

**MATERIALS  
AND  
METHODS**

## MATERIALS AND METHODS

On the basis of genetic divergence of the existing genetic stocks of multivoltine (Subbarao *et al.*, 1991) & bivoltine (Sen, 1993) mulberry silkworms, *Bombyx mori* L. as well as and per'se performance, 5 multivoltine lines /breeds viz., Nistari, CB5, G, Raj & B and 5 bivoltine breeds viz., P5, NB18, JD6, SF19 & KS(O) (Photographs ; TABLE : MATER-1) were crossed in the following fashion :

- |                        |   |                            |
|------------------------|---|----------------------------|
| 1. Multivoltine breeds | = | 5 x 5 diallel fashion      |
| 2. Bivoltine breeds    | = | 5 x 5 diallel fashion      |
| 3. Multi x bivoltine   | } | 5 x 5 Line x Tester design |
| 4. Bi x multivoltines  |   |                            |

All the hybrids and their parents each with 3 replications were reared during (1) MAY - JUNE (DRY SUMMER), (2) AUG. - SEPT. (WET SUMMER) (3) OCT. - NOV. (AUTUMN) and (4) JAN. - FEB. (SPRING) commercial seasons for two consecutive years i.e. 1991 and 1992 following the standard techniques of rearing (Krishnaswami, 1978, 1979). Each replication consisted of 300 silkworms which was retained during III instar from each individual Disease free laying (DFL).

Performance data in respect of No. of eggs deposited by each mother moth constituting Fecundity (FEC), Hatching percent

(HAT%), larval period in days (LP), wt. of 10 larvae in grams (LW), pupation rate per 10,000 larvae (PUP), yield per 10,000 larvae (from the larvae counted during III instar) by weight in grams (YIELD), single cocoon weight in grams (SCW), single cocoon shell weight in grams (SSW), cocoon shell percent (SR%) and filament length in meters (FL) were recorded.

The following are the formulae on which few parameters were calculated -

$$1. \text{ Hatching percent (HAT\%)} = \frac{\text{No. of eggs hatched}}{\text{Total No. of eggs}} \times 100$$

$$2. \text{ Pupation rate (PUP) per 10000 larvae} = \frac{\text{No. of good cocoons with live pupae + double cocoon}}{\text{Total No. of worms retained in III instar}} \times 10000$$

$$3. \text{ Cocoon shell percent (SR\%)} = \frac{\text{Average cocoon shell wt. (gms)}}{\text{Average weight of cocoon (gms)}} \times 100$$

\* Average cocoon and shell weight were obtained by weighing randomly 20 male and 20 female cocoons separately.

The mean performance of all the four combinations reared during four seasons for two consecutive years was presented in TABLE - MATER - 2. [Table revealed that bivoltine hybrids could not turnout well during MAY - JUNE, and AUG. - SEPT. But they performed well during OCT. - NOV. and JAN. - FEB. because of favourable seasons. During MAY - JUNE the performance of Multi x bivoltine hybrids proved well whereas during AUG. - SEPT. only multi x multi hybrids thrived better because multivoltine hybrids were robust against high temperature combined with high humidity]. On the basis of above performance the following combinations against specific season were considered for the present investigation.

1. Bivoltine hybrids - OCTOBER - NOVEMBER and  
JANUARY - FEBRUARY.
2. Multivoltine hybrids - AUGUST - SEPTEMBER
3. Multi x Bivoltine hybrids - MAY - JUNE

The genetic architecture of these breeds as well as hybrids have been covered and discussed.

The data obtained during two consecutive years for different seasons were subjected to different types of bio-metrical analysis:

TABLE : MATER : 1 : QUALITATIVE TRAITS OF DIFFERENT SILKWORM BREEDS.

(Rao et al.,1992; chattopadhyay et al.,1993;  
Ghosh et al., 1993; Gupta et al.,1994)

NAME OF THE SILKWORM BREED	SOURCE OF COLLECTION/ ORIGIN	LARVAL MARKING	COCOON COLOUR	COCOON SHAPE	VOLTINISM
NISTARI	West bengal	Marked Larvae	Golden yellow	Spindle	Multi voltine.
CB5	Evolved at CSR&TI, BHB.	Marked Larvae	Golden yellow	Ellip.	Multi voltine.
RAJ	Bangladesh	Plain	White	Spindle	Multi voltine.
G	Evolved at CSR&TI, BHB.	Marked Larvae	Golden yellow	Oval	Multi voltine.
B	Evolved at CSR&TI, BHB.	Marked Larvae	Golden yellow	Ellip.	Multi voltine.
P5	Pampore	Marked Larvae	White	Dumble	Bivol tine
NB18	CSR&TI, MYSORE	Plain	White	Peanut	Bivol tine
JD6	RSRS, D.DUN.	Marked Larvae	White	Ellip.	Bivol tine
SF19	RSRS, D.DUN.	Marked Larvae	White	Ellip.	Bivol tine
KS(O)	RSRS, KALIMPONG.	Marked Larvae	White	Oval	Bivol tine

TABLE : MATER : 2 : SEASONAL PER'SE PERFORMANCE OF VARIOUS  
HYBRIDS (A VARAGE OF 2 YEARS)

