4	17.3	662	2.68	86
BC6	Dec.2002	462	97	20:06	♂ 86.7	1.25	24.9	20.0	749	2.39	87
					♀ 81.4	1.50	25.4	16.9	651	2.55	86
SF1	Jan.2003	486	97	18:06	♂ 90.5	1.27	21.9	17.3	738	2.33	87
					♀ 86.1	1.37	23.0	16.8	635	2.57	86
SF2	Mar.2003	491	98	18:06	♂ 79.3	1.18	23.6	20.1	717	2.30	86
					♀ 63.6	1.36	23.8	17.5	634	2.60	85
SF3	May 2003	521	96	19:06	♂ 92.5	1.25	24.3	19.5	733	2.36	87
					♀ 78.8	1.54	24.8	16.1	664	2.61	86
SF4	Jul.2003	452	98	20:00	♂ 94.7	1.15	21.3	17.7	708	2.29	87
					♀ 89.3	1.39	24.5	17.6	621	2.71	86
SF5	Sept.2003	513	97	21:06	♂ 82.0	1.11	22.0	19.7	755	2.24	86
					♀ 79.4	1.36	21.5	15.8	664	2.60	85
SF6	Nov.2003	498	97	19:06	♂ 88.3	1.06	17.5	16.6	721	2.33	87
					♀ 87.4	1.26	20.1	16.0	655	2.75	8
AVG		488	96	19:09	♂ 89.6	1.22	22.8	18.7	730	2.34	87
					♀ 81.9	1.44	23.6	16.4	639	2.62	86

percentage ranged from 89.3 (BC_2) to 98.2% (SF_2). Total larval span ranged from 18 days 6 hrs (BC_2, BC_3, SF_1 and SF_2) to 20 days 6 hrs (BC_6). The survival percentage in male ranged from 79.3 (SF_2) to 96.5% (F_1) whereas in female it ranged from 62.9 (BC_4) to 93.3% (BC_3). Cocoon weight in male ranged from 1.06 (SF_6) to 1.51 g (BC_2) while in female it ranged from 1.26 (SF_6) to 1.71 g (BC_2). The cocoon shell weight in male and female ranged from 17.5 (SF_6) to 26.0 cg (BC_2) and 20.1 (SF_6) to 28.0 cg (BC_2) respectively. The cocoon shell ratio of male ranged from 16.6 (SF_6) to 20.9% (BC_5) where as in female SR % ranged from 14.8 (BC_2) to 17.6 (SF_4). Cocoon filament length in male ranged from 708 to 752 m while in female it is from 601 (BC_4) to 664 m (SF_3, SF_5). Denier of filament in male ranged from 2.24 (SF_5) to 2.41 (BC_5) whereas in female denier ranged from 2.52 (BC_4) to 2.75 (SF_6) and neatness in male and female ranged from 85 to 87 points in different generations.

General Combining Ability of new Line (GCA)
The general combining ability of BL67 (SL) with recurrent parent, BL67 and local control, Pure Mysore and eight bivoltine testers for twelve characters is given in Table 2. Analysis of GCA effects revealed that BL67 (SL) and BL67 exhibited significant GCA effects for six out of twelve characters studied. Pure Mysore showed significant GCA effects for two characters *i.e.*, fecundity and cocoon weight. Among the bivoltine testers, CSR_2 (SL) and CSR_2 were found good general combiners exhibiting significant GCA effects for six characters each (Table 2).

Specific combining ability (SCA)
The specific combining ability effects for eight multivoltine x bivoltine hybrids have been presented in Table 3. None of the hybrid expressed SCA effects for all the characters. One hybrid *viz.* BL67(SL) × CSR_2 (SL) was found good specific combiner expressing significant SCA effects for four out of twelve characters followed by two hybrids BL67(SL) × NB_4D_2, BL67(SL) × CSR_8 for three characters each.

Heterotic effects
Manifestation of heterotic effects over mid parent value (MPV) and better parent value (BPV) in eight hybrids has been given in Table 4 and 5. Two hybrids *viz.* BL67 (SL) × CSR_3 and BL67(SL) × CSR_2 (SL) were found as good heterotic hybrids which expressed significant hybrid vigour over mid parent value for eight out of twelve characters followed by BL67(SL) × CSR_{19}, possessing significant hybrid vigour over mid parent value for seven characters (Table 4). Significant heterotic effects for six characters were observed in the hybrid BL67(SL) × CSR_2 (SL) over BPV followed by BL67(SL) × CSR_{19} for five characters (Table 5).

