Int. J. Indust. Entomol. Vol. 11, No. 2, 2005, pp. 119~124

International Journal of
Industrial Entomology

Heterobeltiotic Genetic Interaction between Congenic and Syngenic Breeds of Silkworm, *Bombyx mori* L.

A. K. Verma, G. K. Chattopadhyay*, M. Sengupta, S. K. Das and A. Sarkar

Silkworm Breeding, Genetics and Molecular Biology Laboratory, Central Sericultural Research and Training Institute, Berhampore-742101, West Bengal, India.

(Received 13 July 2005; Accepted 7 November)

To determine the level of heterosis, higher cocoon shell weight multivoltine congenic lines (Con. L) and bivoltine syngenic lines (Syn. L) of silkworm were used for crosses. First filial generations (F_1s) expressed heterobeltiotic genetic interaction at significant magnitude ($p < 0.01$) for single cocoon shell weight (SCSW). The other linked characters *viz.*, single cocoon weight (SCW) and yield by weight per 10, 000 larvae were also significantly higher ($p < 0.01$) than the better parental lines. All the hybrids showed significant improvement for these aforesaid characters over standard heterosis (Standard check). The reeling parameters *viz.*, filament length, raw silk, neatness, cohesion-strokes etc, also showed improvement among the hybrids than check in congenial environment. Overall results suggested that the cross between congenic and syngenic lines provide better heterosis with good quality silk than conventional hybrids and may be used for commercial exploitation.

Key words: Silkworm, Breeding, Syngenic line (Syn. L), Recurrent backcross line (RBL) Congenic line (Con. L), Heterobeltiotic, Multigenic

Introduction

In studies of heterosis, emphasis has been given to partially or predominantly out breeding species or breed particularly for crop and livestock improvement. This is applicable for silkworm *Bombyx mori* too. This practice has resuscitated in the field of sericulture in Japan (Harada, 1949, 1961; Yokoyama, 1974). It is well known that heterotic hybrids, in general, show greater vigour, faster growth and development, better yield, higher tolerance to disease, higher adaptability to unfavourable environmental situation and produce uniform and stable crops. It is also well acknowledged that multivoltine breeds, those were basically tropical origin; generally laid non-hibernating eggs, have higher germ load tolerance with low quality silk. On the other hand, high yielding univoltine / bivoltine races or breeds or strains (laying hibernating eggs) produce better quality silk and have higher yield potentiality under temperate situation but show low germ load tolerance and low adaptability (Chattopadhyay and Chatterjee, 1990) in tropical situation. It has become a major impediment to adopt bivoltine sericulture in tropical country like India. Since 1970, various workers have attempted to overcome the problems by making different hybrids of silkworm and used them at field level for screening season and region specific as well as suitable for adverse climatic situation (Sengupta and Datta, 1973; Datta, 1984; Bhargava *et al.*, 1993; Das *et al.*, 1994; Rao *et al.*, 1998; Datta *et al.*, 2000). But the key problem of tropical silkworm breeds or strains is they have highly heterogenic gene pool resulting in higher quantitative and qualitative variation in hybrids leading to a deficiency in the desired result. Keeping this in view, an attempt has taken to develop different syngenic (Syn.) and congenic (Con.) breeds (Chattopadhyay *et al.*, 2001a, b, c) and thereafter its use in hybridizing programme to understand its heterotic effects in F_1. The results are discussed in this context.

*To whom correspondence should be addressed.
Central Sericultural Research and Training Institute, Berhampore-742101, West Bengal India. Tel: +91-3482-253962-213; Fax: +91-3482-251046; E-mail: gkc54 @ rediffmail.com

Materials and Methods

Syngenic line development

Continuous selection was done phenotypically (larval marking, cocoon colour and cocoon shape), physiologi-

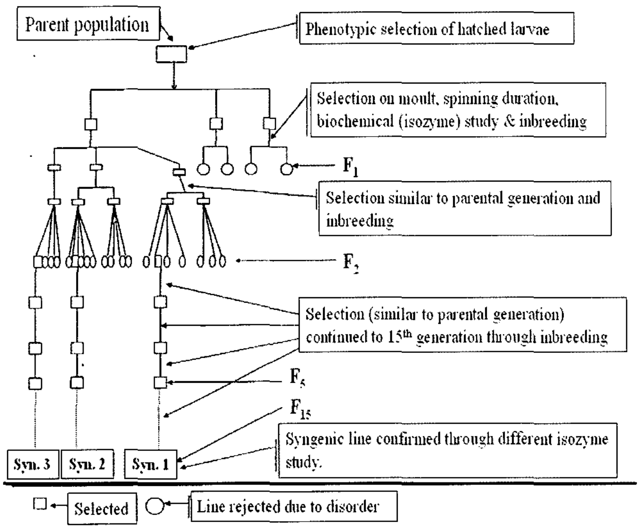

Fig. 1. Scheme for development of syngenic line in silkworm, *Bombyx mori* L.