SEASONS	FEC (No.)	HAT. (%)	L.P. (days)	LW (G)	PUP	YIELD	SCW (g)	SSW (g)	SR (%)	FL (mts.)
MULTI X MULTI (Av. of 20 crosses)										
MAY-JUN	382.16	94.30	20.45	23.61	7792.71	8022.80	1.013	0.150	14.78	524.31
AUG-SEP	401.76	95.86	18.95	27.16	8431.94	9645.50	1.160	0.169	14.47	528.28
OCT-NOV	392.43	93.57	20.99	27.80	8807.53	10382.77	1.181	0.175	14.78	488.57
JAN-FEB	469.08	95.27	25.27	29.22	9059.99	11517.33	1.269	0.192	15.10	575.99
BI X BI (Av. of 20 crosses)										
MAY-JUN	509.21	87.42	23.07	26.35	1458.67	1482.05	1.008	0.173	17.16	616.92
AUG-SEP	418.47	96.00	21.81	31.92	3918.67	5190.69	1.334	0.266	19.94	884.37
OCT-NOV	482.05	91.36	23.97	34.87	7423.85	10150.46	1.282	0.237	18.34	941.84
JAN-FEB	489.01	90.70	25.91	41.13	8918.65	14807.08	1.657	0.328	19.80	880.24
MULTI X BI (Av. of 25 crosses)										
MAY-JUN	366.63	94.26	21.36	28.17	6659.55	7847.79	1.177	0.193	16.39	628.32
AUG-SEP	377.73	95.07	19.47	30.96	6922.60	9784.88	1.412	0.246	17.35	717.37
OCT-NOV	427.07	89.70	22.15	37.04	8687.21	13732.13	1.588	0.285	17.70	865.33
JAN-FEB	471.24	96.63	25.05	40.94	9206.12	16337.29	1.774	0.313	17.66	814.29
BI X MULTI (Av. of 25 crosses)										
MAY-JUN	393.28	95.18	20.31	27.66	6866.71	7694.43	1.120	0.187	16.68	636.16
AUG-SEP	418.11	93.84	18.59	31.30	7422.57	9931.99	1.306	0.225	17.22	684.91
OCT-NOV	428.72	87.02	20.32	29.17	8515.24	11218.84	1.399	0.250	17.69	786.73
JAN-FEB	518.49	93.08	24.67	35.92	9254.24	15491.59	1.671	0.287	17.14	797.80

- I. The graphic and component analysis of diallel populations were carried out by method of Jinks (1954) and Hayman (1954, a) respectively. Heritability in narrow sense was carried out as suggested by Mather and Jinks (1971) and heritability in broad sense was worked out as per Verhalen and Murray (1969).
- II. Combining ability effects were calculated by Model-1, method-2 (parents, F<sub>1</sub>s and reciprocals) of Griffing (1956).
- III. Combining ability of multivoltine x bivoltine strains in the form of "Line x Tester" design was worked out as suggested by Kempthorne (1957).
- IV. Statistical procedures adopted for calculating genotypic and phenotypic co-efficient of variations were similar as described by Burton (1952) and Allard (1960). Heritability and genetic gain (genetic advance) were calculated as per the formulae derived by Lush (1949).
- V. The phenotypic and genotypic correlations were worked out by using the formulae suggested by Miller et al., (1982).
- VI. Path co-efficient analysis as applied by Dewey and Lu (1959) was utilised to partition the correlation coefficients into direct and indirect effects of yield components.

The details are as follows :

Testing of Hypothesis for diallel population :

i) The validity of assumptions of diallel cross analysis was tested using "t<sup>2</sup>" test. Consequence of the hypothesis of diallel cross analysis was the consistency of Wr - Vr i.e. independent of r and the failure of hypothesis would up-set this consistency. Heterogeneity of Wr - Vr was thus a good indication of such failure. To provide a test which gave equal weight to both Wr and Vr, the axes of the graph were related through 45° so that the co-ordinate of the point became proportionate to Wr and Vr and Wr - Vr. Testing of the significance of regression in the axes zero was done by the "t" test. Values of "t<sup>2</sup>" for each character from Wr and Vr values were calculated as.

$$t^2 = \frac{n - 2}{4} \left[ \frac{\{ V(Vr) - V(Wr) \}^2}{\{ V(Vr) - V(Wr) - cov^2(Vr-Wr) \}} \right]$$

The values of "t<sup>2</sup>" were tested for their significance against the tabulated "F" value at a desired level of probability at (P-2) degree of freedom. The significant value of t<sup>2</sup> indicated failure of one or more assumptions.

The weakness of this test was that it only detected variation in Wr, Vr which was correlated with the dominance order of the parents. Variations which merely increased the scatter of the points about the regression line altering its slope, could only be detected by Vr - Wr graph.

(ii) 1-b, Wr, Vr test:

The assumption of non-allelic interaction might be verified by testing the deviation of b, Wr, Vr. From the unity using "t" test at desired level of probability for P-2 degree of freedom. The significant value of 1-b, Wr.Vr. depicted the presence of non-allelic interactions. The regression of Wr, Vr (V, Wr, Vr) and its standard error (S.E.) were estimated as :

$$b.Wr.Vr = \frac{\text{cov. (Wr - Vr)}}{V (Vr)}$$

$$S.E. (b, Wr, Vr) = \sqrt{\frac{V(Wr) - b \{ \text{cov. (Wr.Vr)} \}}{(P-2) V (Vr)}}$$

the slope of byx was tested by the expression

$$t_1 = \frac{byx - 0}{S.E. \text{ of } byx} \quad \text{and} \quad t^2 = \frac{1 - byx}{S.E. \text{ of } byx}$$

$$\begin{array}{c} \text{t - test} \\ \hline t_1 = \frac{b-0}{SE (b)} \end{array} \quad \text{with P -2 d.f.}$$

$$t^2 = \frac{1 - b}{SE (b)}$$

If "t<sub>1</sub>" was significant, the dominance played a vital role in the determination of the characters, t<sub>1</sub> tested the

significance by  $byx$  from zero and  $t_2$  tested the difference of  $byx$  from one i.e. unit slope and detected epistasis, if, significant.

Solution to the problem of the invalidity of diallel cross assumptions :

When the validity of the assumptions on which diallel cross analysis was based were not fulfilled due to significant value of  $t^2$  and/or  $i-b$   $W_r$ ,  $V_r$ , the data of test characters of "p x p" diallel cross table showing invalidity by the assumption could be rearranged by removing one or more interacting arrays, one by one until the remaining diallel cross read the value of  $t^2$  and  $i-b$   $W_r$ ,  $V_r$  to be non-significant. This modified "P'x P'" diallel cross table could then be used for further analysis or processing as already discussed.

