American Journal of Applied Sciences 6 (12): 1981-1987, 2009
ISSN 1546-9239

.

Genetic Distance and Heterosis through Evaluation Index in the Silkworm, *Bombyx mori* (L.)

[1,2]Ebrahim Talebi and [1]G. Subramanya
[1]Department of Studies in Sericulture Science,
University of Mysore, Manasagangotri, Mysore, India
[2]Department of Animal Science, Islamic Azad University, Darab, Fars, Iran

Abstract: Problem statement: Synthesis of new-gene combinations by genetic manipulation is one of the powerful tools in exploiting the commercial qualities of plants and animals. Hybrid performance is evaluated from extensive yield trials that are costly and time consuming. **Approach:** Four silkworm races belonging to two different voltine groups (two multivoltine races namely Pure Mysore and Nistari and two bivoltine races namely C_{108} and NB_4D_2) and the twelve regular and reciprocal hybrids derived from them were reared under standard laboratory condition analyzing six quantitative traits namely cocoon weight, shell weight, shell ratio, filament length, denier and renditta. The data of the pure races was analysed for the estimation of evaluation index to study the genetic divergence between the races, where as evaluation index, heterosis and overdominance effects were studied in twelve hybrid combinations. **Results:** Varied heterotic effects were observed for different traits for hybrid combination. Cocoon weight and shell weight has maximum heterosis over the mid parent in Pure Mysore × Nistari (27 and 42% respectively), whereas C_{108} × Nistari have shown maximum shell ratio (30%) among the hybrids. The maximum filament length for heterosis was observed in the Nistari × Pure Mysore. Heterosis for cocoon weight, shell weight, shell ratio, filament length, denier and renditta based on evaluation index confirmed the above results. **Conclusion:** The investigation indicates that optimum level of genetic divergence between parents is necessary to obtain heterosis in F_1 generation.

Key words: Evaluation index, genetic diversity, heterosis, silkworm

INTRODUCTION

Synthesis of new-gene combinations by genetic manipulation is one of the powerful tools in exploiting the commercial qualities of plants and animals. The heterosis is observed when the animals of different genetic backgrounds are mated and this mating system is called the line crossing or crossbreeding. Perusal of literature related to heterosis manifestation in *Bombyx mori* has been demonstrated by many breeders[3,5,9,24,26,27]. Heterosis, expressed as the improvement in a character shown by a hybrid over their mid- or better parental value, is a vital measure of the genetic progress made in plant, animal and silkworm selection. Doddaswamy *et al.*[3] studied heterosis effect on economic traits of silkworm, *Bombyx mori* L. and reported that heterosis was a result of overdominance and proposed that linked favorable dominant gene also contributed to heterosis. Iftekher *et al.*[8] demonstrated that the degree of heterosis varied considerably for different characters with the maximum heterosis for cocoon shell weight, cocoon weight and thickness of the silk filament. Das *et al.*[2] carried out a series of out-breed crosses to select a suitable F_1 hybrid for better silk production. They observed that heterotic effects were significant for most of the quantitative characters. Farooq *et al.*[5] have found that the hybrid combinations manifested positive heterosis value for the cocoon traits studied. Kumaresan *et al.*[11] observed that heterotic effect for different traits for different hybrid combination in their experiment.

Silkworm breeding programs are based on the development and selection of outstanding hybrids from inbred lines. Developing and selecting inbred lines for performance is quite easy, although time consuming. Hybrid performance is evaluated from extensive yield trials that are costly and time consuming. In any hybrid program, a large number of crosses are made, while only a few good hybrids are obtained. This process is extremely labor intensive, time consuming and tedious and hence often, alternative methods are explored for analyzing the potential parental material. It is an

Corresponding Author: Ebrahim Talebi, Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysore, 570 006, India Tel: +919739766287

already established fact that the amount of yield heterosis obtained by hybrids depends largely on the genetic divergence of the populations from which the parental lines have been extracted[17]. The level of genetic diversity between the two parents being used for crossing has been proposed as a possible predictor of F_1 performance[27]. D^2 statistic by Mahalanobis [14] has been extensively used in predicting the hybrid performance on the basis of morphological traits. However, these methods require extensive field tests and crossing and hence, a need is now felt to determine genetic distance at the molecular level and thus to predict hybrid performance[16].