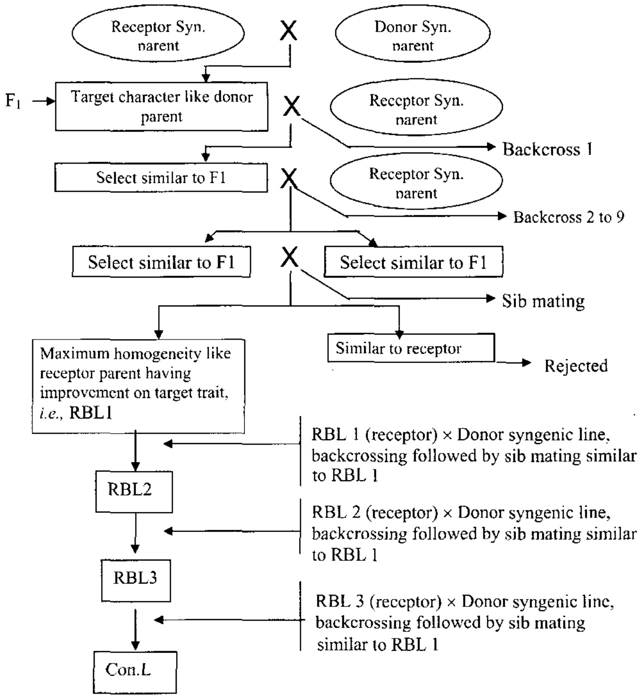

Fig. 2. Scheme for development of congenic breed in silkworm, *Bombyx mori* L.

cally like moulting duration during different stages of development (2nd, 3rd and 4th), maturation period for spinning on multivoltine germplasm (GP) breed CB5 and higher cocoon shell weight bivoltine germplasm breeds JPN and D6. On this basis, few lines were isolated. From each line, 20 to 25 larvae on 5th day of 5th inster were taken to collect digestive juice and haemolymph separately from each larva of each line. Anodic, cathodic amylase, esterase isozyme patterns at pH 7.0 and 8.5 were documented for each sample. Alive larvae possessing the isozyme banding homogeneity for each enzyme were selected together of each line from successive generation and allowed for sib mating for 15 generations to develop syngenic lines (Fig. 1).

Congenic line development

After fixation as syngenic lines, one multivoltine line of CB5 and one bivoltine line of JPN were considered as receptor and donor parents respectively. In F_1, only these cocoons were selected having higher cocoon shell weigh and other phenotipical characters and physiological character like voltinism as receptor parent. Thereafter, consecutive back crosses were conducted for 10 to 15 generations in a similar fashion and finally sib mating was performed between male and female moths emerged from larvae having maximum homogeneity like receptor parent and higher single cocoon shell weight, closer to donor parent resulting in RBL (Fig. 2). As the target character did not appear in RBL, second cross even third and fourth cross (in some cases) was made between donor parent and successively developed RBL as receptor parent. Thereafter same protocol for development of RBL (Fig. 2) was followed in order to develop congenic line, which showed the transgression of target trait as donor parent

For hybridization, three developed higher cocoon shell weight multivoltine congenic breed (Con. *C*, Con. *Gc*, Con. *Ow*) and one inbred multivoltine (Nistari) were used as a female component. The bivoltine males were used from two syngenic lines *viz.,* D6+*p*, D6*p* and NB_4D_2 as one inbred line. Each of the multivoltine and bivoltine breeds differs from the other. The characteristic features of these breeds were depicted in Table 1. Each of the multivoltine Con. L and Nistari females was crossed with males of Syn.L bivoltine and NB_4D_2 males respectively to obtain F_1. Hatched larvae from three disease free layings (DFLs) of each cross were reared on mulberry (*Morus alba*) leaves of S_1 variety following the methodology described by Krishnaswami (1978). After third moult, three hundred larvae were kept and reared for final harvest *i.e.*, cocoons. The cocoon yield by number / 10,000 larvae, cocoon yield by weight (kg) / 10,000 larvae, SCW, SCSW in gram (g), cocoon shell percentage and pupation rate were consider for heterosis study. The cocoon shell percentage was calculated as:

[Single cocoon shell weight (SCSW) ÷ Single cocoon weight (SCW)] × 100

Heterosis was estimated using following formula (Rai, 1979).

