Role of microsatellites in genetic analysis of *Bombyx mori* silkworm: a review [version 1; peer review: 2 approved]

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Abstract

In the genome of *Bombyx mori* Linnaeus (1758), the microsatellites, or simple sequence repeats (SSR), feature among their particular characteristics a high adenine and thymine (A/T) content, low number of repeats, low frequency, and a grouping in "families" with similar flanking regions. Such characteristics may be the result of a complex interaction between factors that limit the size and dispersion of SSR loci—such as their high association with transposons—and mean that microsatellites within this taxon suitable as molecular markers are relatively rare. The determination of genetic profiles in populations and cell lines has not been affected owing to the high level of polymorphism, nor has the analysis of diversity, structure and genetic relationships. However, the scarcity of suitable microsatellites has restricted their application in genetic mapping, limiting them to preliminary identification of gene location of genes or quantitative trait loci (QTLs) related to thermotolerance, resistance to viruses, pigmentation patterns, body development and the weight of the cocoon, the cortex, the pupa and the filament. The review confirms that, as markers, microsatellites are versatile and perform well. They could thus be useful both to advance research in emerging countries with few resources seeking to promote sericulture in their territories, and to advance in the genetic and molecular knowledge of characteristics of productive and biological interest, given the latest technological developments in terms of the sequencing, identification, isolation and genotyping of SSR loci.

Keywords

*Bombyx mori*, silkworm, molecular marker, sericulture, Simple Sequence Repeats, SSR.
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Introduction
Domesticated around 5,000 years ago, the silkworm, Bombyx mori L., is the basis of sericulture, an agroindustrial activity that involves the breeding of silkworms, cultivation of Morus spp. mulberry plants as the sole source of food, and industrial processing of cocoons to produce natural silk yarn. B. mori is an ideal organism as an experimental animal for genetic and biological research. Silkworms are easily reared and produce a genetically uniform population. This, added to their economic importance, has made B. mori one of the most widely studied insects.[3,4]. As a result, genetic materials of agronomic and scientific interest have been identified, characterized and conserved[4] using a variety of molecular markers, prominent among them microsatellites, or simple sequence repeats (SSRs).

Microsatellites are regions of DNA in which a sequence or motif between 1 and 6 bp is repeated in tandem 5 to 100 times, the number of repeats of the same locus being highly variable both inter- and intra-populationally. They are also ubiquitous, distributed uniformly in eukaryotic genomes[5]. SSR loci are usually flanked by regions of unique sequence, which allows PCR amplification with specifically designed primers and determination of the specific genetic profile or genotype of an individual for several loci through the pattern of bands displayed on capillary sequencing equipment[6]. These markers are codominant and highly polymorphic. They have greater reproducibility and band comparability, are less sensitive to contamination by foreign DNA (due to their specificity) and can be amplified using fragmented or partially degraded DNA, because of the reduced size of the loci[7].

These molecular markers have been a useful tool in sericulture, especially in the management, characterization and conservation of materials in germplasm banks[8] and in the development of genetically improved materials[9]. They have been particularly difficult to apply, however, to genetic mapping, mainly due to the low frequency of loci suitable as molecular markers in the B. mori genome. Their contribution in this area has therefore been restricted to preliminary mapping of some genes and QTLs related to characteristics of productive interest[10] or to processes of B. mori body development and pigmentation[11,12].

The review focuses on the description and analysis of the specific characteristics of microsatellites in the B. mori genome, the role these repetitive DNA regions have played as molecular markers in this organism, and how these would play an important role in future in the genetic analysis, conservation and use of silkworm germplasm.

Characteristics of silkworm genome microsatellites
Microsatellite characteristics—distances between loci, abundance, distribution, motif and the average number of repeats they comprise—may vary among taxa[13]. The B. mori microsatellites feature average distances of between 49 and 161 kb[14,15], their genome coverage is only 0.31%[16] and they have a cloning efficiency of between 0.77 and 3.5%[14,15]. Together, such values indicate that microsatellites in the B. mori genome are rare. The pattern is not unusual however, having been observed in other insect species, including several in the Drosophila genus[17,18].