A. Graphic approach :

- (a) Limiting parabola : A limiting parabola was constructed using the equation.

$$W^2f = Vr \times VOLO \text{ i.e. by plotting.}$$

$Wr$  and  $(Vr \times VOLO)^{\frac{1}{2}}$  points.

The points corresponding to  $Wr.Vr$  values of each of the parents must be within the parabola.

- (b) Genetic analysis by means of graph ( $Vr.Wr$ ) : A test which was useful depend on  $Vr.Wr$  graph. The quantity  $Wr - Vr = \frac{1}{2}(D-H_1)$  was expected to be constant for arrays, if the basic assumptions of the diallel cross analysis were valid and environmental effects would be zero. Since  $\frac{1}{2}(D-H_1)$  did not vary with arrays under these conditions,  $Wr = c + Vr$  where  $c = \frac{1}{2}(D - H_1)$  being a constant and the regression of  $Wr$  on  $Vr$  was straight line of unit slop, when  $Vr = 0$  and  $Wr = \frac{1}{2}(D-H_1)$ , this was not a line of unit slop.

When  $Wr - Vr$  varies, then in the  $Vr, Wr$  graph, the  $Wr$  intercept was an indicator of average degree of dominance in the experimental material with partial dominance  $H_1 < D$  and the  $Wr$  intercept was positive, with overdominance  $H_1 > D$  the  $Wr$  intercept was negative.

$$\begin{aligned} \text{If there was no dominance } H_1 &= 4 Uv h^2 \\ &= 0 \text{ and } F = \pm 8 Uvd h^2 = 0. \end{aligned}$$

$$\begin{aligned} \text{Then, } V_r &= D - \frac{1}{4} H - \frac{1}{4} F_r = \frac{1}{4} D \\ &= Uvy (d+h)^2 = \Sigma Uvd^2 \\ W_r &= \frac{1}{4} D + \frac{1}{4}; \quad F_r = \frac{1}{4} D \\ &= 2 \Sigma Uvd (d+h)^2 = 2 \Sigma Uvd^2 \\ \text{or } W_r &= 2 V_r \end{aligned}$$

In such cases all points on the  $V_r.W_r$  graph were estimates of the single point  $W_r = 2 V_r$  and there was no regression. Therefore, the  $W_r.V_r$  graph provided test of significance for the presence of dominance ( $b \neq 0$ ) and the average degree of dominance (sign of  $a$ ) in which " $b$ " was the slope of regression line and " $a$ " was the  $W_r$  intercept.

For a diallel cross with certain value of  $H_1/D$  the points ( $V_r, W_r$ ) were distributed along a corresponding straight line of unit slope inside the limiting parabola.

$$W_r^2 = V_r \times VOLO.$$

### iii) Order of dominance :

The position of  $V_r, W_r$  on the line of regression revealed the relative proportion of dominance and recessive genes in the parental lines (Jinks 1954 and Hayman 1954, a). The parents with

the preponderance of dominant allele would have a low array variance and co-variance ( $V_r$ ,  $W_r$ ) and would lie at upper 2nd of the regression line. If the dominance effects of the gene were un-equal, the position on array point would be weighed in favour of genes with longer dominance effects. It was thus clear that  $W_r$ ,  $V_r$  graph gave fair indication of order of dominance. The parents with high, intermediate and low level of dominance maintained their position on the graph reasonably well. Here, the order of dominance might or might not coincide with the parental measurements. The actual order might be determined by the relative frequency of dominant and recessive genes present in them.

iv) Standardized deviation graph :

Measuring a variate from its mean using S.D. as units, led to a standardized variable  $U = \sqrt{(x - u)/D}$  having (0) zero mean unit variable.

In order to obtain the standardised deviation (S.D.) of  $W_r + V_r$  value for a parent from the mean value of  $W_r + V_r$  averaged overall the parents was divided by the standard deviation of the  $W_r + V_r$  value for all the parents. Similarly, the deviation of  $V_r$  values for a parent from mean value of  $V_r$  (average over all the parents) was divided by the standard deviation of  $V_r$  values for all the parents.

The standardized deviation graph for each test character was constructed and interpreted according to Hayman (1954, b; 1958). The parents having negative sign (-) of the standard deviation of  $W_r + V_r$  showed dominance while those having positive standardised deviation of  $W_r + V_r$ , depicted recessiveness.

High parental performance was shown by parents having positive standardised deviation of  $Y_r$  and low parental performance was shown by the parents having (-) negative S.D. of  $Y_r$ . In a  $W_r + V_r$  and  $Y_r$  co-ordinate, the parents in the first quadrant (++) showed higher parental performance associated with recessive genes. The parent in the second quadrant (+, -) showed lower parental performance associated with recessive genes. The parents in the third quadrant (-, -) showed association of low parental performance with the dominant genes and lastly those in the fourth quadrant (-, +) showed the association of dominant genes with higher parental performance.

#### B. COMPONENT ANALYSIS:

The following genetic components of variation were calculated by adopting the method given by Jinks and Hayman (1953) and Hayman (1954).

$D = 4 \sum uvd^2 =$  component of variation due to additive effect of genes if  $u = v = 0.5$   $D = d^2$

where,  $u =$  proportion of positive genes in the parents.

$v =$  proportion of negative genes in the parents.

$d =$  additive effects and  $u = v = 1$ .

$H_1 = 4 \sum uvh^2$  Component of variation due to dominance effect of genes.

$H_2 = H_1 [1 - (u-v)^2] = 16 \sum U^2 V^2 h^2 =$  Proportion of dominance variance due to the positive ( $u$ ) and the negative ( $v$ ) effects of the genes.

$h^2$  Net dominance effect (expressed as the algebraic sum overall loci in heterozygous phase in all the crosses).

$Fr =$  The covariance of additive and dominance effects in a single array.