Table 1. Characteristic features of parental silkworm breeds

Breed	Characteristics feature
1. Con. *C*	Multivoltine, evolved after introgression of higher shell weight character / gene(s) from bivoltine breed JPN_9 to CB_5 (multivoltine) which was evolved from the multiple crosses and with treatment of physical agent X-ray, EMS and selection. Multivoltine, marked larvae, Golden yellow oval shaped cocoon.
2. Con. *Gc*	Same as Con. *C*, cocoon colour is light green.
3. Con. *Ow*	Same as Con. *C*, cocoon colour is off white.
4. N + *p*	Popular multivoltine in West Bengal. Since time immemorial. Spindle shaped golden yellow cocoons.
5. D6 + *p*	Bivoltine, originally developed from hybrids J1 × A26 and there after selection. Latter on syngenic line was developed at CSR&TI, Berhampore. Marked larvae, peanut shaped whitish cocoons.
6. D6*p*	Same as D6 + *p*, only the larval are Plain.
7. NB_4D_2	Bivoltine, evolved from a Japanese double cross hybrid (Kokko × Seihaku) × (N124 × C124). Cocoons are peanut shaped and whitish.

i) Percent heterosis over

$$MPV = [(F_1 - MPV) \div (MPV)] \times 100$$

$$S.Ed(F_1 - MPV) = \sqrt{3EMS/2r}$$

$$CD = S.Ed \times t_{0.01\&0.05}, \text{ error df}$$

ii) Per cent heterosis over

$$BPV = [(F_1 - BPV) \div (BPV)] \times 100$$

$$S.Ed(F_1 - BPV) = \sqrt{3EMS/2r}$$

$$CD = S.Ed \times t_{0.01\&0.05}, \text{ error df}$$

iii) Per cent heterosis over

$$CPV = [(F_1 - CPV) \div (CPV)] \times 100$$

$$S.Ed(F_1 - CPV) = \sqrt{3EMS/2r}$$

$$CD = S.Ed \times t_{0.01\&0.05}, \text{ error df}$$

MPV = Mid parent value; BPV = Better parent value; CPV = Check parent value; S.Ed. = Standard error difference; EMS = Error mean square; $t_{0.01\ \&\ 0.05}$ error df = Student's 't' values at 1% & 5% level corresponding to error degrees of freedom; r = Replication; CD = Critical difference.

All the reeling parameters assessed by standard methods of Central Silk Technological Research Institute (CSTRI), Bangalore, Karnataka, India.

Results

The mean value and heterosis percentages of different characters in different crosses between higher cocoon shell weight multivoltine congenic and bivoltine syngenic

Table 2. Mean value of rearing performance of parental silkworm breeds and their hybrids in congenial season

Breed / Cross	Cocoon yield/10000 larvae		Pupation	SCW	SCSW	Cocoon shell
	no.	wt.(kg)	(%)	(g)	(g)	(%)
1. Con. *Ow*	9656	13.07	96.56	1.435	0.256	17.86
2. Con. *C*	9178	12.87	93.33	1.463	0.250	17.06
3. Con. *Gc*	9022	12.87	90.67	1.493	0.264	17.68
4. D6*p*	6933	12.13	69.58	1.750	0.360	20.59
5. D6+*p*	6900	10.02	69.33	1.649	0.322	19.51
6. N +*p*	7511	6.72	75.04	0.938	0.109	11.59
7. NB_4D_2	9011	11.50	90.11	1.245	0.245	19.66
8. Con. *Ow* × D6*p*	9678	17.77	97.33	1.893	0.409	21.61
9. Con. *Ow* × D6+*p*	9689	17.64	97.55	1.861	0.372	19.98
10. Con. *C* × D6*p*	9545	17.01	96.33	1.868	0.358	19.15
11. Con. *C* × D6+*p*	9189	16.46	92.44	1.869	0.347	18.56
12. Con. *Gc* × D6*p*	9233	17.43	93.55	2.082	0.413	19.82
13. Con. *Gc* × D6+*p*	9255	18.29	93.22	1.994	0.392	19.67
14. N+*p* × NB_4D_2 (Check)	9500	14.35	96.22	1.457	0.247	16.95