SSR loci with mononucleotide-like motifs represent 60% of the microsatellites of the B. mori genome, high compared to other insect species[13], and are formed by a low number of repeats, nine on average among the different repeat motifs. Loci of more than 15 repeats are scarce, except for mononucleotide motifs[16,17]. Harr and Schlötterer[19] report that Drosophila melanogaster Meigen (1830) also has short SSR loci on average (<15 repeats), because the greater the length, the greater the tendency of the D. melanogaster microsatellites to mutations that decrease the number of repeats instead of increasing it. This is speculated to be the result of interaction between factors related to DNA repair and replication mechanisms.

The B. mori genome has a comparatively high composition of regions rich in adenine and thymine (A/T). Moreover, the microsatellites that include a high proportion of these bases are the most abundant, constituting approximately 40% of the dinucleotide motifs and 28.3% of the total[20]. Such a predominance has not been seen in other organisms[21,22].

Transposons appear to play a key role in the evolution of microsatellites in the B. mori genome[23]. Of the B. mori SSR loci, 35% are grouped into “families” with flanking regions similar in sequence. This is due to their association with transposons[23], mobile genetic elements capable of replicating themselves and associated regions. Transposons account for 35–43.6% of the D. melanogaster genome[24]. For Meglécz et al.[19], this high association suggests one of two scenarios: transposons might favor the development of microsatellites in their vicinity, or promote their formation at the moment of transposition. It is possible, however, that they have no part to play in the genesis. The SSR loci could develop independently, but have a structure that favors the insertion of mobile elements[25].

The characteristics of the B. mori microsatellites—low frequency, low average number of repeats, and grouping in families—are common to the Lepidoptera order. They explain the difficulties in isolating single copy microsatellites[7,18,25,26], but the advance in high-throughput sequencing techniques and graph-based cluster analysis[27], as well as screening against transposons elements in the isolation procedure[28] may in future facilitate identifying suitable microsatellites for genetic studies in species in this taxon.

Zhang[21] suggested that the characteristics of Lepidoptera microsatellites imply that, in their respective genomes, most have experienced a recent development and multiplication, in the different lepidopteran species. This may mirror that reported in D. melanogaster[19], characterized because its long SSR loci are of recent origin and have short prevalence periods.

Microsatellite evolution is extremely complex[24]. The characteristics in B. mori and the Lepidoptera, as well as in other organisms[25], appear to result from interaction in the genome between the mutations or events that cause them and the factors that
obstruct or limit their development, with a balance in favor of the latter in silkworm. The key to the low average number of repeats and low frequency of SSR loci in the _B. mori_ genome may be the abundance of A and T bases, and of microsatellites composed of these bases. Regions rich in A/T have a higher frequency of double-strand breaks in the DNA, which can induce and facilitate both the loss of nucleotides during non-homologous recombination and the insertion of transposons able to divide an SSR locus in two and interrupt its development. This could explain why A/T-rich microsatellites in _B. mori_ have a lower average number of repeats, as reported by Zhan et al.

Additionally, the presence in _B. mori_ of an efficient DNA mismatch repair mechanism, the system in charge of correcting the incorrect incorporation of bases, could counteract replication slippage, the major mutational mechanism in explaining the origin and evolution of repetitive DNA regions.

### Applications of microsatellites

#### Construction of DNA profiles

Microsatellites have proved a useful tool for generating band patterns that enable discrimination of silkworm lines. Kim et al. found that 25–28% of the amplified alleles are specific to the lines, due to which a small number of microsatellites, one to three, allow identifying approximately 20% of the analyzed materials without resorting to the genotyping of other loci. Hou et al. and Chandrankanth et al. likewise indicate that the analyzed genotypes are homozygous for a substantial part of the microsatellites used. Since each locus is multiallelic, those that amplify as a single band in each line are powerful tools for identification, as demonstrated by Li et al. when discriminating between two closely related lines identical in their morphological characteristics: Dazao and P50.

DNA fingerprinting also represents a tool for the identification of insect cell lines. Between 3 and 8 microsatellites have therefore been used to generate profiles of cell lines susceptible to the nuclear polyhedrosis virus of _B. mori_ (BmNPV), developed to study replication and expression mechanisms of the virus. McIntosh et al. obtained stable DNA profiles even after performing 200 subcultures by using coding regions (aldolase, prolactin receptor, interleukin-1β) as molecular markers. Microsatellites are less stable, however, due to their high mutation rate—between $10^4$ and $10^6$ per locus per generation. Thus, for future identification of cell lines, it is advisable to evaluate and select SSR loci that present relatively low mutation rates.