Studies on cocoon size variability
The results of cocoon shape of eight multivoltine × bivoltine hybrids are presented in Table 1. It is clear that the cocoon length ranged from 3.36 to 3.52 cm. Indicating the highest value for the hybrid BL67(SL) × CSR_8 (3.52 cm) and the lowest value for the hybrid BL67(SL) × CSR_3

Table 2. General combining ability effects of lines and testers utilized in the study

Line/Tester	Fecundity	Larval span	Pupation rate	Yield/ 10,000 larvae	Cocoon Wt.	Shell Wt.	Shell ratio	Filament length	Raw silk	Reelability	Neatness	Denier
						Lines						
BL67(SL)	-122.22	-5.00**	2.40**	-0.10	-0.09	-0.01	0.65**	35.96**	0.72**	0.55*	0.23	-0.01
BL67	90.40**	-8.00**	-1.45	-0.05	0.03	0.01**	0.34**	24.50**	0.65**	-0.63	0.10	-0.01
Pure Mysore (PM)	31.82**	13.00	-0.95	0.15	0.06**	-0.00	-0.99	-60.46	-1.37	0.08	-0.33	0.02
						Testers						
CSR_2	-5.18	1.00	1.14	0.35	-0.01	0.00	0.43**	-28.60	0.17	0.11	-0.31	-0.05
CSR_3	7.71	1.00	0.42	0.53*	0.12**	0.03**	0.36**	19.74	0.10	0.31	0.52*	0.11*
CSR_4	20.15	1.00	0.58	0.45	-0.00	-0.01	-0.55	-1.60	0.34*	-0.12	-0.14	0.09
CSR_{18}	-3.51	-7.00**	0.53	-0.29	0.00	0.00	0.21	31.18**	-0.42	1.06*	0.35	-0.18
CSR_{19}	-19.96	1.00	4.41**	0.95**	-0.04	0.00	0.76**	4.40	-0.07	-0.63	0.02	-0.01
CSR_2(SL)	-4.63	1.00	2.14*	1.17**	0.09**	0.02**	0.03	26.18	0.78**	-0.62	0.02	0.10*
NB_4D_2	-6.74	1.00	-2.81	-1.21	-0.04	-0.02	-0.45	-19.15	0.25	-0.17	0.35	-0.00
CSR_8	12.15	1.00	-5.36	-1.94	-0.11	-0.04	-0.79	-32.15	-1.15	0.06	-0.81	-0.05

*>5% and **>1%

Table 3. Specific combining ability of miltivoltine × bivoltine hybrids

Hybrid	Fecun-dity	Larval span	Pupation rate	Yield/ 10,000 larvae	Cocoon Wt.	Shell Wt.	Shell ratio	Filament length	Raw silk	Reela-bility	Neat-ness	Denier
BL67(SL) × CSR_2	62.56	-1.00**	-0.95	0.14	-0.003	-0.017	-0.82	-13.07	0.77**	0.98	0.44	0.02
BL67(SL) × CSR_3	-7.00	-1.00**	0.94	0.36	-0.071	-0.011	0.21	40.60	1.74**	0.68	0.10	-0.04
BL67(SL) × CSR_4	-0.78	-1.00**	-1.90	-1.20	-0.038	-0.014	-0.34	-46.07	-0.65	1.91*	-0.23	-0.14
BL67(SL) × CSR_{18}	51.56	7.00	3.22	1.07*	0.030	0.007	-0.13	-121.51	-1.02	-0.83	-0.73	0.22
BL67(SL) × CSR_{19}	29.00	-1.00**	-2.23	-0.83	-0.007	0.004	0.38	20.93	0.14	0.16	0.10	0.20
BL67(SL) × CSR_2(SL)	-39.00	-1.00**	-7.28	-2.02	0.064*	0.015	0.38	51.49*	-0.40	-0.59	0.10	-0.25**
BL67(SL) × NB_4D_2	-117.00	-1.00**	5.83**	1.46**	-0.010	-0.001	0.06	-14.18	-0.62	-0.03	-0.23	0.07
BL67(SL) × CSR_8	20.89	-1.00**	2.38	1.02*	0.036	0.016	0.27	81.82**	0.04	-1.28	0.44	-0.08

*>5% and **>1%

Table 4. Heterosis percentage in miltivoltine × bivoltine hybrids over Mid Parent Value (MPV).

