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Review Article

Genetic Divergence, Implication of Diversity, and Conservation of Silkworm, *Bombyx mori*

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Genetic diversity is critical to success in any crop breeding and it provides information about the quantum of genetic divergence and serves a platform for specific breeding objectives. It is one of the three forms of biodiversity recognized by the World Conservation Union (IUCN) as deserving conservation. Silkworm *Bombyx mori*, an economically important insect, reported to be domesticated over 5000 years ago by human to meet his requirements. Genetic diversity is a particular concern because greater genetic uniformity in silkworm can increase vulnerability to pests and diseases. Hence, maintenance of genetic diversity is a fundamental component in long-term management strategies for genetic improvement of silkworm which is cultivated by millions of people around the worlds for its lusture silk. In this paper genetic diversity studies carried out in silkworm using divergent methods (quantitative traits and biochemical and molecular markers) and present level of diversity and factors responsible for loss of diversity are discussed.

1. Introduction

Sericulture is a unique field of agriculture, because silkworms are reared on an extensive scale in rearing houses and their silk cocoons are utilized as fine material for clothing. Like agriculture, sericulture also requires a continuous flow of productive silkworm breeds and host plant varieties to meet the ever-changing demand of people involved in the industry besides the consumer sector. To meet all these requirements, the breeder needs very wide and inexhaustible genetic resources to meet the ever-changing demands from various sectors. Considering the great economic importance of *Bombyx mori*, silk producing countries, such as China, Japan, India, Russia, Korea, Bulgaria, and Iran, have collected number of silkworm breeds suitable for a wide range of agro-climatic conditions. More than 4000 strains are maintained in the germplasm of *B. mori* and 46 institutes are involving silkworm genetic resources maintenance, which includes univoltine, bivoltine, and polyvoltine strains. These different genotypes display large differences in their qualitative and quantitative traits that ultimately control silk yield. It was estimated that silkworm genome consists of about $4.8' 108$ bp; its genetic information volume is about one-sixth of human being. There are over 450 morphological, physiological, and biochemical characters recorded at present, among them 300 (including multiallele) had been located on 27 groups of the total 28 chromosomes [1]. Apart from a rich biodiversity of geographical races, there are also a large number of mutants for a variety of characters present in *B. mori* [2].

2. Genetic Divergence in Silkworm

Study on genetic diversity is critical to success in any crop breeding and it provides information about the quantum of genetic divergence and serves as a platform for specific breeding objectives [3]. Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety or population of a species [4]. It results from the many genetic differences between individuals and may be manifest in differences in DNA sequence, in biochemical characteristics (e.g., in protein structure or isoenzyme properties), in physiological properties (e.g., abiotic stress

resistance or growth rate), or in morphological characters [5]. Genetic diversity has been conventionally estimated on the basis of different biometrical techniques (Metroglyph, D^2 divergence analysis, and principal component analysis) such as phenotypic diversity index (H), or coefficient of parentage utilizing morphological, economical, and biochemical data [6–9].

The genetic diversity of *B. mori* is derived from hybridization of different geographical origins, mainly the Japanese, Chinese, European, and Indian strains, which have distinct traits. Among these four geographical strains, silkworm of temperate origin produces a higher quantity of good, finer, stronger silk fiber, whereas the tropical strains are hardy, tolerant to pathogen load, and resistant to diseases. However, the tropical strains produce low amounts of silk, which is coarser and weaker [10]. To help the breeders in the process to identify the parents that nick better, several methods of divergence analysis based on quantitative traits have been proposed to suit various objectives. As most of the desirable characters in silkworm are quantitative nature, multivariate statistical methods have been employed to measure the genetic diversity among the stocks. Among them, D^2 analysis of Mahalanobis [10] using Tocher's optimization method [11] occupies a unique place and an efficient method to gauge the extent of diversity among genotypes, which quantify the difference among several quantitative traits. It is being used by most of the workers and has been found as an extremely useful tool for estimating genetic divergence in the silkworm [12–16]. Table 1 summarizes genetic divergence study carried out by several workers in silkworm. Using this method, silkworm genotypes were formed into different clusters indicating presence of distinct divergence among the genotypes. Though divergence reported to exist among genotypes and mixed trend of clustering observed. Jolly et al. [13] subjected forty-nine silkworm breeds for this analysis and reported that these breeds were found to form three distinct clusters indicating the presence of distinct diversity among the breeds. Subba Rao et al. [14] and Govindan et al. [15] reported that breeds derived from the same parents were included in different clusters showing variation among the breeds derived from the same source. On the other hand, the breeds derived from the same source were included in the same cluster showing close affinity between advanced sister lines [12, 14, 15] and those of differing genetic background occupied in single cluster indicated uniformity in selection procedures [12]. However, genotypes of temperate and tropical origin formed into separate clusters indicating environmental influence on the expression of characters [17]. Though theoretically geographical diversity is important factor, it is not the whole determining factor for genetic divergence [18, 19]. All these studies were aimed to identify suitable parents for breeding programme and recommended to cross the genotypes from different clusters [20–22] for yield improvement. Though many characters in silkworm are subjected for divergence study, characters, namely, fecundity larval weight, single cocoon weight, cocoon shell weight, and filament length only, contributed about 97% to the total genetic divergence [12, 13, 16, 23, 24].