Small populations are particularly vulnerable to inbreeding and loss of genetic diversity through genetic drift[25]. Levels of genetic diversity within and divergence among populations are expected to vary with degree of fragmentation. As geographic distance between fragments or time since isolation increases, genetic diversity within small populations is expected to decrease and populations will diverge[7].

Prediction of hybrid performance has been of primary interest to essentially all hybrid breeding programs and has attracted an enormous amount of effort. The present studies were undertaken to prediction and estimate the level of heterosis and overdominance among F_1 hybrids of two bivoltine (C_{108} and NB_4D_2) and two multivoltine (Pure Mysore and Nistari) races of silkworm, *Bombyx mori* L. This information's would be useful to investigate the performance and relationship of F_1 hybrids and parents and to select suitable parents and population for designing an effective silkworm breeding programme.

MATERIALS AND METHODS

The silkworm races in the present study includes, two bivoltine races NB_4D_2 and C_{108} and two multivoltine races Nistari and Pure Mysore races which were drawn from the germplasm bank of the Department of Studies in Sericulture Science, University of Mysore, Mysore, India. The F_1 hybrids of regular and reciprocals along with parents were reared in replicates of three each following the rearing methodology described by Narasimhanna and Krishnaswamy[20]. The larvae were fed with M-5 variety of mulberry (*Morus indica*) leaves. The performance of all the genotypes were studied by analyzing six economic characters namely cocoon weight, shell weight, shell ratio, filament length, denier and renditta. Evaluation index (e_i and e'_i) was computed for all the traits except larval duration and renditta by the following formula suggested by Mano *et al.*[15]:

$$e_i = [(A-B)\times 10/C]+50$$

where as for the larval duration and renditta traits the modified formula of Mano *et al.*[15] was adapted as: follow:

$$e'_i = [(B-A)\times 10/C]+50$$

Where:
A = Value of particular genotype for a trait
B = Mean value
C = Standard deviation
10 = Standard unit
50 = Fixed value

The percent increase or decrease of F_1 hybrids over mid parent as well as better parent was calculated to estimate possible heterotic effects for above mentioned parameters[6]:

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

Where:
MPV = Mid Parent Value
BPV = Better Parent Value

The 't' test was manifested to determine whether F_1 hybrid means were statistically different from mid parent and better parent means as follows[26]:

$$t_{ij} = (F_{1ij} - MPV_{ij})/\sqrt{(3\times EMS/8)}$$

The 't' value for overdominance was calculated following the formula:

$$t_{ij} = (F_{1ij}-MPV_{ij})/\sqrt{(EMS/2)}$$

Where:
F_{1ij} = The Mean of the ij^{th} F_1 cross
MPV_{ij} = The mid parent for the ij^{th} cross
BPV_{ij} = The better parent values for ij^{th} cross
EMS = Error mean square

To estimate significant differences among mean value and evaluation index of parents and F_1 hybrids, the data were subjected by analysis of variance using SAS software[21]. Evaluation index was used to determine genetic relationships among pure races. The cluster analysis was carried out using the UPGMA method (Unweighted Pair Group Method Algorithm) developed by Sokal and Michener[22].

RESULTS

The mean value of four parental races and 12 F_1 hybrids for six cocoon characters namely cocoon weight, shell weight, shell ratio, filament length, denier and renditta are presented in Table 1 and relevant statistical analysis through ANOVA is presented in Table 2. It is evident from the data presented in Table 1 and 2 that all the six traits measured revealed high significant difference ($p<0.01$) except the trait denier.

The dendrogram produced on Unweighted Pair Group Method with Arithmetic means (UPGMA) and cluster analysis indicated that the two bivoltines C_{108} and NB_4D_2 formed into one cluster, where as, the two multivoltines Pure Mysore and Nistari formed into another separate cluster (Fig. 1). The evaluation index revealed significant difference between the six cocoon characters and rank value in parents (Table 3). The distance between C_{108} and NB_4D_2 was 0.33 and distance between Pure Mysore and Nistari was 0.44. The distance between two clusters calculated revealed a value of 1.196 (Table 4).

