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Analysis of Expression of Heterosis on Fecundity, Hatchability of Eggs in New Breeding Lines of Bombyx *mori* L

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Abstract: The present study was designed to evolve bivoltine races for our tropical climatic conditions by utilizing the silkworm races of known genetic background (KA, NB_{18} and PM) in different hybrid combinations and to inbreed them over generations followed by backcrossing and adopting appropriate selection at different generations with an objective of the viability and productivity. The isolated bivoltine lines (R_1 and R_2) were reared in different seasons of the year along with their parental races to evaluate their stability in the expression of commercial characters such as fecundity and hatchability. The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx *mori* L. were selected for the present breeding programme. Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters. Heterosis in F_1 generations of crosses including NB_{18} and PM were determined over Mid-parental value (MPV) and Better parent value (BPV). Significant test for heterosis was performed using standard analysis of variance (ANOVA) table. Based on the results of our study it was concluded that the metric variables like fecundity and hatchability are found to be complexly related being influenced by the physiological conditions of an organism also.

***Keywords* - Bombyx *mori* L, Bivoltine, Kalimpong-A (KA), Pure Mysore (PM), Fecundity, Hatchability**

I. INTRODUCTION

Among insects, Drosophila and domesticated mulberry silk worm Bombyx *mori* have been used extensively for various experimental purposes. The silk worm Bombyx *mori* for a long time has been under the patronage of man due to its economic value, and this has been an object of research since medieval times.[1] The sericulture probably was practiced in China since 2255 BC and gradually spread to South Korea, Japan and by the North Eastern route to India in 140 BC via the so called 'Silk Road', a road ultimately leading to world culture.[2] Since the birth of silkworm genetics, extensive investigations have been carried out with reference to improve the economic characters. These results together with silkworm breeding experiments contributed much to the establishment of contemporary silkworm breeds of high economic values.

In India, Sericulture is an agro based cottage industry providing gainful employment mainly to the weaker sections of the society. Because of its employment potential and profitability, low investment cost and high foreign exchange earnings, sericulture could become an important factor in the economic development of our country. Therefore, studies on genetics, breeding and biochemical aspects of silkworm will have a direct impact on the upliftment of sericulture industry.

There are many species of silkworm which produce cocoons of superior filament quality and containing larger quantities of silk. Among them Bombyx *mori* L. of the family Bombycidae is the only species widely used for commercial rearing. India holds unique position in the world by producing all the four commercially known varieties of silk namely, mulberry, eri, Tasar and muga. India ranks second in raw silk (12,665 tonnes) production in the world. The mulberry silk production in India accounts for 90.7% (11,487 tonnes) while non-mulberry silks (eri 624 tonnes 4.9%, Tasar 484 tonnes) 3.8% and muga (70 tonnes 0.5%) account for 9.3% of the total raw silk production. The major share of the Indian mulberry silk production is from Karnataka accounting for 54.095% (6214 tonnes).

Systematic breeding experiments of silkworm evolved during the past few years have enabled the breeders to synthesise desirable genotypes of known genetic constitution with a main objective of increasing the productivity and viability. Application of conventional hybridization methods with appropriate selection have contributed a great deal in increasing quality and quantity of silk. Sericulturally advanced countries such as China and Japan have achieved a remarkable breakthrough in increasing the unit production of high grade silk by evolving silkworm races suitable to their agroclimatic conditions.[3] In India several attempts have been made during the last two decades to produce suitable, high productive bivoltine races and also multivoltine races to our tropical climatic conditions but with little success.[4-8] Further, in the absence of suitable bivoltine races, the locally available multivoltine and bivoltine races are being used for the production of commercial hybrids. Therefore, the unit production and the quality of silk produced by the commercial hybrids remains poor. Hence, it is essential to produce better bivoltine races for commercial exploitation under tropical climatic conditions. Moreover, voltinism, moultinism, viability, productivity, resistance or susceptible to disease and tolerance to unfavourable environmental conditions assume a special importance in the efforts to produce suitable silkworm races.

With this viewpoint, the present study was undertaken to evolve bivoltine races for our tropical climatic conditions by utilizing the silkworm races of known genetic background (KA, NB_{18} and PM) in different hybrid combinations and to inbreed them over generations followed by backcrossing and adopting appropriate selection at different generations with an objective of the viability and productivity. The isolated bivoltine lines (R_1 and R_2) were reared in different seasons of the year along with their parental races to evaluate their stability in the expression of commercial characters.