$F =$  The Mean of "Fr" over the arrays. It indicated the relative frequency of dominant and recessive alleles in the parents.

It might take the negative (-) sign if there were an excess of recessive alleles or the positive (+) sign indicating excess of

dominant alleles. The value of  $F$  would be zero if dominant and recessive alleles of each gene was distributed equally among parents and the relationship of  $H_1$ ,  $H_2$  and  $h^2$  would be

$$H_1 = H_2 = h^2$$

The components of variance were derived by constructing a group of equation based on the following parameters derived from the diallel table :

VOLO =  $V_p$  = variance of the parental array.

$V_r$  = Variance of the  $r$ th array

$V_{1L_1} = V_r$  = Mean of array variance

$W_r$  = The covariance between the parents and their offsprings in an array ( $r$ th array).

WOLO =  $W_r$  = Mean covariance between the parents and offspring of all the arrays.

VOL1 =  $V_m$  = The variance of array means.

$ML_1 - MLO$  = The difference between the mean of the parents and mean of their progenies.

$E$  = The expected environmental component of variation, which was observed from analysis of variance.

The expected values of these components of variation were calculated by substituting the values in the following equations:

$$VOLO = VP = D + E$$

$$V_1L_1 = Vr = \frac{1}{2} D + \frac{1}{2} H_1 - \frac{1}{2} F + (n+1) \cdot \frac{E}{2n}$$

$$WOLO_1 = Wr = \frac{1}{2} D - \frac{1}{2} F + \frac{E}{n}$$

$$VOL_1 = Vm = \frac{1}{2} D + \frac{1}{2} H_1 - \frac{1}{2} H_2 - \frac{1}{2} F + \frac{E}{2n}$$

Where,

$$E = \text{Error } \text{MSS}/r = Me$$

$$D = VOLO - E$$

$$H_1 = VOLO - 4WOLO_1 + 4 V_1L_1 - (3n-2) \cdot \frac{E}{n}$$

$$H_2 = 4 V_1L_1 - 4 VOL_1 - 2E$$

$$h^2 = 4(ML_1 - MLO)^2 - 4(n-1) \cdot \frac{E}{n^2}$$

$$Fr = 2 (VOLO - WOLO_1 + V_1L_1 - Wr - Vr) - 2(n-2) \cdot \frac{E}{n}$$

$$F = 2 VOLO - 4 WOLO_1 - 2(n-2) \cdot \frac{E}{n}$$

n = No. of parents

r = No. of replications.

#### Estimation of standard Errors of genetic components :

The standard errors, to test the significance of the six components (D, H<sub>1</sub>, H<sub>2</sub>, h<sup>2</sup>, F, & E) of variation listed above were calculated using the formula  $\frac{1}{2} \{ \text{var} (Wr - Vr) \} = s^2$  and the terms of the main diagonal of covariance matrix given by Hayman (1954) as corresponding multipliers.

Therefore, the standard errors of the components of variation would be -

$$\text{S.E. (D)} = s^2 (n^5 + n^4) / n^5$$

$$\text{S.E. (H)}_1 = s^2 (n^5 + 41n^4 - 12n^3 + 4n^2) / n^5$$

$$\text{S.E. (H)}_2 = s^2 (36n^4) / n^5$$

$$\text{S.E. (h)}^2 = s^2 (16n^4 + 16n^2 + 32n + 16) / n^5$$

$$\text{S.E. (F)} = s^2 (4n^5 + 20n^4 - 16n^3 + 16n^2) / n^5$$

$$\text{S.E. (E)} = s^2 n^4 / n^5$$

Where, n = number of parents.

#### Test of significance of components of variation :

The significance of various statistics was tested by "t" test at (n-2) degree of freedom. Here n = number of parents.

$$t = \frac{\text{Parameter}}{\text{S.E. of parameters}}$$

#### Proportion of genetic components:

The proportion of the genetic components were worked out according to the procedure given below :

##### 1) Degree of dominance

The degree of dominance in  $F_1$  was calculated using the formula  $(H_1/D_1)^{\frac{1}{2}}$  (Hayman 1954)

If degree of dominance = 0 (No dominance)  
 = 1 (complete dominance)  
 > 1 over dominance  
 < 1 > 0 partial dominance

2) Proportion of genes with positive and negative effects in the parents:

It was calculated by the ratio -  $H_2/4H_1$ . It indicated the mean product of  $u_i, v_i$  averaged over all the parents of a diallel set of crosses. When  $u$  and  $v$  were symmetrically distributed, i.e.  $u = v = 0.5$ , the ratio would give the value of  $H_2/4H_1 = 0.25$ .

3) Proportion of dominant and recessive genes in the parents :

It was calculated by the following formula :

$$\frac{KD}{KR} = \frac{(4 DH_1)^{\frac{1}{2}} + F}{(4 DH_1)^{\frac{1}{2}} - F} \quad \text{for } F_1$$

If,  $\frac{KD}{KR} = 1$  (equality of dominant and recessive genes)

> 1 - excess of dominant genes.

< 1 - excess of recessive genes.

4) Number of groups of genes which control the character and exhibit dominance :

It was calculated by the following formula :  $\frac{h^2}{H^2}$

It was an approximate measure of groups of genes exhibiting dominance

Estimation of Heritability :

Heritability in narrow sense was defined as the ratio of additive and/additive x additive genetic variance to the total phenotypic variance. For  $F_1$  it was calculated using the formula given by Mather and Jinks (1971) :

$$\text{Heritability (ns)} = \frac{(\frac{1}{2})D + (\frac{1}{2})H_1 - (\frac{1}{2})H_2 - (\frac{1}{2})F}{(\frac{1}{2})D + (\frac{1}{2})H_1 - (\frac{1}{2})H_2 - (\frac{1}{2})F + E}$$

$h^2(\text{bs})$  was calculated following Verhalen and Murray (1969).

