

ECOLOGY, BEHAVIOR AND BIONOMICS

Evaluation of Economically Important Traits from Sixteen Parental Strains of the Silkworm *Bombyx mori* L (Lepidoptera: Bombycidae)

DANIELA B ZANATTA, JULIANA P BRAVO, JOICE F BARBOSA, ROXELLE E F MUNHOZ, MARIA A FERNANDEZ

Depto. de Biologia Celular e Genética, Univ. Estadual de Maringá, 87020-900, Maringá, PR; danizanatta@gmail.com; jupbravo@gmail.com; joicefelipes@gmail.com; roxellemunhoz@gmail.com; aparecidafernandez@gmail.com

Edited by Jorge Braz Torres – UFRPE

Neotropical Entomology 38(3):327-331 (2009)

Avaliação de Características Economicamente Importantes de Dezesseis Linhagens do Bicho-da-Seda *Bombyx mori* L (Lepidoptera: Bombycidae)

RESUMO - A classificação e caracterização de linhagens de *Bombyx mori* L é importante para a sericultura, uma vez que essa atividade é sustentada pelo constante desenvolvimento de novos híbridos da espécie. Neste trabalho, 16 linhagens parentais de *B. mori* do banco de germoplasma da Universidade Estadual de Maringá - UEM, e da Associação dos Criadores de Bicho-da-Seda de Nova Esperança e Regiões Sericícolas do Paraná - ACESP, foram avaliadas em relação às características biológicas e produtivas consideradas economicamente importantes. As linhagens C122-B e C121-A, de origem Chinesa, e as HA-A e HA-B, Japonesas, apresentaram o maior peso de casulo (CW), o qual é associado ao teor de seda (RSP). Os resultados apresentados neste trabalho podem ser utilizados em vários programas de melhoramento visando à produção de linhagens e híbridos geneticamente superiores.

PALAVRAS-CHAVE: Germoplasma, sericultura, seda

ABSTRACT - The classification and characterization of silkworm strains are important for sericulture, which is supported by the constant development of new hybrids. In this study, 16 parental strains of *Bombyx mori* L from the germplasm banks of the Universidade Estadual de Maringá - UEM, and Associação dos Criadores de Bicho-da-Seda de Nova Esperança e Regiões Sericícolas do Paraná - ACESP, were evaluated regarding biological and productive traits economically important. The Chinese C122-B and C121-A, and the Japanese HA-A and HA-B strains yielded the highest cocoon weight, which is related to the raw silk percentage. Our data will be useful in breeding programs for the production of superior silkworm strains and hybrids.

KEY WORDS: Germplasm, sericulture, silk

The silkworm *Bombyx mori* L is an insect of great importance not only to the sericultural economy but also to biological studies, and has become the most studied model system in lepidopteran genetic research. Silkworms have been reared for over 5000 years in China (Nagaraju & Goldsmith 2002) for silk production purposes, and an estimated 4310 silkworm germplasm strains, comprising geographical strains, inbred lines, and mutants are thought to be available worldwide (Goldsmith *et al* 2005).

Silk production has increased steadily over the years, with a production of 125,000 metric tons of silk in 2006-2007 (Deccan Herald 2007). The silk production in Brazil only accounts for 2.4% of the world silk production, with 89% of the silk production in the state of Paraná. These values have been increasing every year, making this an economically viable option for Brazilian producers (SEAB 2009).

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. Hence, these geographical strains are very valuable genetic stocks for further improvement of silkworm strains (Goldsmith 2005).

Several studies have been conducted to determine the best strain for silkworm breeding programs (Raju & Krishnamurthy 1993, Porto & Okamoto 2003, Porto *et al* 2004, Rao *et al* 2006). With this purpose, it is necessary to survey all the important sericultural characteristics related

to every silkworm life-cycle stage. During the different developmental stages of the silkworm, life traits affect the qualitative aspects of silk yield (Ohi *et al* 1970). Chatterjee *et al* (1993) reported that 21 traits of the *B. mori* contribute to silk yield either quantitatively or qualitatively.

An extensive study is needed to improve existing strains for commercial purposes and to develop new strains through breeding programs aimed at improving silk productivity, adaptability to local environments, and disease resistance/tolerance capabilities (Sen *et al* 1999, Li *et al* 2001). Several studies related to the use of productivity markers (Fragoso *et al* 2003, Marco Jr *et al* 2005) and morphological dissimilarity (Aagaard *et al* 2002, Pilgrim *et al* 2002, Dujardin & Le Pont 2004, Arruda *et al* 2007) as indicators of the best strains for breeding.

