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

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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 C108 and NB4D2) 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 C108 × 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 F1 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. 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.[9] 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 F1 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
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\(_4\)D\(_2\)) 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\(_4\)D\(_2\) 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 (\(e\) and \(e'\)) was computed for all the traits except larval duration and renditta by the following formula suggested by Mano *et al.*\(^{15}\):

\[
e_i = [(A-B)\times10/C]+50
\]

where as for the larval duration and renditta traits the modified formula of Mano *et al.*\(^{15}\) was adapted as:

\[
e'_i = [(B-A)\times10/C]+50
\]

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\(^{6}\):

\[
\text{MPV} = \left(\frac{(F_{ij}-\text{MPV})}{\text{MPV}}\right)\times100
\]

\[
\text{BPV} = \left(\frac{(F_{ij}-\text{BPV})}{\text{BPV}}\right)\times100
\]

Where:

- \(\text{MPV}\) = Mid Parent Value
- \(\text{BPV}\) = 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\(^{26}\):

\[
t_{ij} = \frac{(F_{ij}-\text{MPV})}{\sqrt{\left(3\times\text{EMS}/8\right)}}
\]

The ‘t’ value for overdominance was calculated following the formula:

\[
t_{ij} = \frac{(F_{ij}-\text{MPV})}{\sqrt{\left(\text{EMS}/2\right)}}
\]

Where:

- \(F_{ij}\) = The Mean of the ij\(^{th}\) F\(_1\) cross
- \(\text{MPV}_i\) = The mid parent for the ij\(^{th}\) cross
- \(\text{BPV}_j\) = The better parent values for ij\(^{th}\) cross
- \(\text{EMS}\) = 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 F1 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.

Table 1: Mean value of parents and F1 progenies for six quantitative traits

| Races/hybrids     | Cocoon weight (g) | Shell weight (g) | Shell ratio (%) | Filament length (m) | Denier | Renditta (kg) |
|-------------------|-------------------|------------------|-----------------|---------------------|--------|--------------|
| C108              | 1.95c             | 0.317b           | 18.42bc         | 1054.63bc           | 2.18   | 7.595bc      |
| C108 × NB,D1      | 2.10d             | 0.377a           | 20.85a          | 1166.40b            | 2.18   | 7.093a       |
| C108 × Nistari    | 1.55d,e           | 0.290bc          | 18.63c          | 838.23def           | 2.17   | 7.727bcd     |
| C108 × pure Mysore| 1.61d,b           | 0.300b           | 18.73b          | 730.15fk            | 2.68   | 7.900hcd     |
| NB,D1             | 1.76b             | 0.315b           | 19.98b          | 910.30de            | 2.12   | 8.760bcd     |
| NB,D1 × C108      | 2.02a             | 0.373a           | 20.42a          | 1247.70b            | 2.46   | 7.485b       |
| NB,D1 × Nistari   | 1.63d,b           | 0.297b           | 18.13b          | 978.10cank          | 1.98   | 7.473c       |
| NB,D1 × Pure Mysore| 1.61d,b           | 0.290bc          | 17.86bc         | 817.10def           | 1.87   | 10.427b      |
| Nistari           | 1.14d             | 0.137c           | 12.13c          | 524.65g             | 2.05   | 10.095bc     |
| Nistari × C108    | 1.57d,e           | 0.277bc          | 17.52abc        | 848.47def           | 2.03   | 8.697bcd     |
| Nistari × NB,D1   | 1.49d,e           | 0.253c           | 17.13abc        | 784.50k             | 1.85   | 9.733bcd     |
| Nistari × pure Mysore| 1.30f             | 0.205d           | 15.00d          | 647.73f             | 2.37   | 8.905b       |
| Pure Mysore       | 1.04d             | 0.153c           | 14.91f          | 408.60d             | 1.92   | 10.563a      |
| Pure Mysore × C108| 1.67c             | 0.297b           | 18.12b          | 792.53k             | 2.17   | 9.517bcd     |
| Pure Mysore × NB,D1| 1.44f             | 0.253c           | 17.43bc         | 712.17fg            | 2.16   | 9.173bcd     |
| Pure Mysore × Nistari| 1.39f             | 0.207d           | 14.96d          | 624.46gh            | 2.11   | 10.140bc     |

Means having the same letters do not differ significantly.