Hybrid	Fecun-dity	Larval span	Pupation rate	Yield/ 10,000 larvae	Cocoon Wt.	Shell Wt.	Shell ratio	Filament length	Raw silk	Reela-bility	Neat-ness	Denier
BL67(SL) × CSR_2	-17.58	0.62	5.15*	45.53**	21.00**	23.53**	2.45	-3.19	2.19	4.87**	-1.88	3.36
BL67(SL) × CSR_3	-22.19	-1.25	7.85**	38.41**	22.62**	26.40**	4.07**	13.05**	8.04**	3.24**	-1.33	15.95
BL67(SL) × CSR_4	-19.65	0.62	4.42*	38.91**	19.78**	19.70**	-0.05	0.23	-2.89	-2.39	-2.08	1.91
BL67(SL) × CSR_{18}	-13.26	0.62	8.62**	59.78**	32.73**	46.14**	7.70**	3.57	-8.22	3.17**	-2.26	12.88
BL67(SL) × CSR_{19}	-14.61	1.25	6.35**	56.21**	27.30**	40.41**	9.55**	20.73**	3.06	2.32*	-1.32	16.91
BL67(SL) × CSR_2(SL)	-31.14	-1.22**	-1.94	48.89**	34.01**	47.33**	9.93**	8.43*	-1.73	5.49**	-1.88	-2.37*
BL67(SL) × NB_4D_2	-50.21	1.25	7.76**	40.17**	15.24**	19.73**	4.84**	4.40	3.29	1.88	-0.76	8.94
BL67(SL) × CSR_8	-10.16	0.62	7.50**	61.31**	32.14**	39.53**	4.03*	11.20**	-7.01	1.15	-1.52	2.28

*>5% and **>1%

Table 5. Heterosis percentage in miltivoltine × bivoltine hybrids over Better Parent Value (BPV).

Hybrid	Fecun-dity	Larval span	Pupation rate	Yield/ 10,000 larvae	Cocoon Wt.	Shell Wt.	Shell ratio	Filament length	Raw silk	Reela-bility	Neat-ness	Denier
BL67(SL) × CSR_2	-28.61	0.00	4.82	32.66**	18.20**	4.79	-11.36	-18.25	-16.84	1.06	-5.09	-7.52
BL67(SL) × CSR_3	-28.21	-3.57**	6.10*	35.24**	17.06**	2.91	-12.03	-2.14	-12.24	0.94	-4.20	11.57
BL67(SL) × CSR_4	-28.82	0.00	3.17	28.92**	17.48**	2.18	-13.45	-12.77	-19.20	1.88	-4.95	-9.33*
BL67(SL) × CSR_{18}	-20.49	0.00	7.56**	56.01**	27.37**	33.92**	-4.57	-3.27	-22.08	0.04	-5.29	6.66
BL67(SL) × CSR_{19}	-15.95	1.25	5.70*	54.45**	21.38**	25.81**	-5.82	13.75**	-12.28	-0.78	-4.04	9.36
BL67(SL) × CSR_2(SL)	-36.44	1.25	5.70*	54.45**	21.38**	25.81**	-2.72	13.75**	-20.15	-3.12	-5.09	-13.54**
BL67(SL) × NB_4D_2	-55.98	1.25	7.59**	25.23**	9.77**	0.96	-7.73	-7.56	-9.96	-1.64	-2.97	-1.00
BL67(SL) × CSR_8	-12.31	0.00	1.34	49.15**	21.83**	33.21**	-8.50	-4.06	-21.70	-1.64	-3.89	-6.53

* > 5% and ** > 1%

Table 6. Cocoon size variation in multivoltine × bivoltine hybrids of silkworm, *Bombyx mori*

Sl. No.	Combination	Cocoon length (cms)	Cocoon width (cms)	Length/width ratio	Coefficient of variation (%)
1	BL67(SL) × CSR_2	3.51 ± 0.17	2.05 ± 0.14	176.18 ± 11.16	6.34
2	BL67(SL) × CSR_3	3.42 ± 0.13	2.04 ± 0.06	167.62 ± 6.55	3.91
3	BL67(SL) × CSR_4	3.36 ± 0.12	2.07 ± 0.10	162.79 ± 9.29	5.71
4	BL67(SL) × CSR_{18}	3.40 ± 0.17	2.02 ± 0.09	168.18 ± 8.21	4.88
5	BL67(SL) × CSR_{19}	3.49 ± 0.13	1.88 ± 0.10	186.36 ± 10.70	5.74
6	BL67(SL) × CSR_2(SL)	3.53 ± 0.10	1.84 ± 0.08	191.90 ± 9.71	6.06
7	BL67(SL) × NB_4D_2	3.52 ± 0.13	1.86 ± 0.06	189.61 ± 9.02	4.76
8	BL67(SL) × CSR_8	3.42 ± 0.11	1.79 ± 0.05	191.20 ± 8.37	4.38

(3.36 cm). The mean value for cocoon width ranged from 1.79 to 2.07 cm with the highest value of 2.07 cm for BL67(SL) × CSR_3 and the lowest of 1.79 cm for BL67 (SL) × CSR_{19}. The standard deviation of these hybrids ranged from 6.55 to 11.16 with maximum observed for BL67(SL) × CSR_2 and the minimum observed for BL67 (SL) × CSR_2. Co efficient of variation for cocoon length/ width ratio of eight hybrids ranged from 3.91 to 6.34 with minimum for the hybrid BL67(SL) × CSR_2.