3. Diversity in Silkworm

Genetic diversity is most often characterized using data that depict variation in either discrete allelic states or continuously distributed (i.e., quantitative) characters, which lead to different possible metrics of genetic diversity [25]. Genetic diversity can be assessed among different accessions/individuals within the same species (intraspecific), among species (interspecific). and between genus and families [26]. It plays an important role in any breeding either to exploit heterosis or to generate productive recombinants. The choice of parents is of paramount importance in any kind of breeding programme; hence, the knowledge of genetic diversity and relatedness in the germplasm is a prerequisite for crop improvement programmes. Genetic diversity is also an essential aspect in conservation biology because a fundamental concept of natural selection states that the rate of evolutionary change in a population is proportional to the amount of genetic diversity present in it [27]. Decreasing genetic diversity increases the extinction risk of populations due to a decline in fitness. Genetic diversity also has the potential to affect a wide range of population, community, and ecosystem processes both directly and indirectly. However, these effects are contingent upon genetic diversity being related to the magnitude of variation in phenotypic traits [28].

In general, cocoon colour and cocoon shape, larval, marking, and quantitative traits have been used for differentiation of silkworm genotypes and, based on that, parents are being selected. However, recent advent of different molecular techniques led breeders to estimate genetic diversity on the basis of data generated by different molecular markers, which provided a means of rapid analysis of germplasm and estimates of genetic diversity, which were often found to corroborate phenotypic data. These molecular markers are broadly categorized as biochemical and molecular markers.

3.1. Biochemical Markers. Application of isoenzymes and other molecular markers helps to estimate genetic diversity much more accurately than that of morphological traits. Electrophoresis identifies variation (alleles) at loci that codes for enzymes (usually termed isozymes or allozymes). One advantage of allozyme loci is that they are codominant and heterozygotes can be scored directly. Understanding the genetic constitution of an individual in the population of races and allelic variations through isozyme studies is known to reflect the differential catalytic ability of allelic genes and their significant role in the adaptive strategy of the genotypes [29].

The diversity study carried out in silkworm through protein profiles, enzymes, and isozymes are summarized in Table 2. Isozymes like esterase, acid phosphatase, alkaline phosphatase, amylase, phosphoglucomutase, aspartate aminotransferase, malate dehydrogenase, glucose 6 phosphate dehydrogenase, and carbonic anhydrase have been used by various authors to study diversity in silkworm genotypes [30–43]. Among the different isoenzymes analyzed, esterase was most preferred because of its diverse substrate specificity and polymorphic expression followed by acid phosphatase [36, 37, 44]. Eguchi et al. [32] found four

Table 1: Genetic divergence study reported in silkworm.

SL number	Reference number	Number of genotypes used and clusters formed	Measures of genetic diversity	Conclusion
1	[89]	49 and 3	Mahalanobis (1936) and Tocher (1956)	(1) Presence of distinct diversity. (2) Breeds derived from the same parents were included in different clusters. (3) Breeds derived from the same source were included in the same cluster.
2	[23]	32 and 7	Mahalanobis (1936) and Tocher	Geographical diversity did not contribute much to genetic diversity.
3	[14]	15 and 5	Mahalanobis (1936) and Tocher (1956)	(1) Enough diversity present. (2) Suggested for making crosses between different clusters.
4	[116]	50 and 5	Mahalanobis (1936) and Tocher (1956)	Cluster III was the largest, consisting of 34 strains. The clusters are compared for various features influencing silk production.
5	[15]	18 and 8	Mahalanobis (1936) and Tocher (1956)	Breeds derived from the same ancestry were included in different clusters and those of different genetic background occupied a single cluster.
6	[20]	25 and 6	Mahalanobis (1936) and Tocher (1956)	The genetically divergent parents were grouped into four classes.
7	[18]	30 and 5	Mahalonobis' D^2 values (Ward's minimum variance)	Geographical diversity though important is not the determining factor for genetic divergence.
8	[24]	24 and 7	Mahalanobis (1936) and Tocher	Genotypes of temperate and tropical origin formed separate clusters.
9	[117]	11 and 3	Mahalanobis (1936) and Tocher (1956)	The intracluster distance ranged from 0.00 to 1689.37 implying the prevalence of substantial amount of intracluster diversity.
10	[21]	22 and 6	Mahalonobis' D^2 values (Ward's minimum variance.)	There is no relation between geographical diversity and genetic diversity.
11	[118]	65 and 9	Mahalonobis' D^2 values (Ward's minimum variance)	Breeds in the optimum distance obtained cluster can be used in the conventional silkworm breeding programme to improve silk quality.
12	[119]	47 and 12	Mahalonobis' D^2 values (Ward's minimum variance)	Geographic diversity had no association with genetic diversity.
13	[120]	51 and 2	UPGMA	Clusters of individuals exhibited high internal (within clusters) homogeneity and high external (between clusters) heterogeneity.
14	[121]	16 and 3	Mahalanobis (1936), UPGMA	The strains of the same origin did not group together, demonstrating they can have different biological and development performance.
15	[122]	8 and 5	Mahalanobis (1936), UPGMA	Genetic distance and not the geographic diversity is to be considered while identifying parents for hybridization programme.
16	[16]	21 and 7	Mahalanobis (1936) and Tocher (1956)	Silkworm genotypes originating from the same geographical regions fell in one cluster.
17	[17]	56 and 8	Mahalanobis (1936) and Tocher (1956)	Silkworm genotypes originating from different geographical regions fell in one cluster while those originating from a single region fell in different clusters.
18	[123]	51 and 4	Hierarchical agglomerative clustering UPGMA	Inclusion of genotypes of the same origin in different clusters clearly indicates the presence of considerable genetic diversity among the populations.
19	[19]	19 and 3	The hierarchical cluster analysis using Euclidian distance	Cluster analysis and conformity with the variability in the performance of the genotypes for different traits. Geographic diversity had no association with genetic diversity.
20	[124]	4 and 2	UPGMA method (Sokal and Michener)	The optimum level of genetic distance is necessary to obtain heterosis.