Table 3. Heterosis calculated in percentage for the cocoon yield (number and weight), pupation rate, single cocoon weight, Single cocoon shell weight and cocoon shell percentage between congenic multivoltine and syngenic bivoltine silkworm during favourable season

Hybrids	Cocoon yield (no.)			Cocoon yield (wt.)			Pupation rate		
	MPH	BPH	CH	MPH	BPH	CH	MPH	BPH	CH
Con. *Ow* × D6*p*	16.68^{NS}	0.23^{NS}	1.87^{NS}	41.03**	35.99**	23.87*	17.18*	0.80^{NS}	1.16^{NS}
Con. *Ow* × D6+*p*	17.05^{NS}	0.35^{NS}	1.99^{NS}	52.86**	35.00**	22.98*	17.62*	1.04^{NS}	1.39^{NS}
Con. *C* ×D6*p*	18.49*	4.00^{NS}	0.47^{NS}	36.10**	32.22**	18.58^{NS}	18.27*	3.21^{NS}	0.12^{NS}
Con. *C* × D6+*p*	14.31^{NS}	0.12^{NS}	-3.28^{NS}	43.84**	27.91*	14.72^{NS}	13.66^{NS}	-0.95^{NS}	-3.93^{NS}
Con. *Gc* × D6*p*	15.74^{NS}	2.34^{NS}	-2.81^{NS}	39.45**	35.48**	21.50*	16.76^{NS}	3.18^{NS}	-2.77^{NS}
Con. *Gc* × D6+*p*	16.26^{NS}	2.59^{NS}	-2.56^{NS}	59.88**	42.17**	27.50**	16.53^{NS}	2.82^{NS}	-3.12^{NS}
N+*p* × NB4D2	15.00^{NS}	5.43^{NS}		57.52**	24.76^{NS}		16.52^{NS}	6.78^{NS}	

Hybrids	Single cocoon weight			Single cocoon shell weight			Cocoon shell %		
	MPH	BPH	CH	MPH	BPH	CH	MPH	BPH	CH
Con. *Ow* × D6*p*	18.85**	8.15**	29.90**	32.74**	13.60**	65.73**	12.46**	5.00*	27.56**
Con. *Ow* × D6+*p*	20.69**	12.86**	27.73**	28.72**	15.65**	50.66**	6.96**	2.44^{NS}	17.92**
Con. *C* × D6*p*	16.28**	6.74**	28.21**	17.29**	-0.72^{NS}	44.84**	1.75^{NS}	-6.98**	13.02**
Con. *C* × D6+*p*	20.12**	13.34**	28.28**	21.52**	7.91**	40.58**	1.55^{NS}	-4.82*	9.57**
Con. *Gc* × D6*p*	28.42**	18.99**	42.92**	32.21**	14.57**	67.15**	3.57*	-3.74^{NS}	16.95**
Con. *Gc* × D6+*p*	26.95**	20.94**	36.88**	33.98**	21.98**	58.91**	5.78**	0.83^{NS}	16.07**
N+p × NB4D2	33.51**	17.03**		39.72**	0.89^{NS}		8.46**	-13.80**	

MPH = mid parent heterosis, BPH = better parent heterosis, CH = check heterosis.
* = significant at 5% level ($p < 0.05$), ** = significant at 1% level ($p < 0.01$), NS = non significant.

breeds along with check are depicted in Tables 2, 3 and 4. It was observed that cocoon yield by number, pupation rate and cocoon shell percentage did not reflect any significant heterotic effect (Table 3).

Cocoon yield by weight

The heterosis is found to be positive in all the three cases *i.e.*, MPH, BPH and CH. It ranges from 14.72 (CH) to 59.88 (MPH). All the six hybrids showed significant heterosis ($p < 0.01$) over MP and BP and four hybrids over check. The best hybrid for this character was Con. *Gc* × D6 + *p* (Table 3). The average yield / 10000 larva of the hybrid were 18.29 kg against 12.9 kg in BP and 14.4 kg in check (Table 2).

Single cocoon weight

The desired heterosis for this character is positive. It ranges from 6.74 (BPH) to 42.98 (CH). The best hybrid in case of BPH is also the same as above *i.e.*, Con.*Gc* × D6 + *p* (20.94).

Single cocoon shell weight

The range of desired heterosis in respect of SCSW is 7.91 (BPH) to 67.15 (CH). Hybrids Con. *Gc* × D6 + *p* and Con. *Gc* × D6*p* showed better improvement for this character with highest MPH and BPH (33.98 and 21.98) in the first one. The average SCSW of the former one is 0.392 g against 0.322 g in BP and 0.247g in check (Table 2 and 3). The overall performance of the hybrid Con. *Gc* × D6 + *p* was found best among the six hybrids and check under congenial atmosphere.