Discussion

Tanaka (1916) observed presence of sex chromosomes in male (ZZ) and female (ZW) silkworm. Hashimoto (1933) demonstrated determination of female sex by W chromosome. Tazima (1941) a Japanese scientist was the first to introduce sex-limited larval markings by translocation using X-ray radiation from second chromosome to W-sex chromosome. By utilizing sex limited genetic stocks, several Japanese scientists developed numerous sex-limited breeds and they were commercially exploited (Yamashita, 1978; Mano, 1984). Kimura *et al.* (1971) for the first time have developed cocoon colour sex-limited bivoltine breed in Japan by gamma irradiation. Later, Yamamoto (1989) developed sex-limited cocoon colour races of silkworm by chromosome manipulation. In India, Sengupta (1968) introduced sex-limited larval markings to two bivoltine breeds *viz.*, J112 and C110 from a Russian sex-limited strain, Sanish18. In the present study one multivoltine breed, MY_1(SL) maintained at CSR&TI, Mysore was used as donor parent and BL67(SL) was developed. Performance of the sex-limited line at different generations of breeding is given in Table 1. It is clear from the Table that pupation rate is slightly higher in male than female over the generations in BL67(SL). This may be due to hyperploidy. Iyengar *et al.* (1975) have reported possible differences in survival and yield in the hybrids of C.Nichi, HS6 and Pure Mysore. Nagaraju *et al.* (1988, 1989) have developed a polyvoltine breed with sex-limited larval markings and reported sex ratio and female viability in sex-limited strains does not differ from that of normal strains. Mundukur *et al.* (1999) have developed a multivoltine strain SL-KSPM with sex-limited larval markings similar to Pure Mysore. Ravindra Singh *et al.* (2001) evolved few polyvoltine breeds with sex-limited cocoon colour. Suresh Kumar *et al.* (2002) have developed a bivoltine hybrid, CSR_{18} × CSR_{19} with sex-limited larval markings tolerant to high temperature and are authorized for commercial exploitation. Recently, Rao *et al.* (2003) have introgressed sex-limited larval markings to a productive polyvoltine strain, MDPSY suitable to Andhra Pradesh conditions.

Studies on General Combining Ability (GCA) and Specific Combining Ability (SCA) showed that the sex-limited BL67 showed significant effects for six characters and resembles with normal breed. Studies on SCA and hybrid vigour revealed that the hybrid BL67(SL) × CSR_2 (SL) showed its superiority by expressing significant SCA effects for four characters and significant positive heterosis for eight and six characters for hybrid vigour MPV and BPV respectively. Expression of hybrid vigour was very high in some economic characters like cocoon yield and cocoon shell weight in BL67(SL) × CSR_{18} (59.78%). All eight hybrids manifested significant heterosis over mid and better parent for cocoon yield and cocoon weight. Manifestation of hybrid vigour has been studied for the evaluation and identification of prospective multivoltine × bivoltine hybrids (Datta *et al.*, 1984; Noamani *et al.*, 1990; Nagaraju *et al.*, 1989; Ravindra Singh *et al.*, 2000, 2001, Raghavendra Rao *et al.*, 2002).

Of late, silkworm breeders are giving more importance to cocoon size variability as uniform cocoons are of immense use to the silk industry in evaluation and identification of silkworm parents/hybrids with uniform filament size. Studies on cocoon size variability showed that the hybrid BL67(SL) × CSR_2(SL) produced more uniform size cocoons than the other hybrids by showing standard deviation of 6.55 and co-efficient of variation of 3.91. Cocoon size variability has been studied in F_1 hybrid between polyvoltine × bivoltine (Ravindra Singh *et al.*, 2001; Raghavendra Rao *et al.*, 2002).

The selected hybrid BL67(SL) × CSR_2(SL) compared with the commercial multivoltine × bivoltine F_1 hybrid, PM × CSR_2. The new hybrid showed improvement in yield and gain observed was for cocoon shell weight, cocoon shell ratio, filament length, raw silk percentage and neatness. The present study indicates that the newly developed sex-limited multivoltine breed is found promising with good combining ability with improved qualitative and quantitative characters over commercial hybrid, PM × CSR_2. By considering the superiority of the new hybrid is being evaluated with the farmers. After completion of trails with the farmers, the hybrid will be subjected to Race Authorization Test for commercialization.

Acknowledgements

The authors thank Sri Umapathy, Technical Assistant and Sri V. Sitharaman Senior Technical Assistant of Multivoltine Breeding Laboratory for their help during silkworm rearing and hybrid evaluation.

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