Table 2: Analysis of variance (mean square values) for six cocoon characters in parents and F1 hybrids

| SOV        | df    | Cocoon weight | Shell weight | Shell ratio | Filament length | Denier | Renditta |
|------------|-------|---------------|--------------|-------------|-----------------|--------|----------|
| Genotype   | 15    | 0.22972**     | 0.013495**   | 9.721**     | 1221.477**      | 0.1213S | 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/hybrids     | Cocoon weight | Shell weight | Shell ratio | Filament length | Denier | Renditta | Mean Rank |
|-------------------|---------------|--------------|-------------|-----------------|--------|----------|-----------|
| C108              | 60.61a        | 58.94e       | 60.70c      | 48.50d          | 60.35d | 58.12a   | 1         |
| NB,D1             | 56.41b        | 58.43a       | 60.53c      | 56.14d          | 49.63  | 56.80ab  | 56.85c    | 1         |
| Nistari           | 42.63b        | 40.56d       | 37.04e      | 43.35f          | 50.14  | 41.59b   | 43.45b    | 2         |
| Pure Mysore       | 40.37b        | 42.16b       | 48.19b      | 39.81f          | 51.66  | 41.28b   | 44.78b    | 2         |

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

The dendrogram produced on Unweighted Pair Group Method with Arithmetic means (UPGMA) and cluster analysis indicated that the two bivoltines C108 and NB,D2 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 C108 and NB,D2 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).

Fig. 1: Dendrogram representing genetic distances and three clusters among the four races 1983
**DISCUSSION**

These results indicated that being inbred population and differing voltmism, 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×D2 and C108 have been placed together in another cluster. Sreekumar et al.[12] 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 voltmism (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 F1 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 F1 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[19].

**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.[8] using B103×KA and B103×KA crosses.

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

| Hybrids                        | Ht   | OD     | Ht   | OD     | Ht   | OD     | Ht   | OD     | Ht   | OD     | Ht   | OD     |
|--------------------------------|------|--------|------|--------|------|--------|------|--------|------|--------|------|--------|
| Pure Mysore × C108             | 0.30**| -0.185**| 0.28**| -0.068*| 0.11**| -0.047| 0.10**| -0.031| 0.08**| -0.026**| 0.07**| -0.017**|
| Pure Mysore × NB×D2            | 0.05**| -0.162**| 0.11**| -0.054| 0.06**| -0.154**| 0.05**| -0.053| 0.04**| -0.057**| 0.04**| -0.057**|
| Pure Mysore × Nistari          | 0.06**| -0.178**| 0.09**| -0.062| 0.07**| -0.057**| 0.06**| -0.055| 0.04**| -0.055**| 0.04**| -0.055**|
| Nistari × C108                 | 0.22**| -0.154**| 0.13**| -0.083| 0.11**| -0.059| 0.10**| -0.049| 0.08**| -0.048**| 0.08**| -0.048**|
| Nistari × NB×D2                | 0.09**| -0.093**| 0.07**| -0.048| 0.06**| -0.094**| 0.06**| -0.048| 0.05**| -0.048**| 0.05**| -0.048**|
| Nistari × Pure Mysore          | 0.07**| -0.162**| 0.10**| -0.049| 0.06**| -0.094**| 0.06**| -0.048| 0.05**| -0.048**| 0.05**| -0.048**|
| C108×Pure Mysore               | 0.12**| -0.154**| 0.07**| -0.045| 0.06**| -0.094**| 0.06**| -0.048| 0.05**| -0.048**| 0.05**| -0.048**|
| C108×Nistari                   | 0.06**| -0.178**| 0.09**| -0.062| 0.07**| -0.057**| 0.06**| -0.055| 0.04**| -0.055**| 0.04**| -0.055**|
| NB×D2×C108                    | 0.14**| -0.154**| 0.11**| -0.093| 0.09**| -0.048**| 0.08**| -0.048| 0.07**| -0.048**| 0.07**| -0.048**|

