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Expression of Heterotic Genetic Interaction among Multivoltine Recurrent Backcross/Congenic Lines for Higher Shell Weight of Silkworm, *Bombyx mori* L.

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Manifestation of heterotic genetic interaction was studied in different hybrids made between multivoltine recurrent backcross (RBL)/congenic lines (Con. L) during unfavourable season when temperature and relative humidity are >30°C and 86%, respectively. A few number of silkworm race or strain or breed like Nistari (N+*p* or N*p*) can sustain the temperature above 30°C and RH above 86%. The present heterosis study screened a hybrid *i.e.*, CB_5*Lm5*RBL1×M_6DPC-*LmE*[1]RBL and its reciprocal provided heterobeltiotic effect on survival by number and pupation rate at a magnitude of 20% (p<0.01) and yield by weight of 10% (p<0.01). Beside all the hybrids expressed heterosis over check - Nistari (N+*p*) with better quality silk. Therefore, aforesaid hybrid may be useful for utilization at commercial level during adverse seasons of West Bengal.

Key words: Silkworm, Breeding, Syngenic, Recurrent Backcross Line (RBL), Congenic Line (Con. L), Heterosis, Heterobeltiotic, Genetic interaction

Introduction

A number of workers reported heterotic genetic interaction in hybrid of number of plants (Cregan and Busch, 1978; Borghi *et al.*, 1988; Singh and Singh, 1971) and live stock. Same study was also conducted in silkworm, *Bombyx mori* by different worker (Harada, 1949, 1961; Yokoyama, 1974), which has resuscitated in the field of sericulture in Japan. In general, heterotic hybrids 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. Multivoltine breeds, those are basically of tropical origin, lay 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, different workers (Sengupta and Datta, 1973; Datta, 1984; Subba Rao *et al.*, 1990; Bhargava *et al.*, 1993; Rao *et al.*, 1998) attempted to overcome the problems by making various silkworm hybrids and used them at field level for screening season and region specific as well as suitable for adverse climatic situation. 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 desire result. Keeping this in view, an attempt has been taken to develop different syngenic (Syn.) and congenic (Con.) breeds (Chattopadhyay *et al.*, 2001a, b) and thereafter its use in hybridizing programme to understand its heterotic effect in F_1. The results are discussed in this context.

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Materials and Methods

Syngenic line development

Continuous selection was done phenotypically (larval marking, cocoon colour and cocoon shape), physiologically like moulting duration during different stages of development (2^{nd}, 3^{rd} and 4^{th}), maturation period for spinning on multivoltine germplasm (GP) breeds CB_5, M_6DPC and higher shell weight bivoltine germplasm breeds JPN_9 and D_6. On the basis of aforesaid characters, some lines were isolated initially. From each line, 20 to 25 larvae on 5^{th} day of 5^{th} instar were taken to collect digestive juice and haemolymph separately from each larva of each line. Anodic, cathodic amylase and 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).

