



Acta Scientiarum. Biological Sciences

ISSN: 1679-9283

eduem@uem.br

Universidade Estadual de Maringá
Brasil

Ronqui, Ludimilla; Fernandez, Maria Aparecida; Takasusuki, Maria Claudia Colla Ruvolo
Genetic Analysis of Izoenzymes Polymorphisms in Silkworm (*Bombyx mori* L.) Strains
Acta Scientiarum. Biological Sciences, vol. 35, núm. 2, abril-junio, 2013, pp. 249-254
Universidade Estadual de Maringá
Maringá, Brasil

Available in: <http://www.redalyc.org/articulo.oa?id=187126298014>

- How to cite
- Complete issue
- More information about this article
- Journal's homepage in redalyc.org

redalyc.org

Scientific Information System

Network of Scientific Journals from Latin America, the Caribbean, Spain and Portugal

Non-profit academic project, developed under the open access initiative



Genetic Analysis of Isoenzymes Polymorphisms in Silkworm (*Bombyx mori* L.) Strains

Ludimilla Ronqui^{1*}, Maria Aparecida Fernandez² and Maria Claudia Colla Ruvoletto Takasusuki²

¹Departamento Interdisciplinar de Tecnologia e Ciências, Fundação Universidade Federal de Rondônia, Av. Tancredo Neves, 3450, 76872-862, Campus Ariquemes, Ariquemes, Rondônia, Brazil. ²Departamento de Biologia Celular e Genética, Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *Author for correspondence. E-mail: ludmilla@unir.br

ABSTRACT. This work carried out to evaluate the polymorphism in the silkworm of different lineages using the isoenzymes electrophoresis to detect biochemical markers and to investigate the genetics of populations for those lineages. They were used as samples individual extracts of silk glands of second day old larvae of the fifth instar, originating from seven Japanese lineages and eight pure Chinese lineages maintained by the Cocamar-Cooperativa Agroindustrial de Maringá. The isozymes acid phosphatase (ACP), alkaline phosphatase (AKP) and carbonic anhydrase (CA) they were submitted to the electrophoresis in starch gels 14%. The esterases (EST) were analyzed in polyacrylamide gels to 10% and stained with α and β -naphthyl acetate. The total of 21 loci was detected, and 04 (19.05%) they are polymorphic, *Est-11*, *Acp*, *Akp*, *Ca*. The fixation index (F_{is}) for the analyzed isozymes it was 0.0751, indicating excess of homozygotes. The value of F_{st} (0.6128) it shows that the lineages are well differentiated. The dendrogram obtained with the values of genetic distance didn't separate the Chinese and Japanese lineages analyzed totally. That preliminary evaluation of the lineages of *B. mori* shows that they present genetic material that it can be used in breeding programs that have the purpose of producing hybrid for silk production.

Keywords: silkworm, genetic polymorphism, isoenzymes.

Análise genética de polimorfismos bioquímicos em linhagens do bicho da seda (*Bombyx mori* L.)

RESUMO. Este trabalho teve como objetivo avaliar o polimorfismo em lagartas do bicho-da-seda de diferentes linhagens utilizando a eletroforese de isoenzimas para detectar marcadores bioquímicos e investigar a genética de populações para essas linhagens. Foram utilizados como amostras extratos individuais de glândulas sericígenas de lagartas do segundo dia da quinta idade, de sete linhagens japonesas e oito linhagens chinesas puras mantidas pela Cocamar-Cooperativa Agroindustrial de Maringá. As isozimas fosfatase ácida (ACP), fosfatase alcalina (AKP) e anidrase carbônica (CA) foram avaliadas por meio de eletroforese em géis de amido de milho a 14%. As esterases (EST) foram analisadas por meio de eletroforese vertical em géis de poliacrilamida a 10% e coloração com α e β -nftil acetato. Foram observados 21 locos, dentre os quais quatro (19.05%) são polimórficos, *Est-11*, *Acp*, *Akp*, *Ca*. O índice de fixação (F_{is}) para as isozimas analisadas foi 0.0751, indicando excesso de homocigotos. O valor de F_{st} (0.6128) permite sugerir que as linhagens estão bem diferenciadas. O dendograma obtido a partir dos valores de distância genética não separou totalmente as linhagens chinesas e japonesas analisadas. Essa avaliação preliminar das linhagens de *B. mori* mostra que elas apresentam material genético que pode ser utilizado em programas de cruzamentos que tenham a finalidade de produzir híbridos para produção de seda.

