



RESEARCH ARTICLE - BEES

Genetic Variability of Stingless Bees *Melipona mondury* Smith and *Melipona quadrifasciata* Lepeletier (Hymenoptera: Apidae) from a Meliponary

JR KOSER¹, FO FRANCISCO², G MORETTO¹

1 - Universidade Regional de Blumenau, Blumenau, Santa Catarina, Brazil.

2 - Instituto de Biociências, Universidade de São Paulo, São Paulo, São Paulo, Brazil.

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Corresponding author

Jaqueline Reginato Koser

Departamento de Ciências Naturais

Universidade Regional de Blumenau

Blumenau, Santa Catarina, Brazil

E-Mail: jaquelinekoser@gmail.com

Abstract

The species of stingless bees *Melipona mondury* Smith and *Melipona quadrifasciata* Lepeletier are native to the Atlantic Forest. These species are sensitive to environmental changes and due to habitat loss they are endangered in several Brazilian states. This study aimed to evaluate the genetic variability of populations of these two species at the meliponary of the Regional University of Blumenau through the use of heterospecific microsatellite primers. We collected one worker from 19 colonies of *M. mondury* and from 25 colonies of *M. quadrifasciata*. We found low levels of genetic variability for both species, which may be explained by queen philopatry, intraspecific reproductive parasitism, and/or artificial maintenance of hives. If natural populations of these species are also presenting low genetic variability they might be endangered.

Introduction

The strictly Neotropical stingless bee genus *Melipona* (Hymenoptera: Apidae) comprises more than sixty species in Brazil (Camargo & Pedro, 2013) and is one of the most important insects for pollination in natural and in cultivated areas (Heard, 1999; Slaa et al., 2006). The species *Melipona* (*Michmelia*) *mondury* Smith, 1863 inhabits the Atlantic Forest biome, from Bahia to Rio Grande do Sul states (Camargo & Pedro, 2013). The species *Melipona* (*Melipona*) *quadrifasciata* Lepeletier, 1836 has a wide distribution in southern and southeastern Brazil (Camargo & Pedro, 2013) where it is widely cultivated and especially valued in tomatoes production (Santos et al., 2009; Sarto et al., 2005). In addition, its propolis has medicinal properties (Mercês et al., 2013). Both species are endangered in several Brazilian states (Machado et al., 1998; Marques et al., 2002; Mikich & Bérnils, 2004; Santa Catarina, 2011) due to habitat loss caused by deforestation (Brosi et al., 2007; Brown & Oliveira, 2014).

Nowadays, the Atlantic Forest is extremely fragmented, and bee species suffer from the negative impacts of the interrup-

tion of gene flow and decrease in genetic diversity (Freiria et al., 2012). One way to prevent local extinction of these bees is maintaining hives in free-foraging wooden boxes in meliponaries (apiaries for stingless bees). Meliponiculture for crop pollination and for honey extraction has been an encouraging economic practice aligned with sustainable development and educational purposes in several countries (Cortopassi-Laurino et al., 2006).

However, little is known about the genetic variability of hives of native bees in meliponaries (Carvalho-Zilse et al., 2009). Such knowledge is essential for the development of conservation strategies and rational exploitation of native species (Cortopassi-Laurino et al., 2006; Alves et al., 2011). Our aim was to characterize the genetic variability of *M. mondury* and *M. quadrifasciata* maintained in the meliponary of the Regional University of Blumenau.

Material and Methods

We collected one worker from each of 19 colonies of *M. mondury* and from 25 colonies of *M. quadrifasciata*



acquired from beekeepers from Vale do Itajaí region, in the Santa Catarina state, Brazil and maintained currently at the meliponary of the Regional University of Blumenau (mRUB) (Fig. 1) localized at 26° 54' 21.81" S 49° 04' 48.53" W (Fig.2). Colonies of *M. quadrifasciata* have been maintained at mRUB since 1998 and colonies of *M. mondury* since 2003. These hives were extracted directly from nature in geographically close cities and have not been artificially divided.



Figure 1. Meliponary of the Regional University of Blumenau (mRUB).