Table 2: Genetic divergence study reported in silkworm using enzymes, protein, and isozymes.

SL number	Reference number	Number of genotypes and clusters	Measures of genetic diversity	Conclusion
1	[32]	—	Esterase used for polymorphism	Polymorphism noticed among genotypes.
2	[125]	—	Protein profiles used for genetic diversity	Divergence arises internally after a relatively long amino terminal sequence which appears to be conserved. A plausible explanation for the observed genetic variability is the occurrence of relatively large unequal crossing-over exchanges in the repetitive domain of the fibroin gene.
3	[126]	—	Esterase used for polymorphism	Polymorphism noticed among genotypes.
4	[127]		Acid phosphatase used for polymorphism	Polymorphism observed among genotypes.
5	[128]	20	Enzymes	Rich genetic diversity among genotypes.
6	[129]	10	Esterase used for polymorphism	Polymorphism noticed among genotypes.
7	[36]	12 and 6	Nei and Li (1978) [130] and Yeh et al. (1999) [131]	Rich genetic diversity among genotypes.
8	[132]	8 and 2	Enzymes and UPGMA	Genetic diversity noticed among genotypes.
9	[133]		Nei and Li (1978)	The protein profile of different breeds has indicated the polymorphism and genetic diversity among silkworm breeds.
10	[37]	15	—	Esterase exhibited polymorphism among the bivoltine breeds.
11	[134]	6 and 3	Protein	Genetic differentiation among populations of different races.
12	[44]	12 and 2	Nei and Li (1978) UPGMA	The mean value of FST (0.2224) calculated on the base of the established polymorphism showed that 22.24% of the genetic variability was observed between the different strains, which corresponds to the level of the interstrain genetic differentiation.
13	[40]	21 and 8	Nei and Li (1978) UPGMA	Genetic variations were observed and they can be identified by relating with their morphology and geographical origins
14	[135]		Nei and Li (1978) UPGMA	Protein profiles studied and presence of rich genetic diversity among germplasm stocks. Different origin accessions established a close relationship indicating close affinity in protein pattern.
15	[41]	15	Esterase used for polymorphism	Variation in esterase pattern was observed among genotypes.
16	[43]	10 and 2	Nei (1978) by UPGMA dendrogram (Sneath and Sokal, 1973)	A perusal of genetic diversity within and among strains indicated that 34.72% of the observed variation occurred among strains and the rest of the variation (65.28%) within strains. Their rich genetic diversity needs to be exploited in conservation and breeding programme.
17	[136]	4	(Swofford and Selander, 1981)	The lower degree of observed heterozygosity and the higher degree of homozygotes proved the inbreeding effect.
18	[42]	15 and 3	Nei (1978) and UPGMA	Japanese and Chinese strains could not be totally separated by the isoenzyme system analysis. The results indicate that, in spite of the genetic distance and differentiation among the lineages, they cannot be separate just with the isozymes alleles. The high FST value (0.6128) allows the conclusion that the lineages are differentiated.

fundamental types of esterase and about 70% of the Japanese, Chinese, and European races investigated belong to A type and 20% to 0 type, while B type was found only in Chinese races. Yoshitake et al. [45] analyzed polymorphism pattern of esterase and acid phosphates in 300 strains of silkworm and concluded that distribution of acid phosphatase and esterase was similar in European and Japanese strains and there was resemblance between Chinese and European strains. A higher degree of interstrain variability was reported on the acid phosphatase [43, 44] and esterase [36, 37, 41, 42]. Acid phosphatase is also found to be a suitable marker for analyzing the inter- and intrastrain diversity and the strain

differentiation [44]. Isozyme analysis in different silkworm genotypes by different authors indicated rich genetic diversity between the genotypes and results were mainly used to separate populations and strains in order to use them in selection programs.