II. MATERIALS AND METHODS

Silkworm varieties and rearing

The pure races of bivoltine Kalimpong-A (KA) spinning oval white cocoons, New Bivoltine-18 (NB_{18}) spinning dumbbell white cocoons and multivoltine Pure Mysore (PM) spinning pointed yellow cocoons of mulberry silkworm Bombyx mori L. were selected for the present breeding programme. These races were obtained from their respective seed areas and are reared in cytogenetics laboratory, Jnana Bharathi campus, Bangalore University.

The disease free layings were prepared as described by Narasimhanna, (1988),[9] and were incubated at 25°C and relative humidity of 75%. On 8th day composite layings were prepared (10-20 layings were prepared 100-200 eggs were collected from each laying). The hatched worms were reared according to the method described by Krishnaswamy (1978).[10] Ms variety of mulberry leaves were used in rearing. The worms were reared in mass up to III instar, after III moult 300 worms were collected in three replicates in order to evaluate the rearing performance. Standard temperature and humidity were maintained in the rearing house. The quantitative traits such as fecundity (total number of eggs laid by single female moth) and hatching percentage (total number of eggs hatched in a laying) were evaluated to assess the performance breeding lines of Bombyx *mori* L.

Breeding

Single and three way crosses were made by using the above said three races. The first single cross involved KA females and PM males. The second single cross involved NB_{18} females and PM males. During the course of breeding selection was made at the egg, larva, pupa and cocoon stages to fix the desirable traits. F_5 progenies of the respective crosses were back crossed to their respective bivoltine males to improve commercial characters.

Evolutions of new lines R_1 and R_2

Females of KA and NB_{18} were crossed with males of PM. The composite layings of F_1 hybrid were brushed and reared under standard laboratory conditions. The selection parameters explained earlier were applied to choose the seed cocoons for the preparation of F_2 layings. The replicates showing higher pupation rate were selected for intra family selection of cocoons. Further, segregation with respect to cocoon colour and built was noticed. Only white oval in case of KAxPM and dumbbell white in case of NB_{18}xPM qualifying the parameter of selection were choosen for breeding in subsequent generations. The females of F_5 were backcrossed to the males of KA and NB_{18} respectively in both the lines and reared up to 11 generations. At the end of the 11th generation the lines R_1 and R_2 were extracted with higher effective rate of rearing (ERR) than their respective better parents, with shorter larval period and with moderate cocoon productivity character in case of R_1 and R_2.

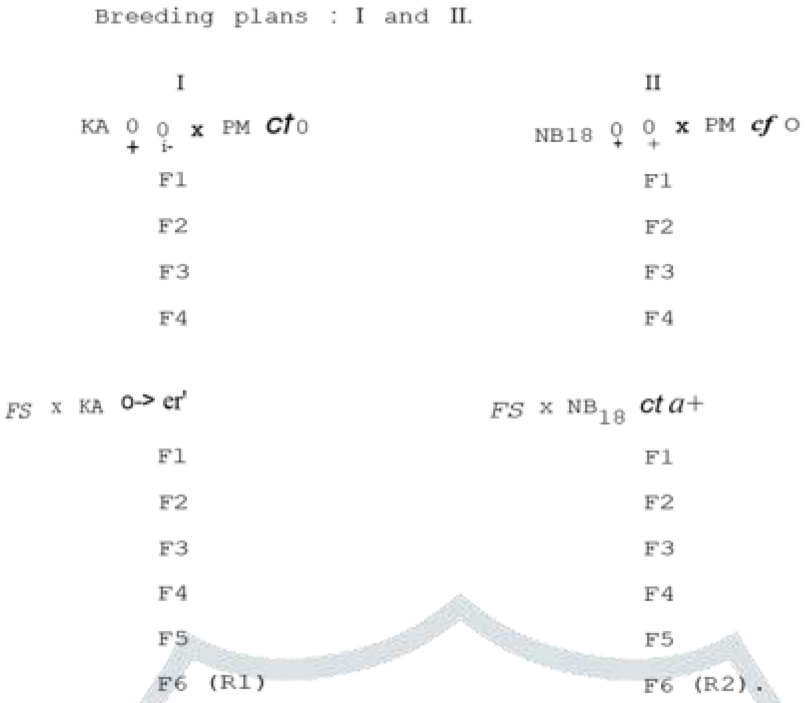

Figure 1: Breeding Plan

Statistical Analysis

Heterosis in F_1 generations of crosses including NB_{18} and PM were determined over Mid-parental value (MPV) and Better parent value (BPV). Heterosis was determined as follows;

% Heterosis over MP = (F1 – MPV/MPV) X 100
% Heterosis over BP = (F1 – BPV/BPV) X 100
Where;
MP: Mid Parent
BP: Better Parent

Significant test for heterosis was performed using standard ANOVA table. To test the generation effect on the rearing performance standard regressions of different parameters on the generation number were worked out. To compare the generation performance of crosses, the data were transformed into standard normal varieties. This is due to the fact that per second comparison of the absolute value does not show the inherent trend in the data.