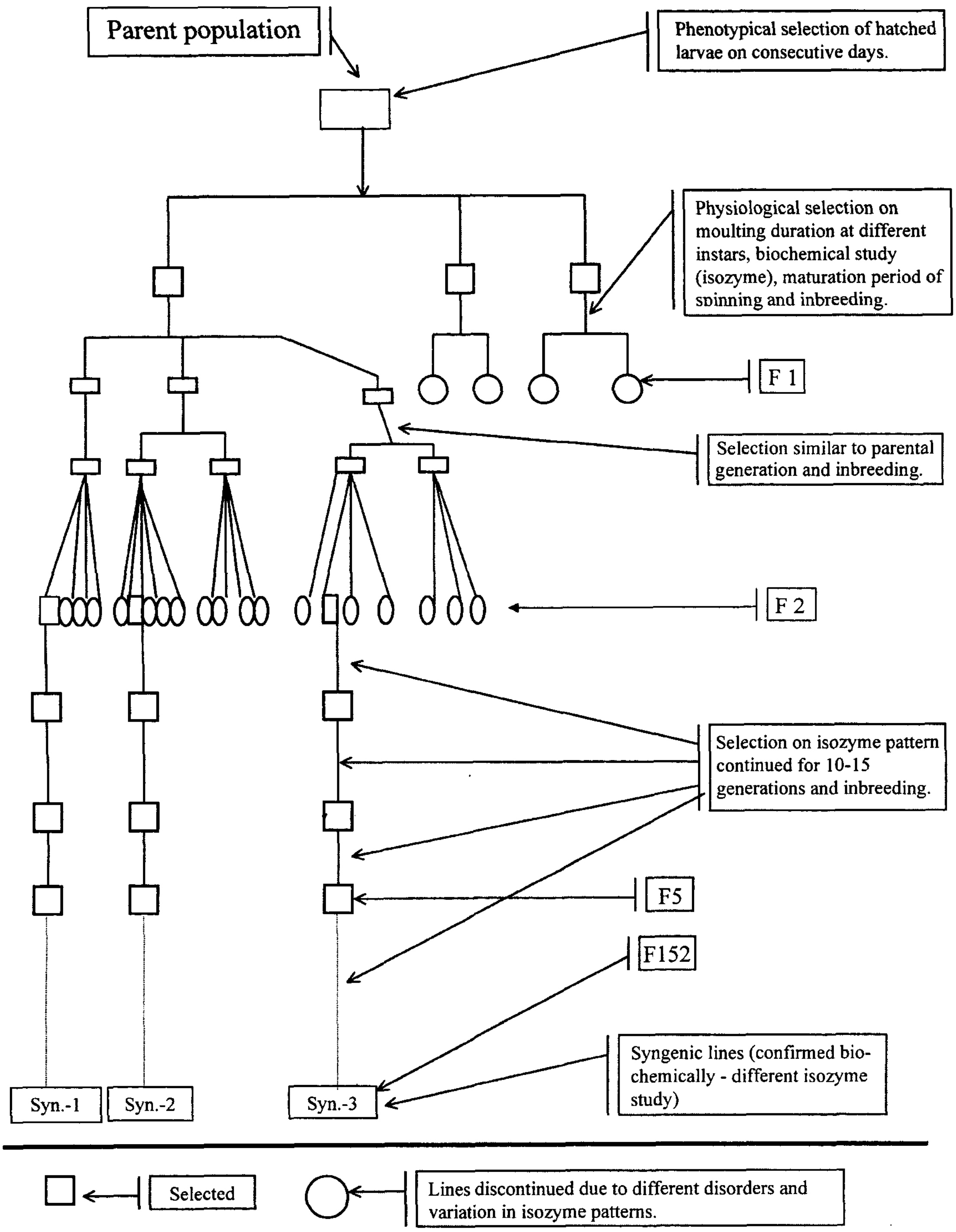

Fig. 1. Scheme for development of syngenic lines in silkworm *Bombyx mori* L.

Recurrent backcross/congenic line development

For this, two multivoltine syngenic lines, *viz.*, CB_5*Lm*5 and M_6DPC*Lm*E[1] used as receptor and two bivoltine syngenic lines JPN_9 and D_6*Lmp* were considered as donor parents respectively. In F_1, only those cocoons were selected having higher cocoon shell weight, other phenotipical and physiological characters like larval marking, voltinism as receptor parent. Thereafter, consecutive back crosses with receptor 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 SCSW, closer to donor parent resulting in RBL (Fig. 2). As the target character did not appear fully in RBL, second even third and fourth crosses are required between donor and developed successive RBL as receptor