3.2. Molecular Markers. Molecular diversity studies assess all levels of genetic structure and species specific complex components [46]. The detection and exploitation of naturally occurring DNA sequence polymorphisms have wide potential applications in animal and plant improvement programmes as a means for varietal and parentage identification facilitate genetic diversity and relatedness estimations in germplasm [47]. The results obtained from different molecular markers may themselves be quite different from those obtained by using biochemical markers such as isozymes or morphological characters. The molecular markers, namely, RAPD, RFLP, ISSR, and SSR, have been effectively utilized in analyzing the genetic diversity and phylogenetic relatedness in the domesticated silkworm *Bombyx mori* [48–56]. Details of diversity study carried out in silkworm through molecular markers are summarized in Table 3. RAPD based dendrogram resulted in a clear separation of two groups, one comprising of diapausing and other comprising of nondiapausing genotypes [49, 57–60]. Among the diapausing genotypes, all the "Chinese type" genotypes which spin oval cocoons grouped separately, while the "Japanese type" genotypes which spin peanut shaped cocoons were found in another group. Further genotypes, which share the same geographical origin, were grouped in the same cluster [57, 61]. SSR and mtDNA markers analysis revealed considerable genetic diversity among the nondiapausing silkworm genotypes that were developed in India, China, and Bangladesh [62]. The dendrogram constructed analysing RFLP markers revealed two distinct groups as Khorasan native (Iran) and Japanese commercial lines. The distinct clustering of these two sets of strains and lines reflects differences of the geographical origin and morphological, qualitative, and quantitative traits associated with them [54]. Kim et al. [63] made phylogenetic analysis using the individual or the nine concatenated intronic sequences which showed no clustering on the basis of known strain characteristic such as voltinism, moultinism, egg colour, blood colour, cocoon colour, or cocoon shape. Furthermore, the tree obtained by them using the nine concatenated intronic sequences comprising 5,897 bp including indels resulted in a similar conclusion. However, Tunca et al. [64] stated moderately low level of diversity among genotypes studied. Supporting this argument recently, Jagadeesh Kumar [65] reported the low level of genetic distance between the breeds on the basis of gene frequency evidenced by the boot strap values in the constructed dendrogram with the help of molecular markers.

On the whole, the diversity study conducted using phenotypic characters and molecular markers had reported adequate genetic variation between genotypes. But these differentiations mostly based on voltinism and geographical origin indicating narrow genetic base between the available genotypes.

4. Status of Genetic Diversity in Silkworm

Zhang et al. [51] reported that genetic distances within Japanese strains are closer than those of Chinese strains and within a strain; the individual polymorphism is significantly higher in wild silkworm than those of domesticated silkworm. According to Liu et al. [66] at the species level, *Antheraea pernyi* and *Bombyx mori* showed high levels of genetic diversity, whereas *Samia cynthia ricini* showed low level of genetic diversity. However, at the strains level, *Antheraea pernyi* had relatively the highest genetic diversity and *B. mori* had the lowest genetic diversity. Analysis of molecular variance (ANOVA) suggested that 60% and 72% of genetic variation resided within strains in *Antheraea pernyi* and *Samia cynthia ricini*, respectively, whereas only 16% of genetic variation occurred within strains in *B. mori*. Similarly, genetic variation was measured using the population size scaled mutation rate which was significantly smaller in domesticated strains (0.011), when compared to the wild strains (0.013) of *B. mori*. The rate of heterozygosity in domesticated strains was reported to be two times lower than that in wild varieties (0.003 and 0.008, resp.). Recently, Yukuhiro et al. [67] analyzed PCR amplified carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) gene fragments from 146 *Bombyx mori* native strains and found extremely low levels of DNA polymorphism. CAD haplotype analysis of 42 samples of Japanese *B. mandarina* revealed four haplotypes. No common haplotype was shared between the two species and at least five base substitutions were detected. These results suggesting that low levels of gene flow between the two species. Further extremely low level of DNA polymorphism in *B. mori* compared to its wild relatives suggested that the *CAD* gene itself or its tightly linked regions are possible targets for silkworm domestication. This information clearly indicates narrow level of genetic diversity in silkworm.

5. Causes for Loss of Genetic Diversity

The existence of genetic variation within a population is crucial for its ability to evolve in response to novel environmental challenges. Genetically variable populations are expected to evolve morphological, physiological, or behavioural mechanisms to cope with the novel conditions [68]. This sorting process not only results in populations that are better adapted to their local environments, but may also, at least in theory, cause a reduction in the genetic variation. Forces that affect genetic variation within populations are effective population size, mutation, genetic drift, gene flow, inbreeding depression, out breeding depression, and natural selection. In silkworm, reduction in genetic diversity might be mainly due to domestication, breeding systems, selection, genetic drift, and inbreeding. In maize, too, selection and drift due to the domestication are the principal factors that influence

Table 3: Molecular diversity reported in silkworm.