III. RESULTS

Fecundity

The F_1 hybrids of KAxPM revealed a mean fecundity of 576 (17.282) eggs which gradually decreased in F_2 to F_5 generations. F_5 females with a fecundity of 487 (20.402) eggs were backcrossed with the males of KA revealed a mean fecundity of 508 (±14.337) eggs in F_1 generation of the three way cross. It was increased in F_2 and gradually decreased F4 and F_5 generations, increased in F6 fixation of the trait leading to the isolation of the line. R_1 spinning white oval cocoons (Table 1 and 2). Further it may be seen from Fig. 1 the line declined from F_1 to F_5 in two-way cross. In three way crosses also it declined from F_1 to F_5 generation. However, the line showed an upward trend from F_5 to F_6 generations.

Table 1: Fecundity and Hatchability characters of silkworm hybrid KAxPM in 5 generations

Generations	Fecundity	Hatchability
F1	576.000 ±17.282	89.000 ±2.160
F2	524.667 ±10.873	89.000 ±2.944
F3	502.667 ±5.249	86.333 ±2.867
F4	486.333 ±10.873	87.333 ±2.055
F5	487.333 ±20.402	85.333 ±1.700

Values are expressed as Mean ± SD

Table 2: Fecundity and Hatchability characters of silkworm hybrid F (KAxPM) x KA in 6 generations

Generations	Fecundity	Hatchability
F1	508.333 ± 14.334	89.000 ± 1.633
F2	558.333 ± 8.498	90.667 ± 2.055
F3	525.333 ± 18.264	85.000 ± 1. 633
F4	509.000 ± 14.900	89.000 ± 2.944
F5	492.667 ± 5.312	91. 333 ± 2.625
F6	533.000 ± 26.944	90.333 ± 0.471

Values are expressed as Mean ± SD

The F_1 hybrids of NB_{18}xPM revealed a mean fecundity of 574 eggs, which remained same in F_2 and gradually decreased from F_3-F_5. The F_5 females were backcrossed to NB males revealed mean fecundity of 536 eggs in F_1 generations. It also decreased in successive generations up to F_6 indicating the fixation of the line R_2, spinning white dumbbell cocoons. Further, this has been expressed in standard deviation units (Tables 3 and 4 Fig. 2). The curve showed a decline from F_1 to F_5 in two-way cross and similar trend was observed in three-way cross also. When the mean performance of KA (550±15.912) with F5 (KA x PM) x KA (5333±26.94) was compared, it is found that these did not vary for or the magnitude was marginally higher in KA (Tables 5 and 2).

Table 3: Fecundity and Hatchability characters of silkworm hybrid NB1axPM in 5 generations

Generations	Fecundity	Hatchability
1	574.333 ± 38.553	91.000 ± 2.000
2	575.667 ± 29.687	84.667 ± 4.726
3	551.667 ± 24.664	85.333 ± 2.082
4	497.333 ± 13.57	88.667 ± 3.215
5	500.667 ± 11.590	91.33 ± 1.528

Values are expressed as Mean ± SD

Table 4: Fecundity and Hatchability characters of silkworm hybrid (NBxPM) x NB in 6 generations

Generations	Fecundity	Hatchability
1	536.333 ± 50.322	88.000 ± 5.568
2	529.000 ± 18.520	90.667 ± 2.082
3	500.667 ± 12.014	90.000 ± 1.000
4	503.333 ± 5.859	91.000 ± 1.732
5	498.333 ± 12.858	90.000 ± 1.000
6	503.333 ± 8.505	90.000 ± 3.000

Values are expressed as Mean ± SD

Table 5: Fecundity and Hatchability characters in bivoltine silkworm race of KA over 12 generations

