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Development of a new multivoltine breed (D^{+p}) of Silkworm (Bombyx *mori* L) having better heterosis of its Economic Characters in the climate of West Bengal, India

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ABSTRACT

A new superior multivoltine breed of *Bombyx mori* L. (D^{+p}) has been developed by crossing between two strains of Nistari Plain (N^{p}) and Nistari Marked (N^{+p}) which are the two most preferred strains of multivoltine breed at the farmer's level of eastern India. Through standard breeding plan this need-based new breed, D^{+p} was developed by adopting three steps: i) Development of Recurrent Backcross Line through backcross breeding for initial eight generations; ii) Improvement of developed RBL following directional selection process for the next twenty generations and ultimately iii) stabilization for next eight generations upto F40 generation at normal environmental conditions. Study of interrelations among twelve quantitative characters related to viability and productivity of the new breed have been evaluated statistically and Heterosis % are also determined. An improved multivoltine breed, D^{+p} suitable for West Bengal climatic conditions was developed having higher heterosis % in respect of single shell weight, yield/100 dfls, fecundity, single cocoon weight, reelability, raw silk, shell percentage and filament length.

Keywords: New Breed, Nistari (D^{+p}), Multivoltine, RBL, Heterosis

1. Introduction

In Bengal delta, the priority should be given to the evolution of multivoltine breeds which are basically of tropical origin, having higher tolerance to climatic vagaries and can produce quality silk. Bivoltine breeds although produce better quality silk under temperate condition it shows lower tolerance and low adaptability in tropical situation [1] and hence is a major impediment to bivoltine sericulture in tropical country. Since 1970, different workers [2, 3, 4, 5, 6, 7] have attempted to overcome problems by raising various multi x bi hybrids and used them at field level for identifying season and region specific hybrids. Again, an attempt has been made by [8, 9, 10] for developing different syngenic lines and congenic breeds of multivoltine with high cocoon shell weight and bivoltine with higher survival.

Indian silk industry especially in the eastern region basically rests on multivoltine and only indigenous multivoltine races and their hybrids were reared for commercial silk production [11] till 1970s. Even today more than 90% of silk produced in India is either from multivoltine x multivoltine or from multivoltine (multi) x bivoltine (bi) hybrids. Though the indigenous races are well acclimatized in fluctuating eco-climatic conditions, they are poor in silk production. So, a number of attempts have been made to improve indigenous races through hybridization with exotic races. However, use of bivoltine breeds as breeding material to improve economic characters over multivoltine breeds has to compromise with the robustness as well as on enhanced number of hibernating eggs [12].

Previously different silkworm breeders tried to develop new multivoltine breeds by crossing multivoltine with exotic bivoltine breeds which were rejected due to poor survivability, occasional production of hibernating eggs and deterioration of heterosis in West Bengal climatic conditions. Hence, there was an urgent requirement for productive multivoltine silkworm races to suit the agro-climatic conditions prevailing in West Bengal. In this direction we had selected two most trusted and stable multivoltine eco-races of Nistari, as parents i.e. N^{+p} and N^{p} which had different quantitative characters and were maintained at Debra Sericulture Complex, Government of West Bengal, Paschim Medenipur.

Application of qualitative biochemical information like isozyme pattern is adopted for characterization in crop plants as well as insects [13, 14, 15, 16] demonstrated allozymic variations in silkworm by electrophoresis. More information is also available in this direction in silkworm, *Bombyx mori* [17, 18, 19]. Gel electrophoresis of proteins and isozymes are powerful tool to distinguish between genotypes [20]. Esterase isozyme is widely used in polymorphism studies because of their high frequency of genetic variants detected in insect populations [21].

According to Mukherjee [22] heterosis has two general modes of expression viz. increase in size, probably due to faster rate of cell activity and cell division and increase in 'biological efficiency' such as reproductive rate and survivality.