#### Analysis of genetic diversity

Sericulture depends on the strategic use of silkworm germplasm to develop hybrids with high yields of silk that resist or tolerate disease and adverse climatic conditions, based on knowledge of the extent and distribution of the genetic diversity available in both the domesticated silkworm _B. mori_ and its wild relative _Bombyx mandarina_ Moore (1872). In this context, between 500 and 700 microsatellites were developed in _B. mori_, of which 5 to 27 markers have been used for analyzing genetic diversity (Table 1).

Miao et al. and Zhan et al. discovered that the genome of _B. mori_ lines with contrasting characteristics is similar, in terms of the low percentage of polymorphic SSR loci, ranging between 17% and 24% compared, for example, with 85% for the European bee, _Apis mellifera_, and 55% in laboratory rats. Together, these data attribute the origin of _B. mori_ to a single domestication event from a reduced population of _B. mandarina_. Xia et al., however, on conducting a complete genome analysis on domesticated lines and wild individuals report that _B. mori_ harbors 83% of the genetic variability of wild populations, indicating that the origin was possibly not limited to a reduced population or a single domestication event.

The low percentage of polymorphic SSR loci found among domesticated lines of _B. mori_ should not be interpreted as evidence of the reduction of genetic diversity with respect to wild populations. The low figure could instead be the result of size homoplasy, a process of change by which convergent mutations cause microsatellites, belonging to different lineages, to have the same length in base pairs.

Size homoplasy is favored when mechanisms are present that neutralize elongation of the microsatellites and limit the number of repeats, since possible alleles are reduced and mutations are more likely to converge in the same length. These conditions appear to be present in the _B. mori_ genome, in which most microsatellites are of reduced size. In 2016, De Barba et al. proposed a new method for genotyping microsatellites using high-throughput sequencing; this would allow direct access to the microsatellite sequences in _B. mori_ and evaluate whether the low percentage of polymorphic loci is due to the presence of size homoplasy.

Although _B. mori_ has not experienced a drastic reduction in genetic diversity compared to _B. mandarina_, the wide genetic distances between domesticated and wild populations indicate that these species have a marked genetic differentiation and distant relationships. This is likely due to the absence of genetic flow, a result of the inability of _B. mori_ to fly and to survive without human intervention. Thus, _B. mandarina_ represents a potential unique source of genetic material for sericulture.

Cluster analyzes to determine the relationships between _B. mori_ lines provide contradictory results. Reddy et al., Qian et al., Thiyagu and Kamble and Chandrakanth et al. report that grouping the materials based on the microsatellites analyzed corresponded to type of volitism, geographical origin, silk productivity, color or shape of the cocoon. However, the groups formed in studies that analyzed a larger sample of germplasm—69 lines on average, compared to 18 in the works cited above—exhibit mixtures of genotypes with variability in these characteristics.
Table 1. Studies of genetic diversity conducted in *B. mori* using microsatellites.

