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Studies on Parthenogenetic Development, Hybrid Vigour and Cocoon Shape Variability in Bivoltine F1 Hybrids of the Silkworm, *Bombyx mori* L.

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Parthenogenetic development, hybrid vigour and cocoon shape variability were studied in bivoltine F1 hybrids of the silkworm, *Bombyx mori* L. Considerable breed differences were observed in parthenogenetic ability. Among the parental breeds, CSR18 exhibited maximum parthenogenetic development (79.65%) followed by CSR4 (67.90%). Among bivoltine F1 hybrids, CSR18 × CSR4 showed highest parthenogenetic development (73.32%) followed by CSR2 × CSR4 (55.43%). Study on hybrid vigour demonstrated that CSR18 × CSR19 expressed maximum significant hybrid vigour for all the seven economic characters over Mid Parent Value (MPV) followed by CSR2 × CSR4 and CSR18 × CSR4 exhibiting significant heterosis for six characters. Cocoon shape measurement study revealed that among ten bivoltine F1 hybrids, cocoons of three hybrids were found comparatively uniform. Importance of this study to know the level of parthenogenetic development, hybrid vigour and cocoon shape variability in different bivoltine F1 hybrids have been discussed.

Key words: *Bombyx mori*, Cocoon shape, Hybrid vigour, Parthenogenetic development

Introduction

Artificial parthenogenesis in domestic silkworm, *Bombyx mori,* was first demonstrated by Tichomirov, a Russian Zoologist in 1886. Later, several workers reviewed the works carried out on parthenogenesis in silkworm (Astaurov, 1957; Chowdhury, 1989; Klymenko, 2001; Ravindra Singh *et al.*, 1997; Strunnikov, 1975; Tazima, 1964). Parthenogenesis in silkworm acquires a special significance in the development of outstanding homozygous genotypes with low phenotypic variation in quantitative characters, increased hybrid vigour, viability and combining ability (Ohkuma, 1971; Strunnikov *et al.*, 1982; Strunnikov, 1986; 1995).

Among various methods used for activation of artificial parthenogenesis in silkworm, the method of hot water treatment of unfertilised eggs, is the most effective and widely used technique. Recently, Yonqiang *et al.* (2001) have studied parthenogenetic development in commercial silkworm races. Perusal of literature revealed little information on parthenogenesis in indigenous and exotic silkworm breeds/hybrids found in India.

Commercial exploitation of hybrid vigour in the mulberry silkworm was first advocated by Toyama in 1911 (Yokoyama, 1956). Nagaraju *et al.* (1996) have reviewed heterosis and cross breeding in silkworm. Recently, manifestation of hybrid vigour has been studied in silkworm (Rao *et al.*, 2001; Ravindra Singh *et al.*, 1998a, 2000, 2001a, 2001b). Uniform cocoon shape is very important from the standpoint of silk production, evolution, evaluation of commercial hybrids as well as to get uniform silk filament (Mano, 1994; Nakada, 1994). In India, during the recent years, some efforts have been made on cocoon shape variability in multivoltine × bivoltine and bivoltine hybrids (Ravindra Singh *et al.*, 1998b, 2001a, 2001b). With the main objectives to know the ability of parthenogenetic development, hybrid vigour and cocoon shape variability in some bivoltine F1 hybrids of the silkworm, *B. mori,* the present study has been undertaken at Central Sericultural Research & Training Institute, Mysore.

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Materials and Methods

Four bivoltine silkworm breeds *viz.*, CSR2, CSR4, CSR18 and CSR19 were used in the present study. Characteristics of the silkworm breeds are presented in Table1. Crosses were made between the breeds. Reciprocals were also studied. For the induction of parthenogenetic development, the method of Astaurov (1967) was followed. Ovarian eggs were obtained from freshly emerged virgin female moths by squeezing out the ovarioles. Eggs were washed in running tap water, dried and kept at 25°C and 85% Relative Humidity (RH) for 12 hrs. Eggs were treated in hot water at 46°C for 18 minutes. Soon after treatment, eggs were transferred to water at room temperature for 10 minutes. Subsequently, the eggs were dried and incubated at 15°C and 85% for 72 hrs. With the appearance of light brownish serosa colour, eggs were treated with hot hydrochloric acid at 46°C (specific gravity 1.075) for 5 minutes. Then eggs were incubated at 25°C and 85% RH till hatching.