Hybridization followed by appropriate selection is a potent experimental procedure to bring together the economic characters of choice from defined sources and to synthesize genotypes of desirable constitution and expression. Hybridization of silkworm races with different genetic constitution can be made many times either by backcrossing or by out-crossing depending on the character that one wants to improve in the breed. The increased productivity of the heterozygotes, and resistance to biotic and abiotic stresses [23], is exploited through the development of hybrid varieties in several crop and animal species [24, 25] and historically it represented one of the most revolutionary advancements in silkworm improvement [26, 27, 28, 29].

2. Materials and Methods

In our present study initially we took Nistari plain race, N^p as mother as it had higher fecundity (426.0 number), survivality percentage (82.4 %), cocoon yield (31.5 kg/ 100dfls), pupation rate percentage (86.04 %), but it was poor in reeling parameters like shell weight (10.6 cg), shell percentage (11.91 %), filament length (378.3 m) and raw silk percentage (9.44 %). Again, Nistari marked race, N^{+p} was selected as father as it had better shell weight (12.9 cg), shell percentage (12.39%) and other reeling parameters (e.g. filament length 405.7 m, raw silk percentage 10.57 %). In our present study initially we took N^p as mother (receiver) and then backcrossed with N^{+p} (donor), for eight successive generations for introgression of higher reeling characters into the new breed and for that a recurrent backcross line (RBL) was developed from generation-1 to generation- 8 during first year [30].

Table 1: Average performance of quantitative traits of parent's i.e. Nistari plain (N^p) and Nistari marked (N^{+p}) (Thangavalu *et al.*, 1994).

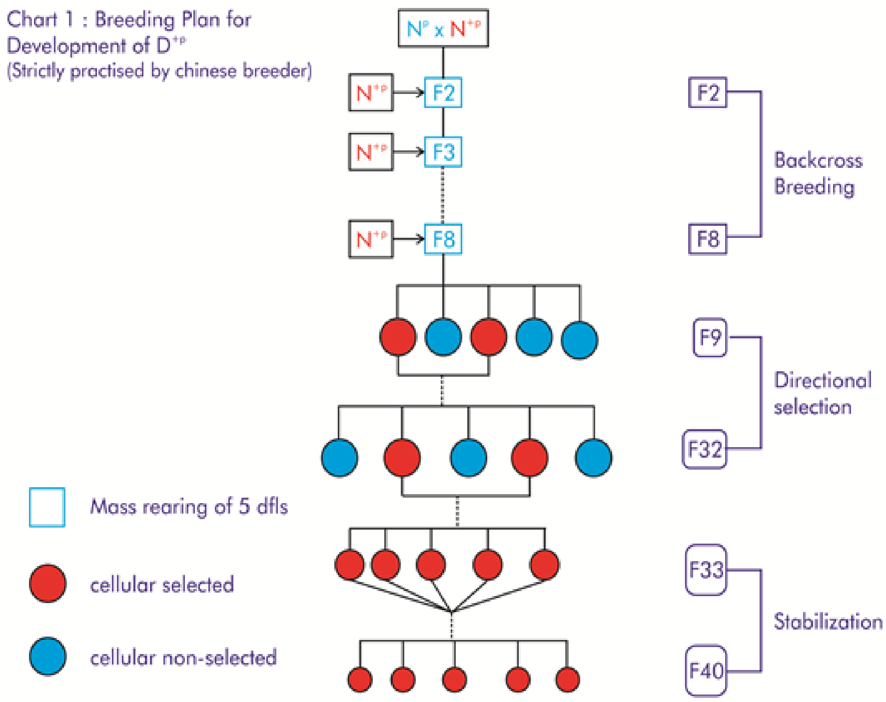
Sl.No.	**Characters**	**Nistari(N^p)**	**Nistari(N^{+p})**
1	Fecundity(numbers)	426	376
2	Hatching (percentage)	88.80	90.05
3	Weight of 10 matured larvae (g)	20.58	19.64
4	E.R.R (percentage)/ Survival (percentage)	82.40	77. 30
5	Yield per 100 d.f.ls (kg)	31.5	27.5
6	Pupation rate (percentage)	86.04	81.44
7	Single cocoon weight (g)	0.890	1.042
8	Single shell weight (cg)	10.60	12.75
9	Shell (percentage)	11.91	12.39
10	Filament length (m)	378. 3	405.7
11	Raw Silk (percentage)	09.44	10. 57
12	Reelability (percentage)	83.65	83.10