Generations	Fecundity	Hatchability
1	521.000 ± 17.568	90.700 ± 1.687
2	512.333 ± 27.157	90.733 ± 0.895
3	495.333 ± 6.018	85.667 ± 0.942
4	487.333 ± 24.689	85.333 ± 2.054
5	552.667 ± 15.627	90.167 ± 1.027
6	555.000 ± 18.239	90.000 ± 0.816
7	576.333 ± 23.342	89.000 ± 2.160
8	521.667 ± 14.974	89.333 ± 1.699
9	523.333 ± 34.778	90.067 ± 0.894
10	491.333 ± 11.556	84.667 ± 3.681
11	514.000 ± 10.424	85.000 ± 1.414
12	495.333 ± 12.256	87.333 ± 2.054

Values are expressed as Mean ± S.D

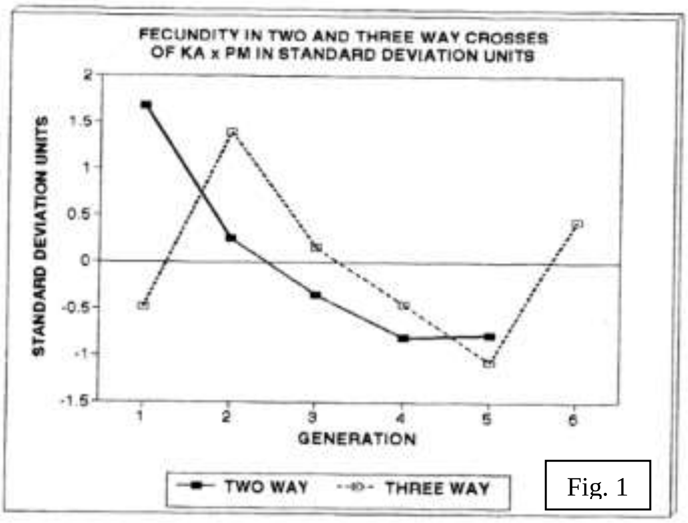

Fig. 1

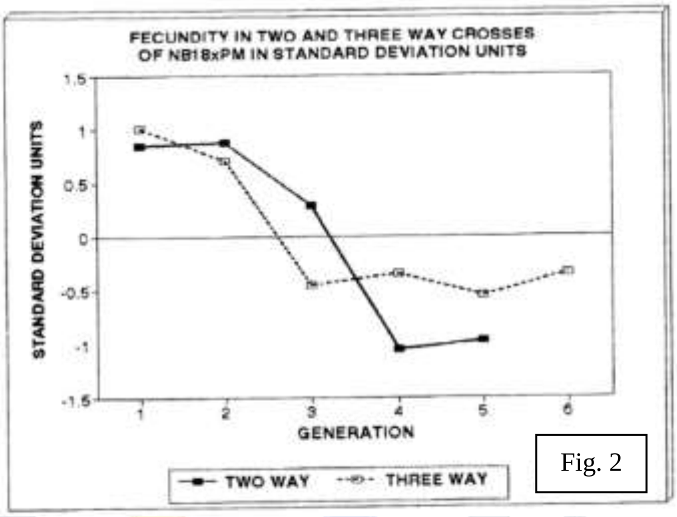

Fig. 2

When the mean performance of NB_{18} (495±12.25) with FS (NB_{18} x PM) X NB18 (503±8.5) was compared, it may be seen the fecundity did not vary but the magnitude was marginally higher 1n the hybrid (Tables 6 and 4). It may be seen from Table 7 that the degree of heterosis calculated over MP value (12.904) was significant, while it was non-significant over better percent (10.56). Heterosis over MP and BP was found to be significant and negative for 3-way hybrid F_5 (KAxPM) x KA (-2.24 and -8.02) (Table 8).

Table 6: Fecundity and Hatchability characters in bivoltine silkworm race of NB_{18} over 12 generations

Generations	Fecundity	Hatchability
1	588.667 ± 17.556	93.000 ± 1.485
2	570.000 ± 24.913	90.033 ± 0.817
3	529.333 ± 20.531	90.053 ± 0.819
4	504.333 ± 18.696	90.900 ± 1.564
5	547.667 ± 24.073	81.700 ± 0.989
6	541.333 ± 26.712	81.033 ± 1.391
7	501.000 ± 12.355	81.700 ± 1.202
8	530.000 ± 20.992	86.000 ± 2.943
9	523.333 ± 34.778	90.067 ± 0.894
10	491.333 ± 11.556	84.667 ± 3.681
11	514.000 ± 10.424	85.000 ± 1.414
12	495.333 ± 12.256	87.333 ± 2.054

Table 7: Heterotic effects in different economic characters of silkworm hybrids KAxPM