<table>
<thead>
<tr>
<th>Reference</th>
<th>SSRs(^1) and alleles</th>
<th>PIC(^1)</th>
<th>Genotypes and groups</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reddy <em>et al.</em>, 1999(^2)</td>
<td>15 and 113</td>
<td>-</td>
<td>13 and 2</td>
<td>Identification of specific alleles of lines with diapause and without diapause.</td>
</tr>
<tr>
<td>Zhang <em>et al.</em>, 2005(^3)</td>
<td>27 and 146</td>
<td>0.57-0.88</td>
<td>12</td>
<td>The diversity in highest in <em>B. mandarina</em> populations, followed by Chinese, Japanese and European lines of <em>B. mori</em>.</td>
</tr>
<tr>
<td>Li <em>et al.</em>, 2005(^4)</td>
<td>26 and 188</td>
<td>0.12-0.89</td>
<td>31 and 7</td>
<td>Groupings with mixtures of genotypes of different types of voltinism.</td>
</tr>
<tr>
<td>Hou <em>et al.</em>, 2007(^5)</td>
<td>35 and 467</td>
<td>0.30-0.92</td>
<td>96</td>
<td>Groups composed of mixtures of genotypes with different types of voltinism and geographic origin. The greatest diversity in lines of Chinese origin.</td>
</tr>
<tr>
<td>Qian <em>et al.</em>, 2007(^6)</td>
<td>11 and 106</td>
<td>-</td>
<td>40</td>
<td>The groupings coincide with differences in geographical origin and voltinism.</td>
</tr>
<tr>
<td>Vijayan <em>et al.</em>, 2010(^7)</td>
<td>7 and 49</td>
<td>0.10-0.40</td>
<td>13 and 2</td>
<td>Genotypes with contrasting characteristics could be crossed to obtain hybrid vigor in their offspring.</td>
</tr>
<tr>
<td>Kim <em>et al.</em>, 2010(^8)</td>
<td>9 and 68</td>
<td>0.06-0.86</td>
<td>54</td>
<td>The groupings do not coincide with phenotypic characteristics of the lines. On detecting 17 unique alleles, it was possible to identify 14 lines.</td>
</tr>
<tr>
<td>Thiyagu and Kamble, 2011(^9)</td>
<td>10 and 139</td>
<td>-</td>
<td>10 and 4</td>
<td>The grouping coincided with differences in geographical origin and silk productivity of the lines evaluated.</td>
</tr>
<tr>
<td>Kim <em>et al.</em>, 2012(^10)</td>
<td>8 and 76</td>
<td>0.34-0.82</td>
<td>85</td>
<td>On detecting 22 unique alleles, it was possible to identify 19 lines. The groups formed do not coincide with known phenotypic characteristics.</td>
</tr>
<tr>
<td>Chu and Peng, 2013(^11)</td>
<td>22</td>
<td>0.0-100</td>
<td>23 and 2</td>
<td>The lines were divided into 2 groups.</td>
</tr>
<tr>
<td>Chandrakanth <em>et al.</em>, 2014(^12)</td>
<td>15 and 54</td>
<td>0.16-0.75</td>
<td>10 and 4</td>
<td>The groupings coincide with the geographical origin, and the subdivision coincides with the color or shape of the cocoon.</td>
</tr>
<tr>
<td>Furdui <em>et al.</em>, 2014(^13)</td>
<td>5 and 31</td>
<td>0.35-0.67</td>
<td>15</td>
<td>20% of the genetic variance is between genotypes. Lines with wide genetic distances and contrasting characteristics are useful for developing new hybrids.</td>
</tr>
<tr>
<td>Kim <em>et al.</em>, 2014(^14)</td>
<td>8 and 73</td>
<td>0.37-0.77</td>
<td>78</td>
<td>The groupings do not coincide with the phenotypic characteristics of the lines. On detecting 19 unique alleles, it was possible to identifying 16 lines.</td>
</tr>
</tbody>
</table>

\(^1\) SSRs: microsatellite loci used (Simple Sequence Repeats)  
\(^2\) PIC: polymorphic information content (PIC) ranking of microsatellites within the study

Silk yield or survival rate in various environmental conditions\(^15-35\). This would alter their phenotypic characteristics but, due to backcrossing, they would maintain a similar—or even the same—genotype, depending on the microsatellites analyzed.

Various pure lines may also share an origin in the same ancestral population of *B. mandarina*, but would have been selected to express different phenotypes, maintaining a high similarity at the genotypic level\(^35-39\). This might also explain why pure lines with similar characteristics are not always grouped, given that they would have a distant relationship, but would have been selected to express similar traits\(^31-37\).

Traditional genetic improvement in *B. mori* implies the use of phenotypic characteristics or geographical origin to differentiate and select parents with contrasting characteristics with which to perform crosses. However, due to the scenarios already mentioned, these characteristics do not always make it possible to accurately determine genetic relations between materials\(^36\). Microsatellites thus represent an important tool for making accurate estimates of genetic diversity and relationships in order to develop genetically improved materials (hybrids), bearing in mind that, comparatively, performance is better than that of other markers such as RAPDs, RFPLs and ISSRs\(^36\). Microsatellites not only represent a marker with a good performance in analysis of genetic diversity in *B. mori*. They constitute a versatile tool that can be adjusted to meet the research requirements and the resources available. For example, they can potentially be genotyped with high-throughput sequencing for greater accuracy\(^46\) and even be identified and analyzed simultaneously with SNPs to strengthen inferences about diversity, structure and genetic relationships\(^41\). However, they can also be identified by means of polyacrylamide gels or capillary sequencing equipment when fluorescently labeled\(^1\). As such, they provide accessible and profitable information for managing germplasm...
banks and promoting regional initiatives in developing countries that do not have the resources to access cutting-edge technology, yet view sericulture as an opportunity to generate employment and improve the conditions of the rural population.