All the rearings were performed within natural environmental conditions of the rearing room, where we had collected the most important twelve expressed quantitative characters like fecundity (numbers), hatching percentage, weight of ten matured larvae (g), ERR percentage, pupation rate (percentage), yield/100dfls (kg), cocoon weight (g), shell weight (cg), shell percentage, filament length (m), reelability percentage and raw silk percentage.

Subsequently, as per modern breeding methods, through directional selection for twenty four generations (i.e. from generation-9 to generation-32) in the year 2nd,3rd and 4th year, we tried to improve the quantitative characters by giving selection pressure on the above mentioned twelve quantitative characters. During last eight generations for stabilization (i.e. generation-33 to generation-40) the rearing was done without imposing any selection pressure on these 12 characters [31].

In our present experiment we studied the genetic expression of twelve multivoltine quantitative characters of egg, larva, and cocoon and post cocoon stages at each generation, by providing scientific rearing techniques with improved leaves (S_{1635}), in relation to prevailing four major abiotic factors like maximum and minimum temperature as well as humidity for five years within rearing room. The relation between twelve quantitative expressed characters and four abiotic factors, their season wise performance in five years was studied through ANOVA and the values were assessed at 5 % level. Again, correlation coefficient among the expression of twelve silkworm quantitative characters and studies on heterosis percentage over mid-parent values (MPH) and better-parent values (BPH) of the developed breed were also calculated. Finally for confirming phenotypic improvement of the newly developed breed genetically, an isozyme study of haemolymph of both parents and the newly developed breed through esterase and acid phosphatase isozyme pattern was assessed [32].

Breeding Schemes:



3. Results and Discussion

3.1. Fecundity

While studying the correlations among twelve economic parameters it has been observed that fecundity (*P*<0.0001) correlated with larval weight (0.5935), yield of cocoons (0.7475) and single cocoon weight (0.5911). Studies on expression of heterosis of fecundity revealed that the heterosis over BPV (better parent value) and MPV (mid parent value) were recorded as 13.30 % and 21.20 % respectively. This improvement ranked fifth place among 12 quantitative characters in respect of BPH.

Table 2: Correlation Coefficient among twelve Quantitative Traits of Developed breed (D^{+p}) of Silkworm, *Bombyx mori* L.

	1. Fecundity	2. Hatching%	3. Larval wt.	4. ERR%	5.Yield /100dfls	6. Pupation rate%	7. S.C.wt.	8. S.S.wt.	9. Shell%	10. Fl. length	11. Raw Silk%	12. Reela-bility%
1.Fecundity	X											
2.Hatching%	0.2451 (0.3919)	X										
3.Larval wt.	0.5935 (<0.0001)	0.3881 (0.0437)	X									
4.ERR%	0.1823 (0.1078)	0.6192 (<0.0001)	0.5975 (<0.0001)	X								
5.Yield /100dfls	0.7475 (<0.0001)	0.6533 (<0.0001)	0.6061 (<0.0001)	0.5058 (<0.0001)	X							
6.Pupation rate%	0.2003 (0.0325)	0.5966 (<0.0001)	0.6112 (<0.0001)	0.7602 (<0.0001)	0.6093 (<0.0001)	X						
7.S.C.wt.	0.5911 (<0.0001)	0.1098 (0.5201)	0.5338 (<0.0001)	0.0991 (0.3314)	0.5772 (<0.0001)	0.6778 (<0.0001)	X					
8.S.S.wt.	0.1070 (0.2331)	0.0720 (0.6033)	0.1199 (0.5272)	0.2218 (0.4714)	-0.2228 (0.6516)	0.4183 (0.2591)	0.3340 (0.0150)	X				
9.Shell%	-0.1685 (0.5551)	-0.1115 (0.7781)	-0.2418 (0.7164)	0.0758 (0.5646)	-0.3096 (0.7955)	-0.2984 (0.1566)	-0.3104 (0.0512)	0.6011 (<0.0001)	X			
10.Fl. -length	0.0108 (0.6261)	0.4193 (0.0493)	0.0933 (0.3885)	0.3847 (0.2142)	0.1205 (0.4633)	0.0105 (0.5612)	0.5804 (<0.0001)	0.6881 (<0.0001)	0.6185 (<0.0001)	X		
11.Raw Silk%	-0.1090 (0.4366)	-0.3070 (0.4451)	-0.3002 (0.2311)	0.3371 (0.4017)	-0.0104 (0.5212)	-0.2071 (0.3312)	-0.3272 (0.1526)	0.6775 (<0.0001)	0.5508 (<0.0001)	0.7061 (<0.0001)	X	
12.Reela-bility%	0.0719 (0.1901)	0.3370 (0.4314)	0.0869 (0.5269)	0.5370 (<0.0001)	0.1322 (0.2451)	0.0369 (0.5015)	0.0637 (0.8651)	0.4036 (0.8029)	0.1336 (0.3096)	0.4221 (0.0172)	0.3754 (0.1288)	X