The parthenogenetic development of the eggs was identified with the appearance of reddish-brown/dark pigmentation in the serosa cells. The ratio of reddish-brown/ dark pigmented eggs and total number of eggs treated was expressed as percentage of parthenogenetic eggs. Rearing of F1 hybrids along with parental breeds was carried out simultaneously as per Krishnaswami (1978). Three replications were maintained in each combination/parent and 300 larvae were retained after third moult. Data were recorded for larval span, 5th instar larval span hatchability, effective rate of rearing (ERR), cocoon weight, shell weight and shell ratio. Heterosis over mid parent value (MPV) and better parent value (BPV) was calculated by using the following formulae:

1. Heterosis over (MPV) = $\frac{\text{F1}-\text{MPV}}{\text{MPV}} \times 100$

2. Heterosis over (BPV) = $\frac{\text{F1}-\text{BPV}}{\text{BPV}} \times 100$

In order to know the cocoon shape variability in bivoltine F1 hybrids, 100 cocoons from each combination were randomly picked up and three cocoon shape variables *viz.*, cocoon length, cocoon width and length/width ratio were determined with the help of vernier callipers and by using the following formula:

$$\frac{\text{Length}}{\text{Width}} \times 100$$

Cocoon shape variabilty was determined by using uniformity test on the basis of Standard Deviation (SD). Ratio between cocoon length and cocoon width was calculated for each cocoon and its SD and Coefficient of Variation (CV%) were considered for cocoon uniformity. Hybrids possessing less SD and CV% were considered comparatively uniform in cocoon shape.

Results

Comparative parthenogenetic development in bivoltine silkworm breeds/ F1 hybrids

Parthenogenetic development in the ovarian eggs of silkworm breeds and ten F1 hybrids has been given in Table 2 and 3, respectively. Among the parental breeds, CSR18 (Chinese type) exhibited highest percentage of parthenogenesis (79.65%) followed by CSR4 (Japanese type) and CSR2 (Chinese type) exhibiting 67.90% and 48.15% respectively. Breed difference of parthenogenesis is given in Table 2.

A great deal of variation was observed on the occur-

Table 1. Characteristics of Bivoltine Silkworm breeds used in the present study

Breed	Origin	Fecundity	Larval pattern	Larval span (D:H)	Pupation rate (%)	Cocoon colour & shape	Cocoon wt. (g)	Shell wt. (g)	Shell ratio
CSR2	Mysore	567	Plain	23:12	90.90	White oval	1.870	0.468	25.0
CSR4	Mysore	518	Plain	24:00	88.40	White dumbbell	1.860	0.410	21.9
CSR18	Mysore	525	Sex-limited	22:00	93.00	White oval	1.590	0.361	22.7
CSR19	Mysore	484	Sex-imited	22:00	92.30	White dumbbell	1.510	0.327	21.7

D denotes days; H denotes hours.

Table 2. Parthenogenetic development in bivoltine silkworm breeds

Sl. no.	Breed	No. of reddish brown/ dark pigmented eggs	No. of non-pigmented eggs	Total no. of eggs treated	% of parthenogenicity
1	CSR2	1123	1209	2332	48.15
2	CSR4	1902	899	2801	67.90
3	CSR18	2283	583	2866	79.65
4	CSR19	135	2540	2675	5.04

Table 3. Parthenogenetic development in different bivoltine F1 hybrids of the silkworm, *Bombyx mori*