The Universidade Estadual de Maringá - UEM and the Associação dos Criadores de Bicho-da-Seda de Nova Esperança e Regiões Sericícolas do Paraná - ACESP, in Brazil, maintain a silkworm germplasm bank that includes stocks from Chinese and Japanese origins, of which little is known about the genetic contents (Fernandez *et al* 2005). In order to maintain these genetic resources, the classification and characterization of these strains is very important. In our study, 16 *B. mori* strains were evaluated for several economically important traits and for the feasibility of using these strains for breeding programs.

Material and Methods

The experiments were carried out on 16 *B. mori* parental strains maintained at the UEM/ACESP germplasm bank and were analyzed for biological characteristics including genetic influence related to production performance. The larval and cocoon strain patterns, which reflects their different geographical origins are described in Table 1.

For the experiments, 180 silkworm larvae from each strain were raised in rearing houses under controlled environment and hygienic conditions. All strains received the same nutritional and rearing treatments.

For each strain, we analyzed 60 5-day-old 5th instars, 60 6-day-old pupae and 60 cocoons. The 5-day-old 5th instars were placed into plastic bags, briefly exposed to -20°C and evaluated for: silk gland weight (SGW), body weight (LBW) and body length (LBL). For the cocoons, the following parameters were measured: length (CL), weight (CW), width (CWd), shell weight (SW) and raw silk percentage (RSP). The RSP value was calculated using the formula: raw silk percent (RSP) = 30 cocoons shell weight (g)/30 cocoons weight (g) x 100; 24% was deducted from the result (silk wiring loss).

The experiment was analyzed considering a random design, with 16 treatments and three replicates. The data were submitted to analysis of variance (Fischer's test) and Scott-Knott's test at 5% of significance level.

The morphological dissimilarity among the silkworm strains of the germplasm bank was calculated from the generalized distance of Mahalanobis (1936), which is an extension of the Euclidian distance. The grouping methods allowed us to subdivide an original group of observations

Table 1 ACESP/UEM germplasm bank silkworm strains features.

Strain	Origin	Larval pattern	Cocoon
C14	China	Plain	Short oval
C21	China	Plain	Short oval
C24-2	China	Multi-lunar	Oval and peanut
C24-A	China	Plain	Oval and peanut
C25-B	China	Plain	Short oval and slight peanut
C121-A	China	Plain	Oval
C122-B	China	Plain	Oval
C209	China	Plain	Short oval and slight peanut
HA-A	Japan	Marked	Long peanut
HA-B	Japan	Marked	Long peanut
J1	Japan	Marked	Peanut
M11-A	Japan	Plain	Peanut
M12-B	Japan	Plain	Peanut
M18-2	Japan	Plain	Peanut
M19-2	Japan	Multi-lunar	Peanut
M102	Japan	Marked	Long peanut

into several subgroups in such a way that we obtained homogeneity inside the subgroups and heterogeneity among the subgroups. The method employed for grouping, UPGMA, uses the average distance among all the equal genotypes for the formation of each group (Cruz & Regazzi 2001). The statistical analyses were calculated using the GENES program (Cruz 2001).

Results and Discussion

The characteristics analyzed in this study (SGW, LBW, LBL, CL, CW, CWd, SW and RSP) play an important role in the selection of specialized strains and are related to the silkworm cocoon and silk yarn (Tables 2 and 3).

During the larval stage, the silkworm accumulates energy reserves for cocoon production and for the last stage of its life-cycle, of which larva body weight (LBW) is a very important characteristic, especially at the end of 5th instar. Strains C121-A, C209, M11-A, M18-2, and M102 showed the best performance when comparing the LBW and LBL obtained by 5th instars (Table 2). However, only strains M11-A and M102 achieved higher values of SGW, contrary to what has been previously reported, as these parameters were indicated as correlates to predict the silkworm silk production capacity (Porto *et al* 2004).

The mean value obtained for SGW corresponds to 16.5% of the larval body weight (LBW) (Fig 1), much lower than the 32-38% reported by Porto *et al* (2004), but in this case, seven to 8-day-old 5th instars were analyzed instead. This time difference (two to three days) is important because the silk production is faster at the end of the 5th instar, which can be reflected in an increase in the SGW weight. The cocoon

Table 2 Mean values of cocoon length (CL), silk gland weight (SGW), larval weight (LW) and body length (LBL) of 16 parental silkworm strains.