$$\text{Heritability (bs)} = \frac{(\frac{1}{2})D + (\frac{1}{2})H_1 - (\frac{1}{2})H_2 - (\frac{1}{2})F}{(\frac{1}{2})D + (\frac{1}{2})H_1 - (\frac{1}{2})H_2 - (\frac{1}{2})F + E}$$

1. Combining ability analysis :

The present study was undertaken as per Griffing (1956, b) (Model 1 and Method 2). The statistical model for analysis of variance of diallel table for measuring  $y_{ij}$  was

$$y_{ij} = \mu + g_i + g_j + s_{ij} + r_{ij} + 1/bc \sum \sum e_{ijkl}$$

$$i, j = 1, 2, \dots, n$$

$$k = 1, 2, \dots, b$$

$$l = 1, 2, \dots, c$$

where,  $y_{ij}$  was the mean of  $i \times j$ th genotype over  $k$  and  $l$

$\mu$  was the population mean

$g_i$  was the general combining ability (gca) effect of  $i$ th parent.

$g_j$  was the gca effect of  $j$ th parent.

$S_{ij}$  was the interaction i.e. - specific combining  
ability effect

$r_{ij}$  was the reciprocal effect

$1/bc \sum \sum e_{ijkl}$  was the mean error effect.

The total variability might be partitioned into components like variance due to gca, sca, reciprocal and error.

$$SS \text{ due to gca} = \frac{1}{4}n \cdot \sum (y_{i.} + y_{.i})^2 - \frac{2}{n^2}y^2$$

$$SS \text{ due to sca} = \frac{1}{4} \sum \sum y_{ij} (y_{ji} + y_{ji}) - \frac{1}{4}n \sum (y_{.j} + y_j)^2 + \frac{1}{n^2}y^2 \dots$$

$$SS \text{ due to reciprocals} = \frac{1}{4} \sum \sum (y_{ij} - y_{ji})^2$$

$$MS \text{ (error)} = \frac{MS \text{ (errors)}}{r} \quad \text{Where, } r = \text{replications}$$

Analysis showing the expectations of Mean sum of squares was as follows :

Source	Df	sum of squares	MSS	MS(Expected)
GCA	$P-1$	$SS(g)$	$M_g$	$\sigma^2 + \{2p/(p-1)\} \sum g^2 i$
SCA	$P(P-)/2$	$SS(s)$	$M_s$	$\sigma^2 + \{2/(\sum p(p-1))\} \sum \sum S^2 ij$
Reciprocal	$P(P-1)/2$	$SS(r)$	$M_r$	$\sigma^2 \{4/(P(P-1))\} \sum \sum r^2 ij$
Error	$\{(r-1)(p-1)(p+1)\}/r$		$M'e$	$\sigma^2$

Where,  $P$  = No. of parents;  $r$  = No. of replications &  $M_g$ ,  $M_s$ ,  $M_r$  &  $M_e$  were mean sum of squares due to gca, sca, reciprocals and error respectively. The mean sum of squares for gca, sca were obtained by dividing  $SS(g)$  and  $SS(s)$  with their respective degree of freedoms (df),  $M'e$  by dividing error mean square obtained from the general analysis with the number of replication. For "F" test each mean sum of squares was tested against  $M'e$  and  $n-1$  and  $n-2$  degree of freedom.

For testing of overall differences :-

- i) Test of gca effect :  $F = M_g/M'e$
- ii) Test of sca effect :  $F = M_s/M'e$
- iii) Test of reciprocal effect :  $F = M_r/M'e$

The general combining ability (gca) of an inbred line was defined as the average performance of the hybrids which this line produces with other lines chosen from a random mating population. In general, such an effect gave the epistatic interaction of digenic type and directed by  $\sigma^2$  gca is given by  $\sigma^2$  gca =  $\frac{1}{2} \sigma^2 A + \frac{1}{2} \sigma^2 AA$ .

The specific combining ability (sca) refers to a pair of inbred lines involved in a cross. It indicated crosses in which earlier crosses do relatively better or worse than would be

expected on the basis of average performance of the two lines involved. Its existence indicated non-additive genetic effects and its variance  $\sigma^2 \text{ sca}$  was given by  $\sigma^2 \text{ sca} = \sigma^2 \text{ D} + \frac{1}{2} \sigma^2 \text{ AA} + \frac{1}{2} \sigma^2 \text{ AD} + \sigma^2 \text{ DD}$ . It might also be seen that total genotypic variance was (summation) related to these two variances i.e.  $\sigma^2 \text{ G} = 2 \sigma^2 \text{ gca} + \sigma^2 \text{ sca}$ .

Estimates of gca = These effects could be obtained as follows :

gca effect of ith parent :  $g_i = 1/(p+2) (X_i + i_i - 2/pX \dots)$

Estimates of sca = These effects could be obtained as follows :

$S_{ij} = X_{ij} - 1/p+2 (X_i + X_{ii} + X_j + X_{jj}) + 2/(p+1)(p+2) X \dots$

Estimates of variance due to general and specific combining ability:

These variances were obtained by the following formulae :

$$\sigma^2 g = 1/(p+2) \left[ Mg - \frac{Me + p (P-1) MS}{C} - Y \right]$$

$$\sigma^2 S = P^2/2C (MS - Me')$$

Where,  $\sigma^2 g$  = variance due to (gca) General Combining Ability

P = number of parents

C =  $P^2 - P + 1$ ,  $2s$  = variance

$\sigma^2 S$  = Variance due to sca

Standard Error of the Estimates :

Standard error of an estimate was calculated as the square roots of the variance of the estimates. The variance of the various estimates were calculated as follows :

$$\text{Variance (Xij)} = S^2 = Me'$$

$$\text{Variance (u)} = \frac{2}{P(P+1)} S^2$$

$$\text{Variance (gi)} = \frac{2}{P(P+2)} S^2$$

$$\text{Variance (sij)} = \frac{P^2+P+2}{(P+1)(P+2)} S^2 \quad (i = j)$$

Test of significance of gca & sca effects :

Each gca & sca value was tested against zero for its significance by "t" test.

$$t = \frac{g_i - 0}{\text{S.E. (g}_i)} \quad \& \quad "t" = \frac{s_{ij} - 0}{\text{S.E. (s}_{ij})} \quad \text{respectively}$$

the "t" value thus obtained was tested at error degree of freedom.