Palavras-chave: bicho-da-seda, polimorfismos genéticos, isoenzimas.

Introduction

The world production of silk is around 635 thousand tons, only the China produces 70%, following by India with 20% (ANN, 2008). Brazil participates in 3.2% of that production, arriving to 10.300 tons. Paraná State is the largest producing of Brazil silk, it was responsible for 89% of the harvest 2006/2007 (IAPAR, 2008).

The silkworm silk can be classified according to their geographical origin, such as Japanese, Chinese,

Indian or European. This insect has morphological and physiological differences correlated with the production of silk because of its origin and, therefore, different breeds ent origins have been, used in crosses, called arrays, as a source of characters for selection in favor of sericulture (PORTO et al., 2004).

The silkworm has been used as model for genetic studies since the discovery of the Mendelian inheritance in the beginning of the last century, due

to its large size, easy of maintenance in the laboratory, and the economic importance (NAGARAJU, 2000). According to this author, *Bombyx mori* is, after *Drosophila melanogaster*, the second most important model insect in genetic studies. Over 400 mutations have been mapped in 28 linkage or chromosomes groups in successful genetic studies with the species (NAGARAJU, 2000). Besides, hundreds of geographical races and genetically improved strains are maintained in different countries where sericulture is in vogue. These races and strains differ not only in well characterized Mendelian traits but also in not so well-studied complex or quantitative traits such as body size, feeding duration, thermal tolerance, and disease resistance (NAGARAJU, 2000).

Many of the studies with *B. mori* are related with molecular biology including the cloning of many silkworm genes as e.g., genes of the enzymes sorbitol dehydrogenase (NIIMI et al., 1996), trealase (SU et al., 1996); homeoproteins such as antennapedia (UENO et al., 1996 apud NAGARAJU, 2000); diapause hormone (SATO et al., 1993); sericin (MICHAILLE et al., 1990), heavy chain fibroin (MITA et al., 1994) and light chain fibroin (YAMAGUCHI et al., 1989).

Since 1995, different molecular markers such as RAPD (PROMBOON et al., 1995) AFLP (TAN et al., 2001) and microsatellites (MIAO et al., 2005) are being used to detect polymorphisms and construct linkage maps for silkworm.

Aside from the different molecular markers available nowadays, biochemical markers such as isoenzymes have generated a broad array of practical information for the identification of hybrid species and natural and cultivated populations of diverse live organisms. Compared with other markers, these analyses are relative simple, fast and cheap (TEIXEIRA et al., 2004).

Polymorphism of nonspecific esterases from pupal haemolymph was analyzed, as well as of phosphoglucosomutase from different organs of larvae, pupae and imago, from eight introduced from Bulgaria were analysed by Staykova (2008).

The observation of genetic polymorphisms and molecular markers of those lineages can contribute with an improvement in the handling and consequently with the silk production in the northwest of Paraná State.

This study aimed to evaluate the genetic polymorphism in larvae of Japanese and Chinese silkworm (*B. mori* L.) lineages through isoenzymes, to detect molecular markers and investigate the population genetics of these strains.

Material and methods

Two-day-old larvae of the 5th instar of different *Bombyx mori* lineages were provided by ACESP (Associação dos Sericicultores de Nova Esperança e Regiões Sericícolas), Paraná, Brazil. Of the 15 lineages under study, seven were of Japanese and eight of Chinese origin (Table 1).

Table 1. *Bombyx mori* lineages and their origin.

Lineages	Origem	Lineages	Origem
Line1	Japonesa	Line8	Chinesa
Line2	Japonesa	Line9	Japonesa
Line3	Chinesa	Line10	Chinesa
Line4	Japonesa	Line11	Japonesa
Line5	Japonesa	Line12	Chinesa
Line6	Chinesa	Line13	Japonesa
Line7	Chinesa	Line14	Chinesa
		Line15	Chinesa

The larvae were deep-frozen at -20°C in duly closed and identified flasks for the laboratory analysis.