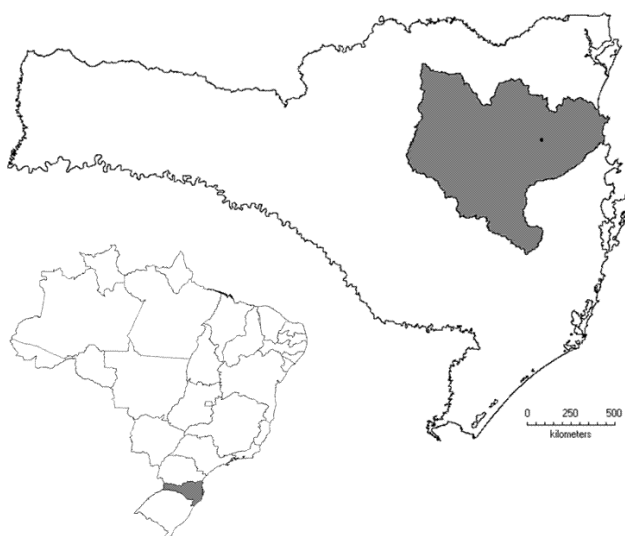


Figure 2. Localization of Santa Catarina state showing the Vale do Itajaí region. Meliponary localization (●).

DNA extraction was performed according to Anderson and Fuchs (1998). For both species, we used 10 microsatellite primer pairs developed for the species *M. bicolor* (Mbi11, Mbi28, Mbi32, Mbi33, Mbi88, Mbi218, Mbi233, Mbi254, Mbi259 and Mbi522) (Peters et al., 1998) and 11 primer pairs developed for *M. mondury* (Mmo03, Mmo06, Mmo08, Mmo10, Mmo11, Mmo15, Mmo19, Mmo20, Mmo21, Mmo22 and Mmo24) (Lopes et al., 2010b). PCR reactions and the annealing temperatures were performed according to Peters et al. (1998) for the Mbi primers and ac-

cording to Lopes et al. (2010b) for the Mmo primers. PCR products were separated by electrophoresis in 12% polyacrylamide gels and stained with silver nitrate for visualization.

The program Arlequin v.3.5.1.3 (Excoffier & Lischer, 2010) was used to calculate allelic richness (\hat{A}), observed heterozygosity (H_o), expected heterozygosity (H_e), percentage of polymorphic loci (PPL) and F_{IS} with 10,000 permutations. Hardy-Weinberg Equilibrium (HWE) and Linkage Disequilibrium (LD) were computed using Genepop v.4.1.4 (Rousset, 2008). P -values were adjusted with Bonferroni correction (Rice, 1989). The frequencies of null alleles were computed using Cervus 3.0.6 (Kalinowski et al., 2007).

Results

Genetic variability was low for *M. mondury*, but no inbreeding was detected (Table 1). Loci Mbi254 and Mmo19 deviated from HWE even after Bonferroni correction ($P < 0.0167$). LD was detected between Mbi254 and Mmo19 after Bonferroni correction ($P = 0.0062$). The presence of null alleles with a frequency of 29% was found in the locus Mbi254, a heterospecific primer (Table S1). The loci Mmo19 and Mmo21, both specific primers for *M. mondury*, were not affected by null alleles (Table S1).

Melipona quadrifasciata also showed low genetic variability although higher than *M. mondury* (Table 1). Significant inbreeding was detected. Primers developed for *M. bicolor* were more polymorphic in *M. quadrifasciata* than those developed for *M. mondury* (Table S2). Deviation from HWE was detected for the loci Mbi218, Mbi233 and Mmo21 after Bonferroni correction ($P = 0.0000$). LD was not detected (all $P > 0.05$). For *M. quadrifasciata*, the frequency of null alleles was higher than 25% in the following heterospecific primer pairs: Mbi218, Mbi233, Mmo03, Mmo11 and Mmo21 (Table S2). The loci Mbi11, Mbi88, Mbi254 and Mbi259 were not affected by null alleles (Table S2).

Table 1. Genetic variability for *Melipona mondury* and *Melipona quadrifasciata* based on microsatellite data. \hat{A} : allelic richness; F_{IS} : inbreeding coefficient; H_e : expected heterozygosity; H_o : observed heterozygosity; N: sample size; PPL: percentage of polymorphic loci.

Species	N	\hat{A}	H_o	H_e	PPL	F_{IS}	P -value
<i>M. mondury</i>	19	1.60	0.105	0.102	15%	-0.00629	0.569307
<i>M. quadri-</i> <i>fasciata</i>	25	2.22	0.129	0.189	50%	0.33493	0.000000

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Discussion

Our results indicate low genetic variability in the populations of *M. mondury* and *M. quadrifasciata* maintained at the mRUB. Low genetic variability in native stingless bees is also documented in studies using molecular markers such as RAPD (Tavares et al., 2001), mitochondrial polymorphism (Brito et al., 2013), ISSR (Inter Single Sequence Repeats) (Nascimento et al., 2010; Miranda et al., 2012), and microsatellites (Francisco et al., 2006; Tavares et al., 2007; Carvalho-Zilse et al., 2009; Francini et al., 2009; Alves et al., 2011; Duarte et al., 2011).

