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Evaluation and Selection of Potential Parents Based on Selection Indices and Isozyme Variability in Silkworm, *Bombyx mori*, L.

S. M. Moorthy*, S. K. Das, P. R. T. Rao[1], S. Raje Urs[2] and A. Sarkar

Central Sericultural Research and Training Institute, Berhampore-742 101, West Bengal, India
[1]Basic Tasar Silkworm Seed Organisation, Bilaspur-495 001, India
[2]Seri Biotech Research Laboratory, P.O. Carmelram, Kodathi, Bangalore-560 035, India

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In order to find out the appropriate parents for the breeding programme, twelve bivoltine and three multivoltine silkworm breeds were evaluated on the basis of multivariate selection index and isozyme analysis. Of which, four [CSR2, D6 (P), SK3, SK4] bivoltine and two multivoltine (Nistari, Cambodge) breeds were selected and breeding initiated to develop higher survival bivoltine silkworm breed suitable for tropical conditions. Among two isozyme (Esterase and acid phosphatase) analyzed, only esterase exhibited polymorphism among the bivoltine breeds. No polymorphism was observed among multivoltine in respect of esterase as well as acid phosphatase.

Key words: Silkworm, Esterase, Acid phosphatase, Selection index

Introduction

In the tropical belts of Eastern India, multivoltine breeds with poor economic character, but with shorter larval duration, higher survival and crop stability are dominating. On the other hand traditional bivoltine breeds having high qualitative and quantitative characters become poor, when they are reared under widely fluctuating climatic conditions of the region (Das, 1994). As a result, farmers do not generally accept these conventional bivoltine breeds/hybrids. Hence, it is felt essential to develop bivoltine breed suitable for this region through introgression of multivoltine gene(s). However, the success of developing lines depends on the selection of appropriate parents, their effective utilization in desirable combinations and choice of mating system to obtain genetic variability. Parents selection may be based on per se performance, multivariate analysis, character complementation and geographical & parental distances (Baenziger and Peterson, 1992). Similarly application of qualitative biochemical information like isozyme pattern and protein profiles is adopted for characterization in crop plants as well as in insects (Khandelwal *et al.*, 2004; Patnaik *et al.*, 1994). Eguchi and Yoshitake (1967) demonstrated allozymic variations in silkworm by electrophoresis. More information is also available in this direction in silkworm, *Bombyx mori* (Eguchi *et al.*, 1965; Egorova *et al.*, 1985; He, 1995). In the present study the parents were evaluated on the basis of per se performance, multivariate analysis and isozymes.

Materials and Methods

Twelve bivoltine and three multivoltine silkworm breeds were taken from the germplasm bank and considered for this study. The morphological and the physiological traits of the bivoltine and multivoltine breeds have been depicted in Table 1. The bivoltine breeds were reared in simulating congenial climatic conditions during March-April, August-Sep and natural climatic conditions during Nov-Dec and Feb-March for two years (Temperature: 24-28°C; Relative humidity: 65-85%) and the multivoltine breeds were reared under prevailing room temperature and relative humidity during April-May, June-July & August-September for two years (Temperature: 28-32°C; Relative humidity: 65-93%). The rearing was conducted following the standard methodology suggested by Krishnaswamy (1978). Data collected on the standard economic parameters were subjected

*To whom the correspondence addressed.
Central Sericultural Research and Training Institute, Berhampore-742 101, West Bengal, India. Tel: +91-3482-253962-213; Fax: +91-3482-250146; E-mail: moorthysm@hotmail.com

Table 1. Characteristic of bivoltine and multivoltine breeds

Breed	Evolved / collected	Voltinism	Larval marking	Cocoon colour	Cocoon shape
CSR-2	CSR&TI, Mysore, India	Bivoltine	Plain	White	Oval
CSR-4	CSR&TI, Mysore, India	Bivoltine	Plain	White	Dumbbell
SK-3	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Oval
SK-4	CSR&TI, Berhampore, India	Bivoltine	Marked	White	Dumbbell
O3	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Oval
O4	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Oval
D6(P)	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Dumbbell
D6(M)	CSR&TI, Berhampore, India	Bivoltine	Marked	White	Dumbbell
D7	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Dumbbell
Chinese (PN)	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Dumbbell
MJ2(OW)	CSR&TI, Berhampore, India	Bivoltine	Marked	White	Dumbbell
MC4(O)	CSR&TI, Berhampore, India	Bivoltine	Plain	White	Oval
Nistari (M)	CSR&TI, Berhampore, India	Multivoltine	Marked	Yellow	Spindle
NK-4	CSGRC, Hosur, India	Multivoltine	Plain	Yellow	Spindle
Cambodge	CSGRC, Hosur, India	Multivoltine	Plain	Yellow	Spindle