Sl. no.	Hybrid	No. of reddish brown/ dark pigmented eggs	No. of non-pigmented eggs	Total no. of eggs treated	% of parthenogenicity
1	CSR2 × CSR4	841	676	1517	55.43
2	CSR4 × CSR2	667	1006	1673	39.86
3	CSR18 × CSR19	1046	855	1901	55.02
4	CSR19 × CSR18	561	1290	1851	30.30
5	CSR2 × CSR18	923	1007	1930	47.82
6	CSR18 × CSR2	1311	981	2292	57.19
7	CSR4 × CSR19	481	1195	1676	28.69
8	CSR19 × CSR4	162	1353	1515	10.69
9	CSR4 × CSR18	741	819	1560	47.50
10	CSR18 × CSR4	1496	547	2043	73.32

rence of parthenogenesis in the ovarian eggs of different bivoltine F1 hybrids. Highest percentage of parthenogenetic development was observed in CSR18 × CSR4 (73.22%) followed by CSR18 × CSR2 (57.19%) and CSR2 × CSR4 (55.43%) respectively. Least parthenogenecity percentage was found in CSR19 × CSR4 (10.69%). There was no hatching from parthenogenetic eggs of the bivoltine silkworm breeds and their F1 hybrids. Percent parthenogenetic development in bivoltine F1 hybrids is shown in Table 3.

Rearing performance of bivoltine silkworm breeds / hybrids

Average rearing performance of four bivoltine silkworm breeds and ten F1 hybrids is given in Table 4.

Fecundity: Among parental breeds, maximum fecundity was recorded in CSR 19 (563) followed by CSR2 (551) and CSR4 (528) respectively, Among the hybrids, highest fecundity was recorded in CSR2 × CSR4 (564) followed by CSR18 × CSR4 (558) and CSR18 × CSR19 (539), respectively.

Larval span: Larval span in parental breeds ranged from 486 hrs in CSR4 and CSR18 to 504 hrs in CSR2. All the bivoltine F1 hybrids showed an equal larval span of 486 hrs except CSR18 × CSR19 which showed shortest larval span of 482 hrs.

Fifth instar larval span: Minimum fifth instar larval span was observed in CSR19 (125 hrs) whereas longest 5th instar span was found in CSR2 (140 hrs). Among the F1 hybrids, 6 hybrids showed a 5th instar larval span of 126 hrs and 4 hybrids showed 120 hrs.

Yield/10,000 larvae by number: Among parental breeds, CSR18 recorded the highest yield/10,000 larvae of 9,016 followed by CSR2 (8,922) and CSR19 (8,725) respectively. Among F1 hybrids, CSR4 × CSR18 recorded highest yield/10,000 larvae of 9,733 followed by CSR18 × CSR4 (9,638) and CSR4 × CSR19 (9,411).

Yield/10,000 larvae by weight: CSR2 registered highest cocoon yield/10,000 larvae by weight of 14.480 kg followed by CSR18 (13.980 kg). Among F1 hybrids, CSR2 × CSR4 registered highest cocoon yield of 16.760 kg followed by CSR4 × CSR2 (16.360 kg) and CSR4 × CSR18 (16.270 kg) respectively.

Cocoon weight: Among bivoltine silkworm breeds, highest cocoon weight was recorded in CSR2 (1.620 g) followed by CSR18 (1.550 g). Among F1 hybrids, CSR2 × CSR4 recorded maximum cocoon weight of 1.850 g followed by CSR4 × CSR2 (1.810 g) and CSR2 × CSR18 (1.780 g) respectively.

Cocoon shell weight: Maximum cocoon shell weight was observed in CSR2 (0.380 g) followed by CSR18 (0.320 g). Among F1 hybrids, maximum cocoon shell weight was found in CSR2 × CSR4 and CSR4 × CSR2 (0.390 g) followed by CSR2 × CSR18 (0.380 g) and CSR18 × CSR2 and CSR19 × CSR4 recorded an equal cocoon shell weight of 0.370 g.

Cocoon shell ratio: Highest cocoon shell ratio of 23.46% was recorded in CSR2. Among bivoltine F1 hybrids, CSR18 × CSR19 registered highest cocoon shell ratio of 22.36% followed by CSR19 × CSR18 (21.87%). Two hybrids *viz*., CSR4 × CSR18 and 18 × CSR4 recorded an equal cocoon shell ratio of 21.68%.