KAxPM	Fecundity	Hatchability
MP	12.904*	-2.394*
BP	10.557	-2.97*

*Significance at 5%

Table 8: Heterotic effects in different economic characters of silkworm hybrids F5 (KAXPM) X KA

F_5 (KAXPM) X KA	Fecundity	Hatchability
MP	2.24*	1.42
BP	-8.02**	-1.30

*Significance at 5%; ** Significance at 1%

The hybrids of the single cross NB18xPM did not show significance over MP and BP. Similar trend was observed in respect of three-way cross also (Table 9 and 10). Regression of fecundity on generation number was significant only for NB_{18}. This implies that environment has a significant role in the expression of this trait in NB_{18} whereas the role of environment is not significant in KA and PM.

Table 9: Heterotic effects in different economic characters of silkworm hybrids NB18 XPM

NB_{18} XPM	Fecundity	Hatchability
MP	4.235	-1.675
BP	-2.435	-2.151

Table 10: Heterotic effects in different economic characters of silkworm hybrids F5 (NB18XPM) X NB18

F_5 (NB_{18}XPM) X NB_{18}	Fecundity	Hatchability
MP	2.32	1.72
BP	-2.07	-3.650

Hatchability

The F_1 hybrids of KAxPM revealed a mean hatchability of 89% (=2.160) which remained same in F_2 which gradually decreased to 85% (=1.700) in F_5 generation. The FS females were back crossed to KA males with a hatchability percent of 90 (=1.027) revealed 89% (=1.633) and it increased in F6 to 90% (Table 1 and 2). Further it may be seen from the table 11 and Fig. 3 which is expressed in terms of standard deviation units (Table 9 and Fig. 3). The line declined from F_1 to F_5 in two-way cross and similar trend was noticed 1n three way cross also which revealed the fixation of the trait leading to the isolation of the line R_1.

The F_1 hybrids of NB_{18}xPM revealed a mean hatchability percent of 91 (=2.000) which decreased 1n successive generations F_5 females' hatchability and again increased in FS to 91% (=1.732). The F_5 females were backcrossed to NB_{18} males with of 81.7 (±4.133) revealed 88% hatchability in F_1 and was found to be 90% in succeeding generations with F6 (Table 3 and 4). Further this may be seen from the table 12 and Fig. 4, the curve showed an upward trend from F_3 generation onwards in two-way cross while in three way cross it was found to be moving towards fixation leading to the isolation of the line R2.

When the mean performance of KA (90.3=1.24) with Fs (KA x PM) x KA (90.3=0.47) was compared. Their performance 1s same. (Tables 5 and 2). When the mean performance of NB18 (87.3±2.0) with F5 (NB18 x PM) x NB18 (90.0±3.0) was compared, the magnitude was marginally higher in hybrid (Tables 6 and 7). The hybrids of KAxPM and Fs (KAxPM) x KA did not show significant heterosis over MP and BP. Similar trend was observed in respect of NB_{18}xPM and F5 (NB_{18}xPM) x NB_{18} (Tables 7, 8, 9, and10). Regression of hatching percent on generation number was not significant in any of races under study.

Table 11: Performance of important silkworm character in two and three-way hybrid of KAxPM

	Generations	Fecundity	Hatchability
KAx PM	1	1.6766	0.5714
	2	0.2564	0.5714
	3	-0.3523	-0.3810
	4	-0.8042	-0.0238
	5	-0.7765	-0.7381
F_5 (KAxPM) x KA	1	-0.4800	-0.0762
	2	1.3984	0.4953
	3	0.1586	-1.4477
	4	-0.4550	-0.0762
	5	-0.0686	0.7238
	6	0.4466	0.3810

Values are expressed in S.D units

Table 12: Performance of important silkworm character in two and three-way hybrid of NBxPM

	Generations	Fecundity	Hatchability
$NB_{18}xPM$	1	0.8520	0.7623
	2	0.8850	-0.9619
	3	0.2906	-0.7804
	4	-1. 0551	0.1270
	5	-0.9725	0.8530
F 5 ($NB_{18}xPM$) x NB1	1	1.0084	-0.7695
	2	0.7066	0.2858
	3	-0.4596	0.0220
	4	-0.3499	0.4177
	5	-0.5557	0.0220
	6	-0.3499	0.0220