Strain	CL (cm)	SGW (cm)	LBW (g)	LBL(cm)
C14	2.8 ± 0.04 e	0.5 ± 0.03 b	3.6 ± 0.10 a	6.1 ± 0.04 b
C21	2.9 ± 0.04 e	0.5 ± 0.03 b	3.4 ± 0.20 b	6.1 ± 0.10 b
C24-2	3.0 ± 0.04 d	0.5 ± 0.05 a	3.7 ± 0.11 a	6.1 ± 0.15 b
C24-A	3.0 ± 0.05 d	0.5 ± 0.03 a	3.5 ± 0.09 b	6.0 ± 0.19 b
C25-B	2.9 ± 0.02 e	0.5 ± 0.00 a	3.5 ± 0.06 b	5.9 ± 0.12 b
C121-A	3.0 ± 0.04 d	0.4 ± 0.01 b	3.6 ± 0.09 a	6.4 ± 0.37 a
C122-B	3.0 ± 0.02 d	0.4 ± 0.04 b	3.5 ± 0.14 b	6.4 ± 0.13 b
C209	2.9 ± 0.09 e	0.5 ± 0.02 b	3.7 ± 0.14 a	6.3 ± 0.19 a
HA-A	3.5 ± 0.03 b	0.5 ± 0.13 b	3.4 ± 0.14 b	6.1 ± 0.26 b
HA-B	3.7 ± 0.08 a	0.4 ± 0.03 a	3.3 ± 0.08 b	6.1 ± 0.10 b
J1	3.3 ± 0.04 c	0.5 ± 0.01 a	3.6 ± 0.07 a	6.2 ± 0.10 b
M11-A	3.4 ± 0.07 c	0.6 ± 0.02 a	3.8 ± 0.08 a	6.3 ± 0.17 a
M12-B	3.4 ± 0.02 c	0.5 ± 0.03 a	3.5 ± 0.01 a	5.9 ± 0.15 b
M18-2	3.4 ± 0.03 c	0.5 ± 0.01 b	3.8 ± 0.03 a	6.7 ± 0.14 a
M19-2	3.4 ± 0.01 c	0.5 ± 0.02 a	3.5 ± 0.12 b	6.4 ± 0.21 a
M102	3.5 ± 0.14 b	0.5 ± 0.04 a	3.7 ± 0.13 a	6.3 ± 0.14 a

Mean and standard deviation followed by different letters in the same column are different by the Scott-knott test ($P < 0.05$)

length (CL) confirms the slender shape of the Japanese strain cocoons (Table 2), and cocoon width (CWd) was the only

trait similar to all 16 strains, with an overall average of 26 mm (data not showed).

Table 3 Mean values of cocoon weight (CW), shell weight (SW) and raw silk percent (RSP) for 16 parental silkworm strains.

Strain	CW (g)	SW (g)	RSP (%)
C14	1.2 ± 0.03 c	0.3 ± 0.01 c	17.6 ± 1.00 c
C21	1.4 ± 0.09 b	0.3 ± 0.02 b	18.7 ± 0.19 b
C24-2	1.5 ± 0.09 a	0.3 ± 0.04 b	16.9 ± 0.83 c
C24-A	1.6 ± 0.11 a	0.4 ± 0.03 b	17.7 ± 1.35 c
C25-B	1.5 ± 0.10 b	0.3 ± 0.03 b	18.1 ± 0.84c
C121-A	1.4 ± 0.07 b	0.3 ± 0.03 b	19.7 ± 1.20 a
C122-B	1.3 ± 0.09 c	0.3 ± 0.01 b	20.6 ± 0.82 a
C209	1.4 ± 0.07 b	0.3 ± 0.01 c	17.2 ± 0.71 c
HA-A	1.7 ± 0.12 a	0.4 ± 0.02 a	19.1 ± 1.10 b
HA-B	1.7 ± 0.03 a	0.4 ± 0.01 a	18.3 ± 0.46 b
J1	1.4 ± 0.06 b	0.3 ± 0.01 b	18.5 ± 0.66 b
M11-A	1.4 ± 0.02 b	0.3 ± 0.00 b	18.6 ± 0.24 b
M12-B	1.4 ± 0.06 b	0.4 ± 0.01 b	18.1 ± 0.77 c
M18-2	1.4 ± 0.00 b	0.3 ± 0.02 c	17.3 ± 0.87 c
M19-2	1.3 ± 0.06 c	0.3 ± 0.02 c	18.3 ± 0.33 c
M102	1.6 ± 0.08 a	0.3 ± 0.01 b	16.8 ± 0.22 c

