Offspring Analysis in a Polygyne Colony of *Melipona scutellaris* (Hymenoptera: Apidae) by Means of Morphometric Analyses

by

Carlos Alfredo Lopes de Carvalho[1], Wyratan da Silva Santos[2], Lorena Andrade Nunes[3], Bruno de Almeida Souza[4], Gislene Almeida de Carvalho Zilse[5] & Rogério Marcos de Oliveira Alves[6]

ABSTRACT

In the few cases of polygyne colonies in *Melipona* the presence of active queens is common, generating offspring from different maternal origins. One of the techniques employed to identify maternity of the offspring is morphometric analysis, which allows inter- and intraspecific groups to be discriminated. The objective of this study was to identify the maternal source of *Melipona scutellaris* workers from a polygyne colony with five queens using wing morphometric analysis. The right forewings and hindwings of 209 workers were used. The workers came from a brood disk extracted from the colony and taken to a B.O.D. incubator maintained at 28±1°C and 75% relative humidity. Conventional and geometric morphometry analyses were made. Groups were discriminated, indicating that the material analyzed had different maternal origins, with predominance of one queen, which was responsible for 57% of the progeny. Cluster analysis allowed to confirm that the use of conventional morphometry can identify offspring groups from existing queens in a *M. scutellaris* polygyne colony. However, such identification was not possible with geometric morphometry.

[1] Centro de Ciências Agrárias, Ambientais e Biológicas, UFRB, C. Postal: 118, 44380-000, Cruz das Almas-BA. Brazil. E-mail: calfredo@ufrb.edu.br
[2] National Post-Doctorate Program, CAPES, Graduate Program in Agricultural Science/CCAAB/ UFRB, Cruz das Almas-BA
[3] Escola Superior de Agricultura "Luiz de Queiroz"/USP, Graduate Program in Entomology. Piracicaba-SP, Brazil. E-mail: lanunes@esalq.usp.br
[4] Bee Research Center, Embrapa Meio-Norte, 64006-220, Teresina-PI Brazil. E-mail: bruno@cpamn. embrapa.br.
[5] Bee Research Group – GPA; Instituto Nacional de Pesquisas da Amazônia – INPA, 69060-000. Manaus-AM, Brazil. E-mail: gislene@inpa.gov.br
[6] Instituto Federal Baiano, Catu-BA, Brazil. Graduate Program in Agricultural Science, CCAAB/ UFRB, 44380-000, Cruz das Almas-BA. Brazil.

INTRODUCTION

The occurrence of several queens simultaneously in the same colony is termed polygyny. In the order Hymenoptera it represents an early stage in their evolutionary sociability and can be found in a large number of wasp and bee species (Velthuis *et al.* 2001, 2006).

Polygyny is very rare among eusocial bees and is transient whenever it occurs (Velthuis *et al.* 2001, 2006). For example, during the replacement process of an old queen with a young one, both queens may live side by side for a short period, even laying eggs concurrently. Such situation was reported by Carvalho-Zilse & Kerr (2004) in *Melipona scutellaris*, by Velthuis *et al.* (2006) in *Melipona bicolor*, and was observed in the meliponine experimental rearing facility at Universidade Federal do Recôncavo da Bahia by the authors CALC and RMOA in *Melipona asilvai*, always with two queens.

Until then, polygyny studies in meliponines were conducted mainly with *M. bicolor,* by recording the insects with a camera in order to study the provisioning and oviposition processes in brood cells.

Lasting polygyne colonies in the genus *Melipona* with more than two queens were found exclusively in *M. bicolor* by Bego (1983). Recently, Carvalho *et al.* (2010) reported the presence of a natural polygyne colony in *M. scutellaris*, with five active queens.

In spite of advances in the recording and studying of the polygyne condition of *Melipona* colonies, information regarding the interaction among those various queens and the existing brood in those colonies are still scarce; in addition, techniques generally used involve a considerable amount of financial resources.

Morphometric analysis techniques have been used because they present robust data, especially because of the development of various pieces of software, and provide a considerable reduction in the costs involved. Such analysis takes into account the morphometric patterns of individuals, and its application can discriminate inter- and intraspecific groups (Monteiro & Reis 1999).

The objective of this study was to identify the maternal source of *M. scutellaris* workers from a polygyne colony with five queens using both traditional and geometric wing morphometric analysis.

MATERIAL AND METHODS

A brood disk from the *M. scutellaris* polygyne colony reported by Carvalho *et al.* (2010) was removed and taken to a B.O.D. incubator maintained at 28±1°C and 75% moisture; 209 workers emerged from the disk. Five days after emergence the workers were sacrificed and preserved in absolute alcohol.

The morphometric analyses were conducted based on characters obtained from the right fore- and hindwings of all 209 workers. Motic 2.0 ML software was used to capture wing images. Wings were arranged between two slides, one at a time, with a digital camera attached to a stereoscopic microscope at 7.5X magnification.