Sl number	Reference number	Number of genotypes and Cluster	Measures of genetic diversity	Conclusion
1	[48]	13 and 2	RAPD	Silkworm genotypes were clustered into two groups, one consisting of six diapausing and the other of seven nondiapausing genotypes. RAPD technique could be used as a powerful tool to generate genetic markers that are linked to traits of interest in the silkworm.
2	[57]	13	RAPD and banded krait minor satellite DNA	The RAPD based dendrogram resulted in a clear separation of two groups, one comprising diapausing and the other nondiapausing genotypes. The clustering pattern of RFLP obtained was comparable to the phenogram resulting from RAPD analysis.
3	[49]	5 and 3	RAPD	Some of the DNA fragments were strain specific and some could differentiate the multivoltine from the bivoltine strains or vice versa.
4	[50]	13 and 2	SSR	Detailed analysis of silkworm strains with microsatellite loci revealed a number of alleles ranging from 3 to 17 with heterozygosity values of 0.66–0.90. Along with strain specific microsatellite markers, diapause and nondiapause strain-specific alleles were also identified
5	[137]	13 and 2	ISSR and RAPD	The highest diversity index was observed for ISSRPCR (0.957) and the lowest for RAPDs (0.744). Differentiated diapause and nondiapause strains
6	[138]	31 and 7	SSR	The average heterozygosity value for each SSR locus ranged from 0 to 0.60, and the highest one was 0.96 (Fl0516 in 4013). The mean polymorphism index content (PIC) was 0.66 (range of 0.12–0.89). SSR markers are an efficient tool for fingerprinting cultivars and conducting genetic-diversity studies in the silkworm
7	[139]	20 and 6	RAPD	Multivoltine Silkworm has more genetic diverse than bivoltine
8	[51]	12	SSR	Within a strain, the individual polymorphism of wild silkworm was significantly higher in abundance than those of domesticated silkworm
9	[140]	5	RAPD	The genetic distances between the clusters and within the clusters estimated 6 percent variability between the 4 races and Nistari. RAPDs are very efficient in the estimation of genetic diversity in populations that are closely related and acclimatized to local environmental conditions.
10	[52]	29 and 4	CAP	Considerable genetic diversity observed. Grouped strains roughly according to their geographical origin.
11	[55]	96	SSR	The mean polymorphism index content was 0.71 (range of 0.299–0.919). UPGMA cluster analysis of Nei's genetic distance grouped silkworm strains based on their origin.
12	[141]	6 and 2	AFLP	Higher degree of genetic similarity within Japanese commercial lines than the Iranian native strains. The distinct clustering of these two sets of strains and lines reflects differences of the geographical origin and morphological, qualitative, and quantitative traits associated with them.
13	[61]	7 and 2	AFLP	The genetic similarity estimated within and among silkworms could be explained by the pedigrees, historical and geographical distribution of the strains, effective population size, inbreeding rate, selection intensity, and gene flow.
14	[64]	6	RAPD	The genetic diversity in studying strains was moderately low. Estimates of gene diversity in populations were higher in total (Ht) as compared to those within population diversity (Hs).
15	[142]	20 and 6	ISSR	In selected mutant genetic stocks, the average number of observed alleles was (1.7080 ± 0.4567), effective alleles (1.5194 ± 0.3950), and genetic diversity (Ht) (0.2901 ± 0.0415). ISSR is a valuable method for determining the genetic variability among mutant silkworm strains.

Table 3: Continued.

Sl number	Reference number	Number of genotypes and Cluster	Measures of genetic diversity	Conclusion
16	[63]	25 and 3	Intronic sequences	The degree of sequence divergence in some introns is very variable, suggesting the potential of using intronic sequences for strain identification.
17	[58]	12	RAPD, ISSR, and RFLP-STS	RAPD generated 93.6%, ISSR was 84.62, and RFLP was 75.6% polymorphism. Ability to discriminate bivoltine and multivoltine.
18	[143]	3 and 2	RAPD	The diversity within the populations (Hs) was 0.1334 and the magnitude of differentiation among the populations (GST) was 0.2968.
19	[56]	14	ISSR	ISSR markers has generated 92 percent were polymorphic,diapausing and non-diapausing silkworm stocks could be distinguished by specific marker.
20	[144]	8 and 2	ISSR, RAPD, and isozymes	Sufficient polymorphism and genetic diversity observed.
21	[145]	30 and 2	ISSR	PCA analysis helped to visualize the two major clusters which included the multivoltines and bivoltines separately. The grouping of bivoltines in the PCA analysis clearly showed higher similarity among bivoltines as compared to the multivoltines.
22	[62]	13 and 2	SSR and mtDNA	The heterozygosity generated by the seven pairs of SSR primers varied from 0.098 to 0.396. Considerable genetic diversity is present among the 13 silkworm genotypes.
23	[146]	30 and 2	ISSR	The grouping of bivoltines in the PCA analysis clearly showed higher similarity among bivoltines as compared to the multivoltines.
24	[66]	*A. Pernyi*-3 *S. cynthia ricini*-12 *B. mori*-12	RAPD	At the species level, *A. pernyi* and *B. mori* showed high levels of genetic diversity, whereas *S. cynthia ricini* showed low level of genetic diversity. However, at the strain level, *A. pernyi* had relatively the highest genetic diversity and *B. mori* had the lowest genetic diversity.
25	[147]	14 and 2	RAPD, ISSR	High polymorphisms (70.91 and 74.70%) were revealed by ISSR and RAPD markers.
26	[59]	4 and 2	RAPD	Multivoltine silkworm races are genetically more distant than the two bivoltine silkworm. Genetic distances among the multivoltine and bivoltine silkworm were 0.52 and 0.27, respectively.
27	[60]	9 and 3	RAPD	The average genetic distance between the samples was 0.53. The average genetic distance from analyzed samples proved to be relatively high.
28	[148]	8 and 6	RAPD	Genetic distances varied from 0.28889 (B75.2-C1.4) to 0.92437 (A1.2-A1.3) with an average of 0.58497. Silkworms group a high genetic diversity.
29	[65]	5 and 2	ISSR	Artificial selection during seven continuous generations generally caused lesser genetic distance between the breeds.
	[149]	6 and 3	ISSR	This marker could not discriminate same geographical races correctly.
30	[150]	10 and 3	RAPD	The genotypes were grouped based on voltinism and bivoltines are subgrouped based on silk productivity nature of silkworm breeds.
31	[151]	10	SSR	Sufficient polymorphism and genetic diversity observed. The genotypes were grouped based on voltinism and subdivided based on cocoon shape and cocoon colour.