Table 2. Mean value of rearing performance of bivoltine breeds and ranking

Breeds	Fecundity (No)	Larval Period (days)	Larva Wt (g)	Pupation %	Yield/ 10000 Larvae (wt) (kg)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell %	Filament length (m)	Denier	Selection index	Rank
CSR-2	447	25.64	33.75	73.68	10.619	1.422	0.305	21.47	1041	2.89	93	1
CSR-4	433	24.97	39.72	54.45	6.965	1.399	0.293	21.01	1054	2.91	126	2
D6(P)	553	25.81	40.67	73.09	11.314	1.548	0.307	19.84	1047	2.86	153	3
O4	461	26.19	38.19	64.72	9.046	1.455	0.288	19.87	990	2.79	204	4
D6(M)	452	25.67	39.14	69.27	10.201	1.445	0.289	20.05	938	2.65	215	5
O3	492	25.81	38.33	72.77	10.737	1.444	0.282	19.55	970	2.74	239	6
D7	419	24.36	37.79	79.41	10.163	1.411	0.280	19.91	917	2.62	250	7
SK-3	476	25.78	32.39	84.85	11.794	1.337	0.265	19.90	995	2.77	250	8
SK-4	496	24.44	34.85	77.79	9.736	1.389	0.273	19.68	847	2.65	314	9
MC4(O)	429	25.42	37.38	72.02	10.330	1.409	0.255	18.16	892	2.64	427	10
Chinese (PN)	343	24.48	30.33	78.48	8.904	1.140	0.233	20.49	625	2.20	559	11
MJ2(OW)	410	25.11	34.52	72.72	9.283	1.299	0.216	16.63	768	2.53	758	12
CD at 5%	55.080	0.6566	1.9137	9.9132	1.5801	0.0856	0.0178	0.8319	149.6820	0.2807		
Criterion I	3	-3	3	3	3	3	3	3	3	0		
Criterion II	7	6	7	8	8	9	10	10	10	5		

for ANOVA and multivariate analysis (Smith, 1936) based on the criterion I and II for ranking of the breeds. While ranking, the character *viz.*, single cocoon shell weight (SCSW), shell %, filament length for bivoltine and pupation % and yield/10000 Larvae -Wt (kg) for multivoltine were given equal and more weightage (Table 2,3). The selected bivoltine breeds were crossed with multivoltine breed and prepared eight (F_1) bi×multi combinations, which were reared during the adverse (May-June) season (Temperature: 28-33°C; Relative humidity: 71-93%). The heterosis was estimated using the formula suggested by Rai (1979). Gel electrophoresis study of esterase and acid phosphatase isozyme was done in the haemolymph of silkworms following the methodology suggested by Harris and Hopkinson (1977) and Eguchi *et al.* (1988) respectively.

Result and Discussion

It is well accepted that success of breeding depends on

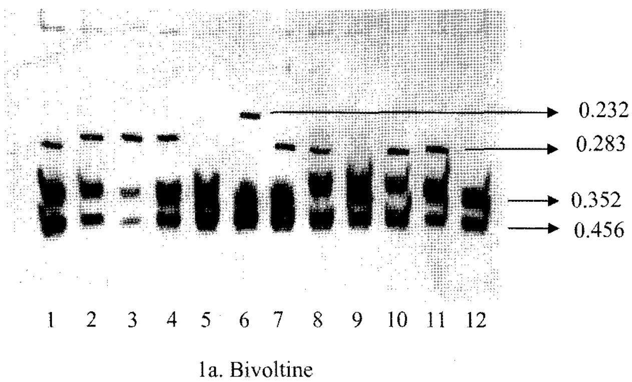
Table 3. Mean value of rearing performance of multivoltine breeds and ranking