For the electrophoresis the silkworms were dissected and the silk glands removed under a stereomicroscope in a dissection dish.

Esterase (EST - EC 3.1.1.1)

Native polyacrylamide gels (10%, T= 30.8%; C = 2.6%) were used together with 5% stacking gels (T= 10.5%; C = 4.8%) (LAPENTA et al., 1995).

Electrophoresis was performed for 4.5 hours at 4°C , at a constant voltage of 200 V. Running buffer was 0.1M Tris-glycine, pH 8.3.

The samples were homogenized in centrifuge tubes (1.5 mL) in 150 μL β -mercaptoethanol and 40 μL carbon tetrachloride with a glass stick and immediately centrifuged at 12000 xG for 15 minutes to 2°C . Of the supernatant, 20 μL were used with 20 μL of 0.1 M Tris-HCl solution pH 8.8 containing 10% glycerol. This solution (15 μL) was then applied on the gel.

After the electrophoresis the gel was incubated for 30 min. in 50 mL sodium phosphate buffer solution at 0.1 M pH 6.2. Then the gel was incubated for ± 1 hour at 38°C in staining solution (50 mL phosphate buffer 0.1 M pH 6.2; 5 mL N-propanol; 0.06 g Fast Blue RR Salt; 0.03 g α -naphthyl acetate and 0.02 g β -naphthyl acetate) (LAPENTA et al., 1995).

Starch gel

Horizontal electrophoresis was carried out using the starch gel technique according Ruvolo-Takasusuki et al. (2002). After the electrophoretic running the starch gel was cut in three homologous parts, used for the staining of three enzyme systems (Acid phosphatase, alkaline phosphatase and carbonic anhydrase).

The buffer system used for the gel was Tris-HCl pH 7.5 0.02M and for the run Tris-HCl at 0.3 M pH 7.5.

Acid phosphatase (ACP – EC 3.1.3.2)

The acid phosphatase (ACP) activity was identified using 0.5 mL 1 M magnesium chloride, 0.05 M sodium acetate buffer pH 5.5, 0.04 g Fast Blue RR Salt and 3.5 mL 1% α - naphthyl phosphate.

Alkaline phosphatase (AKP – EC 3.1.3.1)

For the visualization of alkaline phosphatase (AKP), a solution was used containing 0.5 mL 1 M magnesium chloride, 0.05M Tris-HCl buffer pH 7.5, 0.04 g Fast Blue RR Salt, and 3.5 mL 1% α -naphthyl phosphate.

Carbonic anhydrase (CA – EC 4.2.1.1)

Fluorescein diacetate (10 mg) was dissolved in 0.5 mL acetone and mixed in 100 mL sodium phosphate buffer 0.1 M, pH 6.5. Gel was soaked in that solution and incubated at 37°C for 40 minutes, and was observed under long wave ultraviolet light.

Analysis of population genetics

After the revelation of the isozyme activity regions, the phenotypes of the discovered loci were recorded. The gene frequencies as well as the measure of the genetic variability were directly estimated, to determine the deviations from the expected genotypic frequency according to the Hardy-Weinberg equilibrium. Furthermore the similarity coefficients and genetic distances for all analyzed strains were calculated, using software Popgene 1.31 (YEH et al., 1999) and Genepop 4.1.1 (RAYMOND; ROUSSET, 1995).

Results and discussion

In the analyses in four enzyme systems with 15 *B. mori* L. lineages, (eight Chinese and seven Japanese lineages) 21 loci were observed. Four of these were polymorphic (19.05% polymorphism) with the polymorphic loci Est-11A (0.8974) and rarest Est-11B (0.1026), AcpA (0.300), AcpB (0.3917), AcpC (0.3083), AkpA (0.2500), AkpB (0.5333), AkpC (0.2167), CaA (0.4750), CaB (0.5250). Table 2 shows the frequencies of the loci for analyzed lineages.