the amount and distribution of genetic variation in crop genomes as compared to their wild progenitors in maize [69].

5.1. Domestication. Over the past 12 000 years, humans have sampled, selected, cultivated, travelled through, and colonized new environments, thus inducing a plethora of bottlenecks, drifts, and selection. Plant breeders have accelerated the whole process by selecting preferred genotypes [46]. In the broadest sense, alteration and narrowing of crop genetic diversity began with the first domestication of wild plants/animals. Domestication represents a relatively recent evolutionary event, occurring over the past 13,000 years after the Neolithic revolution [70, 71]. This process frequently leads to the improvement of economically important traits and the diversification of morphological traits in domesticated species compared to their wild ancestors. Silkworm domestication, which is a relatively recent evolutionary event, may have generated a large number of alterations and diversification in the structure of an evolutionarily conserved morphogenetic gene [72]. There is an assumption that the process of domestication and selection has resulted in drastic narrowing of the genetic variation and homozygosity in mulberry silkworm which has been domesticated over 5000 years ago. Xia et al. [73] compared the whole genome sequencing of 29 *B. mori* strains and 11 Chinese *B. mandarina* individuals by 1.50 billion short reads and concluded that *B. mori* was clearly genetically differentiated from *B. mandarina*. At the same time, based on the high level of conservation of genetic variability, the authors estimated that a large number of *B. mandarina* individuals were used for domestication (i.e., the population bottleneck during silkworm domestication might not have been severe.) Therefore, gene flow limited to *B. mori* could have occurred for many genes during silkworm domestication. Recently, Yu et al. [74] and Guo et al. [75] reported decreased level of genetic variation in *B. mori* genes or regions compared to those in Chinese *B. mandarina* in the domestication targeted gene. About 40.7% or 49.2% of the genetic diversity of wild silkworm was lost in domesticated silkworm [74]. Study conducted with *B. Mandarina* and *B. mori* by Guo et al. [75] reveals that diversity of *B. mori* is significantly lower than that of *B. mandarina*. Further gene DefA showed signature of artificial selection by all analysis methods and might experience strong artificial selection in *B. mori* during domestication resulting less diversity [75]. However, when analysing the carotenoid binding protein (CBP) genes in *B. mori* identified large copy number variations and retrotransposon associated structural differences in CBP from *B. mori*, which were absent from *B. mandarina*, and concluded that domestication can generate significant diversity of gene copy number and structure over a relatively short evolutionary time.

5.2. Breeding Too Causes Loss of Variability. Breeding systems and life history traits govern the transmission of genes between generations and have been long recognized as impacting the genetic diversity and population genetic structure [76–78]. Breeding is a strong force in the reduction of genetic diversity [79] and views the introduction of modern varieties as evidence of genetic erosion [80]. Silkworm breeding by definition is the selection of superior genotypes and/or phenotypes over a period of time. During the last decades, development and increased focus on more efficient selection programmes have accelerated genetic improvement in a number of breeds. As a result, highly productive silkworm breeds have replaced local ones across the world [81–84]. This development has led to growing concerns about the erosion of genetic resources. As the genetic diversity of low-production breeds is likely to contribute to current or future traits of interest [85, 86], they are considered essential for maintaining future breeding options.

Selection naturally results in a narrowing of the genetic base of the genotype. Even if the breeder has introduced alleles from indigenous races to his target genotype, he/she must then begin the process of "weeding out" the alleles that are undesirable. This weeding out of undesirable alleles is once again narrowing the genetic base of the line. Practically, a breeder typically uses the best genotypes available and selects superior progeny. The continual use of the best genotypes as parents naturally narrows the gene pool to only those alleles that are available from the elite parents and therefore tends to decrease the genetic variation of the population [87]. There is also a threat or loss of genetic diversity as a result of replacement of wild species by exotic high-yielding varieties. Typically, population size is also a major source of loss of genetic diversity.