Table 1: Mean value of parents and F_1 progenies for six quantitative traits

Races/hybrids	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)	Filament length (m)	Denier	Renditta (kg)
C_{108}	1.95^{a}	0.317^{b}	18.42^{bcd}	1054.63^{abc}	2.18	7.595^{cd}
C_{108} × NB_4D_2	2.10^{a}	0.377^{a}	20.85^{a}	1166.40^{ab}	2.18	7.093^{d}
C_{108} × Nistari	1.55^{cde}	0.290^{bc}	18.63^{a}	838.23^{cdef}	2.17	7.727^{bcd}
C_{108} × pure Mysore	1.61^{bcd}	0.300^{b}	18.73^{a}	730.15^{defg}	2.68	7.900^{abcd}
NB_4D_2	1.76^{b}	0.315^{b}	19.98^{ab}	910.30^{cde}	2.12	8.760^{abcd}
NB_4D_2 × C_{108}	2.02^{a}	0.373^{a}	20.42^{a}	1247.70^{a}	2.46	7.485^{cd}
NB_4D_2 × Nistari	1.63^{bcd}	0.297^{b}	18.13^{ab}	978.10^{bcd}	1.98	7.473^{cd}
NB_4D_2 × Pure Mysore	1.61^{bcd}	0.290^{bc}	17.86^{abc}	817.10^{cdef}	1.87	10.427^{ab}
Nistari	1.14^{h}	0.137^{e}	12.13^{e}	524.65^{hg}	2.05	10.095^{abc}
Nistari × C_{108}	1.57^{cde}	0.277^{bc}	17.52^{abc}	848.47^{cdef}	2.03	8.697^{abcd}
Nistari × NB_4D_2	1.49^{def}	0.253^{c}	17.13^{abc}	784.50^{def}	1.85	9.733^{abcd}
Nistari × pure Mysore	1.30^{g}	0.205^{d}	15.00^{cd}	647.73^{fg}	2.37	8.905^{abcd}
Pure Mysore	1.04^{h}	0.153^{e}	14.91^{d}	408.60^{h}	1.92	10.563^{a}
Pure Mysore × C_{108}	1.67^{bc}	0.297^{b}	18.12^{ab}	792.53^{def}	2.17	9.517^{abcd}
Pure Mysore × NB_4D_2	1.44^{efg}	0.253^{c}	17.43^{abc}	712.17^{efg}	2.16	9.173^{abcd}
Pure Mysore × Nistari	1.39^{fg}	0.207^{d}	14.96^{d}	624.46^{fgh}	2.11	10.140^{abc}

Means having the same letters do not differ significantly

Table 2: Analysis of variance (mean square values) for six cocoon characters in parents and F_1 hybrids

SOV	df	Cocoon weight	Shell weight	Shell ratio	Filament length	Denier	Renditta
Genotype	15	0.22972**	0.013495**	9.721**	122147**	0.1213^{NS}	3.72*
Error	30	0.00409	0.000241	0.552	7370	0.0665	1.60

*: Significant; **: Highly significant; NS: Non-Significant

Table 3: Evaluation index and statistical significance for six cocoon characters

Races	Cocoon weight	Shell weight	Shell ratio	Filament length	Denier	Renditta	Mean	Rank
C_{108}	60.61^{a}	58.94^{a}	54.25^{ab}	60.70^{a}	48.30	60.35^{a}	58.12^{a}	1
NB_4D_2	56.41^{a}	58.43^{a}	60.53^{a}	56.14^{b}	49.63	56.80^{ab}	56.85^{a}	1
Nistari	42.63^{b}	40.56^{b}	37.04^{c}	43.35^{c}	50.14	41.59^{b}	43.45^{b}	2
Pure Mysore	40.37^{b}	42.16^{b}	48.19^{b}	39.81^{d}	51.66	41.28^{b}	44.78^{b}	2