Hybrid vigour studies

Heterosis over mid parent and better parent value for seven characters in different bivoltine F1 hybrids have been shown in Table 5. Maximum significant hybrid vigour for different characters was exhibited by different hybrids. CSR18 × CS19 has shown its superiority by exhibiting maximum significant hybrid vigour over mid parent value for all the seven characters whereas CSR2 × CSR4 and CSR18 × CSR4 showed significant heterosis

Table 4. Comparative rearing performance of bivoltine silkworm breeds/ F1 hybrids (Mean±SD) of 3 replicates

Sl. no.	Breed/Hybrid	Fecundity	Hatching (%)	Total larval span (h)	V instar larval span (h)	Yield / 10,000 larvae		Cocoon wt. (g)	Shell wt. (g)	Shell ratio
						By no.	By wt. (kg)			
1	CSR2	551±14	97.7±0.65	504±0.00	140±2.83	8922 ±489	14.480±1.22	1.620±0.07	0.380±0.03	23.46±0.55
2	CSR4	528±9	98.4±0.29	486±0.00	126±0.00	8466±268	12.360±0.73	1.510±0.06	0.310±0.01	20.52±0.76
3	CSR18	512±21	98.0±0.54	486±0.00	126±0.00	9016±54	13.980±0.12	1.550±0.01	0.320±0.01	20.64±0.26
4	CSR19	563±18	97.6±0.08	492±8.49	125±1.41	8725±34	11.780±0.01	1.460±0.04	0.300±0.01	20.54±0.14
5	CSR2 × CSR4	564±25	97.3±0.19	486±0.00	126±0.00	9227±68	16.760±0.57	1.850±0.04	0.390±0.01	21.08±0.14
6	CSR4 × CSR2	533±9	98.3±0.37	486±0.00	126±0.00	9077±259	16.360±0.57	1.810±0.01	0.390±0.01	21.54±0.34
7	CSR18 × CSR19	539±21	97.0±0.17	482±2.83	120±0.00	9400±135	15.100±0.31	1.610±0.01	0.360±0.00	22.36±0.11
8	CSR19 × CSR18	520±21	97.2±0.74	486±0.00	120±0.00	9189±394	14.780±0.52	1.600±0.01	0.350±0.00	21.87±0.23
9	CSR2 × CSR18	514±35	97.4±0.57	486±0.00	126±0.00	9288±354	16.200±0.11	1.780±0.01	0.380±0.01	21.34±0.26
10	CSR18 × CSR2	483±4	96.4±0.54	486±0.00	126±0.00	9172±195	15.550±0.24	1.740±0.01	0.370±0.00	21.26±0.10
11	CSR4 × CSR19	535±5	97.4±0.19	486±0.00	126±0.00	9411±232	16.110±0.35	1.750±0.05	0.360±0.00	20.57±0.50
12	CSR19 × CSR4	510±5	97.1±0.41	486±0.00	126±0.00	9266±98	15.540±0.11	1.720±0.02	0.370±0.00	21.51±0.18
13	CSR4 × CSR18	518±10	97.3±0.29	486±0.00	120±0.00	9733±98	16.270±0.11	1.660±0.03	0.360±0.03	21.68±0.23
14	CSR18 × CSR4	558±18	97.2±0.57	486±0.00	120±0.00	9638±168	15.600±0.15	1.660±0.02	0.360±0.01	21.68±0.33

Table 5. Heterosis over mid-parent (MPV) and better parent (BPV) values in bivoltine F1 hybrids