Note: Data in parenthesis are ‘p’ values.

3.2. Hatching percentage
However, no significant improvement of hatching percentage was observed during 40 generation of studies and accordingly the heterosis over BPV (better parent value) and MPV (mid parent value) were recorded as 1.39 % and 2.09 % respectively, which was very low.

3.3. Higher matured larval weight (10 numbers):
This character correlated with survival percentage (0.5975), yield/100dfls (0.6061), pupation rate percentage (0.6112) and single cocoon weight (0.5338) significantly at 1% level and as a result improvements in terms of heterosis was observed in the tune of 14.82% and 17.50% in BPH and MPH respectively. This improvement ranked third place among twelve quantitative characters in respect of BPH.

3.4. Survival percentage:
It helped to increase the yield of the cocoons (0.5058), pupation rate percentage (0.7602) and reelability percentage (0.5370) significantly but BPH and MPH were recorded only as 2.71 % and 5.99 % respectively in terms of heterosis was concerned.

3.5. Yield/100 dfls
However, fecundity (0.7475), hatching percentage (0.6533), larval weight (0.6061), survival % (0.5058), increased pupation rate percentage (0.6093) and single cocoon weight (0.5772) also help to increase the yield/100 dfls significantly at 1% level. Due to positive effect of all these factors, heterosis was recorded 23.21 % and 31.56 % respectively in terms of BPH and MPH values. This improvement ranked second place among twelve quantitative characters in respect of BPH

3.6. Pupation rate percentage
Statistical data also revealed that different rearing parameters like hatching percentage (0.5966), matured larval weight (0.6112), ERR percentage (0.7602), yield/100dfls (0.6093) and single cocoon weight (0.6778) helped to increase the higher pupation rate percentage significantly (*P*<0.0001). As the parents were tropical in origin, much improvement could not be obtained in pupation rate percentage and as a result the BPH and MPH values were recorded as 0.81 % and 3.58 % respectively.

3.7. Single cocoon weight
This character was positively correlated with filament length (0.5804) at 1 % level. So, heterosis percentage of single cocoon weight was recorded as 11.90 % and 20.70 % in case of BPH and MPH respectively. This improvement ranked fifth place among twelve quantitative characters in respect of BPH.

3.8. Single shell weight
In our experiment maximum temperature (32.9 °C) and maximum humidity (16.2 °C) during winter might have considerable significant influence to get higher single shell weight. Statistical data also revealed that higher single shell weight helps to increase the shell percentage (0.6011), filament length (0.6881) and raw silk percentage (0.6775) significantly (*P*<0.0001). So, it can be stated that the single shell weight increased and stabilized significantly from the parental value after forty generations and this improvement of character was recorded as heterosis of 24.71 % and 36.13 % in case of BPH and MPH respectively which was the percentage-wise highest improvement in the present study.