Means having the same letters do not differ significantly at 0.05 level of probability

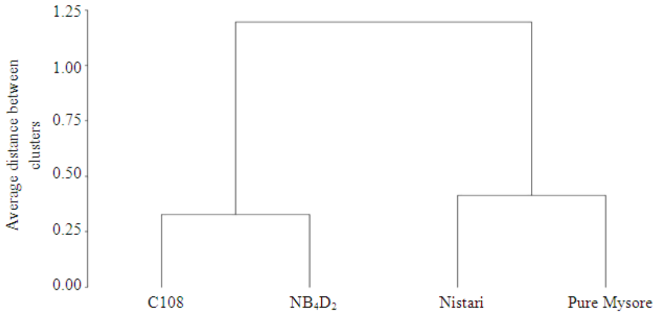

Fig. 1: Dendrogram representing genetic distances and three clusters among the four races

DISCUSSION

These results indicated that being inbreed population and differing voltinism, the higher genetic distance was between multivoltine and bivoltine in the above mentioned races. Pure Mysore and Nistari have been placed together in same cluster as they have higher similarity values among themselves and NB_4D_2 and C_{108} have been placed together in another cluster. Sreekumar *et al.*[23] using DNA profiling revealed that Nistari and Pure Mysore fall in the same cluster. Thus, the UPGMA clustering based on evaluation index showed clear separation of races with specific voltinism (Fig. 1).

Cocoon weight: Positive and significant heterosis over mid parent was observed from 8 crosses and positive and significant heterosis over better parent was observed from 2 crosses out of 12 crosses (Table 5). The present finding are in conformity with the report of Kumaresan *et al.*[11] who demonstrated positive heterosis in F_1 in respect to cocoon weight. Similar reports have been made by [8,10,24].

Shell weight: The eleven crosses exhibited positive and significant estimates for heterotic effects and positive and significant heterosis over better parent was observed from 4 crosses. Doddaswamy *et al.*[3] and Kumaresan *et al.*[11] also reported high heterosis for the shell weight trait. The superiority of F_1 hybrids and their parents with regard to economically important traits such as hatchability, larval duration, cocoon weight and shell weight have also been studied by Katsumata[9].

Shell ratio: Positive and significant heterosis over mid parent was observed from 11 crosses and positive and significant heterosis over better parent was observed from 4 crosses. Similar results were noticed by Murkami[18] in a cross between Combodge female with bivoltine male. Similar report has been made by Farooq *et al.*[4] using B_{103}×KA and B_{104}×KA crosses.

Table 4: Estimation of distance between four pure races

Races	C_{108}	NB_4D_2	Nistari	Pure Mysore
C_{108}	0.00	0.33	1.32	1.94
NB_4D_2		0.00	1.24	1.45
Nistari			0.00	0.41
Pure Mysore				0.00

Table 5: Estimation of heterosis and overdominance for different traits in the silkworm