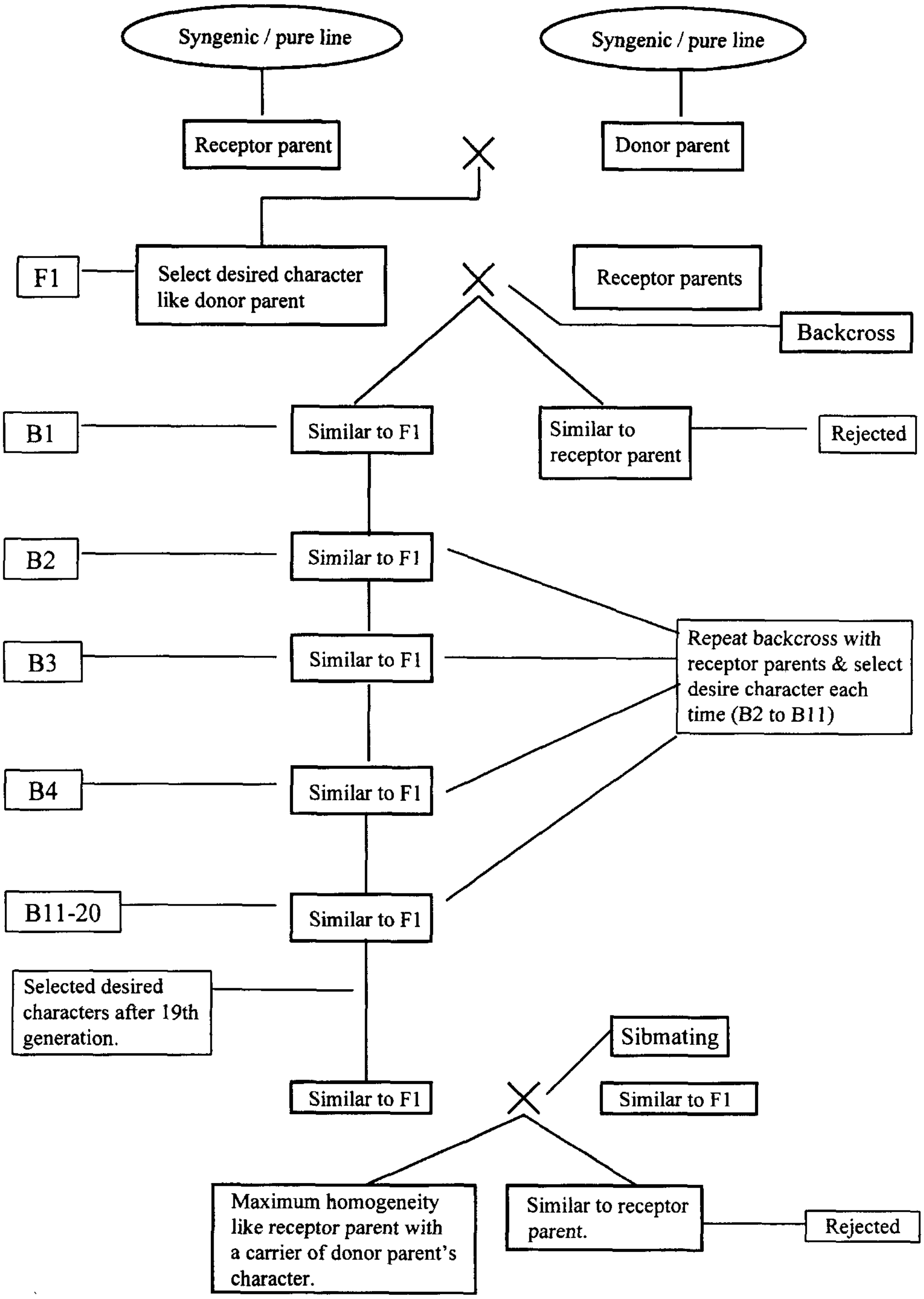

Fig. 2. Scheme for developing recurrent backcross (RB)/congenic line (Con. L.) in silkworm *Bombyx mori* L.