Critical difference of the estimates :

The difference between two estimates were tested by comparing them with the C.D. values.

C.D. = S.E. of difference of the two estimates  $\times t$  (5%) at error degree of freedom.

The standard error of difference of the two estimates was taken as square roots of the variance of the difference of two estimates. The variance of difference of the estimates was calculated as follows :

$$\text{Var. } (g_i - g_j) = 2/(P+2) S^2 \quad (i = j) \text{ for gca estimates.}$$

$$\text{Var. } (s_{ij} - s_{ik}) = 2(P+1)/(P+2) S^2 \quad (i=j.k; j=k)$$

for sca estimates in an array.

$$\text{Var. } (s_{ij} - s_{ki}) = 2 P/(P+2) S^2 \quad (i=j, K, 1; j=k, 1; k=1)$$

for any two sca estimates where P is the number of parents and  $S^2 = Me'$ .

II. Line  $\times$  Tester crosses (multivoltine  $\times$  bivoltine) :

The application of line  $\times$  Tester design (Kempthorne, 1957) was useful for deciding about the relative capacity of a number of female and male parents to produce desirable hybrids

(Arunachalam, 1974). This design provided information about the general and specific combining ability along with the estimation of various types of gene effects. Here unlike diallel design the male and female parents were invariably different. Let 'l' be the line and 't' be the tester, all these 'l' lines were crossed to each of 't' testers and thus  $l \times t$  full sib progenies were produced. These progenies along with parents (lines & testers) were then tested in replicated trials using Completely Randomised Designs (CRD) where 5 lines and 5 testers were used in the present experiment out of which the multivoltines had been considered as lines and bivoltine as testers and vice versa to produce (1) M x bi layings  $(5 \times 5) = 25 + 10$  parents, total entry being 35 and (2) Bi x Multi layings  $(5 \times 5) = 25 + 10$  parents, total entry being 35 and the scheduled parameters were recorded.

Line x Tester design based on

$$y_{ijk} = \mu + g_i + g_j + s_{ij} + r_k + e_{ijk}$$

Where,  $y_{ijk}$  = value of any trait measured of the cross  $i \times j$  in

$\mu$  = population mean effect the  $k$ th replicates

$g_i$  = gca effect of the  $i$ th parent

$s_{ij}$  = sca effect of the cross  $i \times j$

$r_k$  =  $k$ th replication effect

$e_{ijk}$  = environmental effect of  $ijk$ th individual.

The analysis of variance and expectation of mean squares as suggested by Kempthorne (1957) was presented here using which the combining ability effects might be estimated.

ANOVA for combining ability based on L x T design

Source	df	MS	EMS
Males	S-1	MS	$\sigma^2 e + rI + rdy$
Females	d-1	Md	$\sigma^2 + rI + rsy$
Males x females (S-1)(d-1)		MI	$\sigma^2 e + rI$
Error		ME	$\sigma^2 e$

r = No. of replications, S = No. of male parents

d = No. of female parents, y = Cov. (half-sib)

=  $\sigma^2 gca$

2y+I = Cov.(full-sibs), I =  $\sigma^2 sca$

E  $\sigma^2 e$ .

Least square estimates the components of combining ability variances derived by Arunachalam (1974) were given hereunder :

Let a =  $(Me - ME)/r$

b =  $(Md - ME)/r$

c =  $(Mi - ME)/r$

Then,  $\sigma^2_{gca} = y$  &  $\sigma^2_{sca} = X - 2y$  for

$$X (sd - s^2 - d^2) = S(a+c-2b) + d(b+c-2a) - \frac{1}{2} \{ S^2(a+c) + d^2(b+c) - sd(a+b) \}$$

$$y (sd - S^2 - d^2) = \frac{1}{2} \{ S(a+c-2b) + d(b+c-2a) \}$$

Estimates of gca effects :

(a) Lines :

$$g_i = \frac{x_{i\dots}}{tr} - \frac{x_{\dots}}{ltr} \quad \text{Where, } l = \text{no. of lines}$$

$$t = \text{no. of testers}$$

$$r = \text{no. of replications.}$$

(b) Testers :

$$= g_t = \frac{x_{.j}}{lr} - \frac{x_{\dots}}{ltr}$$

Estimates of sca effects :

$$S_{ij} = \frac{x_{ij}}{r} - \frac{x_{i\dots}}{tr} - \frac{x_{.j}}{lr} + \frac{x_{\dots}}{ltr}$$

Standard Errors for combining ability effects :

$$\text{S.E. (gca for line)} = (Me/r \times l)^{\frac{1}{2}}$$

$$\text{S.E. (gca for tester)} = (Me/r \times t)^{\frac{1}{2}}$$

$$\text{S.E. (Sca effect)} = (Me/r)^{\frac{1}{2}}$$

$$\text{S.E. (g}_i - \text{g}_j \text{) line} = (2 Me/r \times l)^{\frac{1}{2}}$$

$$\text{S.E. (g}_i - \text{g}_i \text{) tester} = (2 Me/t \times r)^{\frac{1}{2}}$$

$$\text{S.E. (s}_{ij} - \text{s}_{kl}) = (2 Me/r)^{\frac{1}{2}}$$

#### IV. Estimation of Heterosis:

Heterosis in percent was calculated over Mid parent (MP) and better parent (BP) (over dominance) by using the following formulae.