	Cocoon weight		Shell weight		Shell ratio		Filament length		Renditta		Denier	
Hybrids	Ht	OD	Ht	OD	Ht	OD	Ht	OD	Ht	OD	Ht	OD
Pure Mysore × C_{108}	0.12**	-0.145**	0.270**	-0.063	0.16**	0.104**	0.10	-0.232**	0.06	0.099	-0.06	-0.104
Pure Mysore × NB_4D_2	0.03	-0.182**	0.070	-0.203**	0.06	-0.031	0.09	-0.205*	-0.01	0.132	-0.05	0.105
Pure Mysore × Nistari	0.27**	0.217**	0.420**	0.360**	0.11*	0.003	0.35*	0.214	-0.04	0.040	-0.06	0.129
Nistari × C_{108}	0.02	-0.195**	0.210**	-0.137**	0.23**	0.068	0.10	-0.178*	-0.03	0.173	-0.04	-0.066
Nistari × NB_4D_2	0.03	-0.154**	0.130*	-0.191**	0.14**	-0.047	0.11	-0.125	0.05	0.224	-0.11	-0.127
Nistari × Pure Mysore	0.19**	0.138**	0.422**	0.370**	0.18**	0.073	0.37**	0.229	-0.15	-0.151	0.06	0.022
C_{108}× NB_4D_2	0.07**	0.021	0.180**	0.173**	0.10**	0.048	0.21**	0.130	-0.07	-0.108	0.01	-0.001
C_{108}× Pure Mysore	0.08*	-0.206**	0.280**	-0.057	0.20**	0.065**	0.04	-0.278**	-0.12	0.253*	0.16	0.229
C_{108}× Nistari	0.01	-0.204**	0.280**	-0.089*	0.30**	0.135**	0.08	-0.188**	-0.14	0.265**	0.03	-0.005
NB_4D_2 × C_{108}	0.09**	0.039	0.180**	0.168**	0.07*	0.024**	0.24**	0.161*	-0.01	-0.048	-0.09	-0.103
NB_4D_2 × Pure Mysore	0.15**	-0.083**	0.230**	-0.086*	0.09*	-0.007	0.84**	-0.338**	0.13	0.013	-0.23	-0.227
NB_4D_2 × Nistari	0.12**	-0.074*	0.310**	-0.056	0.20**	0.008	0.39**	0.091	-0.19*	-0.289**	-0.05	-0.069

Ht: Relative heterosis, OD: Overdominance. Tabulated 't' value at 5 and 1% for d.f.$_{30}$ is 2.042 and 2.750, respectively. *: Significant, **: Highly significant

Table 6: Estimation of heterosis and overdominance for evaluation index (different traits) in the silkworm

	Cocoon weight		Shell weight		Shell ratio		Filament length		Denier		Renditta	
Hybrids	Ht	OD	Ht	OD	Ht	OD	Ht	OD	Ht	OD	Ht	OD
Pure Mysore × C_{108}	0.13**	-0.152**	0.20**	-0.050	0.30**	0.189**	0.07	-0.180**	-0.06	-0.134	-0.04	-0.215*
Pure Mysore × NB_4D_2	0.03	-0.197**	0.05	-0.162**	0.11	-0.054	0.06	-0.154**	-0.05	-0.143	0.02	-0.139
Pure Mysore × Nistari	0.31**	0.246**	0.28**	0.232**	0.23**	0.004	0.21**	0.135	-0.07	-0.175	0.07	0.051
Nistari × C_{108}	0.02	-0.210**	0.15**	-0.111**	0.48**	0.124	0.07	-0.135**	-0.01	-0.056	0.02	-0.152
Nistari × NB_4D_2	0.03	-0.165**	0.10*	-0.152**	0.27**	-0.082	0.08	-0.094	-0.13	-0.150	-0.09	-0.213
Nistari × Pure Mysore	0.22**	0.154**	0.28**	0.235**	0.41**	0.148	0.22**	0.144	0.06	-0.068	0.24	0.219
C_{108} × NB_4D_2	0.08**	0.022	0.15**	0.139**	0.17**	0.081	0.16**	0.104*	0.05	0.035	0.07	0.028
C_{108} × Pure Mysore	0.09*	-0.188**	0.21**	-0.046	0.37**	0.257**	0.03	-0.211**	0.20	0.097	0.15	-0.058
C_{108} × Nistari	0.01	-0.219**	0.20**	-0.072*	0.64**	0.247**	0.06	-0.144**	0.08	0.033	0.15	-0.042
NB_4D_2 × C_{108}	0.10**	0.042	0.14**	0.134**	0.12*	0.222**	0.18**	0.125**	-0.13	-0.141	0.01	-0.046
NB_4D_2 × Pure Mysore	0.17**	-0.089**	0.17**	-0.068*	0.15**	-0.039	0.17**	-0.068**	-0.30**	-0.365**	-0.16	-0.290*
NB_4D_2 × Nistari	0.14**	-0.079*	0.23**	-0.045	0.40**	0.012	0.27**	0.069**	-0.04	-0.068	0.23*	0.061

Ht: Relative heterosis, OD: Overdominance. Tabulated 't' value at 5 and 1% for d.f.$_{30}$ is 2.042 and 2.750, respectively. *: Significant, **: Highly significant