Table 1. Mean value of rearing performance of parental silkworm breeds and their hybrids during unfavourable season

Sl. no.	Breed/Cross	Yield/10,000 larvae (no)	Yield/10,000 larvae (wt)	Pupation rate (%)	SCW (g)	SCSW (g)	S.R. (%)
1	CB_5*Lm*5 Con.*C*+	9072	9.721	90.72	1.317	0.234	17.77
2	M_6DPC*LmE*^{l}-RBL	6165	7.796	61.76	1.345	0.213	15.94
3	CB_5*Lm*5Con.*Ow*	5562	5.834	55.62	1.173	0.174	14.92
4	CB_5 *Lm*5-RBL1	2876	3.367	28.94	1.254	0.220	17.55
5	CB_5 *Lm*5-RBL2	947	0.922	9.47	0.980	0.181	18.52
6	CB_5 *Lm*5 Con.*C*+ × M_6DPC*LmE*^{l}-RBL	4308	4.839	44.70	1.318	0.211	16.09
7	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5Con.*C*+	5659	6.546	56.59	1.287	0.206	15.97
8	CB_5*Lm*5Con.*Ow* × M_6DP*LmE*^{l}-RBL	5142	5.894	51.68	1.177	0.188	16.04
9	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5Con.*Ow*	5475	5.993	55.52	1.221	0.219	17.91
10	CB_5*Lm*5-RBL1 × M_6DPC*LmE*^{l}-RBL	7609	8.572	76.45	1.308	0.218	16.73
11	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5-RBL1	7493	8.727	74.78	1.154	0.183	15.84
12	CB_5*Lm*5-RBL2 × M_6DPC*LmE*^{l}-RBL	6155	7.194	61.69	1.403	0.231	16.48
13	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5-RBL2	6502	7.732	66.33	1.346	0.234	17.40
14	N+*p* (Check)	6893	4.763	68.64	0.696	0.086	12.45

Temperature at 30°C and relative humidity at 86%.

parent. After that same protocol for development of RBL (Fig. 2) was followed in order to develop congenic line, which expressed the target character as donor parents.

For hybridization, four higher shell weight multivoltine RBL/Con. L from CB_5*Lm*5 and one from M_6DPC*Lm*E^l (Table 1) and their reciprocal crosses were done and one inbred multivoltine (N+*p*) was used as a check. Hatched larvae from three disease free layings (Dfls) of each cross were reared on mulberry (*Morus alba*) leaves of S_1 variety following the normal schedule (Krisnaswamy, 1978). After third moult, three hundred larvae with three replications were kept and reared for final harvest *i.e.,* cocoons. The cocoon yield by number/10,000 larvae, cocoon yields by weight (kg)/10,000 larvae, SCW, SCSW in gram (g), cocoon shell percentage (SR%) and pupation rate were considered for heterosis study. The SR% was calculated as: [Single cocoon shell weight (SCSW) ÷ Single cocoon weight (SCW)] × 100

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

i) Percent heterosis over MPV = $[(F_1 - MPV) \div MPV] \times 100$

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

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

ii) Per cent heterosis over BPV = $[(F_1 - BPV) \div BPV] \times 100$

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

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

iii) Percent heterosis over CPV = $[(F_1 - CPV) \div CPV] \times 100$

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

$CD = S.\ Ed \times t_{0.01\ \&\ 0.05}$, 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 of different characters in the parents and different crosses between higher shell weight multivoltine RBL/Con. Lines and check are depicted in Table 1. The heterosis percentage *i.e.,* mid parent, better parent and check heterosis is presented in Table 2.

Cocoon yield by number

The desired heterosis over MPV, BPV and CPV is found to be positive and highly significant ($p < 0.01$) in respect of the hybrid CB_5*Lm*5-RBL1 × M_6DPC*Lm*E^l-RBL and its reciprocal. It ranges between 8.709% (CH) and 68.335% (MPH). The BPH is 23.429% in the same hybrid (Table 2). The average cocoon yield number/10,000 larvae is 7609 against 6165 in better parent (Table 1).

