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Preliminary Studies on the Role of α-Esterase Isozymes in Quantitative Traits of Two Multivoltine Silkworm (*Bombyx mori* L.) Races and their F_1 Hybrid

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Heterosis was studied involving two multivoltine silkworm breeds *viz*, APM1 and SLKSPM through rearing and isozyme analysis. A positive significant heterotic effect was observed in fecundity, hatching % and survivability. The heterobeltiosis was observed only in fecundity and hatching %. Isozyme analysis of α-esterase showed variation in loci and allelic expression. The allele with heterozygosity (Est-2^{12}) was observed at the Est-2 locus in F_1 progeny. Est-3 was observed in F_1 progeny, whereas it was completely absent in both parental lines. The present study suggests that the markers (Est-2^{12} and Est-3) targeted for introgression may be useful for the improvement of fecundity and survivability as the phenomenon of heterosis was observed only in F_1 progeny.

Key words: Heterosis, Isozyme, Quantitative traits, Multivoltine, Silkworm races

Introduction

The silkworm breeders are always been concerned in choosing the parental lines which would result in higher heterotic combination without necessarily making all possible crosses among the parents. To predict heterosis, *per se* performance of parents, combining ability, genetic distances and biochemical markers are being used (Virmani, 1994). A trait can respond to selection only when it has heritable genetic variation and is not constrained by genetic correlations with other traits under selection (Falconer and Mackay, 1996). The efficiency of hybrid breeding programme could be increased if superior crosses could be predicted based on a screening of parent inbred lines using biochemical markers. The isozymes marker are generally stable, codominant and not influenced by pleiotrophy and their variations are due to amino acid sequence differences and could be generally parallel to nucleotide sequence changes of DNA of the isozymes genes (Weeden and Wendel, 1989). Esterase isozymes are genetically determined by several loci, and a high frequency of genetic variants is usually detected in insect populations (Selander, 1976). In silkworm *Bombyx mori*, esterases have been studied from the standpoint of genetics. Polymorphism of esterase isozymes observed by their differential mobility on electrophoresis was found in haemolymph, silk glands, fat bodies, midguts, eggs and integuments (Yoshitake and Eguchi, 1965; Yoshitake and Akiyama, 1965; Eguchi *et al*., 1965; Eguchi and Yoshitake, 1967). In the present investigation a preliminary attempt has been made to identify an esterase isozyme marker for predicting heterosis of quantitative traits by using two different multivoltine breeds having different characters.

Materials and Methods

Two multivoltine silkworm breeds *viz.*, APM1 and SLKSPM from the gene bank of Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, India were selected. The characteristic features of the both parental lines are given in Table 1. These parental lines are, a race with carrying *pnd*$^+$ gene (Pigmented non-diapause) highly productive (Donor) and the sex-limited race (in larvae) with low productive (Receptor), so that it could be easy to predict the heterotic effects on their hybrid with linked

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Table 1. Characteristic features of parental silkworm breeds used for the study

Breed	Characteristics feature
SLKSPM	This is the sex-limited breed in larval markings evolved using Pure Mysore as one of the parent by KSSR&DI, Thalaghattapura, Karnataka, India. The cocoons of SLKSPM are oval in shape and greenish yellow in colour. The special characteristic feature of breed is having longer larval duration. It is low productive and spins poor quality silk fiber.
APM1	The eggs laid by the APM_1 are pigmented in nature controlled by the gene $^{pnd+}$ (pigmented non diapause). The cocoons of APM1 are oval in shape and greenish yellow in colour. The breed is one of the multivoltine parent of popular hybrid Swarnandhra ($APM_1 \times APS_8$). It is an elite multivoltine inbred line highly productive and having larval duration, which was evolved using Madagascar hybrid as a donor parental genetic material by APSSR&DI, Andhra Pradesh, India.

genetic markers. In silkworm *B. mori* the pnd^+ gene specific protein is involved in the process of diapause determination, which acts after fertilization, it was observed in diapause and HCL treated diapause eggs but not in non-diapause eggs (Suzuki *et al.*, 1999). The strain SLKSPM was used as a female parent with APM_1 as male counterpart to produce F_1 progeny. The F_2 progeny was obtained by self-matting of F_1 progeny. The rearing of parental lines and F_1 progeny as well F_2 was conducted during favorable season October - December 2005 following method described by Krishnaswamy (1978). The data of 12 important economic traits were recorded from the rearing of parental lines and their F_1 progeny and analyzed statistically for heterosis parameters by using the Indostat Statistical Computer Packages, Hyderabad, India. Heterosis was estimated using the formula described by Rai (1979).