A first explanation of this low variability could be the presence of null alleles. In this study, we found high values of null alleles only in one out of three polymorphic loci for *M. mondury* (Mbi254) and in five out of nine polymorphic loci for *M. quadrifasciata* (Mbi218, Mbi233, Mmo03, Mmo11 and Mmo21). In addition, null alleles might be responsible for the high number of monomorphic loci found in both species.

The use of heterospecific primers may also be related to low genetic diversity. In the stingless bee *Plebeia remota*, the same samples analyzed with heterospecific (Francisco et al., 2006) and specific (Francisco et al., 2013) primers showed high divergent values. The same result was found in *Melipona* bees (Lopes et al., 2010a). Cross-species amplification is one of the advantages of microsatellites, and low variability should always be interpreted with caution when using heterospecific primers. However, data obtained from other populations/species with the same primer pairs we used show higher levels of genetic diversity (Tables S3 and S4) suggesting that the low diversity we observed in this work are not due to heterospecific primers only.

Low variability may also be explained by the species natural biology. Queens of most stingless bee species mate with a single male (monandric) (Peters et al., 1999; Palmer et al., 2002) and are known to nidify near maternal nests (Nogueira-Neto, 1954). Their low dispersion increases genetic drift and inbreeding within sub-populations (Hartl & Clark, 2007). A special concern is the maintenance of alleles of the complementary sex determination system in small populations, because inbreeding can lead to the production of diploid males (Cook & Crozier, 1995; Zayed, 2009; Alves et al., 2011). For other stingless bee species, males are the dispersing sex (Cameron et al., 2004; Carvalho-Zilse & Kerr, 2004; Francisco et al., 2013), but no data is available for *M. mondury* and *M. quadrifasciata*. Nevertheless, the location of mRUB near forest fragments might allow the mating of queens from the meliponaries with males from native forests in the surroundings, decreasing their inbreeding probability.

Managed populations of local bees can be considered a reservoir of genetic diversity if they can interbreed with wild populations (Alves et al., 2011). However, to introduce bees from distant populations or another species that could hybridize with local populations might cause outbreeding depression (Lynch, 1991; Waser et al., 2000).

Recent data from Wenseleers et al. (2011) showed intraspecific reproductive parasitism in *M. scutellaris*. If we speculate that this behavior occurs in other *Melipona* species, colonies that were previously unrelated could become related if sibling queens take over these colonies. In these cases, genetic variability would decrease.

Another explanation to account for the low variability may be related to the artificial maintenance of hives through founder events and bottlenecks as already reported for *Apis mellifera* (Sheppard, 1988; Schiff & Sheppard, 1996; Moritz et al., 2007; Delaney et al., 2009; Jaffé et al., 2010; Meixner et al., 2010) and *M. scutellaris* (Carvalho-Zilse et al., 2009; Alves et al., 2011). However, despite low variability, populations can be successfully maintained if a strong care is dispensed over the nests (Alves et al., 2011).

If natural populations of these species are also presenting low genetic variability they might be endangered. Adaptation to environmental changes is dependent on the genetic variations that exist among members of a population. Without variation, populations are more prone to extinction. Therefore, the analysis of natural populations of these species in south Brazil is crucial. Detecting the male flight range is also important since they can prevent genetic isolation in fragmented populations. If meliponaries were able to assist in maintaining the genetic variability of natural populations, they could be used for research and reintroduction programs.