Mean and standard deviation followed by different letters in the same column are different by the Scott-knott test ($P < 0.05$)

The most important traits to correlate with silk production performance of the silkworm are the cocoon weight (CW), shell weight (SW) and the raw silk percentage (RSP) (Table 3). An initial analysis of all strains showed that the mean values of CW and SW of the HA-A and HA-B Japanese strains were superior, and the lowest values were observed for the C14 Chinese and M19-2 Japanese strains. Therefore, the RSP measure is an estimate that involves CW and SW simultaneously with other factors and defines the cocoon's final value. Strains C121-A (19.7%) and C122-B (20.6%) had the highest RSP values among all analyzed strains. In general, although discrete differences among the Chinese and Japanese strains were observed, they were not significant (Fig 1).

The SW and the SGW determine the potential for silk production because the energy for silk formation is contained within this gland (Porto *et al* 2004). However, Strains C121-A and C122-B generated lower values for SGW (Table 2), but exhibited the greatest values of RSP, indicating the silk gland development may be uneven among different strains, especially when 5th instars were sampled earlier in their development. The CW (1.5 g), SW (0.4 g) and RSP (18.3%) mean values obtained for all 16 strains were higher than in some of the studies available in the literature (Porto & Okamoto 2003, Porto *et al* 2004, Rao *et al* 2006).

Cocoon weight (CW), shell weight (SW) and raw silk percent (RSP) are very important economic silkworm traits. They are highly inheritable, are affected by additive gene action and allow a better response to selection (Petkov & Nguyenvan 1987).

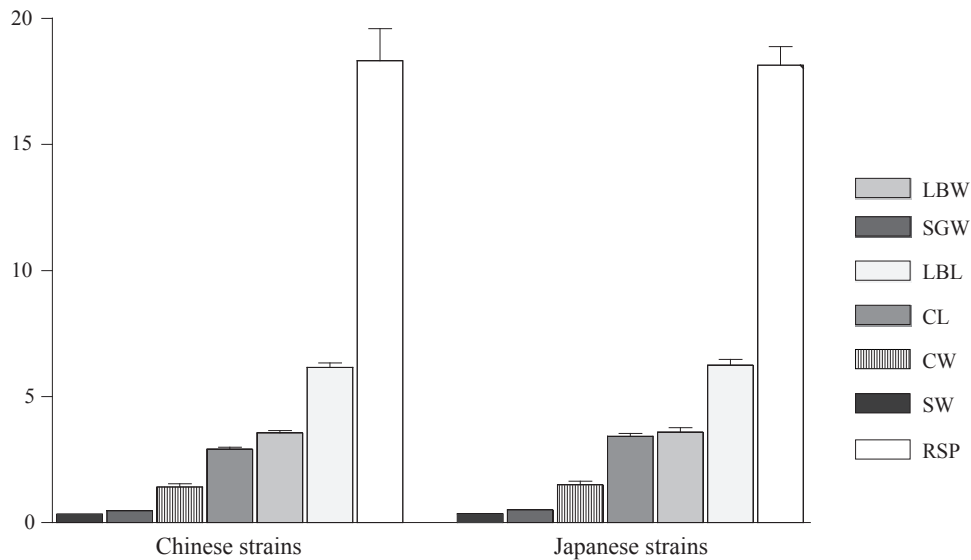


Fig 1 Comparison analyses between economically important traits from Chinese and Japanese *Bombyx mori* strains. SW = Cocoon shell weight (g), SGW = silk gland weight (g), CW = cocoon weight (G), CL = cocoon length (cm), LBW = larval body weight (g) and LBL = body length (cm), and RSP = raw silk percentage. The mean and standard variations were calculated using Prism 3.0 software.

External cocoon traits related to its shape are strongly linked to the origin of the silkworm strains. The Chinese strains have a white larval body and make an oval cocoon type, while the Japanese have a more painted larval body and make a peanut-shaped cocoon (Table 1).

All of the studied strains in here are polyvoltine, are generally more resistant to climatic changes and produce considerably less silk than the mono- or bivoltine strains (Rao *et al* 2006). However, our data indicated that the strains from the ACESP/UEM germplasm bank are also great producers with higher RSP values, which are close to the values reported for bivoltine strains (Goel *et al* 2007).