5.3. Effects of Selection on Diversity. Patterns of diversity in any populations are likely to be affected by selection. Balancing selection due to overdominance (heterozygote advantage), or to frequency dependent selection, may maintain variants in populations, and environmental differences may select for different genotypes in different populations [3]. Purifying selection, however, removes deleterious variants that arise by mutation; such variants are expected to be present at frequencies lower than predicted for the neutral equilibrium. Another form of directional selection occurs when advantageous mutations rapidly reach high frequencies whether they spread throughout a species to fixation or just within a population undergoing adaptation to its local environment [88, 89]. Artificial selection has been widely utilized in the breeding programmes concerning *B. mori*, which is of commercially important insect. Nevertheless, the genetic diversity of silkworm is greatly reduced during systematically extensive selection for a few target traits. In general, selection of superior individuals results in genetic gain, but also loss in genetic diversity and it is strongly dependent on selection method and selection intensity [90]. Selection will have two important consequences: (1) the genetic average value will be changed, thus conventionally it measured a gain, and (2) there will be change in diversity, and this will be measured by relative effective number of families. It is a well-known fact that diversity is affected by directional selection. Directional or disruptive selection will ultimately fix one allele and thereby deplete genetic variation. It has been suggested that directional selection

decreases the level of developmental precision or developmental stability [91] because it may prevent the evolution of canalisation and possibly favour those mechanisms that increase the phenotypic variation [92, 93] showing that systematic selection of parents' results in reduced genetic variation among their offspring. After 4-5 generations with the same selection intensity, the reduction will stabilize. Class example of diversity changes through directional selection and inbreeding in silkworm was reported by Pradeep et al. [94]. They have separated larval populations of Nistari strain based on the shortest larval duration (SLD) and the longest larval duration (LLD) and maintained for 4 more generations. RAPD and ISSR primers generated polymorphic profiles in LLD and SLD lines. Distinct markers specific to LLD individuals were observed from the 3rd generation and indicated selection induced differentiation of allelic variants for longer larval duration. This finding implies that selection combined with inbreeding could result in lines with different genetic properties following separation from the original parental populations. According to Strunnikov [95], continuous selection and inbreeding could have induced a homozygous state of the recessive gene for longer larval duration, where shorter larval duration is the dominant and fitness character. Though it introduced diversity, because of losing its dominant or fitness characters, chances of survival become vulnerable. Further, as reported by Seidavi [96], the genetic performance of selected population of silkworm for cocoon weight trait after the fourth generation shown increased sensitivity towards environment resulting in poor survival due to selection based on productivity traits indicating effect of selection on diversity.

5.4. Genetic Drift. Genetic drift is the chance changes in allele frequency that result from the random sampling of gametes from generation to generation in a finite population. It has the same expected effect on all loci in the genome [97]. In a large population, on the average, only a small chance change in the allele frequency will occur as the result of genetic drift. On the other hand, if the population size is small, then the allele frequency can undergo large fluctuations in different generations in a seemingly unpredictable pattern and can result in chance fixation (going to a frequency of 1.0) or the loss (going to a frequency of 0.0) of an allele. A classic illustration of how finite population size affects allele frequency was provided by Buri [98]. He looked at the frequency of two alleles at the brown locus that affects eye color in *Drosophila melanogaster* in randomly selected populations of size 16. However around 107 number of populations had 0 to 32 bw^{75} genes in different (19) generations. The total number of populations fixed for one of the two alleles increased at nearly a linear rate after generation 4 and in generation 19 it is nearly equal for the two alleles, with 30 populations fixed for *bw* and 28 fixed for bw^{75}. In silkworm germplasm maintenance centers, at every cycle only 40–60 cocoons are selected from each strain/breed for the next generation, from which around 20 layings are prepared and subsequently only 5-6 layings are brushed for next generation. This size of population is small which may be lead to change in allele frequency as explained by Buri [98].

5.5. Effects of Inbreeding. Loss of genetic diversity among populations occurs due to the synergetic effects of inbreeding and environmental stressors [99]. The negative interaction between inbreeding and environmental stress reflects on population growth rates and inbreeding and environmental effects may interact in their effects on population dynamics. Inbreeding is characterized by an increase in homozygosity resulting in increased expression of recessive deleterious alleles (partial dominance hypothesis) [100] and/or reduced opportunity to express heterozygote superiority (overdominance hypothesis) [101]. Selfing has direct genetic consequences, including its effect on the intensity of inbreeding depression [102] and the partitioning of genetic diversity within and among populations [103]. A consequence of inbreeding is that it makes it much more likely that an individual is homozygous for a rare gene because it is more likely that two related parents simultaneously possess a rare allele and transmit it to their inbred offspring than the two unrelated individuals independently transmit the same rare allele to noninbred offspring. Thus inbreeding seems to reduce fitness because it reveals harmful genes in homozygotes [104].

