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The Heterosis Phenomenon in Mulberry Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae)

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ABSTRACT

The introduction of hybrids and exploitation of heterosis had played a vital role in Indian sericulture industry, which clearly depicted a quantum jump in silk production during the last four decades. A systematic procedure to identify the best combiners by the breeders included combining ability test, line x tester analysis or D^2 analysis for maximum expression of heterosis. The expression of heterosis expressed in crossbreed population was determined by the interaction between genotype and prevailing environmental factors. Except few exceptions, heterosis was found invariably higher in single crosses compared to three-way and double crosses. An attempt was made in this review article to elucidate briefly the concepts of heterosis, causes of heterosis, utilization of heterosis, manifestation of heterosis and its significance in silkworm breeding.

Keywords: *Bombyx mori*, heterosis, hybrid vigour

INTRODUCTION

In the field of biological sciences, it is well known that hybrids become very strong; a fact mentioned by Darwin in his book 'Origin of Species' in 1859. The term heterosis derived from the Greek word 'Heteros' and 'Osis' (heteros = different; osis = conditions) is used to describe the superiority of cross breeds. The phenomenon of heterosis has been commercially exploited in agriculture, horticulture, animal husbandry, poultry and sericulture is not an exception. Genetically, heterosis is the function of a hybrid over the parents resulted from crossing of unlike individuals differing in their one or more parameters. The pioneering works of Toyama a Japanese silkworm-breeding expert on hybridization and use of F1 hybrids for commercial rearing made an epoch in the history of sericulture. The hybrids, which were the crosses of Japanese and Chinese origin, became so popular with the farmers there that by 1919 over 90% of eggs produced were of hybrid origin, reaching 100% by 1928 [1]. Enhanced heterotic expression in various economic traits has been reported in single, three-way and double crosses over their parents [2 - 8].

In this article attempt has been made to discuss briefly the concept of heterosis, utilization of different forms of heterosis and its significance in silkworm (*Bombyx mori)* breeding.

CLASSIFICATION OF HETEROSIS

No other biological phenomenon is so much shrouded in mysteries as heterosis with respect to the probable cause of its manifestation. The various types of heterosis reviewed are individual, maternal and parental heterosis [9]. The heterosis caused by improvement in performance, vigour etc. in an individual (relative to the mean of its parents) that is not attributable to either maternal, parental or sex-linkage effects are called individual heterosis, while heterosis in a population attributable due to using of crossbreed instead of pure breed is referred as maternal / parental heterosis. Heterosis in silkworm is also classified as balanced, mutational and pseudo heterosis [10]. The heterosis resulting from hybridization is referred as balanced or true heterosis, while that resulted from creation of mutation is known as mutational heterosis. The heterosis resulting from more favorable environmental conditions is referred pseudo-heterosis or false heterosis. Mostly, heterosis exploited in sericulture for the production of commercial silk is due to hybridization (true heterosis). Heterosis is also classified based on direction [positive heterosis (+) (Beneficial) or negative heterosis (-) (Non-beneficial)] or function (luxuriant heterosis, adaptive heterosis, selective heterosis and reproductive heterosis) or transmissibility (liable heterosis and fixed heterosis).

Theories of heterosis

The several theories proposed to explain the manifestation of heterosis are -

a) The dominance theory: The dominance theory postulates that the parental lines are homozygous dominant for different favorable loci. This theory is proposed on the basis of the superiority of dominant alleles over the recessive alleles, where genes governing vigor are beneficial and dominant in nature whereas recessives are deleterious and have detrimental effect on heterosis. The dominance theory states that inbreeding in a particular line produces homogygosity for some recessive genes and when crosses are made between such inbred lines the recessive genes of one line get masked by the dominant genes of the other line producing heterotic effect in F1 [11]. In such instances, it is the presence of dominant genes that produces heterosis rather than heterozygotic loci.

b) Over-dominance theory: The over-dominance theory postulates that the heterozygote is superior to homozygote. Various versions of this include ‘enheterosis theory’ (super dominance or over-dominance at the chromosomal level) and the ‘physiological balance theory’. Mostly, the theory of dominance and over-dominance leads to the same expectations. In both the cases, gain on out-breeding and loss (decrease) of vigor on inbreeding is established. Dominance theory is based on the homozygous dominant alleles for different characters present in the parental lines while non-dominance theory is postulated on the basis of effect of allelic differences in heterozygote.