The esterases are a widely distributed isozyme family that is found in bacteria as well as in human beings. Those isozymes present wide specificity to substrate, with a differential distribution in tissues and in the different development stages. In *Diatraea*

saccharalis (Lepidoptera, Pyralidae) eight esterase loci were observed, of which EST-3 was polymorphic (RUVOLO-TAKASUSUKI et al., 2002).

Table 2. Allelic frequency of the polymorphic loci of *Bombyx mori* lineages.

	EST – 11		ACP			AKP			CA	
	A	B	A	B	C	A	B	C	A	B
1*	0.666	0.333	0.000	1.000	0.000	0.250	0.750	0.000	0.500	0.500
2*	---	---	0.000	0.250	0.750	0.000	0.000	1.000	1.000	0.000
3*	---	---	0.000	0.500	0.500	0.000	0.000	1.000	1.000	0.000
4*	0.250	0.750	0.500	0.000	0.500	1.000	0.000	0.000	0.500	0.500
5*	---	---	0.000	0.750	0.250	0.000	1.000	0.000	0.000	1.000
6*	1.000	0.000	0.500	0.500	0.000	1.000	---	0.000	0.000	1.000
7*	1.000	0.000	0.500	0.000	0.500	0.000	1.000	0.000	0.000	1.000
8*	1.000	0.000	0.750	0.250	0.000	0.000	1.000	0.000	0.875	0.125
9*	1.000	0.000	0.000	0.500	0.500	0.000	1.000	0.000	0.000	1.000
10*	1.000	0.000	0.750	0.250	0.000	0.000	1.000	0.000	0.750	0.250
11*	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
12*	1.000	0.000	0.500	0.500	0.000	0.000	0.750	0.250	0.500	0.500
13*	---	---	0.000	0.250	0.750	0.250	0.250	0.500	1.000	0.000
14*	1.000	0.000	0.000	0.125	0.875	0.250	0.250	0.500	0.000	1.000
15*	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000

*lineages.

The mean heterozygosity observed for all loci was 0.0350, while the expected heterozygosity was 0.0929 (Table 3). These results indicate low heterozygosity for the analyzed loci and suggest that these lineages can be crossed with others for genetic improvement.

Table 3. Observed and expected heterozygosity of isoenzymes for 15 loci of *Bombyx mori* lineages.

	EST – 11		ACP		AKP		CA		Mean	Mean
	HO	HE	HO	HE	HO	HE	HO	HE		
1*	0.6667	0.5333	0.0000	0.0000	0.5000	0.4286	1.0000	0.5714	0.5417	0.3833
2*	0.0000	0.0000	0.0000	0.4286	0.0000	0.0000	0.0000	0.0000	0.0000	0.1429
3*	0.0000	0.0000	0.5000	0.5714	0.0000	0.0000	0.0000	0.0000	0.1667	0.1905
4*	0.5000	0.4286	0.5000	0.5714	0.0000	0.0000	1.0000	0.5714	0.5000	0.3929
5*	0.0000	0.0000	0.0000	0.4286	0.0000	0.0000	0.0000	0.0000	0.0000	0.1429
6*	0.0000	0.0000	0.0000	0.5714	0.0000	0.0000	0.0000	0.0000	0.0000	0.1429
7*	0.0000	0.0000	1.0000	0.5714	0.0000	0.0000	0.0000	0.0000	0.2500	0.1429
8*	0.0000	0.0000	0.5000	0.4286	0.0000	0.0000	0.2500	0.2500	0.1875	0.1696
9*	0.0000	0.0000	0.0000	0.5714	0.0000	0.0000	0.0000	0.0000	0.0000	0.1429
10*	0.0000	0.0000	0.0000	0.4286	0.0000	0.0000	0.0000	0.4286	0.0000	0.2143
11*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
12*	0.0000	0.0000	1.0000	0.5714	0.5000	0.4286	1.0000	0.5714	0.6250	0.3929
13*	0.0000	0.0000	0.5000	0.4286	0.5000	0.7143	0.0000	0.0000	0.3333	0.3810
14*	0.0000	0.0000	0.2500	0.2500	0.5000	0.7143	0.0000	0.0000	0.1875	0.2411
15*	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

*lineages.