Values are expressed in S.D units

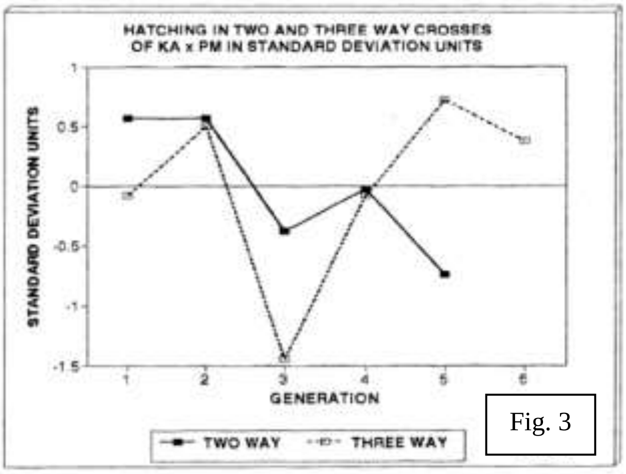

Fig. 3

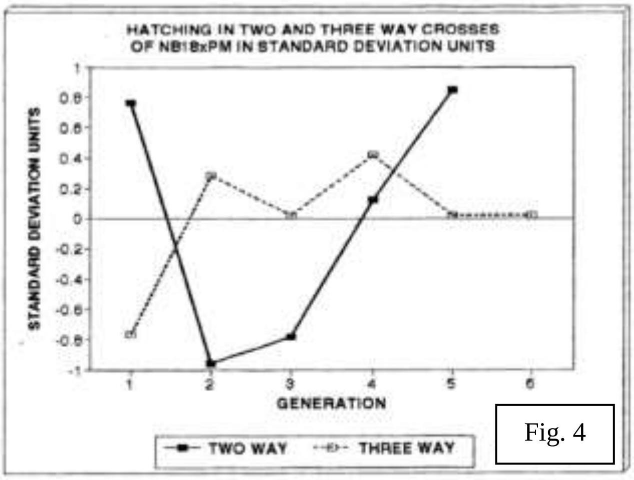

Fig. 4

IV. DISCUSSION

Hybridization as a procedure has been practiced since the beginning of civilization for improving any breed of animal or plant for better performance than the pure races. It provides choice for genetic manipulation for the selection of new and better gene combinations. Utilizing the known genotypes, choice of mating systems and judicious method of selection is of vital importance in the conventional breeding methods such as line breeding and cross breeding. Cross breeding is more dependable than line breeding for evolution of silkworm races. Some races are good in some characters and some are poor in some characters. Cross breeding involves two or more races in order to get desirable characters. Three pure lines with known genotype were used to obtain new promising lines. Multivoltine race Pure Mysore spinning yellow pointed cocoon shows resistance to diseases and is known for its less silk yield.[11] Tropical bivoltines Kalimpong-A (KA) spinning oval white cocoon and NB18 spinning dumbell white cocoon are known for their higher silk yield and are comparatively less resistant to diseases and adverse environmental conditions.[12]

In the present study the author used bivoltine females and multivoltine males. The crosses were made initially and backcrossed to F_5 generations. Analysis of the metric traits viz. fecundity and hatchability revealed the manifestation of varied degrees of heterosis over mid parent and better parent. Owing to the simultaneous segregation of a large number of genes in conjugation with the influence of environmental effects. For instance, comparatively lesser variation in fecundity and hatchability was observed in our study findings. Selection is made to choose intermediate sized cocoons from the batches of high ERR as these individuals are expected to possess maximum fitness value.[13] Accordingly inbreeding and selection over generations resulted in favorable combinations of desired alleles in the progenies leading to the expression of statistically non-significant differences for the metric traits under studies at later generation indicating their fixation in the isolated lines.

The results of the present investigation with regard to the manifestation of heterosis are in agreement with the findings of Osawa and Harada (1944).[14] Harada (1956, 1961) who have pointed that greater the midparent value, lesser will be the effect of heterosis. Overall performance of backcross hybrids are observed to be better than biparental hybrids (KAxPM or NB_{18}xPM) due to the fact that backcross hybrids have 75% of the genetic structure resembles that of either KA or NB_{18} as the case may be. While the biparental hybrid contains only 50% of NB_{18} or KA.[15,16] However, degree of heterosis is more prominent in Biparental cross than backcross hybrids.[17] Marginal or no difference in the performance observed in backcross hybrid may be attributed to the fact that these silkworm races have been under selection process more or less the same characters over a lay period in a bid to improve the strain.

V. CONCLUSION

In conclusion, findings of our study delineated that the metric variables like fecundity and hatchability are found to be complexly related being influenced by the physiological conditions of an organism also.

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