c) The epistasis theory: It includes all types of inter locus interactions. Epistatic theory is either 'F1 epistasis’ or 'parental epistasis' [12]. Generalized formulae for various types of genetic interactions including dominance modifications, dominant epistasis, recessive epistasis, duplicate genes, recessive suppressor and complementary genes are reported [13]. It is also stated that heterosis is derivations of either complementary or duplicate gene interactions.

d) Biochemical theory: Biochemical basis for heterosis accords with the assumption that the primary heterotic effect is concern with growth substances such as regulatory proteins and hormones, the predominant activity of which is registered in the early part of the developmental cycle of the silkworm. Greater metabolic efficiency in mitochondria of the heterotic hybrid was observed while no such changes occurred in those not exhibiting heterosis.

Initial arguments about the mechanism of heterotic effect supported the dominant gene hypothesis. But there were several objections to the theory -

- Amassing all favorable alleles could possibly develop a completely homozygous superior line.
- If dominance was the major contributor to heterosis, the F2 frequency distribution should be skewed and be reflected in the binomial of (3/4 + 1/4)n. However, the linkage between favourable and unfavourable genes would tend to reduce or eliminate such skewedness.
- Single gene loci could show an over dominance effect from dominance in breeding trials have not been very successful. Current thinking has arrived at a blend of both theories. There is no doubt that for some loci, dominance is the major interaction contributing to the heterosis. However, as an explanation of the heterosis mechanism, there are enough inconsistencies to consider other alternatives.

Before starting hybridization studies it is imperative for breeders to adopt following strategies to obtain better heterosis leading to fruitful and desired results -

(a) Collection of endemic and exotic genotypes of both multivoltine and bivoltine silkworm.

(b) Evaluation and documentation of the collected genotypes and studies on their genotypic and phenotypic stability.

(c) Clustering / grouping of genotypes morphologically and genetically both through diallel analysis besides study on region, season and environmental stability.

In India, utilization of hybrid vigour started during 1920s. However, this did not contribute to the rapid progress in productivity as both the parental strains involved in the hybrid were multivoltine with poor qualitative and quantitative characters. During 1940 attempts was made to improve the Indian indigenous multivoltine breeds viz., Nistari and Chotopolu by hybridization with a few Italian breeds, which resulted new breeds viz., Nismo, Ichhot and Iton reported to be better than Nistari [2]. Cross breeding of silkworm races, which differed in voltinism and quantitative characters were initiated during 1960s by utilizing multivoltine race Pure Mysore and exotic bivoltine races such as J112, C108, J122 x C122, J124, Sanish, Azarbaizan and NN6D. Even though, these hybrids were found to be superior but the goal of achieving increased productivity could not be fully realized.

First systematic hybridization following the diallel crossing method in India, was reported in 1964 [14] involving 5 genetically pure multivoltine races. Since then various crossing systems like diallel, line x tester, three-way crosses and double crosses were attempted by various workers for the utilization of hybrid vigor at commercial level [15-16]. Almost all the economic traits expressed hybrid vigour in the hybrids exhibiting superiority over inbred lines. The characters viz., fecundity, larval weight, single cocoon and shell weight, cocoon-shell ratio, cocoon yield / 100 Dfls, ERR by number and weight are found to improve substantially in F1 hybrids. In addition, reduced mortality in F1 hybrids, less renditta, better filament length adds to the advantage to the silk reeling industry. The heterosis varies from season to season, crosses to crosses, characters to characters and sexes to sexes besides voltinism to voltinism. If the parental genotypes posses high value for the characters, the degree of heterosis will be less for that particular trait in the hybrid. Heterosis is calculated over check parent value (CPV), mid-parent value (MPV) or better parent value (BPV).

If the value for a particular trait is high in F1, the calculated heterosis will be positive but if F1 value is less then the heterosis will be negative and negative heterosis should not be exploited except for larval duration and renditta as short larval duration and less renditta are the beneficial characters in sericulture.