The fixation index (F_{is}) for the analyzed isozymes was 0.0751, indicating homozygous excess and that there is probably no inbreeding in the lineage (Table 4). The high F_{st} value (0.6128) allows the conclusion that the lineages are differentiated (Table 4).

Table 4. Values of the Fixation index (F_{is}) and differentiation degree (F_{st}) of *Bombyx mori* strains.

Locus	F_{is}	F_{st}	F_{it}
Est-11	- 0.2655	0.5857	0.4757
Acp	0.3014	0.4075	0.5861
Akp	0.1429	0.7569	0.7916
Ca	- 0.4444	0.7140	0.5868
Mean	0.0751	0.6128	0.6419

Silkworm hybrids are obtained by single crosses of two silkworm parent races, normally Japanese crossed with Chinese races, to obtain the so-called direct hybrids (KRISHNASWAMI et al., 1979). Another form of hybrid production is the mating of two F₁ hybrids produced by different combinations of Japanese and Chinese *B. mori* races, in other words, double cross hybrids or tetra-parental hybrids (NAGARAJU, 2002). Shows the Japanese and Chinese strains with lowest variability, which are considered pure and are therefore indicated for the development of hybrids with promising results regarding the performance in the silk production.

The values obtained by the genetic distance of Nei (1978) show that the strains are distinct. The results showed that the strain 5 and strain 15 are identical and can be considered a single lineage.

The dendrogram based on the distance of Nei (1978) by the UPGMA method (Figure 1) shows that the 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.

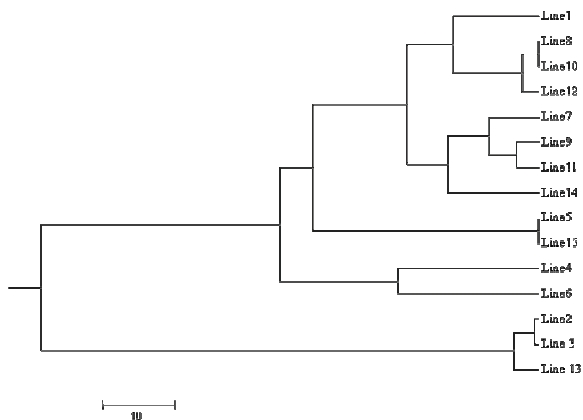


Figure 1. Dendrogram of genetic distance constructed by the UPGMA method based on Nei (1978) between Chinese and Japanese *Bombyx mori* lineages.

The matrices that compose these strains are maintained by crosses that may reduce the genetic variability. It would therefore be important to continuously evaluate the polymorphism degree of the strains, to avoid a marked increase of homozygosity. This would result in the expression of deleterious genes that can cause high matrix mortality.

Several studies have been developed with molecular markers in silkworm to detect the polymorphism degree, develop linkage maps for genes related to silk production and to map economic relevant genes.

The first linkage map by the Amplified Fragment Length Polymorphisms (AFLP) marker in *B. mori* was developed by Tan et al. (2001). For the AFLP mapping 47 progenies of a backcross population were genotyped. The authors found a total of 1248 (60.7%) polymorphisms detected with 35 primer combinations with adaptor *PstI/TaqI*. Since each one of the primer combinations generated an average of 35.7 polymorphic markers, a total of 44% of the polymorphic markers was consistent with the segregation ratio of 1:1 at a significance level of $P = 0.05$. The total AFLP map length was 6512 cM. The genetic distances between two neighboring markers varied from 0.2 to 47 cM, with a mean of 18.2 cM. The *od* linked gene to the sex was located between the markers P1T3B40 and P3T3B27 at the end of group 3, indicating that the linkage group AFLP 3 was on the Z chromosome (sexual).

The domesticated silkworm, *B. mori*, is a herbivorous insect and a model organism for Lepidoptera. Chinese and Japanese scientists made great efforts to accomplish the sequencing project of the whole silkworm genome, which offers *Bombyx* researchers an opportunity to identify peptides using proteomic method (MITA et al., 2004).

The mtDNA of some insects has been completely sequenced, including the mtDNA-arrays of four races of *B. mori*: (1) Xiafang (LU et al., 2001), (2) Backokjam (LEE et al., 2002) (3) C108 (YUKUHIRO et al., 2002), (4) Aojuku (ITOH et al., 2002). These sequences were used to determine the average size of mtDNA from *B. mori* in 15,650 bp.