Table 3: Heterosis percentage over mid and better parent values (MPH & BPH) of newly developed breed (D^{+p}).

S. No	Traits	MPV	BPV	D^{+p}	MPH	BPH
1	Fecundity(numbers)	401	426	486.27	21.20	13.30
2	Hatching (percentage)	89.43	90.05	91.30	2.09	1.39
3	10 larval wt.(g)	20.11	20.58	23.63	17.50	14.82
4	E.R.R/Survival (percentage)	79.85	82.40	84.63	5.99	2.71
5	Yield/100 dfls (kg)	29.50	31.50	38.81	31.56	23.21
6	Pupation rate (percentage)	83.74	86.04	86.73	3.58	0.81
7	Single Cocoon wt.(g)	0.966	1.042	1.166	20.70	11.90
8	Single School wt. (cg)	11.68	12.75	15.90	36.13	24.71
9	Shell (percentage)	12.15	12.39	13.63	12.18	10.01
10	Filament length (m)	392	405.7	429.6	9.59	5.89
11	Raw Silk (percentage)	10.01	10.57	11.68	16.58	10.41
12	Reelability (percentage)	82.38	83.65	92.82	12.67	10.96

Note: BPV-Better parent values, **MPV-** Mid parent values

3.9. Shell percentage:
Again statistical data on correlation of quantitative characters revealed that Shell percentage helped to increase single shell weight (0.6011), filament length (0.6185) and raw silk percentage (0.5508) significantly (*P*<0.0001). So it can be revealed that the Shell percentage had significant improvement during this breeding process and after forty generations this value become fixed in the newly developed breed where the heterosis over BPH and MPH values were recorded as 10.01 % and 12.18 % respectively. This improvement ranked eighth place among twelve quantitative characters in respect of BPH.

3.10. Higher filament length:
This character had significant correlation with single cocoon weight (0.5804), shell weight (0.6881), Shell percentage (0.6184) and raw silk percentage (0.7061) at 1 % level. Here the BHP and MPH values were recorded as 5.89% and 9.59 % respectively and this improvement ranked ninth place among twelve quantitative

characters in respect of BPH.

3.11. Raw silk percentage

It also had significant correlation with single shell weight (0.6775), Shell percentage (0.5508) and filament length (0.7061) at 1 % level. So, it can be revealed that the raw silk percentage had significant improvement during this breeding process and after forty generations this value became fixed in the newly developed breed where heterosis percentage of better parent and mid parent value were calculated as 10.41 % and 16.58 % respectively. This improvement ranked seventh place among twelve quantitative characters in respect of BPH.

3.12. Reelability

This can be revealed that the reelability had significant improvement during this breeding process after forty generations and this value became fixed in the newly developed breed with the improvement of heterosis percentage of parents of 10.96 % and 12.67 % in BPH and MPH respectively. This improvement ranked sixth place among twelve quantitative characters in respect of BPH. Finally following modern breeding technique we got a new multivoltine breed i.e. D^{+p}, which having interacted with natural environmental factors expressed 12 quantitative silkworm breed characters, which were expressed according to prevailing environmental factors in West Bengal eco-zone. To know whether these quantitative improvements in the new breed (D^{+p}) were phenotypically or genotypically different, an isoenzyme study of both parents and newly developed breed were undertaken by gel electrophoresis of esterase and acid phosphatase. The existence of polymorphism in the acid phosphatase isozyme pattern clearly indicated genetic diversity in the new breed (D^{+p}) from its parents [32].

4. Conclusion

Thus, an improved multivoltine breed, D^{+p} suitable for West Bengal climatic conditions was developed having higher heterosis % in respect of single shell weight, yield/100 dfls, fecundity, single cocoon weight, reelability, raw silk, shell percentage and filament length and these economic characters have a significant influence among each other.

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