Table 7: Estimation of heterosis and overdominance for mean of evaluation index in the silkworm

Hybrids	Ht	OD	
C_{108} × NB_4D_2	0.10*	0.088	e_i≥50
NB_4D_2 × C_{108}	0.08	0.067	
C_{108} × pure Mysore	0.15**	-0.025	
NB_4D_2 × Nistari	0.20**	-0.023	
C_{108} × Nistari	0.17**	-0.052	
Pure Mysore × C_{108}	0.09	-0.080	
Nistari × C_{108}	0.11*	-0.105*	
Pure Mysore × NB_4D_2	0.02	-0.124*	e_i<50
Nistari × NB_4D_2	0.04	-0.156**	
NB_4D_2 × pure Mysore	0.01	-0.135**	
Nistari × pure Mysore	0.22**	0.155*	
Pure Mysore × Nistari	0.16*	0.095	

Ht: Relative heterosis, OD: Overdominance; Tabulated 't' value at 5 and 1% for d.f.$_{30}$ is 2.042 and 2.750, respectively; *: Significant, **: Highly significant

Table 8: Correlation between different mean value and evaluation index with distance

Mean value		Evaluation index	
Traits	Distance	Traits	Distance
Cocoon weight	-0.195	Cocoon weight	-0.197
Shell weight	-0.049	Shell weight	-0.051
Shell ratio	0.384	Shell ratio	0.387
Filament length	-0.326	Filament length	-0.325
Denier	0.197	Denier	0.158
Renditta	-0.178	Renditta	0.196

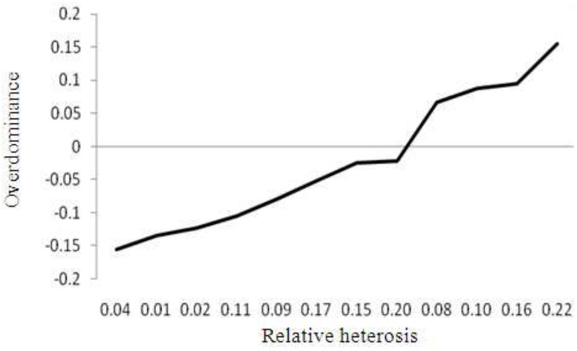

Fig. 2: Relative heterosis and overdominance graph for evaluation index

Filament length: Positive and significant heterosis over mid parent was observed from 6 crosses and positive and significant overdominance was observed from 1 cross out of 12 crosses. Also, Nagaraju *et al.*[19] observed heterosis for filament length in some crosses in their research.

Renditta: No positive and significant heterosis was observed but positive and significant overdominance was observed from 2 crosses out of 12 crosses. These results are in conformity with the report of Malik[13] who has observed neither significant heterosis nor overdominance about the denier trait.

Denier: No positive heterosis and overdominance heterosis were observed.

The estimate of heterosis of F_1's over mid and better parent(s) for mean value of the six traits and evaluation index value are presented in Table 5 and 6, respectively. Estimation of heterosis and overdominance for evaluation index was similar with estimation of heterosis and overdominance based on mean value. C_{108} × NB_4D_2, C_{108} × Pure Mysore, C_{108} × Nistari, NB_4D_2 × Nistari and Nistari × C_{108} showed positive and significant evaluation index average and over than 50. Nistari × Pure Mysore and Pure Mysore × Nistari exhibited positive and significant evaluation index average but under 50 (Table 7). Figure 2 revealed positive correlation between relative heterosis and overdominance.

CONCLUSION

It is concluded from present studies that cross C_{108} × Pure Mysore, NB_4D_2 × Nistari, C_{108} × Nistari, C_{108} × NB_4D_2 and Nistari ×C_{108} could be further evaluated for selecting high cocoon characters genotypes due to its highest heterotic value for important evaluation index.

As well as, evaluation index is a valuable method for determining genetic variability among silkworm races.