Prasad et al. (2005) evaluated the microsatellite frequency and distribution in 21.76-Mb random genome sequences, 0.67-Mb in BAC sequences of the Z chromosome, and 6.3-Mb EST (“Expressed Sequence Tags”) sequences of *B. mori*. In this study microsatellites were estimated in 0.31% of the silkworm genome.

In 13 *B. mori* strains analyzed by Prasad et al. (2005), 23 polymorphic microsatellite loci were found with variation of 2 to 14 alleles, with a mean heterozygosity value of 0.54. Only 36 (18.2%) of 198 microsatellite loci were polymorphic between the two divergent silkworm populations and 10 (5%) loci had no alleles. The map based on these polymorphic markers presented eight linkage groups. The microsatellite loci were most conserved in the closest ancestor, *B. mandarina*, followed by the wild silkworm, *Antheraea assama*.

The analysis of 15 microsatellite loci of 13 *B. mori* strains revealed 3 to 17 alleles, with a heterozygosity between 0.66 and 0.90 (REDDY

et al., 1999). These microsatellites enabled the authors to obtain alleles for strains with and lineages without diapause.

Chatterjee and Pradeep (2003) identified molecular markers associated with silkworm growth and yield parameters. RAPD profiles were generated with 7 UBC primers for 14 silkworm lineages originated from China, Japan, India, and Russia and statistically analyzed. By multiple regressions analysis a significant association of 45 markers was detected with the larval development, the growth indices and the four relevant cocoon yield parameters for silk production.

Miao et al. (2005) constructed a dense linkage map of *B. mori* using 518 Simple Sequence Repeat (SSR) sequences or microsatellites, as part of an international genome program. Six representative strains and 2500 (CA)_n and (CT)_n microsatellite markers were used in the study.

These markers revealed a polymorphism of 17 - 24%, indicating the high homozygosity degree resulting from the long-standing history of inbreeding of silkworm. Those studies with molecular markers indicate that the genome of the silkworm is being studied broadly, for the development of better lineages of those insects.

The analyzed isozimas showed that the 15 Japanese and Chinese lineages maintained by ACESP-Brazil are genetically differentiated, possess a high polymorphism degree and can be used for production of hybrid and to do part of a future improvement program for those lineages.

Besides the improvement the isozymes may contribute to the understanding of the expression of functional genes, in different tissues or organs and in different phases of the development in *B. mori*.

Conclusion

Through the analysis of isoenzyme found an excess of homozygotes, results showed that the line 5 and line 15 are identical and can be considered a single lineage.

The genotypes of the study strains can be used in future studies of genetic improvement to develop superior hybrids for silk production.

Acknowledgements

We thanks the Secretaria de Estado da Ciência, Tecnologia e Ensino Superior-FUNDO PARANÁ, for the financial support.