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References

- Alves, D.A., Imperatriz-Fonseca, V.L., Franco, T.M., Santos-Filho, P.S., Billen, J., & Wenseleers, T. (2011). Successful maintenance of a stingless bee population despite a severe genetic bottleneck. *Conservation Genetics*, 12: 647-658. doi: 10.1007/s10592-010-0171-z
- Anderson, D.L. & Fuchs, S. (1998). Two genetically distinct populations of *Varroa jacobsoni* with contrasting reproductive abilities on *Apis mellifera*. *Journal of Apicultural Research*, 37: 69-78.
- Brito, R.M., Francisco, F.O., Franoso, E., Santiago, L.R. & Arias, M.C. (2013). Very low mitochondrial variability in a stingless bee endemic to Cerrado. *Genetics and Molecular Biology*, 36: 124-128. doi: 10.1590/S1415-47572013000100018
- Brosi, B.J., Daily, G.C. & Ehrlich, P.R. (2007). Bee community shifts with landscape context in a tropical countryside. *Ecological Applications*, 17: 418-430. doi: 10.1890/06-0029
- Brown, J.C. & Oliveira, M. (2014). The impact of agricultural colonization and deforestation on stingless bee (Apidae: Me-

- liponini) composition and richness in Rondônia, Brazil. *Apidologie*, 45: 172-188. doi:10.1007/s13592-013-0236-3
- Camargo, J.M.F. & Pedro, S.R.M. (2013). Meliponini Lepeletier, 1836. In Moure, J.S., Urban, D. & Melo, G.A.R. (Orgs). *Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region - online version*. Available at <http://www.moure.cria.org.br/catalogue>. Accessed Jan/18/2014.
- Cameron, E.C., Franck, P. & Oldroyd, B. P. (2004). Genetic structure of nest aggregations and drone congregations of the south-east asian stingless bee *Trigona collina*. *Molecular Ecology*, 13: 2357-2364. doi: 10.1111/j.1365-294X.2004.02194.x
- Carvalho-Zilse, G.A. & Kerr, W.E. (2004). Substituição natural de rainhas fisogástricas e distância de vôo dos machos em Tiuba (*Melipona compressipes fasciculata* Smith, 1854) e Uruçu (*Melipona scutellaris* Latreille, 1811) (Apidae, Meliponini). *Acta Amazonica*, 34: 649-652. doi: 10.1590/S0044-59672004000400016
- Carvalho-Zilse, G.A., Costa-Pinto, M.F.F., Nunes-Silva, C.G. & Kerr, W.E. (2009). Does beekeeping reduce genetic variability in *Melipona scutellaris* (Apidae, Meliponini)? *Genetics and Molecular Resources*, 8: 758-765.
- Cook, J.M. & Crozier, R.H. (1995). Sex determination and population biology in the Hymenoptera. *Trends in Ecology and Evolution*, 10: 281-286. doi: 10.1016/0169-5347(95)90011-X
- Cortopassi-Laurino, M., Imperatriz-Fonseca, V.L., Roubik, D.W., Dollin, A., Heard, T., Aguilar, I., Venturieri, G.C., Eardley, C. & Nogueira-Neto, P. (2006). Global meliponiculture: challenges and opportunities. *Apidologie*, 37: 275-292. doi: 10.1051/apido:2006027
- Delaney, D.A., Meixner, M.D., Schiff, N.M. & Sheppard, W.S. (2009). Genetic characterization of commercial honeybee (Hymenoptera: Apidae) populations in the United States by using mitochondrial and microsatellite markers. *Annals of the Entomological Society of America*, 102: 666-673. doi: 10.1603/008.102.0411