**Linkage maps**

Determination of the relative positions of microsatellite markers in the chromosomes of the *B. mori* genome began with the low-density linkage map developed by Prasad et al. The medium density one constructed by Miao et al. followed, with an average distance between markers of 6.3 cM and 29 linkage groups. Zhan et al. subsequently increased the density of this map using new lines and mapping populations of *B. mori*, decreasing the average distance between markers to 4.8 cM (Table 2).

The density achieved with the linkage map of SSR markers is below the results expected by the authors due to the high homology (low percentage of polymorphic loci) between the loci of the *B. mori* lines used to generate the mapping populations. Nevertheless, the resolution is sufficient to carry out the preliminary gene screening (Table 3) and identification of QTLs.

Exclusive linkage maps for the Z chromosome were developed by Nagaraja et al. and Miao et al. with the purpose of contributing to the identification of genes related to control of the duration of larval stages, diapause, moltinism, body size and color, etc., and to analyze the role played by characteristics linked to sex in evolutionary processes in Lepidoptera, and differentiation of geographic races.

**Identification of genes and QTLs**

Identification of SSR markers linked to genes was carried out in order to understand the molecular basis of characteristics of agronomic and scientific interest. The SSR loci identified by Miao et al. and Zhan et al. enabled the identification of genes related to characteristics such as thermotolerance, resistance to the Z strain of the virus of densonosis in *B. mori*, tolerance to fluorinated compounds, and absence of wing scales; studies that were used to develop improved lines with marker-assisted selection. Genes related to pigmentation patterns were also identified, in cocoons and larvae, and in development processes such as the formation of extremities, thoracic segments, cell adhesion and regulation of molting (Table 3).

Microsatellites have also allowed tracking of QTLs in *B. mori* related to weight of cocoon, cortex, pupa and filament, which are mainly located on chromosome 1 where they are strongly linked to SSR loci (LOD>1.0), contribute significantly to phenotypic variation (30 %) and have a simultaneous effect on the above characteristics. The location of the QTLs in chromosome 1 was delimited to a region of 290 kb, in which 12 candidate genes were identified that will allow study of the molecular mechanisms underlying these characters of agronomic interest in *B. mori*. Additionally, Gao et al. discovered that Bombyx mori Nuclear Polyhedrosis Virus (BmNPV) resistance is a polygenic characteristic.

**Conclusions and future work**

The microsatellites in *B. mori* have particular characteristics such as low frequency, low average number of locus repeats and grouping into “families”. These are shared by Lepidoptera

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**Table 2. Data summary of linkage maps with microsatellites in *B. mori***

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of LGs</th>
<th>Individuals analyzed</th>
<th>Number of markers</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Others</td>
</tr>
<tr>
<td>Prasad et al., 2005</td>
<td>8 [1]</td>
<td>60 BF1</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Miao et al., 2005</td>
<td>29 [26]</td>
<td>189 BF1</td>
<td>547</td>
<td>518</td>
</tr>
<tr>
<td>Nagaraja et al., 2005</td>
<td>13</td>
<td>55 BC1M</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Miao et al., 2008</td>
<td>1</td>
<td>188 BC1M</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Zhan et al., 2009</td>
<td>30 [28]</td>
<td>188-190 BF1</td>
<td>692</td>
<td>692</td>
</tr>
</tbody>
</table>

1LGs: linkage groups
2SSRs: microsatellite loci (Simple Sequence Repeats)
3Numbers in square brackets indicate linkage groups assigned to established linkage groups
4BF1: backcross
5BC1M: backcross with F1 male
and seem to indicate that factors or mechanisms exist within the genome of this taxon that limit growth and stability of these repetitive DNA regions and their used as single copy loci molecular markers. Such as an abundance of loci SSR rich in A/T susceptible to double strand breaks and loss of repeats, as well as the possible existence of efficient DNA repair mechanisms that avoid the incorrect incorporation of bases (DNA mismatch repair) and the high association with transposons that would cause the formation of groups of loci with high similarity in their flanking regions.