Cocoon yield by weight

The heterosis for this character is also highly significant in

CB_5*Lm*5-RBL1 × M_6DPC *Lm*E[l]-RBL and its reciprocal. It is 53.58% (MPH), 9.958% (BPH) and 79.99% (CH) and 56.351%, 11.942% and 83.238% respectively in the aforesaid hybrid and it reciprocal (Table 2). The average yield/10,000 larvae of these hybrids are 8.572 kg and 8.727 kg against 7.796 kg in better parent (Table 1).

Pupation rate

This is the most important character during adverse season when temperature ranges from 27°C to 33.5°C and RH from 76 to 93%. Here, again CB_5*Lm*5-RBL1 × M_6-DPC*Lm*E[l]-RBL and its reciprocal express highly significant heterosis over MP, BP and CH. The BPH percentages are 23.792 and 21.094%, respectively (Table 2). The average pupation rate of the hybrids are 76.45% and 74.78%, respectively against better parent (M6DPC*LmE*[l]RBL) 61.76%.

Other characters

Other characters like single cocoon weight (SCW), single cocoon shell weight (SCSW) and silk percentage (SR%) do not express any significant heterotic effect except CB_5*Lm*5RBL2 × M_6DPC*Lm*E[l] RBL and its reciprocal over MPH and BPH for SCW and SCSW (Table 2).

Reeling parameters

Reeling parameters *viz.*, filament length, denier, raw silk

Table 2. Heterosis percentage in hybrids (multivoltine RB/Con.L × RB/Con.L) on cocoon yield parameters

Sl. no.	Hybrids	Yield/10000 by no.			Yield/10000 by wt.		
		MPH	BPH	CH	MPH	BPH	CH
1	CB_5 *Lm*5 Con.*C*+ × M_6DPC*LmE*[l]-RBL	−43.453**	−52.515**	−37.502**	−44.750**	−50.219**	1.603**
2	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*C*+	−25.725**	−37.627**	−17.907**	−25.260**	−32.659**	37.444**
3	CB_5*Lm*5Con.*Ow* × M_6DP*LmE*[l]-RBL	−12.299**	−16.584**	−25.398**	−13.512**	−24.393**	23.761**
4	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*Ow*	−6.631**	−11.193**	−20.576**	−12.069**	−23.132**	25.826**
5	CB_5*Lm*5-RBL1 × M_6DPC*LmE*[l]-RBL	68.335**	23.429**	10.387**	53.580**	9.958**	79.990**
6	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL1	65.776**	21.553**	8.709**	56.351**	11.942**	83.238**
7	CB_5*Lm*5-RBL2 × M_6DPC*LmE*[l]-RBL	73.105**	−0.151**	−10.702**	65.038**	−7.722**	51.050**
8	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL2	82.864**	5.477**	−5.668**	77.380**	−0.821**	62.346**
	Hybrids	Pupation rate			SCW		
1	CB_5 *Lm*5 Con.*C*+ × M_6DPC*LmE*[l]-RBL	−41.374**	−50.733**	−34.879**	−0.964**	−1.983**	89.277**
2	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*C*+	−25.778**	−37.627**	−17.556**	−3.294**	−4.289**	84.825**
3	CB_5*Lm*5Con.*Ow* × M_6DP*LmE*[l]-RBL	−11.944**	−16.317**	−24.705**	−6.513**	−12.469**	69.028**
4	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*Ow*	−5.396**	−10.093**	−19.105**	−2.992**	−9.172**	75.395**
5	CB_5*Lm*5-RBL1 × M_6DPC*LmE*[l]-RBL	68.578**	23.792**	11.384**	0.667**	−2.727**	87.841**
6	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL1	64.903**	21.094**	8.955**	−11.211**	−14.204**	65.677**
7	CB_5*Lm*5-RBL2 × M_6DPC*LmE*[l]-RBL	73.222**	−0.108**	−10.121**	20.705**	4.338**	101.484**
8	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL2	86.250**	7.405**	−3.361**	15.802**	0.099**	93.298**
	Hybrids	SSW			SR%		
1	CB_5 *Lm*5 Con.*C*+ × M_6DPC*LmE*[l]-RBL	−5.419**	−9.539**	144.753**	−4.549**	−9.456**	29.210**
2	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*C*+	−7.864**	−11.878**	138.426**	−5.241**	−10.113**	28.273**
3	CB_5*Lm*5Con.*Ow* × M_6DP*LmE*[l]-RBL	−2.879**	−11.746**	117.978**	3.964**	0.648**	28.862**
4	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5Con.*Ow*	12.729**	2.437**	153.009**	16.082**	12.380**	43.882**
5	CB_5*Lm*5-RBL1 × M_6DPC*LmE*[l]-RBL	0.624**	−0.804**	152.160**	−0.109**	−4.691**	34.351**
6	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL1	−15.603**	−16.801**	111.497**	−5.385**	−9.725**	27.256**
7	CB_5*Lm*5-RBL2 × M_6DPC*LmE*[l]-RBL	16.778**	8.013**	166.782**	−4.344**	−10.999**	32.369**
8	M_6DPC*LmE*[l]-RBL × CB_5*Lm*5-RBL2	18.703**	9.794**	171.181**	1.016**	−6.013**	39.786**