- Duarte, O.M.P., Gaiotto, F.A., Souza, A.P., Mori, G.M. & Costa, M.A. (2011). Isolation and characterization of microsatellites from *Scaptotrigona xanthotricha* (Apidae, Meliponini): A stingless bee in the Brazilian Atlantic Rainforest. *Apidologie*, 43: 432-435. doi: 10.1007/s13592-011-0109-6
- Excoffier, L. & Lischer, H.E.L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10: 564-567. doi: 10.1111/j.1755-0998.2010.02847.x
- Francini, I.B., Sforça, D.A., Sousa, A.C.B. & Campos, T. (2009). Microsatellite loci for an endemic stingless bee *Melipona seminigra merrillae* (Apidae, Meliponini) from Amazon. *Conservation Genetics Resources*, 1: 487-490. doi: 10.1007/s12686-009-9113-9
- Francisco, F.O., Brito, R.M. & Arias, M.C. (2006). Allele number and heterozygosity for microsatellite loci in different stingless bee species (Hymenoptera: Apidae, Meliponini). *Neotropical Entomology*, 35: 638-643. doi: 10.1590/S1519-566X2006000500011
- Francisco, F.O., Santiago, L.R. & Arias, M.C. (2013). Molecular genetic diversity in populations of the stingless bee *Plebeia remota*: A case study. *Genetics and Molecular Biology*, 36: 118-123. doi: 10.1590/S1415-47572013000100017
- Freiria, G.A., Ruim, J.B., Souza, R.F.D. & Sofia, S.H. (2012). Population structure and genetic diversity of the orchid bee *Eufriesea violacea* (Hymenoptera, Apidae, Euglossini) from Atlantic Forest remnants in southern and southeastern Brazil. *Apidologie*, 43: 392-402. doi: 10.1007/s13592-011-0104-y
- Hartl, D.L. & Clark, A.G. (2007). *Principles of Population Genetics*. Sunderland: Sinauer Associates, 545 p.
- Heard, T.A. (1999). The role of stingless bees in crop pollination. *Annual Review of Entomology*, 44: 183-206. doi: 10.1146/annurev.ento.44.1.183
- Jaffé, R., Dietemann, V., Allsopp, M.H., Costa, C., Crewe, R.M., Dall'olio, R., De La Rúa, P., El-Niweiri, M.A.A., Fries, I., Kezic, N., Meusel, M.S., Paxton, R.J., Shaibi, T., Stolle, E. & Moritz, R.F.A. (2010). Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. *Conservation Biology*, 24: 583-593. doi: 10.1111/j.1523-1739.2009.01331.x
- Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, 16: 1099-1106. doi: 10.1111/j.1365-294X.2007.03089.x
- Lopes, D.M., Campos, L.A.O., Salomão, T.M.F. & Tavares, M.G. (2010a). Comparative study on the use of specific and heterologous microsatellite primers in the stingless bees *Melipona rufiventris* and *M. mondury* (Hymenoptera, Apidae). *Genetics and Molecular Biology*, 33: 390-393. doi: 10.1590/S1415-47572010005000017
- Lopes, D.M., Silva, F.O., Fernandes-Salomão, T.M., Campos, L.A.O. & Tavares, M.G. (2010b). A scientific note on the characterization of microsatellite loci for *Melipona mondury* (Hymenoptera: Apidae). *Apidologie*, 41: 138-140. doi: 10.1051/apido/2009067
- Lynch, M. (1991). The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, 45: 622-629.
- Machado, A.B.M., Fonseca, G.A.B., Machado, R.B., Aguiar, L.M.S. & Lins, L.V. (1998). Livro vermelho das espécies ameaçadas de extinção da fauna de Minas Gerais. Belo Horizonte: Biodiversitas, 605 p.
- Marques, A.A.B., Fontana, C.S., Vélez, E., Bencke, G.A.,