Synchronous eggs (100 mg) collected from 5 individual disease free layings (dfls) of parental lines, their F_1 and F_2 progenies were used. The eggs were homogenized in 1 ml of extraction buffer (PBS 7.2 PH) and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was subjected to electrophoresis as described by Laemmli (1970) on 10% native polyacrylamide gel electrophoresis (PAGE). The gels were incubated for α-esterases (E.C.3.1.1.1) for 30 minutes at 37°C (Richardson *et al.*, 1986). The stained gels were visualized under a bright illuminator and photographed using Kodak DC4800 camera in gel documentation system (Biovis, India). The isozymes were numbered in the ascending order from the anode. The locus that specifies the isozyme with the least anodal migration was labeled as 1, the next as 2 and 3 and their alleles were designated as ^{1,2,3} etc.

Results and Discussion

Mean performance for 12 economic parameters of parental lines and their hybrid is presented in Table 2. The pooled ANOVA revealed that the interaction between the hybrid and parents indicated significant variation in fecundity, hatching, larval weight, pupation rate and shell ratio. In the present study, the positive significant hybrid vigour was observed in fecundity, hatching % and pupation rate. The heterobeltiosis was observed only in fecundity and hatching %. The hybrid vigour in fecundity is found to be higher than both parental lines. Besides, the heterosis could be noticed in maximum number of characters, which are found to be better than their female partner. It was well established that the positive heterosis over the mid-parent and better parent are due to dominant or over dominant gene(s) expression of a character (Harada, 1961). The results corroborates the earlier reports of heterosis parameters in silkworm *B. mori* (Bhargava *et al.*, 1993; Banuprakash *et al.*, 1994; Das *et al.*, 1994; Rao *et al.*, 1998; Kumaresan *et al.*, 2003).

During the course of α-esterase isozyme study, polymorphism was observed with two to three bands in the egg sample (Fig. 1). The presence of specific α-esterase band Est-3 was observed in F_1 progeny, whereas it was completely absent in both parental lines. Besides, the allele with heterozygocity was also observed in the Est-2 (Est-2 $^{1\,2}$) locus of F_1 progeny. The study was extended to one more generation (F_2) to study the inheritance pattern of Est-2 allele and Est-3 band. The results revealed that both banding patterns were completely absent in the F_2 progeny. The results suggest that the isozyme α-esterase with heterozygous alleles of Est-2 and special band Est-3 might have tightly linked with fecundity and pupation rate. This could be confirmed through the phenomenon of hybrid vigour that the heterosis was observed only in F_1 progeny. This hybrid vigour is due to the heterozygocity of alleles obtained from the crosses of both selected parents. This heterozygocity needs to be maintained for introgression of molecular factors may be specific metabolic enzymes involved in growth; esterases which are known for growth. The presence of an esterase allele *viz.*, Est-$2^{1\,2}$ and expression of new locus Est-3 in the hybrid indicates

Table 2. Mean performance and heterosis over mid parent and better parent for different economic traits