Hybrid vigour in different crossing systems

Hybrid vigour is at its bests in F1 hybrid which decreases gradually as F1 > F2 > F3 > F4 > F5 so on and the phenomenon of hybrid vigour disappears in about 14 generations in silkworm. Silkworm strains can be crossed in different manners viz., single cross [A x B], three-way cross [(A x B) x C] and double cross [(A x B) x (C x D)]

The quantitative characters in silkworm are highly variable and have a greater economic value. F1 (single cross) hybrids are most commonly used for commercial cocoon production because they represent high heterosis for most of the economic characters. It has been reported that F1 hybrids are less variable than parental lines, three-way and double crosses. In the study of heterosis in the silkworm, many characters have been found to have a relationship with the qualitative and quantitative aspects of silk yield [17]. However, the characters in hybrids showing high manifestation of heterosis are shorter feeding duration and better larval weight, lower rate of mortality and better Effective Rate of Rearing (ERR), higher cocoon weight, heavier shell weight, longer filament length, better pupation rate and raw silk percentage etc.

However, the level of heterosis recorded for different strains by different workers are not consistent. Using 30 BV x 30 BV hybrids, highest manifestation of heterosis for cocoon yield (14.25%) are reported [18] which is a function of both survival rate and cocoon weight (Table 1). While conducting studies on 10 MV *x* 10 MV, the highest heterosis is reported [19] for cocoon yield / 100 Dfls that differs from season to season with highest value in unfavorable season (Table 2).

Table 1. Heterosis for different characters in *Bombyx mori* (mean of 30 BV *x* 30 BV hybrids)

Characters	Heterosis %
Cocoon yield	14.25
Cocoon weight	3.89
Cocoon-shell weight	3.29
Denier	3.08
Larval duration	2.58
Survival rate	0.87

Table 2. Heterosis for different characters in *Bombyx mori* (mean of 10 MV x 10 MV hybrids) [19]

Characters	Heterosis (%)	
	Favorable	Unfavorable
E.R.R. (Number)	10.39	23.84
ERR (Weight)	40.49	69.59
Single cocoon weight	25.54	50.43
Single shell weight	32.22	60.91
Cocoon-shell ratio	2.70	14.25
Yield/100 Dfls	73.37	119.83

Highest heterosis for survival rate in MV x BV crosses is also reported [20]. Studies made by various workers indicated that the degree of heterosis varies steeply for different characters. Such wide differences in the manifestation of heterosis suggests that the parental strains involved in the hybrids differs in their genetic make-up as reflected in their sharp differences in origin, voltinism and quantitative traits such as larval duration, single cocoon weight, single shell weight, filament length etc. [21 – 23]. It is established that heterosis becomes lesser with the increase in mid-parental value. In other words, when the quantitative characters of the parents are improved through selection excessively, they become more homogeneous for genetic components; consequently the heterosis tends to become smaller as found for weight of cocoon and length of silk filament.

Heterosis in three-way crosses

In India, exploitation of using three-way crosses of the silkworm was demonstrated by many workers. Most of the studies [20, 24 - 25] showed that three-way and double cross hybrids are inferior to single hybrids. Such a difference between hybrids of single, three-way and double crosses was interpreted considering the fact that one of the parents involved in three-way and both the parents in double cross hybrids are actually F1 individuals [26]. Further more, the population produced by three-way or double cross hybrids is a mixture of genotypes, all of which could in principal have been produced by single crosses but differs from single cross hybrids in the following three ways [27]-

- If the superiority of single cross is due to epistatic interactions, some of its superiority is lost in three-way and double crosses.
- There is genetic variation within the crosses and consequent loss of phenotypic uniformity.
- The variance between crosses is reduced and the best three-way and double cross hybrids are consequently not as good as the best single cross hybrids.

However, it is clearly demonstrated [28] that three-way cross cocoons results in improved reelability, reduced renditta, lower size deviation and improved tenacity compared to those of the conventional single hybrid cocoons. During hot and humid seasons, when rearing of bivoltine F1 hybrid is unsuccessful at field level and indigenous races gives very low and poor quality yield, three-way crosses can play as an intermediary technology for commercial use. Three-way crosses showed highest heterosis for yield/100 Dfls followed by cocoon-shell weight during unfavorable season (Table 3). It is also established that heterosis for different characters is higher over MPV compared to BPV (Table 4) in the combinations tried by many workers.

