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Author(s)	**Maimoona Kanwal** **Waqar Younus** **Mubashar Hussain**
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An Insight into the Genomics of Mulberry Silkworm: A Review

Maimoona Kanwal[1*], Waqar Younus[2], Mubashar Hussain[1]

[1]Department of Zoology,
University of Gujrat, Gujrat, Pakistan
[2]Department of Biochemistry and Molecular Biology
University of Gujrat, Gujrat, Pakistan

*17111714-010@uog.edu.pk

Abstract

The purpose of this article is to review the genetic basis of mulberry silkworm to understand the mystery of silk production and the silkworm's role as a model organism. Data regarding mulberry silkworm's genetic diversity, genetic bases of silk production, gene mapping and chromosomal properties was reviewed. Findings illustrated that genetic variability exists among mulberry silkworms of different geographical regions. Hence, it acts as an indicator of the genetic bases of silk production since it is higher in males, although sex is primarily determined by females. Studies have revealed that chromosomes in mulberry silkworm are holocentric and gene mapping provides an insight into the accurate location of silk genes on chromosomes. It is concluded that the genetic study of silkworm is useful due to its commercial and economic significance and it is the crucial need of sericulture industry to enhance its output by collecting information about superior silkworm breeds. Hence, further research should be carried out to explore the hidden facts about mulberry silkworm.

***Keywords*:** chromosomes, molecular genetics, silkworm genomics, silkworm domestication, transposable elements

1. Introduction

Mulberry silkworm *Bombyx mori* is a valuable insect due to its role in the production of fine quality silk. Its genetic study is essential to get more silk because it provides an insight into genes concerned with silk production and it leads to the development of high quality silkworm breed [1, 2]. However, silkworm breeders are trying to comprehend the superior genetic traits of this valuable insect for high silk yield [2] and leading countries in this field are China, India, Japan and Korea [3, 4].

Mulberry silkworm is regarded as a pioneer organism in the field of genetics because it provided the earliest verification of Mendel's laws of segregation, that is, genes of silkworm segregate in Mendelian fashion

[5, 6]. Easy handling of silkworm makes it the second most successful organism in laboratories apart from drosophila. Since Lepidoptera contains some agricultural pests, so the genetic study of silkworm is helpful in their control [7, 8].

Various scientists have explored the genes of silkworm [9, 10] and provided an insight into its genetic makeup. The reported genome size of *Bombyx mori* is 495 Mb, while the use of Shotgun sequencing technique revealed the haploid genome size of 530 Mb [11]. Chromosomal study indicated that silkworm has 28 haploid number of chromosomes that are holocentric in nature [12]; during mitotic and certain stages of mitosis they appeared condensed or dot shaped [13]. Chromosome size for silkworm is 17.1 Mb [14], while the total chromosomal length of silkworm is recorded as 132.35 micrometer [9, 10]. Silkworm contains 400 visible phenotypes with 200 linkage groups. Almost 18510 genes are predicted by Silkworm Database (SDB) but only 14623 genes have been counted actually and this number is greater than the Fruit fly [15]. A large quantity of silkworm data is saved online in SDB [7].

2. Genetic Study of Silkworm

Gene mapping of silkworm started in late 19's but the very first genetic map of silkworm was constructed by [14]. Gene mapping has led to the identification of 1018 genetic markers with an approximate interval of 500 kb, and it was carried out in twenty-seven autosomes and one Z chromosome [8]. By using backcross population, linkage map was constructed with 85 STS markers, 80 SSRM markers and 16 single nucleotide polymorphisms. Using this data, another map was constructed with 181 markers [3, 15]. For cocoon and filament characters, fourteen QTL (Quantity, Thickness and Length) were analysed among five linkage groups numbered as 1, 14, 18, 23, 25 [16, 17]. Less than 50000 genes were analysed that led to the construction of two linkage maps. BAC integrated consensus map provided 692 unique microsatellites with a gene density of 6.3 cM [18]. It was reported that low polymorphic sites consisted of 497 genetic markers. All these mapping results have shown that genes of silkworm are orthologous with Honey bee and Weevils [19].

2.1. Study of Silkworm on the Bases of Genetic Diversity

Genetic diversity is seemingly dependent on geographical distribution [19], since silkworms belonging to different climates exhibit different genetic makeup [7]. Patterns of silkworm nuclear diversity are studied

using the neutrality test and the bottleneck intensity of silkworm is 1.5. Genetic diversity is studied using microsatellite and mtDNA as main markers and it has helped in the selection of breeds which are responsible for high quality silk production [20]. By using Rapid Amplification of Polymorphic DNA (RAPD) markers, genetic diversity of three species was analysed and it was found that *Bombyx mori* has only 16% genetic variations with a high value of genetic diversity. Geographical evolution of domestic silkworm on the bases of mitochondrial genes has been studied and it has been found that they have evolved from the wild Chinese silkworm [21, 22]. Selection of eighteen silkworm strains was carried out to check their genetic diversity by PCR. Five primers were selected and 368 amplified bands with 13.25% polymorphism [23].