References

- ANN-Agência Nacional de Notícias. Governo Paraná. Available from: <<http://www.aenoticias.pr.gov.br/modules/news/>>. Access on: Mar. 25, 2008.
- CHATTERJEE, S. N.; PRADEEP, A. R. Molecular markers (RAPD) associated with growth, yield, and origin of the silkworm, *Bombyx mori* L. in India. **Russian Journal of Genetics**, v. 39, n. 12, p. 1612-1624, 2003.
- IAPAR-Instituto Agronômico do Paraná. 2008. Available from: <<http://www.iapar.br/modules/noticias/>>. Access on: Mar. 25, 2008.
- ITOH, M.; HIMIZU, K.; KONOSU, A.; YUKUHIRO, K. ***Bombyx mori* mitochondrial DNA, complete genome, strain**: Aojuku. 2002. Available from: <<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=20126707>>. Access on: Feb. 20, 2012.
- KRISHNASWAMI, S.; NARASIMHANNA, M. N.; SURYANARAYAN, S. K.; KUMAVARAJ, S. Sericulture Manual - 2 Silkworm rearing, **Fao Agriculture Services Bulletin**, v. 2, n. 15, p. 130-138, 1979.
- LAPENTA, A. S.; BICUDO, H. E. M. C.; CERON, C. R.; CORDEIRO, J. A. Esterase patterns of species in the *Drosophila buzattii* cluster. **Cytobios**, v. 84, n. 336, p. 18-29, 1995.
- LEE, J. S.; KIM, Y. S.; SUNG, S. H.; HWANG, J. S.; LEE, D. S.; SHUH, D. S. ***Bombyx mori* mitochondrion, complete genome**. 2002. GenBank. Available from: <<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=8163611>>. Access on: Feb. 20, 2012.
- LU, C.; LIU, Y.; LIAO, S.; ZHOU, Z.; XIANG, Z.; WANG, X.; ZHU, M.; HAN, H. ***Bombyx mori* strain Xiafang mitochondrion, complete genome**. 2001. Available from: <<http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nucleotide&val=15213107>>. Access on: Feb. 20, 2012.
- MIAO, X. X.; XU, S. J.; LI, M. H.; LI, M. W.; HUANG, J. H.; DAI, F. Y.; MARINO, S. W.; MILLS, D. R.; ZENG, P.; MITA, K.; JIA, S. H.; ZHANG, Y.; LIU, W. B.; XIANG, H.; GUO, Q. H.; XU, A. Y.; KONG, X. Y.; LIN, H. X.; SHI, Y. Z.; LUC, G.; ZHANG, X.; HUNG, W.; YASUKOCHI, Y.; SUGASAKI, T.; SHIMADA, T.; NAGARAJU, J.; XIANG, Z. H.; WANG, S. Y.; GOLDSMITH, M. R.; LU, C.; ZHAO, G. P.; HUANG, Y. P. Simple sequence repeat-based consensus linkage map of *Bombyx mori*. **Proceedings of the National Academy of Sciences**, v. 102, n. 45, p. 16303-16308, 2005.
- MICHAILLE, J. J.; GAREL, A.; PRUDHOMME, J. C. Cloning and characterization of the highly polymorphic Ser2 gene of *Bombyx mori*. **Gene**, v. 86, n. 2, p. 177-184, 1990.
- MITA, K.; ICHIMURA, S.; JAMES, T. C. Highly repetitive structure and its organization of the silk fibroin gene. **Journal of Molecular Evolution**, v. 38, n. 6, p. 583-592, 1994.
- MITA, K.; KASAHARA, M.; SASAKI, S.; NAGAYASU, Y.; YAMADA, T.; KANAMORI, H.; NAMIKI, N.;