Heterosis over midparent value (MPV):

$$= \frac{F1 - MP}{MP} \times 100$$

Heterosis over better parent value (BPV):

$$= \frac{F1 - BP}{BP} \times 100$$

Where, F1 mean of the F1 generation

MP = mean of the two parental values and

BP = mean of the better parent of the two involved parents in the cross in question

#### Test of significance of Heterosis :

This test of significance of heterosis was done by the "t" test as given below :

$$"t" \text{ MP} = \frac{F1 - MP}{\text{S.E. of heterosis over mid parent}}$$

$$"t" \text{ BP} = \frac{F1 - BP}{\text{S.E. of heterosis over BP parents.}}$$

$$\text{SE of heterosis over Mid parents was} = \sqrt{s_1^2(1/r+1/2r)}$$

$$\text{B. parent} = \sqrt{s_1^2(1/r + 1/r)}$$

$s_1^2$  Error variance for parents and F1

$r =$  No. of replications.

Calculated 't' value was compared with tabulated value of "t" at error degree of freedom.

#### Genetic parameters :

For calculating the genetic parameters such as phenotypic and genotypic variance phenotypic and genotypic coefficient of variation, heritability in broad sense, genetic advance and genetic advance in %age of means, correlation coefficients and path coefficient analysis (both phenotypic and genotypic) for cocoon yield in Bivoltine and multivoltine populations, the rearing data obtained from 4 seasons for two consecutive years had been pooled separately for multivoltine as well as bivoltine breeds and subjected for statistical evaluations.

#### 1. Analysis of variance (ANOVA) :

ANOVA for each character was computed separately :

Source of variation	d. f.	M. S.
Replicate	23	Mr
Treatment	24	Mt
Error	552	Me
Total :	599	

Statistical constants, range and mean of each quantitative characters were calculated from the raw data. The minimum and maximum values of silkworm strains means were taken as range. Mean was calculated by dividing the grand total by number of strains and number of replications. Other statistical constants such as standard error of mean, standard deviation, critical difference and coefficient of variation were computed for the study of phenotypic variability from the analysis of variance in completely randomised designs (CRD). The mean squares of treatments and for error, divided by number of replications were taken as phenotypic variance and error variance of treatment means respectively.

Genotypic variance was derived by subtracting the error variance of treatments from phenotypic variance. Following formulae were utilized for various calculations :

$$\text{Standard error of means} = \left( \frac{\text{Error mean square}}{\text{No. of replications}} \right)^{\frac{1}{2}}$$

$$\text{Critical difference (CD)} = \left( \frac{2 \times \text{Error mean square}}{\text{No. of replications}} \right)^{\frac{1}{2}} \times 0.05$$

$$\text{Phenotypic standard deviation } (\sigma_{PH}) = \sqrt{\sigma^2_{PH}}$$

Where  $\sigma_{PH}$  is the phenotypic variance.

$$\text{Coefficient of variation (CV)} = \frac{\text{Standard deviation}}{\text{Population Mean}} \times 100$$

$$\text{Phenotypic variance } (\sigma^2_{PH}) = \frac{\text{Treatment mean square}}{\text{No. of replications}}$$

$$\text{Error variance of treatment means } (\sigma^2e/r) = \frac{\text{Error mean square}}{\text{No. of replication}}$$

$$\text{Genotypic variance } (\delta^2g) = \frac{\text{Phenotypic variance } (\sigma^2PH) - \text{Error variance of treatment means } (\sigma^2e/r)}{\text{No. of replications.}}$$

Genetic parameters were studied by working out genetic coefficients of variation, heritability and genetic advance.

Statistical procedures adopted for calculating genetic constants for these characters were similar as described by various workers viz., Burton (1952), Allard (1960).

The genetic coefficient of variation was estimated by dividing the square root of the genetic variance by population mean and multiplying by 100 (Burton, 1952). The formulae being

$$\text{G.C.V} = \frac{\sigma^2g}{X} \times 100$$

Where G.C.V. is the genetic coefficient of variance.  $\sigma^2g$  = genotypic variance and X is the population mean of the character.

Heritability (H), in broad sense (Lush, 1949) was calculated for character from pooled data of all the variances as:

$$H = \frac{\sigma^2g}{\sigma^2PH}$$

Where, H = Heritability,

$\sigma^2g$  = Genotypic variance

$\sigma^2PH$  = Phenotypic variance

The expected genetic gain i.e. genetic advance resulting from selection of 5% superior individuals was estimated by the following formulae suggested by Lush (1949), followed by Johnson, Robinson and Comstock (1955).

$$\begin{aligned} \text{Genetic advance} &= H \times K \times \sigma_{PH} \\ &= \sigma^2_g / \sigma^2_{PH} \times K \times \sigma_{PH} \\ &= \sigma^2_g / \sigma_{PH} \times K \end{aligned}$$

Where  $\sigma^2_g$  was genotypic variance,  $\sigma_{PH}$  was phenotypic standard deviation and K was the selection differential. For the purpose of present study K had the value 2.06 which was the expectation in the case of 5 percent selection in large samples from a normally distributed population (Lush, 1949).

The genetic advance in percent of mean was calculated as below :

$$\text{G.A. in percent of mean} = \frac{\text{Genetic advance}}{\text{Population mean}} \times 100$$

#### Correlation studies:

The phenotypic and genotypic correlation coefficient were worked out by using the formulae suggested by Miller et al., (1982). The general formula for correlation coefficient was expressed as follows :

$$r.X.Y = \frac{\text{cov. X Y}}{(\text{variance X}) \times (\text{variance Y})}$$

Where, cov. X. Y = covariance of traits X & Y.