Parameters	Mean performance of			Heterosis over (%)		Std. Err. Diff.@		ANOVA (Hybrid vs Parents)
	APM1	SLKSPM	SLKSPM × APM1 (F_1)	MPV	BPV	P<0.05	P<0.01	F-Value
Fecundity	596±27.50	512±17.05	717±33.55	29.51#	20.34*	92.07	133.97	16.75$$
Hatching %	94.4±1.41	93.1±1.54	99.1±0.16	5.66#	4.93*	3.93	5.72	9.69$
Wt. of 10 grown larvae (g)	35.9±0.40	23.2±0.39	26.2±1.02	-11.35#	-27.05#	1.76	2.57	19.14$$
Total larval duartion (h)	547.2±8.98	633.6±5.88	604.8±2.24	2.44NS	-4.55*	20.00	29.10	2.76NS
Fifth age larval duration (h)	151.2±2.94	196.8±6.25	163.2±0.80	-6.21NS	-17.07#	11.78	17.13	4.47NS
Cocoon yield (No.)/10,000 larvae	9843.6±21.44	9967.6±21.73	9903.2±6.83	-0.02NS	-0.65*	52.71	76.70	0.011NS
Cocoon yield (Kg)/10,000 larvae	14.7±0.12	11.0±0.55	13.1±0.09	1.88NS	-10.75*	0.95	1.39	0.343NS
Pupation rate (%)	91.7±2.30	93.7±1.12	97.9±0.47	5.63*	4.50NS	4.21	6.13	8.15$
Cocoon weight (g)	1.472±0.05	1.086±0.03	1.327±0.01	3.67NS	-9.92*	0.11	0.16	0.956NS
Shell weight (g)	0.25±0.01	0.16±0.01	0.19±0.001	-6.0NS	-23.58#	0.02	0.03	1.895NS
Shell ratio (%)	16.9±0.42	14.4±0.40	14.2±0.22	-9.15#	-15.83#	0.88	1.27	14.23$$
Cocoon yield/100 dfls (Kg)	58.74±0.47	44.14±2.23	52.408±0.35	1.87NS	-10.78*	3.80	5.53	0.342NS

#CD at significance at P<0.01; *CD at significance at P<0.05
Anova=$significance atP<0.05; $$significance at P<0.01; NS Non-significance

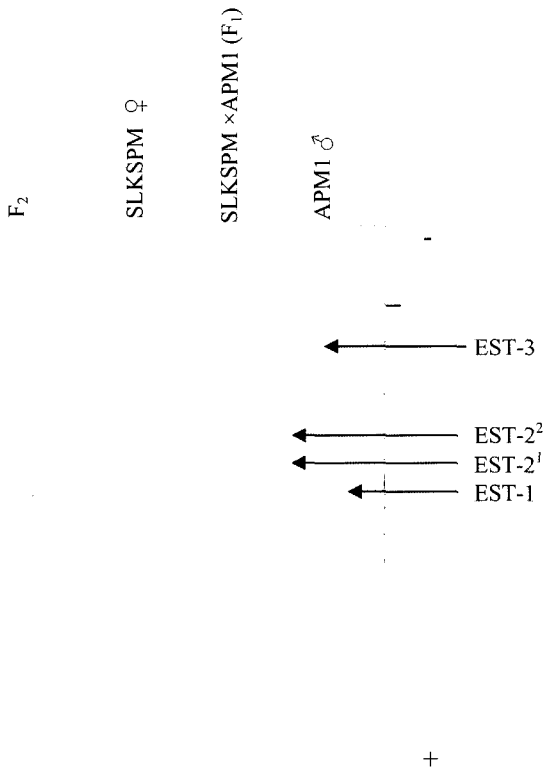

Fig. 1. Esterase isozyme profile in eggs of APM1, SLKSPM and their progenies F_1 and F_2.

the over dominance at a single locus or multiple loci wherein the heterozygous genotypes is superior in their productive traits to either homozygous parental breeds. These allelic variations are found to be a good genetic marker for identifying the productive traits (Hayashi *et al.*, 2002). The study suggests the essentiality to identify specific marker loci, which are tightly linked to those chromosomal segments that determine the expression of the traits of interest (Bernardo, 1992). The results also emphasized possibility of getting heterosis marker by using these *pnd*$^+$ genes carrying genotypes. Moreover, the study suggests that the yolk protein of egg is found to be an ideal sample for predicting heterosis, which can expedite the time and minimizing the man power, besides, minimizing the large number of cross combinations to be made among the parental lines for silkworm breeding. In view of the high heterosis and heterobeltiosis values shown in pupation rate and fecundity indicates the non-additive genetic effect in their expression can be inferred. It is also obvious that the hybrid vigour in the traits is depends on the level of heritability, where the low heritable traits could get more heterotic effects than the highly heritable one, besides the magnitude of heterosis is depended on the genotypes used. The present study suggests that the isozymes markers (Est-2 and Est-3) targeted for introgression were significantly associated with the high fecundity and pupation rate. However this needs to be confirmed by further inheritance study with various parental lines. Further studies are under progress to trace out the role of α-esterase isozymes markers during introgression.

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