Sl. no.	Hybrid		Total larval span	5th instar larval span	Yield/10,000 larvae		Cocoon wt.	Shell wt.	Shell ratio
					By no.	By wt.			
1	CSR2 × CSR4	MPV	−1.82**	−5.26**	6.71**	24.87**	18.05**	13.59**	−3.57
		BPV	0.00	0.00	3.98	15.70**	14.02**	3.19	−9.41
2	CSR4 × CSR2	MPV	−1.82**	−5.26**	4.41	21.89**	15.44**	15.34**	0.15
		BPV	0.00	0.00	1.74	12.94**	11.49**	4.78	−5.92
3	CSR18 × CSR19	MPV	−1.43**	−4.38**	5.97*	17.20**	6.85**	13.66**	6.40**
		BPV	−0.82	−4.00**	4.26	7.98*	3.85	9.91*	5.84*
4	CSR19 × CSR18	MPV	−0.61	−4.38**	3.59	14.72**	6.25**	10.67**	3.98
		BPV	0.00	−4.00**	1.91	5.70	3.27	7.02*	3.44
5	CSR2 × CSR18	MPV	−1.82**	−5.26**	3.59	13.82**	11.98**	7.19**	−4.08
		BPV	0.00	0.00	3.02	11.87**	9.44**	−0.44	−8.91
6	CSR18 × CSR2	MPV	−1.82**	−5.26**	2.26	9.24**	9.73**	6.15	−3.03
		BPV	0.00	0.00	1.73	7.36*	7.24**	−1.42	−7.92
7	CSR4 × CSR19	MPV	−0.61	0.40	9.49**	33.46**	17.55**	18.16**	0.51
		BPV	0.00	0.00	7.87**	30.39**	15.62**	17.01**	−0.11
8	CSR19 × CSR4	MPV	−0.61	0.40	7.80**	28.75**	15.40**	22.43**	6.03**
		BPV	0.00	0.80	6.21*	25.76**	13.50**	21.24**	5.37*
9	CSR4 × CSR18	MPV	0.00	−4.76**	11.34**	23.52**	8.49**	13.00**	3.72
		BPV	0.00	−4.76**	7.95**	16.33**	7.18**	10.32**	2.55
10	CSR18 × CSR4	MPV	0.00	−4.76**	10.26**	18.43**	8.57**	14.80**	4.73*
		BPV	0.00	−4.76**	6.90*	11.54**	7.27**	12.07**	3.54

* and ** denote significantly different at 5% and 1% level, respectively.

Table 6. Uniformity test for cocoon shape in bivoltine F1 hybrids

Sl. no.	Hybrid	Coccon width (cm)	Cocoon length (cm)	Length/width ratio	Coefficient of variation (CV%)
1	CSR2 × CSR4	1.94±0.06	3.30±0.08	170.03±6.39	3.75
2	CSR4 × CSR2	2.00±0.10	3.26±0.10	163.95±8.00	4.88
3	CSR18 × CSR19	1.91±0.07	3.22±0.09	168.83±7.84	4.64
4	CSR19 × CSR18	1.84±0.08	3.20±0.10	173.68±9.42	5.42
5	CSR2 × CSR18	2.11±0.07	3.10±0.10	146.75±5.15	3.51
6	CSR18 × CSR2	2.10±0.09	3.12±0.11	149.16±6.07	4.07
7	CSR4 × CSR19	1.61±0.07	3.25±0.11	201.50±7.78	3.86
8	CSR19 × CSR4	1.63±0.06	3.27±0.11	201.00±8.45	4.20
9	CSR4 × CSR18	1.97±0.06	3.29±0.09	168.05±7.18	4.27
10	CSR18 × CSR4	1.92±0.07	3.21±0.08	166.99±7.26	4.35

Data are mean±SD of 100 cocoons.

for six characters. Highest heterosis for yield/10,000 larvae by number over mid parent value (+11.34%) and better parent value (+7.95%) was exhibited by CSR4 × CSR18. The hybrid CSR4 × CSR19 Showed maximum significant heterosis both over mid parent value (+33.48%) and better parent value (+30.39%) for yield/10,000 larvae by weight. Highest significant heterosis for cocoon weight over mid parent value (+18.05%) was exhibited by CSR2 × CSR4. The hybrid CSR19 × CSR4 exhibited maximum significant heterosis for cocoon shell weight over mid parent value (+22.43%) and better parent value (+21.24%) respectively. Maximum significant heterosis for cocoon shell ratio both over mid parent value (+6.40%) and better parent value (+5.84%) was shown by CSR18 × CSR19.

Studies on cocoon shape measurement

Cocoon shape variability in bivoltine F1 hybrids is presented in Table 6.

Cocoon width: Maximum cocoon width (2.11 cm) was observed in CSR2 × CSR18 whereas minimum 1.61 cm was found in CSR4 × CSR19.