Sericulture practicing countries maintain hundreds of inbred lines of silkworm in germplasm centres for several decades by selection and inbreeding. Sibling mating of the progenies derived from a single brood is preferred for pure stocks so that the original traits of the races are maintained through generations. Generally, breeders try to maintain the original characters of the races/breeds through selection with care to avoid inbreeding depression. However, the effects of inbreeding can accumulate over many generations, as the frequency of slightly deleterious alleles can gradually increase over time due to genetic drift [105, 106]. This is a particular concern in small populations, where natural selection can be inefficient for alleles that have only slight effects on fitness [107]. The rate at which genetic diversity is lost will depend on the population's size and degree of isolation; small, isolated populations can lose genetic diversity within a few generations, whereas large, continuous populations may not lose significant amounts of diversity over thousands of years [108]. In small populations where genetic drift is most rapid, the fixation of common alleles will result in the reduction of genetic diversity. This phenomenon is applicable in silkworm, as different silkworm strains are maintained with small population leading to genetic drift thereby may be reducing genetic diversity. Further, Li et al. [109] when analysing genetic diversity in *B. mandarina* and *B. mori* concluded that the polymorphism level ($\theta\pi$) of mt sequence among Chinese wild population (6.20×10^{-3} nucleotide differences per site) is more than six times that among domesticated varieties (1.14×10^{-3}) and pointing out that the relative larger reduction in polymorphism is most likely caused by inbreeding or population bottlenecking.

6. Broadening the Genetic Diversity

Continuous breeding and selection of silkworm breed for uniformity narrows genetic diversity. One of the approaches

to broaden the genetic diversity is the use of recent advances in molecular biology and biotechnology, which allow the transfer of specific genes from diverse sources to target genotypes. In [110], through transgenic approach, by gene addition, subtraction, and pathway redirection, the genetic constituents of crops can be modified and broadened, resulting in new and improved traits. Another approach of broadening the genetic diversity is by the use of exotic germplasm [111]. It will create genotypes with a diverse range of desirable characteristics. The genetic variation that breeders need to introduce these characteristics is often available only through the exchange of genetic resources. This exchange is necessary because some areas of the world have richer resources of genetic diversity, which will be useful in creation of variation.

7. Germplasm Conservation

Conservation of genetic diversity is essential to the long-term survival of any species, particularly in light of changing environmental conditions. Reduced genetic diversity may negatively impact the adaptive potential for a species. Increasing population size and maximizing genetic diversity are among the primary goals of conservation management [112]. The silkworm germplasm maintenance centres generally follow brushing of "composite population" type of all strains to avoid inbreeding depression as well as genetic erosion and maintain the gene pool as far as possible. Composite laying is defined as collection of a known number of eggs from a known number of individual laying sources that represents the whole population. Though composite layings method can retain gene pool, there is a concern regarding populations, as even slight selection has a drastic effect on genetic variability when the effective population size N is large [113]. In this method, only 250–500 larvae are retained in a strain; improper selection can lead to inbreeding depression and natural selection can be inefficient for alleles that have only slight effects on fitness [107].

8. Strategies Required for Conservation of Silkworm Genetic Resources

(1) The curator of the germplasm bank should carefully verify the available genetic resources and avoid duplicates before collection and introduction of new material.

(2) Development of cost-effective, viable, and cost-economic conservation practices through modification or development of long-term preservation of silkworm genetic resources to reduce the number of crop cycles is required.

(3) Conservation through modern methods includes cryopreservation of sperm, artificial insemination, and induction of synthetic diapause hormones to be explored.

(4) Genetic resources should be categorized as most sensitive and sensitive based on their availability in one place or in more than one place, respectively.

(5) The most sensitive genetic resources should be conserved in more than one place by establishing backup stations under the control of main germplasm station.

(6) Establishment of centers for preservation of endangered/local species under *in situ* condition is required.

(7) Use of silkworm genetic resources for nonsericultural use other than cocoon production needs importance.

9. Conclusion

Though silkworm has been domesticated for hundreds of generations, based on available literature, it is speculated that it has not experienced any major reduction of genetic diversity due to phenotypic selection and breeding. But there is concern that bottlenecks may restrict breeding flexibility and slow response to new opportunities, pests, pathogens, and other practices in the future. To broaden the gene pool of silkworm, exotic elite strains were required to be introduced from various countries. The genomes of introduced exotic germplasm will broaden the gene pool; thereby diversity can be maintained. The original genetic composition of genetic resources should be maintained by avoiding genetic drift and selection process. Maintaining adequate population size can prevent the loss of genetic variability due to genetic drift [114]. Study on effects of inbreeding on inbreeding coefficients in silkworm populations is limited. Hence understanding the effects of inbreeding for various traits can be very crucial points in the management of germplasm. As suggested by Doreswamy and Subramanya Gopal [115] during stock maintenance in germplasm centers, rigid selection for more numbers of generations is required to retain original characteristics of the inbred lines and also reduces the deleterious effects of inbreeding.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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