The characteristics of the SSR loci in *B. mori*, and generally in all Lepidoptera, have made identification and isolation of these markers difficult. They have also been a limitation for applications in genetic mapping. The low frequency and high homology (low percentage of polymorphic loci) of the microsatellites between contrasting lines of *B. mori*, possibly due to the size homoplasy, has not allowed the development of high-density linkage maps. This, together with the absence of mapping models adjusted to this organism, has hindered identification of genes and QTLs, limiting contributions mainly to preliminary mapping, for example of regions related to pigmentation patterns and development processes, as well as to weight of cocoon, cortex, pupa and filament.

These regions of repetitive DNA, however, have shown a high discriminating power between *B. mori* lines due to the high level of polymorphism, the finding of a percentage of single alleles higher than 20%, and the high levels of homozygosity in the materials analyzed, so that a reduced subset of 5–8 SSR loci have made it possible to generate DNA fingerprints, estimating the genetic diversity of domesticated and wild materials and determining the genetic relationships between closely related lines such as Dazao and P50. Although these markers additionally represent a potential tool for identifying *B. mori* cell lines, it is necessary to evaluate and select a subset of microsatellites with relatively low mutation rates that provide stability to the genetic profiles for 200 or more subcultures.

The data indicate that microsatellites will continue to be important for the study, management, conservation and use of silkworm germplasm. They have shown superior performance in these aspects compared to most molecular markers and are versatile – they can be analyzed, depending on resources available

<table>
<thead>
<tr>
<th>Name</th>
<th>LG†</th>
<th>Expression or function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>nsd-Z</td>
<td>15</td>
<td>Resistance to the virus of densonucleosis</td>
<td>Li et al., 2006&lt;sup&gt;60&lt;/sup&gt;</td>
</tr>
<tr>
<td>Seli</td>
<td>24</td>
<td>Yellow cuticle</td>
<td>Miao et al., 2007&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Xan</td>
<td>24</td>
<td>Yellow cuticle</td>
<td>Miao et al., 2007&lt;sup&gt;71&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bmabd-A</td>
<td>6</td>
<td>Additional limb pair</td>
<td>Xiang et al., 2008&lt;sup&gt;72&lt;/sup&gt;</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>Yellow pigmentation in cocoons</td>
<td>Zhao et al., 2008&lt;sup&gt;73&lt;/sup&gt;</td>
</tr>
<tr>
<td>l</td>
<td>9</td>
<td>Prevents absorption of carotenoids</td>
<td>Li et al., 2008&lt;sup&gt;74&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dtf</td>
<td>15</td>
<td>Resistance to fluorinated compounds</td>
<td>Bai et al., 2008&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmiAANAT</td>
<td>18</td>
<td>Larvae and pupae with dark pigmentation</td>
<td>Dai et al., 2010&lt;sup&gt;76&lt;/sup&gt;, Zhan et al., 2010&lt;sup&gt;77&lt;/sup&gt;</td>
</tr>
<tr>
<td>nlw</td>
<td>13</td>
<td>Scaleless wings</td>
<td>Wang et al., 2010&lt;sup&gt;78&lt;/sup&gt;</td>
</tr>
<tr>
<td>KN</td>
<td>8</td>
<td>Thermotolerance</td>
<td>Zhao et al., 2010&lt;sup&gt;79&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ng</td>
<td>12</td>
<td>No glue eggs</td>
<td>Zhao et al., 2011&lt;sup&gt;80&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmAntp</td>
<td>6</td>
<td>Identify thoracic segments</td>
<td>Chen et al., 2013&lt;sup&gt;81&lt;/sup&gt;</td>
</tr>
<tr>
<td>E&lt;sup&gt;20&lt;/sup&gt;-I</td>
<td>6</td>
<td>Control abdominal segment development</td>
<td>Chen et al., 2013&lt;sup&gt;82&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmEP80</td>
<td>10</td>
<td>Egg dehydration</td>
<td>Chen et al., 2013&lt;sup&gt;83&lt;/sup&gt;</td>
</tr>
<tr>
<td>+P</td>
<td>2</td>
<td>Formation of larval marks</td>
<td>Wei et al., 2013&lt;sup&gt;84&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmADC</td>
<td>11</td>
<td>Black pupae</td>
<td>Dai et al., 2015&lt;sup&gt;85&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmLanB1-w</td>
<td>13</td>
<td>Cell adhesion in wing tissues</td>
<td>Tong et al., 2015&lt;sup&gt;86&lt;/sup&gt;</td>
</tr>
<tr>
<td>BmCPG10</td>
<td>5</td>
<td>Regulation of molt</td>
<td>Wu et al., 2016&lt;sup&gt;87&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bmscarface</td>
<td>23</td>
<td>Regulation of body shape</td>
<td>Wang et al., 2018&lt;sup&gt;88&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