Cocoon length: Cocoon length of 3.30 cm was found maximum in CSR2 × CSR4 and minimum 3.10 cm was noticed in CSR2 × CSR18.

Cocoon length/width ratio: Maximum cocoon length/width ratio of 201.50 was found in CSR4 × CSR19 whereas minimum 146.75 was recorded in CSR2 × CSR18.

Standard deviation and coefficient of variation: Among 10 bivoltine F1 hybrids, CSR2 × CSR18 exhibited more cocoon uniformity with 5.15 standard deviation and 3.51% coefficient of variation followed by CSR2 × CSR4 and CSR4 × CSR19 with 6.39 and 7.78 SD and 3.75 and 3.86 CV%, respectively. One hybrid CSR19 × CSR4 showed CV%, of 4.20 though its SD value more than 8, was relatively uniform in cocoon shape.

Discussion

Artificial Parthenogenesis in silkworm is of great interest for the development of hymozygous silkworm breeds with high viability, hybrid vigour, combining ability and less phenotypic variability (Ravindra Singh *et al.*, 1997; Strunnikov, 1983, 1986; Takei *et al.*, 1990). In the present study, considerable differences in parthenogenetic development in the ovarian eggs of different bivoltine silkworm breeds/hybrids have been observed. Similar attempts on parthenogenetic development in silkworm breeds have been reported (Hirokawa, 1990, 1995; Ravindra Singh *et al.*, 1994). Data in the present study revealed that maximum parthenogenesity of 79.65% was exhibited in CSR18 (Chinese type). Shinbo *et al.* (1991) have observed considerable strain differences in parthenogenetic ability and it was found that some breeds *viz.*, Ohkusa and Kokuga showed low parthenogenetic development, whereas N106 and Hinode showed relatively high (40% or more) parthenogenesis.

Manifestation of hybrid vigour was determined in bivoltine F1 hybrids involving productive and robust bivoltine breeds developed at CSRTI, Mysore (Datta *et al.*, 2000, 2001). With the demonstration of package of practices, developed under "Promotion of Popularising the Practical Bivoltine Sericulture Technology" (PPPBST), it is possible to increase bivoltine cocoon production through the rearing of productive CSR hybrids (CSR2 × CSR4 and CSR2 × CSR5) upto 65 – 70 kg/100 dfls and robust CSR hybrid (CSR18 × CSR19) upto 56 kg/100 dfls at farmers level in three southern states *viz.*, Andhra Pradesh, Karnataka and Tamil Nadu (Rajan, 2002).

Study on hybrid vigour revealed that different characters in bivoltine F1 hybrids expressed significant heterosis for various economic characteristics. It was interesting to note that the robust hybrid, CSR18 × CSR19 exhibited significant hybrid vigour over mid parent value for all the seven characters under study. Significant hybrid vigour for cocoon yield, survival rate, cocoon weight, filament length and filament size has been reported in bivoltine hybrids (Subba Rao and Sahai, 1989). Ravindra Singh *et al.* (2000) have reported significant hybrid vigour for cocoon yield/10,000 larvae by weight, cocoon weight and shell weight in bivoltine hybrids. Recently, very high significant heterosis for cocoon yield, cocoon weight and shell weight has been observed in multivoltine × bivoltine hybrids (Rao *et al.*, 2001; Ravindra Singh *et al.*, 2001a, 2001b).

Presently, the silkworm breeders are giving more emphasis on cocoon shape variability, which is very useful to identify suitable silkworm parents/hybrids. Uniform shape cocoons help to get uniform cocoon filament size and thereby make the reeling of the cocoons easier (Mano, 1994). Attempts have been made on cocoon shape variability in silkworm breeds/hybrids (Nakada, 1994; Ravindra Singh *et al.*, 1998, 2001a, 2001b).

The present study gives some information to atleast three areas of research. Firstly, it shows considerable strain differences of parthenogenetic development in some bivoltine silkworm breeds/hybrids. Secondly, hybrid vigour study demonstrated the level of hybrid vigour in different bivoltine F1 hybrids for different economic characters. Finally, the study on cocoon shape variability in bivoltine hybrids may be useful in the identification of suitable parents/hybrids for breeding/commercial exploitation.

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