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

Table 3. Mean value of reeling performance (qualitative parameters) of parental silkworm breeds and their hybrids in unfavourable season

Sl. no.	Breed/Cross	Filament length (m)	Denier	Raw silk (%)	Reelability (%)	Neatness (%)	Evenness (%)	Cohesion (strokes)	Degumming loss (%)
1	CB_5*Lm*5Con.*C*+	650	1.84	10.52	77.44	80	80	60	22.50
2	M_6DPC*LmE*^{l}-RBL	675	1.93	10.11	76.97	80	80	50	21.87
3	CB_5*Lm*5Con.*Ow*	620	1.97	9.02	75.97	80	70	60	22.50
4	CB_5*Lm*5-RBL1	660	1.87	9.69	73.96	80	70	50	22.50
5	CB_5*Lm*5-RBL2	480	2.22	11.23	77.77	80	80	50	22.50
6	CB_5*Lm*5Con.*C*+ × M_6DPC*LmE*^{l}-RBL	600	1.79	10.86	81.44	90	90	50	22.45
7	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5Con.*C*+	660	1.85	10.76	79.79	80	80	40	23.52
8	CB_5*Lm*5Con.*Ow* × M_6DP*LmE*^{l}-RBL	600	1.73	10.52	80.58	90	80	50	22.50
9	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5Con.*Ow*	700	2.02	11.69	84.90	90	90	50	20.00
10	CB_5*Lm*5-RBL1 × M_6DPC*LmE*^{l}-RBL	620	1.79	11.23	83.67	90	90	50	22.50
11	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5-RBL1	650	1.95	10.71	80.73	80	80	50	22.22
12	CB_5Lm5-RBL2 × M_6DPCLmE^l-RBL	715	2.07	10.96	81.56	90	80	50	22.50
13	M_6DPC*LmE*^{l}-RBL × CB_5*Lm*5-RBL2	625	1.81	11.45	84.31	90	90	50	21.05
14	N+*p* (Check)	320	1.75	7.01	75.91	70	70	50	24.50

(%), reelability (%), neatness (%), evenness (%), cohesion stroke, degumming loss etc. in all the hybrids are found to be more or less at par with parents (Table 3).