2.2. Techniques Involved in Genomics Study

The study of silkworm genome has been carried out using a number of techniques like Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), microsatellite and Expressed Sequence Tags (EST) as 2000 cM, 897 cM, 900.2 cM is calculated as the recombination length by AFLP, RAPDs, RFLPs and phenotypic markers, respectively [24, 25]. EST sequence was proposed in 2002 and approximately 47% of silk genes are expressed by it [26]. By Shotgun method, silkworm genome was sequenced in 2004 [25]. High quality silkworm genome was studied in 2007 that included all previous data [23]. Microsatellite were used to analyse the genetic diversity of seven breeds of silkworm. Functional elements in silkworm genome were studied using EST and microarrays [9]. According to gene finder technique, 18510 genes are encoded by silkworm genome; while EST technique has identified only 15% of genes. Using high-throughput paired-end RNA sequencing, 3.3 gigabases (Gb) of sequence was studied with sevenfold coverage of genome. A total of 23,461 results were found with novelty in 5428 regions. Among 14623 predicted regions, 11884 are expressed by genome. Transcriptome analysis revealed that 3247 genes show alternative splicing [10]. Using DNA barcoding, 658 bp mitochondrial Co1 standardized sequence was taken as base and region wise evolutionary history of silkworm was carried out. It was found that all parental breeds were similar to one another while among hybrids, NB7 was distantly related to others [27]. Less than 3000 strains of silkworm have been reported by using these techniques; it illustrates that genetic markers help to distinguish the strains in spite of their environment [4].

2.3. Genes Involved in Silkworm Domestication

Evolutionary insight into the genes of silkworm indicated that the estimated time of its domestication is 7500 years ago, while the termination time is 3984 years ago [10]. Domestication is controlled by 2.9% of total genes and it still contains 83% genes of wild origin [11]; however, no actual gene has been identified. However, according to the estimation, there are 354 candidate genes for domestication [28]. Although, it is also estimated that 33-49% loss of genetic diversity occurs during domestication [29].

2.4. Genetic Bases of Silk Production

The regulation of the synthesis of silk sericin is under control of three genes labelled as Ser1, Ser2 and Ser3, with four introns in each gene [30] and repetitive sequences of 114 bp in one exon. Sericin consists of two types of mRNA (11 and 9.6 kb long), while the former plays the most crucial role. Gene coding silk fibroin is not yet identified but it is thought to produce 104 copies of mRNA that lead to the production of 109 copies of silk fibroin. This amplification is an indication of transcriptional activity [9]. Genes of both silk proteins show homology at 5 flanking sequence regions that coordinate the expression of silk genes [27]. Silk produced by males and females differs quantitatively and qualitatively and genetic study revealed that 210 genes make the bases of this difference [1]. Silk production, being dependent on silk gland, can be enhanced by genetic manipulation of the posterior part of silk gland that is responsible for silk fibroin production [9]. Genetic study indicates that during 5th instar DNA amount is highest in silkworm because in the middle of 5th instar DNA replication dominates [31].

2.5. Study of Sex Chromosomes in Silkworm

Sex determination is important due to major silk production by males [2]. Males are homogenous while females are distinguished as heterogeneous. So, sex determination is done by W chromosomes but until now, feminizing gene (FEM) remained undiscovered. The comparison of W translocated and normal W chromosomes revealed that in zebra strain, the deletion of W specific RAPD markers was observed; while during the study of yellow cocoon strains, eleven W specific RAPD markers were lost. Large number of genes are present on Z chromosomes [12]. ZZ and ZW sex determination system is present in silkworm and W chromosomes perform epistatically [10]. It is also concluded that silkworm and drosophila have common downstream genes. Molecular structure of W chromosomes was determined by Abe

and his colleagues. By using RAPD analysis, they compared male and female DNA. It was analysed that female DNA has more transposable elements due to W chromosomes that are enriched with them [25]. Autosomes have transposable elements and similar sequences were found on W chromosomes. So, they were identified as transposable elements [20]. Larval gonadal tissue was selected to observe chromosomes' behaviour and karyotype. Preferential pairing was studied between ZZ and ZW in 4n oocyte. Two types of results (ZZ+WW & ZW, ZW) were obtained in the ratio of 34:21; hence, preferential pairing was confirmed [13].

Table 1. Overview of Silkworm Genomics

About Genome:	
495Mb	[2]
530Mb	[11]
About Genes:	
18510 predicted genes	[13]
14623 counted genes	[11]
6.3Cm gene density	[18]
2.9% genes in domestication	[5]
33% genetic diversity loss during domestication	[14]
About Chromosomes:	
Holocentric in nature and 28 (haploid)	[25]
Total chromosomal length-132.35	[26]
Chromosomal size- 17.1Mb	[10]
Techniques used in genomic study of silkworm:	
Amplified Fragment Length Polymorphism (AFLP)	[31]
Restriction Fragment Length Polymorphism (RFLP)	[3]
Rapid Analysis of Polymorphic DNA (RAPD)	[9]
DNA barcoding	[27]
Microarrays and Expressed Sequence Tags	[12]

The major drawback in the study of silkworm genetics is the presence of highly repetitive sequences with gaps leading to incorrect gene prediction. It is also limited by the unavailability of full length DNA that acts as a hurdle in the study of gene structure and other properties

[13]. All of this data is summarized below in table 1.

3. Conclusion

Silkworm is used extensively in genetic studies as a Lepidopteran model insect. Chromosomal structure, gene mapping and sex chromosomes are studied by a number of techniques including RFLP, AFLP and RAPD. Males are dominant over females in silk production. Genes involved in silk production are studied to try new breeds for producing superior quality silk. It is concluded that a lot of shortcomings are present in silkworm genomics due to repetitive sequences. So, more work should be performed on it with special reference to silk coding genes, exons and introns. Further investigation should be conducted on silkworm genomics because it is not only helpful in applied research on silkworm but also provides a new way to study comparative biology and control of pests in the order Lepidoptera.

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