Based on the correlation studies, it is important to note that statistical insignificant results were observed between cocoon characters based on mean value and genetic distance and between cocoon characters based on evaluation index and genetic distance (Table 8). Thus authors reiterated that significant difference between the above studies may be used for prediction of heterosis in the silkworm breeding programme similar to the reports of plant breeding programmes[12,16,17]. The optimum level of genetic distance is necessary to obtain heterosis. Arunachalam *et al.*[1] reported that there is an optimum level of genetic divergence between parents to obtain heterosis in F_1 generation and they also reported that it may not be logical to advocate the use of extremely divergent parents to obtain heterotic combinations. As well as, heterosis estimation for mean evaluation index is more important than heterosis estimation for each trait individually and crossing between bivoltine female × multivoltine male is useful than reciprocal crossing.

REFERENCES

1. Arunachalam, V., A. Bandyopadhyay, S.N. Nigam and R.W. Gibbons, 1984. Heterosis in relation to genetic divergence and specific combining ability in ground nut (*Arachis hypogaea* L.) Euphytica, 33: 33-39. DOI: 10.1007/BF00022747
2. Das, S.K., S. Patnaik, B. Ghosh, T. Singh and B.P. Nair, 1994. Heterosis analysis in some three way crosses of *Bombyx mori* L. Sericologia, 34: 51-61. http://cat.inist.fr/?aModele=afficheN&cpsidt=4126510
3. Doddaswamy, M.S, G. Subramanya and E. Talebi, 2009. Studies on some economic traits and biological characters of regular and reciprocal cross between a multivoltine and bivoltine race of the silkworm *Bombyx mori*. J. Entomol. Nematol., 1: 050-055. http://academicjournals.org/JEN/PDF/Pdf2009/September/Doddaswamy%20et%20al.pdf
4. Farooq, M., H.P. Puttaraju, H.U. Dar and A.M. Sofi, 2002. Heterosis in relation to genetic divergence in bivoltine silkworm. *Bombyx mori* L. Indian J. Sericult., 41: 10-18. http://d.wanfangdata.com.cn/NSTLQK_NSTL_QK6335166.aspx
5. Farooq, M., M.A. Malik, G.N. Malik and F.A. Kukiloo, 2006. Combining ability analysis of some bivoltine silkworm (Bombyx mori L.) genotypes. Applied Biological Research, Vol. 8, Issue 1&2. http://www.indianjournals.com/ijor.aspx?target=ijor:abr&volume=8&issue=1and2&article=002
6. Fonseca, S. and F.L. Patterson, 1968. Hybrid vigour in seven parental diallel crosses in common wheat (*Triticum aestivum* L.). Crop Sci., 8: 85-8. http://crop.scijournals.org/cgi/content/abstract/8/1/85
7. Frankham, R., J.D. Ballou and D.A. Briscoe, 2002. Introduction to Conservation Genetics. 1st Edn., Cambridge University Press, Cambridge, pp: 640.
8. Iftekher Islam, M., I. Ara Ali and T. Haque, 2005. Combining ability estimation in popular bivoltine mulberry silkworm, *Bombyx mori* L. Pakistan Journal of Biological Sciences, 8(1): 68-72. http://scialert.net/pdfs/pjbs/2005/68-72.pdf
9. Katsumata, K., 1948. On the study of hybrid vigor in the silkworm. Fuji-San-l.
10. Kamrul Ahsan, Md. And S.M. Rahman, 2008. Genetic variability and correlation analysis in hybrids of mulberry silkworm, *Bombyx mori* L. for egg characters. Univ. J. Zool. Rajshahi Univ., 27: 13-16. http://www.banglajol.info/index.php/UJZRU/article/viewPDFInterstitial/1946/1831
11. Kumaresan, P., R.K. Sinha and K. Thangavelu, 2003. Heterosis studies in some elite multivoltine silkworm (*Bombyx mori* L.) races with popular bivoltine NB_4D_2. Int. J. Ind. Entomol., 7: 221-229. http://www.reportworld.co.kr/data/paper/view/2393/P2392122.html
12. Joshi, S.P., S.G. Bhave, K.V. Chowdari, G.S. Apte and B.L. Dhonukshe, 2001. Use of DNA markers in prediction of hybrid performance and heterosis for a three-line hybrid system in rice. Biochem. Genet., 39: 179-200. DOI: 10.1023/A:1010293325482
13. Malik, M.A., 1992. Studies on the performance and adaptation of bivoltine races of silkworm, *Bombyx mori* L. of Kashmir and evaluation of heterosis in their hybrids under temperate and subtropical climates. Ph.D. Thesis, University of Mysore, Mysore. http://dspace.vidyanidhi.org.in:8080/dspace/handle/2009/2779
14. Mahalanobis, P.C., 1936. On the generalized distance in statistics. Proc. Natl. Inst. Sci. Ind., 2: 49-55. http://www.new.dli.ernet.in/rawdataupload/upload/insa/INSA_1/20006193_49.pdf
15. Mano, Y., S. Nirmal Kumar, H.K. Basavaraja, N. Malreddy and R.K. Datta, 1993. A new method to select promising silkworm breeds/combinations. Indian Silk, 31: 53.
16. Melchinger, A.E., M. Lee, K.R. Lamkey and W.L. Woodman, 1990. Genetic diversity for restriction fragment length polymorphisms: relation to estimated genetic effects in maize inbreds. Crop Sci., 30: 1033-1040. http://crop.scijournals.org/cgi/content/abstract/30/5/1033
17. Moll, R.H., W.S. Salhuana and H.F. Robinson, 1962. Heterosis and genetic diversity in variety crosses of maize. Crop Sci., 2: 197-288.
18. Murakami, A., 1994. Growth phenomenon in *Bombyx mori* L. with special to genetic factors responsible for growth acceleration and moultinism. Indian J. Sericult., 33: 12-15. http://olericulture.org/research/087/482/
19. Nagaraju, J. and T.P. Kumar, 1995. Effects of selection on cocoon filament length in divergently selected lines of the silkworm, *Bombyx mori*. J. Seric. Sci. Jap. 64: 103-109. http://rms1.agsearch.agropedia.affrc.go.jp/contents/JASI/pdf/society/52-0826.pdf