Discussion

Heterosis was found to be superior over mid parent, better parent as well as check-a popular strain Nistari (N + *p*). Heterosis in regards to yield contributing parameters *viz.*, cocoon yield by number (survival), by weight and rate of pupation were found to be positive and statistically significant. The aforesaid two character-cocoon yield by number (survival) and the rate of pupation depend on effective rate of rearing (ERR), on the expression of gene/genes for susceptibility to diseases at homozygous condition (Doira, 1993) and also on high temperature and humidity. Different RBL and Con.L developed on different breed by using two different syngenic lines one as a receptor and other as donor for higher shell weight by continuous inbreeding, backcrossing and sib mating (Chattopadhyay *et al.*, 2001a, b). As a result, there is a possibility in reduction or elimination of number of lethal or deletorious gene (s) from the gene pool through continuous inbreeding. Therefore, the characters cocoon yield by number *i.e.*, survival and rate of pupation increase for heterozygocity appeared after making hybrid and with the increase in yield by number, yield by weight automatically becomes high. The linked character SR% is not expressed any heterosis as it is not directly controlled by any gene/genes. On the other hand, single cocoon shell weight (SCSW) a multigenic character (Tazima, 1964) did not express any heterotic effect due to non-interaction among genes at unfavourable situation. The single cocoon weight depends mainly on the weight of pupa plus SCSW, does not show any positive heterosis due to unfavourable environment. It is well established that positive heterosis over mid-parents and better parent are due to dominant or over-dominant gene(s) expression for a character (Sarawat *et al.*, 1994; Karale and Desai, 2000). This view may get support for yield by number *i.e.*, only but not in other characters in unfavourable season for silkworm. Earlier heterosis study on silk worm, *Bombyx mori* L. by various workers (Bhargava *et al.*, 1993; Das *et al.*, 1994; Banuprakash *et al.*, 1994; Rao *et al.*, 1998) presented heterotic expression on different hybrids for different yield contributing parameters but not by using so specific type of breeds. First time, RBL/Con×RBL/Con lines were used to study for heterosis. This work reflects that especially during hot and humid season of West Bengal, one of the hybrids CB_5*Lm*5RBL1×M_6DPC*LmE*^{l}RBL and its reciprocal can be introduced for commercial use to get higher yield by replacing ruling multivoltine strain or breed.

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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.

Borghi, B., M. Perenzin and R. J. Nash (1988) Agronomic and qualitative characteristics of ten bread wheat hybrid produced using a chemical hybridizing agent. *Euphytica* **39**, 185-194.

Chattopadhyay, G. K. and S. N. Chatterjee (1990) Need for advanced genetical 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. 7.1.4, 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) 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 (2001b) Esterase isozyme polymorphism, specific and nonspecific esterase, syngenic lines development and natural occurrence of a thermostable esterase in tropical silkworm *Bombyx mori* L. *Insect Biochem. Mol. Biol.* **31**, 1191-1199.

Cregan, P. B. and R. H. Busch (1978) Heterosis, inbreeding and line performance in crosses of adopted spring wheats. *Crop Sci.* **18**, 247-251.

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.

Doira, H. (1993) Genetic variation in *Bombyx mori*: Gene mutation and chromosome deficiencies. Silkworm Genetics Division, Institute of Genetic Resources, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, pp. 1-29.

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.

Karale, A. R. and U. T. Desai (2000) Study of heterosis for fruit characters in inter cultivar crosses of pomegranate (*Punica granatum* L.). *Indian J. Genet.* **60**, 191-196.

Krisnaswamy, S. (1978) New technology of silkworm rearing. Bulletin No. 2, CSR & TI, 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.), pp. 461-471, AICCG Publ., Kalyani University.

Sarawat, P., F. L. Stoddard, D. R. Marshall and S. M. Ali (1994) Heterosis for yield and related characters in pea. *Euphytica* **80**, 39-48.

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

Singh, K. B. and J. K. Singh (1971) Potentialities of heterosis breeding in wheat. *Euphytica* **20**, 586-590.

Subba Rao, G., S. K. Sen, B. Ghosh and S. K. Das (1990) Heterosis effect on some new silkworm hybrids evolved by three-cross; in *Perspective in Cytology and Genetics.* Manna, G. K. and S. C. Roy (eds.), pp. 373-378, AICCG Publ., Kalyani University **6**, 373-378.

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

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