Estimates of phenotypic covariance components and genotypic covariance components between two traits were derived from analysis of covariance. The mean sum of product for treatment and error divided by number of replications were taken as phenotypic variance and error covariance respectively for calculating genotypic covariance from its phenotypic covariance. For working out phenotypic coefficient of correlation phenotypic covariance was divided by the square root of their phenotypic covariance. For genotypic correlation coefficient, genotypic covariance was divided by the square root of their respective genotypic variance. The formulae are :

1. Phenotypic correlation coefficient :

$$\text{Phenotypic } r X Y = \frac{\text{Cov. X Y.}}{(\sigma^2\text{PH.X} \times \sigma^2\text{PH Y})^{\frac{1}{2}}}$$

Where cov. X.Y. = phenotypic covariance of two traits say X & Y

$\sigma^2X$  = Phenotypic variance of trait X

$\sigma^2Y$  = Phenotypic variance of trait Y

2. Genotypic correlation coefficient :

$$\text{Genotypic } r X Y = \frac{\text{Genotypic cov.X.Y.}}{(\sigma^2gX \times \sigma^2gY)^{\frac{1}{2}}}$$

Where, genotypic cov.X.Y.= Genotypic covariance of two traits X and Y

$\sigma^2g X$  = Genotypic variance of trait X

$\sigma^2g Y$  = Genotypic variance of trait Y

#### Path coefficient analysis :

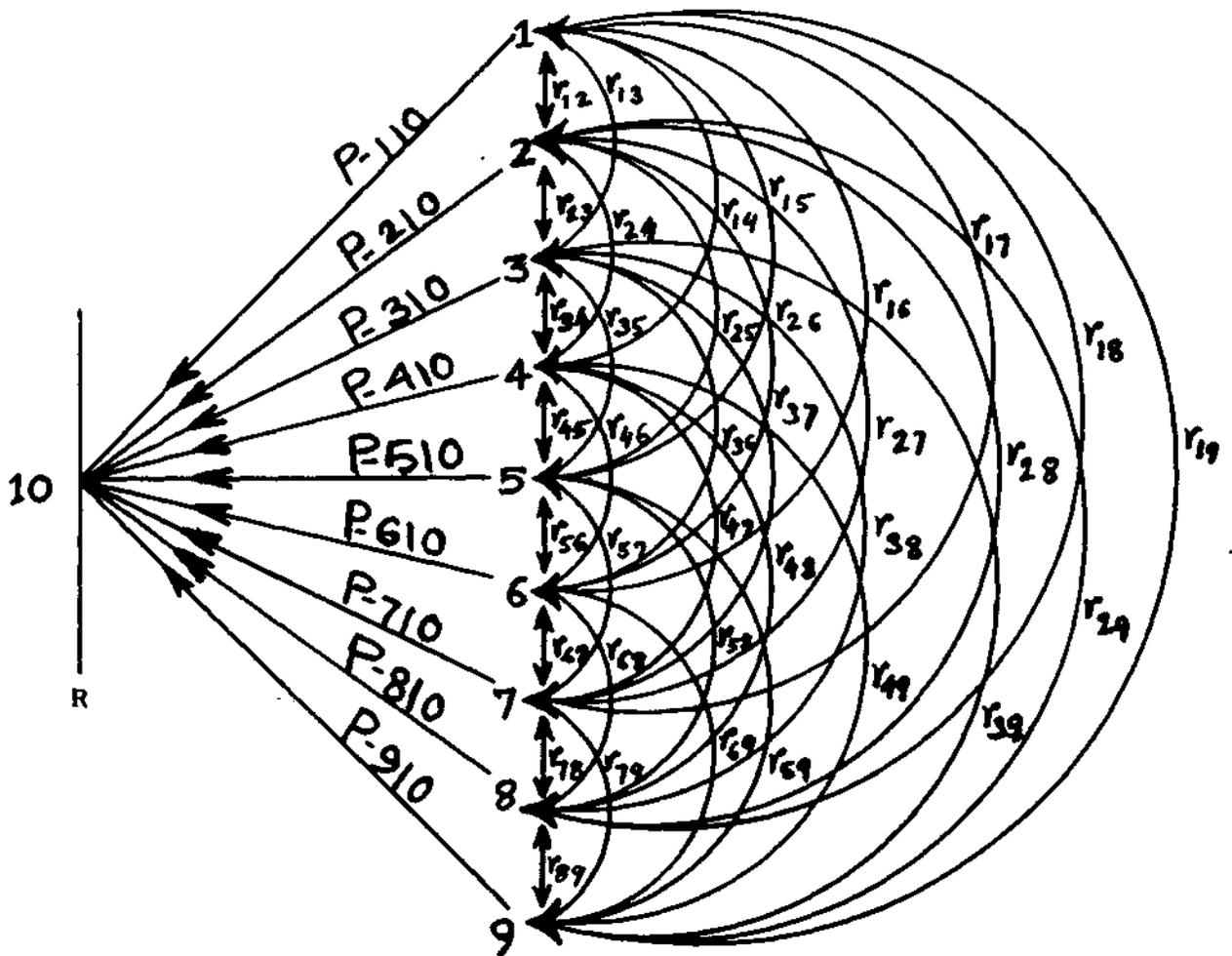
Path coefficient could be defined as the ratio of the standard deviation of the effect due to a given cause to the total standard deviation of the effect. Path coefficient analysis as applied by Dewey and Lu (1959) was utilised to partition the correlation coefficients into direct and indirect effect of the nine yield components such as:

- 1) Fecundity(FEC)
- 2) Hatching%(HAT%)
- 3) Larval period(LP)
- 4) Larval weight(LW)
- 5) Pupation rate(PUP)
- 6) Single cocoon weight(SCW)
- 7) Cocoon shell weight(SSW)
- 8) Cocoon shell percent(SR%)
- 9) Filament length(FL).

A path diagram was also set using the nine characters on the path coefficient analysis :(Fig)

From the figure it was obvious that YIELD was the result of all the components and some other undefined factors designated by R denoting residual factors that influence yield and include sampling errors.

In the path diagram the double arrowed lines indicated mutual association as measured by correlation co-efficient  $r_{ij}$  and the single arrow represents the direct influence as measured by path coefficient  $p_{ij}$ .



Path diagram showing the direct and indirect effects.  
 Unidirectional arrows represent path coefficients  
 (Direct effects) and bidirectional arrows represent  
 correlation coefficients.