†LG: linkage group where the gene is located

Table 3. Genes mapped using microsatellites in *B. mori*. 
and expected reliability, with traditional polyacrylamide gels, with analysis of DNA fragments marked with fluorescence, or with the latest sequencing technologies. The latter, in addition to providing greater precision and automation in genotyping, would also facilitate identification of suitable SSR loci as molecular markers and allow simultaneous analysis with single nucleotide polymorphisms (SNPs), which would complement and strengthen the inferences and analyzes obtained when using them separately.

In this context, microsatellites would play an important role both in supporting the research carried out in B. mori germplasm banks in emerging countries wishing to promote sericulture in their territories, but that do not have the resources to access cutting-edge technologies and in advancing understanding of the complex genetic and molecular mechanisms underlying characteristics of productive and biological interest.

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Data availability
No data are associated with this article.

Grant information
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The authors are grateful to the University of Cauca and the following research groups for the scientific support: Integrated Systems of Agricultural, Forestry and Aquaculture Production (SISINPRO), Geology, Ecology and Conservation (GECO); we are also indebted to the “Technological Development for the Obtaining of Organic and Innovative Products of Natural Silk” project of the General System of Royalties and the Government of Cauca; and we are especially grateful to Colm McLachlan for suggestions related to the English text.

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Maria-Lucia Carneiro Vieira

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I do believe that the review should be useful for readers, it considers the state of the art on the role of microsatellites for the genetic analysis of *Bombyx mori* silkworm. The most recent citation dates from 2018; therefore, I suggest authors to search for more recent articles on other Lepidoptera of agronomic importance. The population of these pests tend to have the same genetic structure as the ones of *Bombyx mori* silkworm? Please make the comparison.

References

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

Are the conclusions drawn appropriate in the context of the current research literature?
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Plant genetics and genomics
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 28 October 2019

https://doi.org/10.5256/f1000research.22017.r54605

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Nalavadi Chandrakanth
Silkworm Breeding and Biotechnology, Central Sericultural Research and Training Institute, Berhampore, India

The authors have scripted the manuscript properly and covered the appropriate and relevant literature necessary for understanding the characteristics of microsatellite and the evolutionary events associated with it in Bombyx mori genome. Further this review has focused on the literature pertaining to the applications of the microsatellite as markers, in genetic analysis studies and in construction of the linkage maps in B. mori. Interestingly, the authors have also discussed about the lacuna of using microsatellite in marker-trait association studies and construction of high density linkage maps in B. mori. The authors have brought out an important point that linkage map construction in silkworm cannot be adjusted to the other models due to their gender based effects and absence of chromosomal cross-linking in the germinal line of the female silkworms. In this line, recently proposed statistical models to analyze QTLs were also reported and explained in this manuscript.

Comments

In the last paragraph of Introduction, it was mentioned that this review will focus on the roles or strategies involving microsatellite markers for conservation of silkworm germplasm. But, in future scope, this part has to be included with effective strategies to conserve the silkworm germplasm using microsatellite markers. In addition, the authors can also report in the future scope about how these microsatellite markers has been employed in breeding programs to improve the genetic materials of different silkworms.

In some parts of the manuscript, the sentences are very lengthy which can be simplified.

Is the topic of the review discussed comprehensively in the context of the current literature?
Yes

Are all factual statements correct and adequately supported by citations?
Yes

Is the review written in accessible language?
Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**
Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Silkworm Breeding and Biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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