Reeling parameters

Particularly the character filament Length attained better improvements in all the hybrids (Table 4) having maximum in Con. *Gc* × D6 + *p* (1060 m) than BP (780 m) and check (853 m). But other reeling parameters *viz.*, denier, raw silk %, reelability %, neatness %, evenness %, cohesion stroke, degumming loss etc. in all the hybrids showed improvement (Table 4).

Discussion

Heterosis was determined to be superior over mid parent, better parent as well as the check-a popular commercial

Table 4. Mean values of reeling performance (qualitative parameters) of parental silkworm breeds and their hybrids in congenial season

Breed / Cross	Filament length (m)	Denied	Raw silk (%)	Reelability (%)	Neatness (%)	Evenness (%)	Cohesion (strokes)	Degumming loss (%)
1. Con. *Ow*	460	1.93	9.02	75.97	80	70	60	22.50
2. Con. *C*	500	2.72	10.52	77.44	80	80	60	22.50
3. Con. *Gc*	500	2.10	9.91	74.13	80	80	50	24.50
4. D6*p*	800	2.51	12.46	83.81	90	90	60	19.85
5. D6+*p*	780	2.56	11.23	80.69	90	80	60	21.77
6. N+*p*	340	1.68	7.01	75.91	70	70	50	24.50
7. NB_4D_2	900	2.10	12.03	81.79	90	90	60	20.57
8. Con. *Ow* × D6*p*	1053	2.79	12.10	85.00	90	90	60	22.50
9. Con. *Ow* × D6+*p*	1007	2.87	12.53	89.67	90	80	50	24.50
10. Con. *C* × D6*p*	1020	2.78	12.28	88.43	90	90	60	24.00
11. Con. *C* × D6+*p*	1020	2.79	12.77	93.24	90	90	60	22.50
12. Con. *Gc* × D6*p*	1040	2.84	11.87	88.57	80	80	50	24.50
13. Con. *Gc* × D6+*p*	1060	2.85	11.87	86.66	80	80	60	24.50
14. N+*p* × NB_4D_2 (Check)	853	2.60	11.37	82.39	80	80	50	24.00

hybrid N × NB_4D_2. It was observed that the yield contributing parameters *viz.*, cocoon yield by number, pupation rate and cocoon shell percentage depicted statistically insignificant positive heterosis. The expression of the aforesaid two characters (cocoon yield by number and pupation rate) depends on effective rate of rearing (ERR) and the linked character cocoon shell percentage is not directly controlled by gene/genes. On the other hand, cocoon yield by weight, single cocoon weight (SCW) and single cocoon shell weight (SCSW) express heterosis over better parent at significant magnitude $p < 0.01$ (Table 3). It was well established that the positive heterosis over the mid-parent and better parent are due to dominant or over-dominant gene(s) expression for a character. But in our work, used parents are quite different as both were developed with a specific character-higher cocoon shell weight. This cocoon shell weight (SCSW) is a multigenic character (Tazima, 1964). With this concept, Chattopadhyay *et al.* (2001a, b, c) have developed multivoltine congenic lines for higher cocoon shell weight through introgression. It was further observed that three to four times cross with donor parent is required to drag the character fully. Therefore, it may be presumed that heterobeltiotic genetic action resulted from the action and interaction among the aggregated favourable genes of syngenic and congenic lines (Chattopadhyay *et al.*, 2001a, b, c). Earlier heterosis study on silkworm, *Bombyx mori* L by various workers (Bhargava *et al.*, 1993; Banuprakash *et al.*, 1994; Das *et al.*, 1994; Rao *et al.*, 1998) presented heterotic expression on different hybrids for different yield contributing parameters. Verma *et al.* (2003) studied the heterotic genetic interaction between multi congenic lines by using some specific type of developed breeds. Genetic interaction between hybrids of congenic and syngenic lines was studied, which suggested that all the hybrids not only provide better heterosis but also produce good quality silk due to the cumulative effect of genes, dominance and genetic diversity of the parents involved in the crosses.

Acknowledgement

The authors are deeply indebted to Central Silk Board, Ministry of Textile, Govt. of India to carry out the work under the project AIG002, entitled 'Utilization of syngenic lines for improvement of shell weight and survival in silkworm *Bombyx mori* L'. The assistance of Shri Tapas Kr. Maitra in data processing and making tables, charts, etc is also acknowledged.