Table 3. Heterosis in three-way crosses (mean of 4 three-way cross involving MV as female and BV hybrids (F1) as male parent)

Characters	June - July	October - November	December - January
Larval period	- 6.52	- 8.33	- 4.62
Survival %	33.39	12.13	3.97
Single cocoon weight	27.18	19.66	19.18
Cocoon-shell weight	44.96	28.41	22.07
Filament length	39.70	18.06	20.95
Yield /100 Dfls	91.06	47.51	18.67

Table 4. Heterosis of different characters in double crosses (mean of three double crosses) [30]

Characters	Heterosis % Helerosis %	
	Mid parent	Better parent
Pupation rate	7.50	4.33
Cocoon weight	12.04	11.68
Cocoon-shell ratio	2.56	0.90
Filament length	10.71	5.94
Raw silk	7.17	5.64

Heterosis in double crosses

The primary objective of rearing double hybrids is to get the desired quantitative and qualitative traits into one combination [29]. It is well known that survival and fecundity are affected greatly with increase in quantitative traits beyond threshold level. Although survival could be maintained in single hybrids, less number of eggs laid by inbred pure mother moths' handicaps them. Unless mother moth is a hybrid, the fecundity cannot be increased. The increase in egg number is possible only with the foundation crosses, which are the parents of double hybrids. In addition, with clear advantage like easy rearing, superior to parental breeds in growth, vigour and other economic characters besides better in yield than single hybrids [30], the double hybrids could be commercially exploited. In general the heterotic manifestation is higher in case of single crosses than in the three-way and double crosses [31] for all the characters except larval weight where higher level of heterosis was found associated. Heterosis over mid-parent and better parent in double hybrids [30] is reported for pupation rate, cocoon weight, shell weight, filament length and raw silk % over foundation crosses with higher values in cocoon weight (Table 4).

Improved silkworm strains which yield longer filament length tends to lay fewer eggs [17]. On the other hand, in double cross hybrids, the eggs number per female moth is invariably more than in single cross hybrids. Hence, the usage of double cross hybrids was to an extent of more than 40% in Japan, while such a use is yet to gain initiative in India. Heterosis in fecundity in double hybrids over foundation crosses is reported [32] considerably high (Table 5).

Table 5. Heterosis in fecundity over foundation crosses

Type of crosses	Season		Average
	Favorable	Unfavorable	
Foundation crosses	479	399	439
Double crosses	509 (6.26%)	522 (30.82%)	516 (17.53%)

Therefore, if the foundation crosses were used at P1 level, it would eventually increase the commercial seed production. This approach may be adopted to bridge the gap of commercial seed production and quality. Also the benefit by the use of double hybrids is -

- Increased crop reliability if foundation crosses are raised and reared at P1 level especially in unfavourable seed crop season.
- Increase in number of eggs up to an extent of 20% promoting an enhanced egg production.
- Seed crop rearers will not hesitate to accept the foundation crosses because of crop assurance.

In general heterotic manifestation is higher in case of single crosses [31] than the three-way and double crosses (Table 6). The instances of negative heterosis in cocoon characters are reported when multivoltine female parents crossed with bivoltine male parents [25, 33]. The performance of heterosis on individual cross basis in different crossing system is reported to have good number of crosses, which failed to exhibit heterosis, and thereby showed negative heterosis [31]. Egg productivity is positively correlated with number of eggs laid and number of effective moths capable of laying reasonable number of eggs but this character is negatively correlated with cocoon shell and raw silk percentage. Therefore, selection of inbred lines for high egg productivity without reducing the cocoon-shell % is difficult task. Hence, breeders are hybridizing two or more inbred lines to get desired result. However, no differences for shell ratio and neatness between pure breeds, foundation cross (F1) and double crosses are reported [30]. The major differences noticed are with respect to pupation rate, cocoon weight, filament length and raw silk recovery. The selection of suitable parents for foundation crosses is very important. If the foundation crosses with high degree of heterosis are used for double hybrid preparation, the hybrid vigour may decline. Therefore, the foundation cross which showed low heterosis should be utilized to have more hybrid vigour in double hybrids.