Breeds	Fecundity (No)	Larval Period (days)	Larval Wt (g)	Pupation %	Yield 10000 Larvae (wt) (kg)	Single Cocoon Weight (g)	Single Shell Weight (g)	Shell %	Selection index	Rank
Nistari (M)	321	23.27	23.18	89.69	8.755	1.010	0.126	12.51	50	1
Cambodge	370	23.71	26.51	73.65	8.097	1.102	0.148	13.49	257	2
NK4	358	23.63	26.31	73.42	7.948	1.132	0.144	12.73	291	3
CD at 5%	23.8	0.1887	1.318	8.307	NS	0.050	0.009	0.528		
Criterion I	3	−3	3	3	3	3	3	3		
Criterion II	7	9	8	10	10	9	6	6		

choice of parents, mating design and appropriate selection procedure. An evaluation of genotypes for diversity and yielding capacity are the prerequisite for any systemic breeding programme. Such an evaluation may be from the phenotypic performance - a product of genetic potential and environment (Ram and Lal, 2002). Further in reality existence of high genetic variability in economic characters is obviously a resource for breeding (Frankel and Brown, 1983). The present investigation also revealed highly significant variation for all the characters among the bivoltine breeds (Table 2) and also among multivoltine breeds except yield /10,000 larvae (wt) (Table 3). In this investigation the bivoltine were reared in congenial climatic conditions, while multivoltine were reared in adverse climatic condition in order to identify the potential productive bivoltine and high survival multivoltine silkworm breeds. Because, it is essential to measure the degree of phenotypic manifestation for the characters of economic importance under similar environmental conditions in order to understand the genetic endowment pertaining to adaptability and productivity of the breeding material (Nirmal kumar and Sreerama Reddy, 1994). According to Falconer (1952) the improvement obtained by selection under favourable conditions will not help in realizing the full potential when the selected strain is transferred to unfavourable conditions. Keeping this view in this experiment bivoltine breeds were reared in uniform climatic conditions not in varied climatic conditions as well as multivoltine were in adverse climatic conditions only to understand the potentiality of the breeds.

Gel electrophoresis of proteins and isozymes are powerful tool to distinguish between genotypes (Cooke, R., 1995). Esterase isozyme is widely used in polymorphism studies because of their high frequency of genetic variants detected in insect populations (Selander, 1976). The esterase isozyme pattern revealed six bands (Fig. 1a) with different relative motilities (0.232 - 0.456) in the bivoltine, where as two bands (Fig. 2a) in acid phosphatase (0.434, 0.464).

However, in multivoltine, the esterase isozyme (Fig. 1b) pattern showed three bands (0.278, 0.374, 0.452) and acid phosphatase (Fig. 2b) with one band (0.304). The existence of polymorphism in the esterase isozyme pattern is clearly indicating genetic diversity among the bivoltine breeds. Egorova *et al.* (1985) also used polymorphic sys-



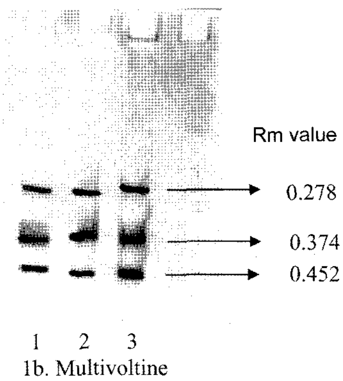
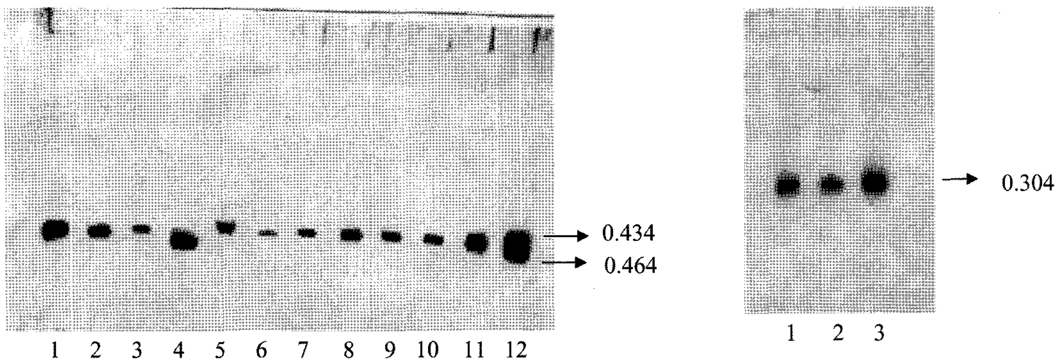