The cluster analysis revealed a clear division into three groups (Fig 2). However, the strains of the same origin did not grouped together, demonstrating they can have

different biological and development performance. Three Chinese strains, C122-B, C14 and C24-A, and two Japanese strains, M18-2 and J1, were grouped together and far from other silkworm strains, indicating they might be suitable for future crossings, maintenance of parental strains, and hybridizations so as to maximize heterosis and to avoid depression inbreeding.

Systematic studies of resource material are very important for the classification and characterization of varieties and also for the selection of promising parents to be utilized in genetic breeding programs. Therefore, characterization of each germplasm bank and access to the maximum amount of information is essential for their appropriate utilization in the future.

In conclusion, the results found in this work showed

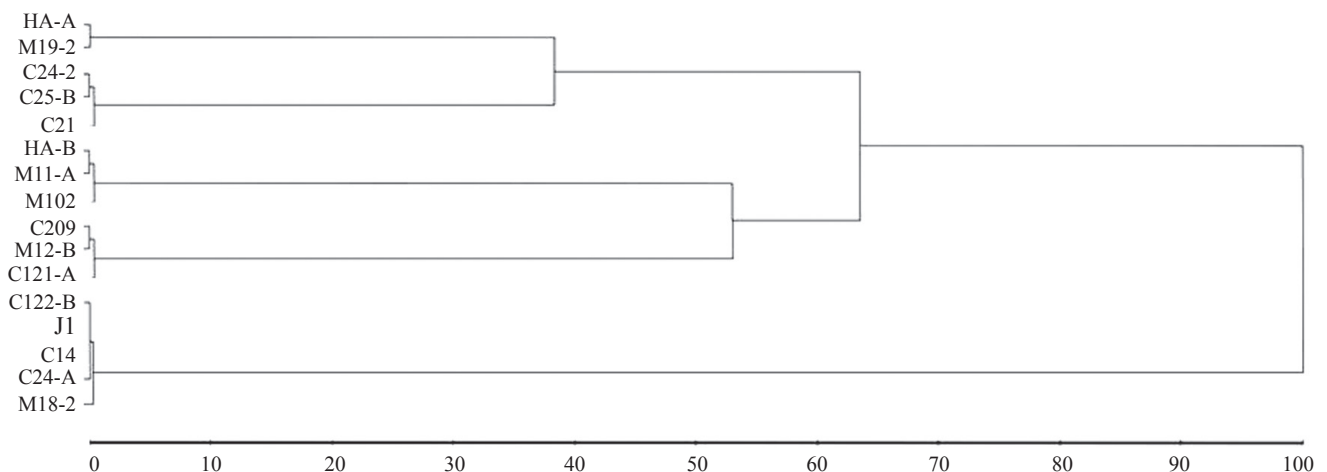


Fig 2 Cluster analysis based on eight traits for 16 silkworm strains according to the Mahalanobis (1936) distance and grouping from UPGMA (Unweighted Pair Group Method Average).

that all 16 parental *B. mori* strains from the UEM/ACESP germplasm bank are good silk producers. Moreover, the Chinese C121-A and C122-B and the Japanese HA-A and HA-B strains are the main candidates for breeding programs for the production of superior silkworm breeds and hybrids.

Acknowledgments

Authors gratefully acknowledge all staff members of the Laboratório de Organização Funcional do Núcleo for their assistance with measurements. We also greatly appreciate the financial support from CAPES, CNPq, FINEP/Fundação Araucária, FINEP/COMCAP/UEM, Secretaria de Estado da Ciência, and Tecnologia e Ensino Superior – FUNDO PARANÁ and grants for the Science and Innovation Santander Banespa 2006 Prize.