Table 6. Heterosis % in different crossing systems

Characters	Single crosses	Three-way crosses	Double crosses
Fecundity	14.13	2.03	9.62
Larval duration	0.33	- 4.22	- 4.26
Larval weight	5.85	25.00	31.82
Yield /10000 larvae	5.40	17.82	23.05
Single cocoon weight	18.63	26.98	25.99
Single-shell weight	66.67	39.18	35.88
Cocoon-shell ratio	52.98	21.62	19.48
Filament length	10.04	31.26	38.43
Denier	16.90	17.50	15.63

HETEROSIS EXPLOITATION

The phenomenon of heterosis or hybrid vigour has been fully exploited in both animals as well as in plant production. The classical example of the application of this phenomenon is corn in agriculture and silkworm in sericulture. Systemic and planned hybridization together with improved farming and rearing practices has helped a great deal to increase the productivity of corn and silk by many folds. In fact Japanese silkworm and American maize are two big stars of hybrid utilization. The exploitation of hybrid vigour in silkworm came to being slightly earlier than in the American maize.

Crossing of inbred lines has made a major contribution of today's high productive silkworm races; ever since the phenomenon of hybrid vigour was discovered and adopted for cocoon production. In this case, the purpose of crossing is to produce a heterotic effect rather than to provide genetic variation for selection. Another purpose of crossing inbred line is to produce superior crossbreed or F1 hybrid. Therefore, heterosis depends on selection as well as on inbreeding and crossing. Since the introduction of heterosis, progress in silkworm breeding has depended on success or failure in identifying better combiners for a cross or a hybrid. One of the most important research activities for development of better hybrid varieties is the identification of inbred lines for specific cross combinations. Good combiners are distinguished by means of the combining ability test / line x tester analysis or D^2 analysis.

HETEROSIS AND ENVIRONMENT

Population maintained in varying environment can be expected to have greater variance and higher fitness than population from a constant environment. The level of heterosis expressed in a cross bred population is determined by interaction between the genotypes and environmental factors prevailing at that time. The concepts of genetic homeostasis in which heterozygous populations are expected to be less influenced by environmental factors in comparison to homozygous populations are reported. High degree of difference in heterosis for various quantitative traits during spring compared to autumn season is also reported [34] which might be due to the influence of environmental factors. Generally, in a good environment (optimum temperature, humidity, air current, rearing space, germfree conditions and high nutritive mulberry leaves), silkworm parental strains perform better. On the other hand, when both the parental lines and hybrids are reared in adverse environment, the performance of hybrids will be much superior to that of parental strains. In such cases, heterosis both over MPV and BPV will be higher. This condition is similar to the 'Greek temple model' [35] for the traits exhibiting greater degree of heterosis in a poor environment (Table 3).

The degree of heterosis over mid-parental value will mostly be less during favourable environmental conditions. For instance, when one of the parents involved is an exotic high yielding strain in MV x BV hybrids, the heterosis over the exotic parent will be negative when the exotic parent and hybrids are raised under similar favourable conditions. On the other hand, when both parental strains and hybrids are raised in unfavourable environmental conditions, performance of hybrids will be much superior to both the parental strains. Under such conditions the heterosis will be higher over both mid-parental and better parental values.

HETEROSIS AND SILKWORM BREEDING

Hybridization followed by appropriate selection bringing together the economic characters of choice from defined sources and to synthesize genotypes of desirable constitution through scientific technique is the main objective of breeding. By utilizing the known and established breeding material, the objective of synthesizing new breeds can easily be realized by the application of appropriate selection pressure for desirable combinations of genes. The reciprocal crossing of two breeds which are good in some characters and poor for some other characters to lead to the segregation of characters at indefinitely large number of loci in F2 generation enabling the breeder to select the desirable combination of characters and reject the individual with undesirable characters.

Evolution of improved breeds through inbreeding of hybrids is well-documented [36]. The distinct genotypic and phenotypic differences between the races utilized in the hybridization produce high degree of phenotypic variability enabling the breeder to step up selection for different characters in the polygenic system [36 - 37] can be exploited by the application of systematic selection and bringing together some advantageous features of the parental races.

Exploitation of heterosis has played a vital role in increasing the silk production to a great extent. Continuous efforts are still being made to understand the mechanisms contributing to heterosis and to develop new hybrid combinations for effective exploitation of heterosis and to increase production and productivity.

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