- Schneider, M. & Reis, R.E. (2002). Lista das espécies da fauna ameaçadas de extinção no Rio Grande do Sul. Porto Alegre: FZB/MCT–PUCRS/PANGEA, 52 p.
- Meixner, M.D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E. & Buchler, R. (2010). Conserving diversity and vitality for honey bee breeding. *Journal of Apicultural Research*, 49: 85-92. doi: 10.3896/IBRA.1.49.1.12
- Mercês, M.D., Peralta, E.D., Uetanabaro, A.P.T. & Lucchese, A.M. (2013). Atividade antimicrobiana de méis de cinco espécies de abelhas brasileiras sem ferrão. *Ciência Rural*, 43: 672-675.
- Mikich, S.B. & Bérnils, R.S. (2004). Livro vermelho da fauna ameaçada no estado do Paraná, <http://www.pr.gov.br/iap> (accessed date: 4October, 2013).
- Miranda, E.A., Batalha-Filho, H., Oliveira, P.S., Alves, R.M.O., Campos, L.A.O. & Waldschmidt, A.M. (2012). Genetic diversity of *Melipona mandacaia* Smith 1863 (Hymenoptera, Apidae), an endemic bee species from Brazilian Caatinga, using ISSR. *Psyche*, 2012: 1-6. doi: 10.1155/2012/372138
- Moritz, R.F.A., Kraus, F.B., Kryger, P. & Crewe, R.M. (2007). The size of wild honeybee populations (*Apis mellifera*) and its implications for the conservation of honeybees. *Journal of Insect Conservation*, 11: 391–397. doi: 10.1007/s10841-006-9054-5
- Nascimento, M.A., Batalha-Filho, H., Waldschmidt, A.M., Tavares, M.G., Campos, A.O. & Salomão, T.M.F. (2010). Variation and genetic structure of *Melipona quadrifasciata* Lepeletier (Hymenoptera, Apidae) populations based on ISSR pattern. *Genetics and Molecular Biology*, 33: 394–397. doi: 10.1590/S1415-47572010005000052
- Nogueira-Neto, P. (1954). Notas bionômicas sobre meliponíneos: III – Sobre a enxameagem. *Arquivos do Museu Nacional*, 42: 419-451.
- Palmer, K.A., Oldroyd, B.P., Quezada-Euán, J.J.G., Paxton, R.J., & May-Itza, W.D.J. (2002). Paternity frequency and maternity of males in some stingless bee species. *Molecular Ecology*, 11: 2107-2113. doi: 10.1046/j.1365-294X.2002.01589.x
- Peters, J.M., Queller, D.C., Imperatriz-Fonseca, V.L. & Strassmann, J.E. (1998). Microsatellite loci from the stingless bees. *Molecular Ecology*, 7: 783-792.
- Peters, J.M., Queller, D.C., Imperatriz-Fonseca, V.L., Roubik, D.W. & Strassmann, J.E. (1999). Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proceedings of the Royal Society B*, 266: 379-384.
- Rice, W.R. (1989). Analyzing tables of statistical tests. *Evolution*, 43: 223-225.
- Rousset, F. (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources*, 8: 103–106. doi: 10.1111/j.1471-8286.2007.01931.x
- Santa Catarina (State), (2011). Conselho Estadual do Meio Ambiente - CONSEMA. Resolução CONSEMA Nº 002, de 06 de dezembro de 2011. Reconhece a lista oficial de espécies da fauna ameaçadas de extinção no estado de Santa Catarina e dá outras providências. http://www.fatma.sc.gov.br/images/stories/biodiversidade/resolucao_fauna__002_11_fauna.pdf. (accessed date: 4October, 2013).
- Santos, S.A., Roselino, A.C., Hrcncir, M. & Bego, L.R. (2009). Pollination of tomatoes by the stingless bee *Melipona quadrifasciata* and the honey bee *Apis mellifera* (Hymenoptera, Apidae). *Genetics and Molecular Research*, 8: 751-757.
- Sarto, M.C.L. del, Peruquetti, R.C. & Campos, L.A.O. (2005). Evaluation of the neotropical stingless bee *Melipona quadrifasciata* (Hymenoptera: Apidae) as pollinator of greenhouse tomatoes. *Journal of Economic Entomology*, 98: 260-266. doi: 10.1603/0022-0493-98.2.260
- Schiff, N.M. & Sheppard, W.S. (1996). Genetic differentiation in the queen breeding population of the western United States. *Apidologie*, 27: 77–86. doi: 10.1051/apido:19960202
- Sheppard, W.S. (1988). Comparative study of enzyme polymorphism in United States and European honey bee (Hymenoptera: Apidae) populations. *Annals of the Entomological Society of America*, 81: 886–889.
- Slaa, E.J., Chaves, L.A.S., Malagodi-Braga, K.S. & Hofstede, F.E. (2006). Stingless bees in applied pollination: practice and perspectives. *Apidologie*, 37: 293-315. doi: 10.1051/apido:2006022
- Tavares, M.G., Dias, L.A.S., Borges, A.A., Lopes, D.M., Busse, A.H.P., Costa, R.G., Salomão, T.M.F. & Campos, L.A.O. (2007). Genetic divergence between populations of the stingless bee urucu amarela (*Melipona rufiventris* group, Hymenoptera, Meliponini): Is there a new *Melipona* species in the Brazilian state of Minas Gerais? *Genetics and Molecular Biology*, 30: 667-675. doi: 10.1590/S1415-47572007000400027
- Tavares, M.G., Ribeiro, E.H., Campos, L.A.O., Barros, E.G. & Oliveira, M.T.V.A. (2001). Inheritance pattern of RAPD markers in *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponinae). *Journal of Heredity*, 92: 279-282. doi: 10.1093/jhered/92.3.279
- Waser, N.M., Price, M.V. & Shaw, R.G. (2000). Outbreeding depression varies among cohorts of *Ipomopsis aggregata* planted in nature. *Evolution*, 54: 485-491.
- Wenseleers, T., Alves, D.A., Franco, T.M., Billen, J. & Imperatriz-Fonseca, V.L. (2011). Intraspecific queen parasitism in a highly eusocial bee. *Biology Letters*, 7: 173-176. doi: 10.1098/rsbl.2010.0819
- Zayed, A. (2009). Bee genetics and conservation. *Apidologie*, 40: 237-262.