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

| Hybrids                        | Cocoon weight | Shell weight | Shell ratio | Filament length | Denier | Rendita |
|--------------------------------|---------------|--------------|-------------|----------------|--------|---------|
| Pure Mysore × C108             | 0.40**        | 0.33**       | 0.28**      | 0.10**         | 0.15** | 0.05**  |
| Pure Mysore × NB×D2            | 0.05**        | 0.18**       | 0.12**      | 0.07**         | 0.16** | 0.02**  |
| Pure Mysore × Nistari          | 0.07**        | 0.16**       | 0.10**      | 0.09**         | 0.13** | 0.01**  |
| Nistari × C108                 | 0.10**        | 0.09**       | 0.08**      | 0.06**         | 0.12** | 0.00**  |
| Nistari × NB×D2                | 0.06**        | 0.05**       | 0.04**      | 0.03**         | 0.09** | 0.00**  |
| Nistari × Pure Mysore          | 0.08**        | 0.06**       | 0.05**      | 0.04**         | 0.09** | 0.00**  |
| C108×Pure Mysore               | 0.09**        | 0.08**       | 0.07**      | 0.06**         | 0.12** | 0.01**  |
| NB×D2×C108                    | 0.11**        | 0.10**       | 0.09**      | 0.07**         | 0.13** | 0.01**  |
| NB×D2 × Nistari               | 0.10**        | 0.11**       | 0.08**      | 0.06**         | 0.12** | 0.01**  |

**Table 4:** Estimation of distance between four pure races

| Races  | C108 | NB×D2 | Nistari | Pure Mysore |
|--------|------|-------|--------|-------------|
| C108   | 0.00 | 0.33  | 1.32   | 1.94         |
| NB×D2  | 0.00 | 1.24  | 1.45   |
| Nistari| 0.00 | 0.41  |
| Pure Mysore | 0.00  |
Table 7: Estimation of heterosis and overdominance for mean of evaluation index in the silkworm

| Hybrids                      | Ht    | OD   |
|------------------------------|-------|------|
| $C_{108} \times NB_{D2}$     | 0.10* | 0.088|
| $NB_{D2} \times C_{108}$     | 0.08  | 0.067|
| $C_{108} \times Pure Mysore$ | 0.15**| -0.025|
| $NB_{D2} \times Nistari$     | 0.20**| -0.023|
| $C_{108} \times Nistari$     | 0.17**| -0.052|
| Pure Mysore $\times C_{108}$ | 0.09  | -0.080|
| Nistari $\times C_{108}$     | 0.11* | -0.105*|
| Pure Mysore $\times NB_{D2}$ | 0.02  | -0.124** e$<50$
| Nistari $\times NB_{D2}$     | 0.04  | -0.156** e$<50$
| $NB_{D2} \times pure Mysore$ | 0.01  | -0.135** e$<50$
| Nistari $\times pure Mysore$ | 0.22**| 0.155*|
| Pure Mysore $\times Nistari$ | 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 | 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.\[10\] 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. $C_{108} \times NB_{D2}$, $C_{108} \times Pure Mysore$, $C_{108} \times Nistari$, $NB_{D2} \times Nistari$ and $Nistari \times C_{108}$ showed positive and significant evaluation index average and over than 50. $Nistari \times Pure Mysore$ and $Pure Mysore \times Nistari$ 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 $C_{108} \times Pure Mysore$, $NB_{D2} \times Nistari$, $C_{108} \times Nistari$, $C_{108} \times NB_{D2}$ and $Nistari \times C_{108}$ 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.
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