Fig. 1. Esterase isozyme pattern in the haemolymph of *Bombyx mori* L:
(a) 1. CSR2; 2. CSR4; 3. O3; 4. O4; 5. SK3; 6. SK4; 7. MJ2; 8. MC4 (O); 9. D6 (M); 10. D6 (P); 11. D7; 12. Chinese (PN).
(b) 1. Nistari; 2. NK4; 3. Cambodge



2a. Bivoltine 2 b. Multivoltine

Fig. 2. Acid phosphatase isozyme pattern in the haemolymph of *Bombyx mori* L:
(a) 1.CSR2; 2. CSR4; 3. O3; 4. O4; 5. SK3; 6. SK4; 7. MJ2; 8. MC4 (O); 9. D6 (M); 10. D6 (P); 11. D7; 12. Chinese (PN).
(b) 1. Nistari; 2. NK4; 3. Cambodge.

tem of silkworm haemolymph esterase as a criterion in the parent selection.

Various methods are being studied in order to judge the superiority of the breeds to the common index. Such selection indices are regularly used in plant and animal breeding programme (Smith, 1936; Arunachalam and Bandopadhyay, 1984), which make possible to select for a character by selecting simultaneously for two or more characters related to it and giving appropriate weightage to different characters. Considering the correlation between shell weight, shell% and filament length (Singh *et al.*, 1998; Kumarasan *et al.*, 2000), for selecting productive bivoltine the important silk contributing parameters *viz.*, shell weight, shell percentage and filament length was taken and given equal as well as more weightage and giving less weightage to other characters (Moorthy *et al.*, 2006). But for selecting healthy multivoltine parents, pupation (%) and yield/10000Larve (wt-kg) were considered, and given equal as well as more weightage to these parameters. As because in order to breed the varieties with good performance of comprehensive characters, complementing in defects and advantages of both parents should be considered in selecting the crossing parents.

Finally on the basis of ranking, CSR-2 (Oval) and D6(P) (Dumbbell) were selected as a productive bivoltine , both were superior than others in shell weight, shell percentage

Table 4. Mean performance of the parent and hybrids

Breed / Combination	Fecundity (No)	Larval period (days)	Pupation %	Yield / 10000 Lar. (Wt-kg)	SCW (g)	SSW (g)	Shell %
CSR2 × Nistari	349	19.41	79.85	10.768	1.420	0.260	18.31
CSR2 × Cambodge	345	19.25	79.39	9.340	1.255	0.219	17.45
D6(P) × Nistari	339	19.66	79.07	11.717	1.516	0.274	18.08
D6(P) × Cambodge	338	19.83	77.25	11.508	1.497	0.255	16.99
SK3 × Nistari	460	19.41	83.39	11.052	1.403	0.238	16.92
SK3 ×Cambodge	424	19.08	79.17	10.220	1.333	0.220	16.53
SK4 ×Nistari	434	19.50	86.86	12.218	1.273	0.224	17.57
SK4 ×Cambodge	445	19.41	89.43	11.550	1.223	0.210	17.19
Bivoltine parent							
CSR2	351	21.12	13.37	1.619	1.547	0.304	19.68
D6(P)	408	22.66	12.00	0.180	1.430	0.293	20.48
SK3	424	19.83	17.60	1.500	1.237	0.241	19.47
SK4	489	20.33	21.13	2.403	1.151	0.209	18.15
Multivoltine parent							
Nistari	359	19.66	76.09	6.077	0.809	0.109	13.52
Cambodge	377	20.00	55.83	4.495	0.919	0.116	12.65