References

Banuprakash, K. G., R. Govindan and M. C. Devaiah (1994) Heterosis observed in the hybrids between some improved multivoltines and bivoltines of *Bombyx mori* L. *Insect Sci. Applic.* **15**, 313-321.

Bhargava, S. K., V. Thiagarajan and R. K. Datta (1993) Hybrid vigour in the silkworm, *Bombyx mori* L. *G. it. Ent.* **6**, 449-453.

Chattopadhyay, G. K. and S. N. Chatterjee (1990) Need for advanced gentical research in silkworm *Bombyx mori* L; in *A Workshop Manual- Cloning and characterization of animal* *gene*. Gupta, P. K. and R. K. Mondal (eds.), pp. 107, DBT Laboratory, Department of Biochemistry, Bose Institute, Calcutta University, India.

Chattopadhyay, G. K., A. K. Sengupta, A. K. Verma, S. K. Sen and B. Saratchandra (2001a) Utilization of congenic line in silkworm breeding; in *Perspectives in Cytology and Genetics.* Manna, G. K and S. C. Roy (eds.), Vol. 10, pp. 717-724, AICCG Pub., Kalyani Univ., India.

Chattopadhyay, G. K., A. K. Sengupta, A. K. Verma, S. K. Sen and B. Saratchandra (2001b) Transgression of shell weight - a multigenic trait, through development of congenic breed in tropical silkworm, *Bombyx mori* L. *Sericologia.* **41**, 33-42.

Chattopadhyay, G. K., A. K. Sengupta, A. K. Verma, S. K. Sen and B. Saratchandra (2001c) Esterase isozyme polymorphism, specific and nonspecific esterase, syngenic lines development and natural occurrence of a thermo stable esterase in tropical silkworm *Bombyx mori* L. *Insect Biochem. Mol. Biol.* **31**, 1191-1199.

Das, S. K., S. Pattnaik, B. Ghosh, T. Singh, B. P. Nair, S. K. Sen and G. Subba Rao (1994) Heterosis analysis in some three-way crosses of *Bombyx mori* L. *Sericologia* **34**, 51-61.

Datta, R. K. (1984) Improvement of silkworm races (*Bombyx mori* L.) in India. *Sericologia* **24**, 393-415.

Datta, R. K., H. K. Basavaraja, N. M. Reddy, N. Nirmal Kumar, M. M. Ashan, N. Suresh Kumar, M. Ramesh Babu and K. P. Jayaswal (2000) Breeding of new productive bivoltine hybrid CSR12 × CSR6 of silkworm, *Bombyx mori* L. *Int. J. Indust. Entomol.* **3**, 127-133

Harada, C. (1949) On the hybrid effect of economical characters in the silkworm. *J. Seric. Sci. Jpn* **18**, 120-121.

Harada, C. (1961) Heterosis of the quantitative characters in silkworm. *Bul. Seric. Expt. Stat. Jpn* **17**, 50-52.

Krisnaswamy, S. (1978) New technology of silkworm rearing. Bulletin No. 2, C.S.R and T. I, Mysore, India. pp.1-18.

Rai, B. (1979) Heterosis Breeding. Agro-biological Publication, Delhi, India.

Rao, P. R. T., B. Ghosh, S. M. Moorthy, S. K. Das, G. C. Roy, A. K. Sengupta and S. K. Sen (1998) Combining ability, gene action and heterosis through introgressive hybridization in *Bombyx mori* L; in *Perspective in Cytology and Genetics.* Manna, G. K. and S. C. Roy (eds.), Vol.9, pp. 461-471, AICCG Pub., Kalyani Univ., India.

Sengupta, K. and R. K. Datta (1973) Studies on evolution of superior multivoltine breeds of silkworm, *Bombyx mori* L. XII International Silk Congress, Barcelona, pp. 1-16.

Tazima, T. (1964) Genetics of Silkworm. pp. 174-179, Logo Press.

Verma, A. K., G. K. Chattopadhyay, M. Sengupta, A. K. Sengupta, S. K. Das and S. Raje Urs (2003) Expression of heterotic genetic interaction among multivoltine recurrent backcross / congenic lines for higher shell weight of silkworm *Bombyx mori* L. *Int. J. Indust. Entomol.* **7**, 21-27.

Yokoyama, T. (1974) Utilization of heterosis in Japanese sericulture. *Indian J. Genet.* **34a**, 206-210.