References

- Aagaard K, Hindar K, Pullin A, James C H, Hammarstedt O, Balstad T, Hanssen O (2002) Phylogenetic relationships in brown argus butterflies (Lepidoptera: Lycaenidae: *Aricia*) from north-western Europe. *Biol J Linn Soc Lond* 75: 27-37.
- Arruda V M, Alves Jr V V, Moraes M M, Netto J C B, Suárez Y R (2007) Análise morfológica da glândula de veneno de *Apis mellifera* L. (Hymenoptera: Apidae) em populações de Mato Grosso do Sul. *Neotrop Entomol* 36: 203-209.
- Chatterjee S N, Rao P R M, Jayaswal K P, Singh R, Datta R K (1993) Genetic variability in mulberry silkworm, *Bombyx mori* L., breeds with low silk yield. *Indian J Seric* 32: 69-86.
- Cruz C D, Regazzi A J (2001) Modelos biométricos aplicados ao melhoramento genético. 2a ed rev Viçosa, UFV, Imprensa Universitária, 390p.
- Cruz C D (2001) Programa Genes: aplicativo computacional em genética e estatística. Viçosa, UFV, Imprensa Universitária, 648p.
- Deccan Herald. Available in silk demand, supply gap 8,000 tonnes. Available in: <<http://www.deccanherald.com/Content/Oct262007/state2007102632414.asp>>. Accessed in 27 November, 2007.
- Dujardin J, Le Pont F (2004) Geographic variation of metric properties within the neotropical sandflies. *Infec Genet Evol* 4: 353-359.
- Fernandez M A, Ciferri R R, Patussi E V, Pereira M F, Felipes J, Bravo J P, Zanatta D B, Gouveia F de S, Balani V A (2005) Utilização da biotecnologia na sericultura brasileira. *Biotecnol Cienc Desenvol* 35: 56-61.
- Fragoso D B, Guedes R N C, Ladeira J A (2003) Seleção na evolução de resistência a organofosforados em *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae). *Neotrop Entomol* 32: 329-334.
- Goel A K, Chandrashekharaiah, Reddy Y S (2007) Development and characterization of productive bivoltine inbred lines of silkworm *Bombyx mori* L. *Indian J Anim Res* 41: 157-166.
- Goldsmith M R, Shimada T, Abe H (2005) The genetics and genomics of the silkworm, *Bombyx mori* L. *Annu Rev Entomol* 50: 71-100.
- Li M, Yao W, Hou Q, Lin C Q, Chen K P (2001) Studies of some special characters in the silkworm (*Bombyx mori* L.) germplasm in China. *Sericologia* 41: 527-535.
- Mahalanobis PC (1936) On the generalized distance in statistics. *Proc Natl Inst Sci India* 2: 49-55.
- Marco Jr P, Latini M O, Resende D C (2005) Thermoregulatory constraints on behavior: patterns in a neotropical dragonfly assemblage. *Neotrop Entomol* 34: 155-162.
- Nagaraju J, Goldsmith M R, (2002) Silkworm genomics-progress and prospects. *Curr Sci* 83: 415-425.
- Ohi H, Miyahara J, Yamashita A (1970) Analysis of various practically important characteristics in the silkworm in early breeding generations of hybrids. Variation among strains, correlation between parents and offspring as well as relation between each character. *Tech Bull Seri Exp Stat* 93: 39-49.
- Petkov N, Nguyenvan L (1987) Breeding genetic studies on some lines of the silkworm, *Bombyx mori* L. *Genetika-i- Seleksiya* 20: 384-354.
- Pilgrim E M, Roush S A, Krane D E (2002) Combining DNA sequences and morphology in systematics: testing the validity of the dragonfly species *Cordulegaster bilineata*. *Heredity* 89: 184-190.
- Porto A J, Okamoto F (2003) Desempenho produtivo de quatro raças do bicho-da-seda (*Bombyx mori* L.) e seus cruzamentos. *Bol Ind Anim* 60: 179-184.
- Porto A J, Okamoto F, Cunha E A, Otsuk I P (2004) Caracterização de oito raças do bicho-da-seda (*Bombyx mori* L.). *Ci Rur* 34: 259-264.
- Raju P J, Krishnamurthy N B (1993) Breeding of two bivoltines, MG511 and MG512, of silkworm, *Bombyx mori* L., for higher viability and silk productivity. *Sericologia* 33: 577-587.
- Rao C G, Seshagi S V, Ramesh C, Ibrahim B K, Nagaraju H, Chandrashekharaiah (2006) Evaluation of genetic potential of the polyvoltine silkworm (*Bombyx mori* L.) germplasm and identification of parents for breeding programme. *J Zhejiang Univ Sci B* 7: 215-220.
- SEAB (2009) Câmara setorial da seda. Relatório Takii. Safra 2007/2008. Available in: <http://www.seab.pr.gov.br>. 11/III/2009.
- Sen R, Ahsan M M, Datta R K (1999) Induction of resistance to *Bombyx mori* nuclear polyhedrosis virus, into a susceptible bivoltine silkworm breed. *Indian J Sericult* 38: 107-112.