Table 4a. Heterosis percentage in hybrids

Hybrids	Fecundity (No)		Larval period (Days)		Pupation %		Yield / 10000 L (Wt-kg)	
	MPH	BPH	MPH	BPH	MPH	BPH	MPH	BPH
CSR2 × Nistari	−1.59	−2.78	−4.80**	−8.09**	78.50**	4.94	179.83**	77.20**
CSR2 × Cambodge	−16.62*	−27.65**	−6.38**	−8.88**	129.42**	42.19**	205.48**	107.76**
D6(P) × Nistari	−11.51	−16.75*	−7.09**	−13.24**	102.53**	3.93	274.55**	92.81**
D6(P) × Cambodge	−23.57**	−29.12**	−7.03**	−12.50**	167.16**	38.36**	392.31**	155.99**
SK3 × Nistari	17.52*	8.56	−1.68	−2.10	78.01**	9.60	191.74**	81.87**
SK3 × Cambodge	−5.88	−11.10	−4.18**	−4.58**	115.62**	41.81**	240.95**	127.35**
SK4 × Nistari	2.32	−11.24	−2.50**	−4.09**	78.69**	14.16	188.16**	101.06**
SK4 × Cambodge	−7.79	−8.92	−3.72**	−4.51**	132.40**	60.19**	234.88**	156.93**

Hybrids	SCW (g)		SSW (g)		Shell %	
	MPH	BPH	MPH	BPH	MPH	BPH
CSR2 × Nistari	20.57**	−8.17**	25.81**	−14.57**	10.28**	−6.97**
CSR2 × Cambodge	1.78	−18.86**	4.12	−28.04**	7.94**	−11.35**
D6(P) × Nistari	35.39**	6.01	36.48**	−6.37	6.38*	−11.68**
D6(P) × Cambodge	27.44**	4.68	24.43**	−13.08**	2.58	−17.03**
SK3 × Nistari	37.13**	13.42**	35.81**	−1.38	2.54	−13.13**
SK3 × Cambodge	23.65**	7.76*	23.32**	−8.58	2.93	−15.11**
SK4 × Nistari	29.85**	10.57**	40.67**	7.09	10.96**	−3.19
SK4 × Cambodge	18.16**	6.25	29.30**	0.64	11.64**	−5.28

**Significant at 1% level
*Significant at 5% level

and filament length (Table 2). Also these breeds were developed from Chinese × Chinese and Japanese × Japanese hybrids confirming their ability to provide better silk. Similarly SK-3 (Chinese origin with oval) and SK-4 (Japanese origin, Dumbbell type), the other two bivoltine were also selected as parents due to their geographical origin, even though they have not faired well in ranking, but it is well known that distant blood relation gives better heterosis. The esterase isozyme pattern also proved genetic diversity among the selected bivoltine and judging the selection. On the other hand, in multivoltine, Nistari along with Cambodge (Table 3) have been selected as initial parents. Nistari is better known for adaptability to this region (Das, 1994) proving the result of selection. But, cambodge the other selected multivoltine, compared with other breed NK4, it is showing better performance. However, isozyme pattern did not reveal any polymorphism among the multivoltine breeds indicating narrow genetic base among multivoltine, yet they showed the differences in the quantitative traits. Kumaresan *et al.* (2000) also selected some best parental silkworm strains of multivoltine based on the variability in quantitative traits and selection indices. Further the selected bivoltine and multivoltine parents were crossed and prepared eight Bi × Multi combinations. In this study Bi × Multi crosses was considered as a breeding material to develop bivoltine breed suitable for tropical region as suggested by He and Oshiki (1984). They reported that resistance to adverse environment was greater in this form. The performance of the selected parents, hybrids (Bi × Multi) and their heterosis values are presented in the Table 4a. All the hybrids were shown highly significant heterosis values for larval period, pupation%, yield/10000larvae (wt), cocoon weight and shell weight except SK3 × Nistari for larval period, CSR2 × Cambodge for cocoon weight and shell weight. Generally, in a good environment the silkworm parental strains register high silk yield and its attributes (Ueda *et al.*, 1969; Rapussas and Gabriel, 1976). As a result, mid parent value (MPV) is close to hybrid mean values with a little difference (Nagaraju *et al.*, 1996, Rao *et al.*, 1998). However in our study highly significant heterosis was observed for most of the characters, because when the parental strains and the hybrids are raised in an adverse environment, the mean of the hybrids will be much higher than the mean of the parental strains (Nagaraju *et al*, 1996). In such cases the heterosis over both MPV and better parent values are higher (Cunningham, 1987). In conclusion, it can be inferred that this study helped to identify the genetic potential of the breed and to obtain a wide genetic base with acceptable levels of viability and productivity, which can yield superior segregants enabling effective selection during the course of evolution of lines suitable for tropical regions.

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