20. Narasimhanna, M.N. and S. Krishnaswamy, 1972. Improved techniques of silkworm rearing. Proceeding of the Seminar on Sericulture, (SS’72) CSRTI., Mysore, pp: 1-11.
21. SAS, SAS/ETS, 2004. User’s Guide (Version 9.1), SAS Institute Inc., Cary, NC., USA., ISBN: 1590472446, pp: 2170.
22. Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38: 1409-1438. http://pacscl.exlibrisgroup.com:48996/F/?func=direct&doc_number=000007157
23. Sreekumar, S., S.K. Ashwath, S. Chitra, H.K. Basavaraja and S.B. Dandin., 2008. DNA profiling of a few indigenous and evolved silkworm breeds of India using microsatellite markers. Ind. J. Sericult., 47: 204-213. http://www.inserco.org/uk/pub_bibli_5.php
24. Talebi, E. and G. Subramanya, 2009. Diallel analysis of bivoltine and multivoltine races for six quantitative traits. J. Applied Sci., 2: 331-339. http://ozelacademy.com/OJAS_v2n3_8.pdf
25. Templeton, A.R. and B. Read, 1994. Inbreeding: One Word, Several Meanings, Much Confusion. In: Conservation Genetics, Loeschcke V., J. Tomiuk and S.K. Jain (Eds.). Birkhä user Verlag, Basel, Switzerland, ISBN: 3-7643-2937-9-4, pp: 91-105.
26. Wynne, J.C., D.A. Emery and P.H. Rice, 1970. Combining ability estimation in Arachis hypogaea L. II. Field Performance of F_1 hybrids. Crop Sci., 10: 713-715. http://crop.scijournals.org/cgi/content/abstract/10/6/713
27. Zhang, Q.F., Y.J. Gao, S.H. Yang, R. Ragab and M.A. Saghai, 1994. A diallel analysis of heterosis in elite hybrid rice based on RFLPs and microsatellites. Theor. Applied Genet., 89: 185-192. http://www.springerlink.com/content/p85k3302405n483j/