- KITAGAWA, M.; YAMASHITA, H.; YASUKOCHI, Y.; OKUDA-KADONO, K.; YAMAMOTO, K.; AJIMURA, M.; RAVIKUMAR, G.; SHIMOMURA, M.; NAGAMURA, Y.; SHIN-I, T.; ABE, H.; SHIMADA, T.; MORISHITA, S.; SASAKI, T. The genome sequence of silkworm, *Bombyx mori*. **DNA Research**, v. 11, n. 1, p. 27-35, 2004.
- NAGARAJU, J. Recent advances in molecular genetics of the silk moth, *Bombyx mori*. **Current Science**, v. 78, n. 2, p. 151-161, 2000.
- NAGARAJU, J. Application of genetic principles for improving silk production. **Current Science**, v. 83, n. 4, p. 409-414, 2002.
- NEI, M. Estimation of average heterozygosity and genetic distance from a small number of individuals. **Genetics**, v. 89, n. 3, p. 583-590, 1978.
- NIIMI, T.; YAMASHITA, O.; YAGINUMA, T. Structure of the *Bombyx* sorbitol dehydrogenase gene: A possible alternative use of the promoter. **Insect Molecular Biology**, v. 5, n. 4, p. 269-280, 1996.
- PORTO, A. J.; OKAMOTO, F.; CUNHA, E. A.; OTSUK, I. P. Characterization of eight races of silkworm silk (*Bombyx mori* L.). **Rural Science**, v. 34, n. 1, p. 259-264, 2004.
- PRASAD, M. D.; MUTHULAKSMI, M.; MADHU, M.; ARCHAK, S.; MITA, K.; NAGARAJU, J. Survey and analysis of microsatellites in the silkworm, *Bombyx mori*: frequency, distribution, mutations, marker potential and their conservation in heterologous species. **Genetics**, v. 169, n. 1, p. 197-214, 2005.
- PROMBOON, A.; SHIMADA, T.; FUJIWARA, F.; KOBAYASHI, M. Linkage map of random amplified polymorphic DNAs (RAPDs) in the silkworm, *Bombyx mori*. **Genetics Research Cambridge**, v. 66, n. 1, p. 1-7, 1995.
- RAYMOND, M.; ROUSSET, F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenism. **Journal of Heredity**, v. 86, n. 3, p. 248-249, 1995.
- REDDY, K. D.; ABRAHAM, E. G.; NAGARAJU, J. Microsatellites in the silkworm, *Bombyx mori*: Abundance, polymorphism, and strain characterization. **Genome**, v. 42, n. 8, p. 1057-1065, 1999.
- RUVOLO-TAKASUSUKI, M. C. C.; MACHADO, M. F. P. S.; CONTE, H. Esterase-3 polymorphism in the sugarcane borer *Diatraea saccharalis* (Lepidoptera, Pyralidae). **Genetics and Molecular Biology**, v. 25, n. 1, p. 61-64, 2002.
- SATO, Y.; OGUCHI, M.; MENJO, N.; IMAI, K.; SAITO, H.; IKEDA, M.; ISOBE, M.; YAMASHITA, O. Precursor polyprotein for multiple neuropeptides secreted from the suboesophageal ganglion of the silkworm *Bombyx mori*: characterization of the cDNA encoding the diapause Hormone precursor and identification of additional peptides. **Proceedings of the National Academy of Sciences**, v. 90, n. 8, p. 3251-3255, 1993.
- STAYKOVA, T. Genetically-determined polymorphism of nonspecific esterases and phosphoglucomutase in eight introduced breeds of the silkworm, *Bombyx mori*, raised in Bulgaria. **Journal of Insect Science**, v. 8, n. 18, p. 1-8, 2008. Available from: <<http://www.insectscience.org/>>. Access on: Aug. 18, 2008.
- SU, Z. H.; SATO, Y.; YAMASHITA, O. Purification, cDNA cloning and northern blot analysis of trehalase of pupal midgut of the silkworm, *Bombyx mori*. **Biochimica et Biophysica Acta**, v. 1173, n. 2, p. 217-224, 1996.
- TAN, Y. D.; WAN, C.; ZHU, Y.; LU, C.; XIANG, Z.; DENG, H. W. An amplified fragment length polymorphism map of the silkworm. **Genetics**, v. 157, n. 3, p. 1277-1284, 2001.
- TEIXEIRA, A. S.; CHAVES, L. S.; YUYAMA, K. Esterases no exame da estrutura populacional de camucamu (*Myrciaria dubia* (Kunth) Mc Vaugh-Murtaceae). **Acta Amazonica**, v. 34, n. 1, p. 75-88, 2004.
- YAMAGUCHI, K.; KIKUCHI, Y.; TAKAGAI, T.; KIKICHI, A.; OYAMA, F.; SHIMURA, K.; MIZUNO, S. Primary structure of the silk fibroin light chain determined by cDNA sequencing and peptide analysis. **Journal of Molecular Biology**, v. 210, n. 1, p. 127-139, 1989.
- YEH, F. C.; BOYLE, T. Y. Z.; XIYAN, J. M. **POPGENE Version 1.31**: Microsoft Window-based freeware for population genetic analysis. Edmonton: University of Alberta and Center for International Forestry Research, 1999.
- YUKUHIRO, K.; SEZUTSU, H.; ITOH, M.; SHIMIZU, K.; BANNO, Y. Significant levels of sequence divergence and gene rearrangements have occurred between the mitochondrial genomes of the wild mulberry silkworm, *Bombyx mandarina*, and its close relative, the domesticated silkworm, *Bombyx mori*. **Molecular Biology and Evolution**, v. 19, n. 8, p. 1385-1389, 2002.

Received on April 8, 2011.

Accepted on June 13, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.