Geometric morphometry

In the geometric morphometry analysis, 15 anatomical landmarks were defined for the forewing (Fig. 1) and 10 for the hindwing (Fig. 2); landmarks were recorded with TpsDig version 2.12 software (Rohlf 2008).
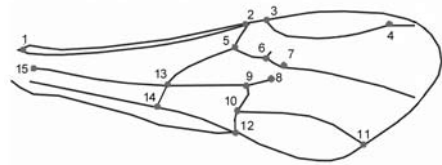

Fig. 1 - Anatomical landmarks used in the geometric morphometry analysis of *Melipona scutellaris* forewings.

Conventional morphometry

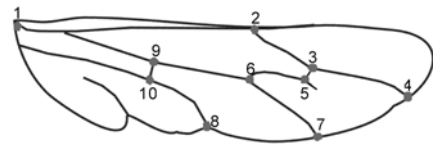Measurements from the variables described in Figs. 3 and 4 were used for the conventional morphometry analysis.

Fig. 2 - Anatomical landmarks used in the geometric morphometry analysis of M*elipona scutellaris* hindwings.

Statistical analysis

The data were analyzed using canonical variables analysis (CVA), principal components analysis (PCA), and cluster analysis using Mahalanobis' D^2 distance and the UPGMA method.

RESULTS AND DISCUSSION

Using the geometric morphometry method, based on CVA analysis for wing shape, the data analyzed were not significant, i.e., individuals from the *M. scutellaris* polygyne colony did not present differences from one another. Group structuring as a function of wing size, which often occurs in certain

species (Diniz-Filho & Malaspina 1995; Diniz-Filho *et al.* 1998; Nunes *et al.* 2007, 2008), does not seem to occur with the variation in wing shape. This could be related to evolutionary restrictions associated with aerodynamic aspects of this structure that is adapted for flight (Araújo *et al.* 2000).
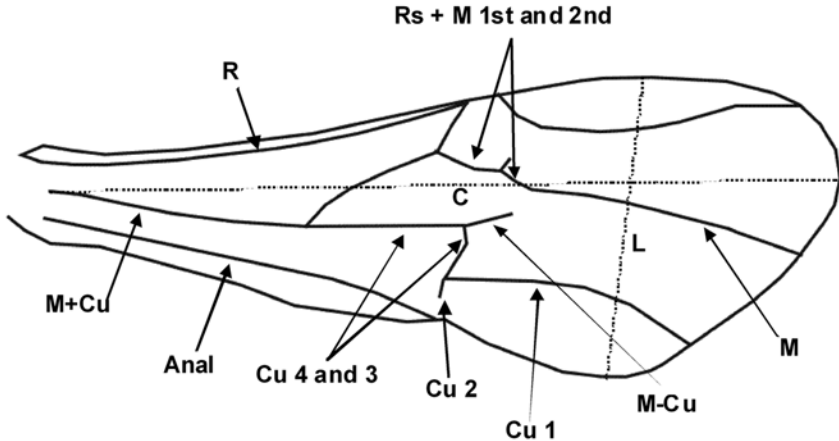

Fig. 3 - Morphology and variables used in the conventional morphometry analysis of *Melipona scutellaris* forewings. (L) width and (C) maximum length of the wing and length of the following veins: (R) radial, (RS+M1st) radial sector + 1st median, (RS+M2nd) radial sector + 2nd median, (M) median, (M-Cu) median-cubital, (Cu1) cubital 1, (Cu2) cubital 2, (Cu3) cubital 3, (Cu4) cubital 4, anal, (M+CU) median + cubital.
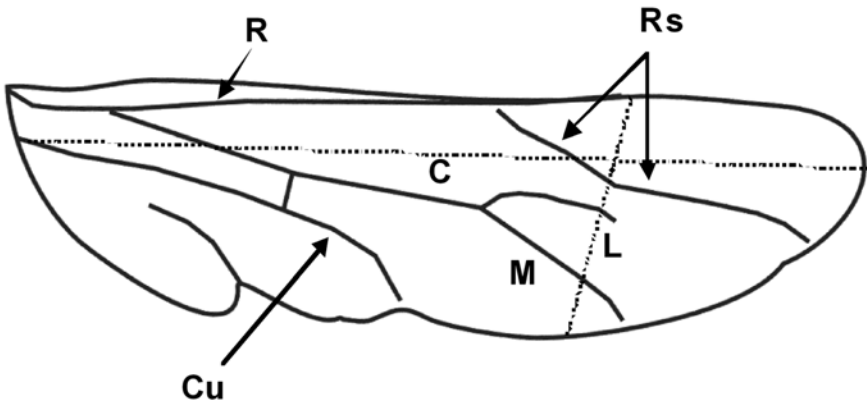

Fig. 4 - Morphology and variables used in the conventional morphometry analysis of *Melipona scutellaris* hindwings. (L) width and (C) maximum length of the wing and maximum length of the following veins: (R) radial, (M) median, (Cu) cubital, (PRs1) radial sector 1, and (PRs2) radial sector 2.

As for size analysis using conventional morphometry, some group structuring (UPGMA) was observed with more significant and concise results. Those groups (a total of five) consisted of 3, 2, 9, 77, and 118 individuals, respectively (Fig.5).
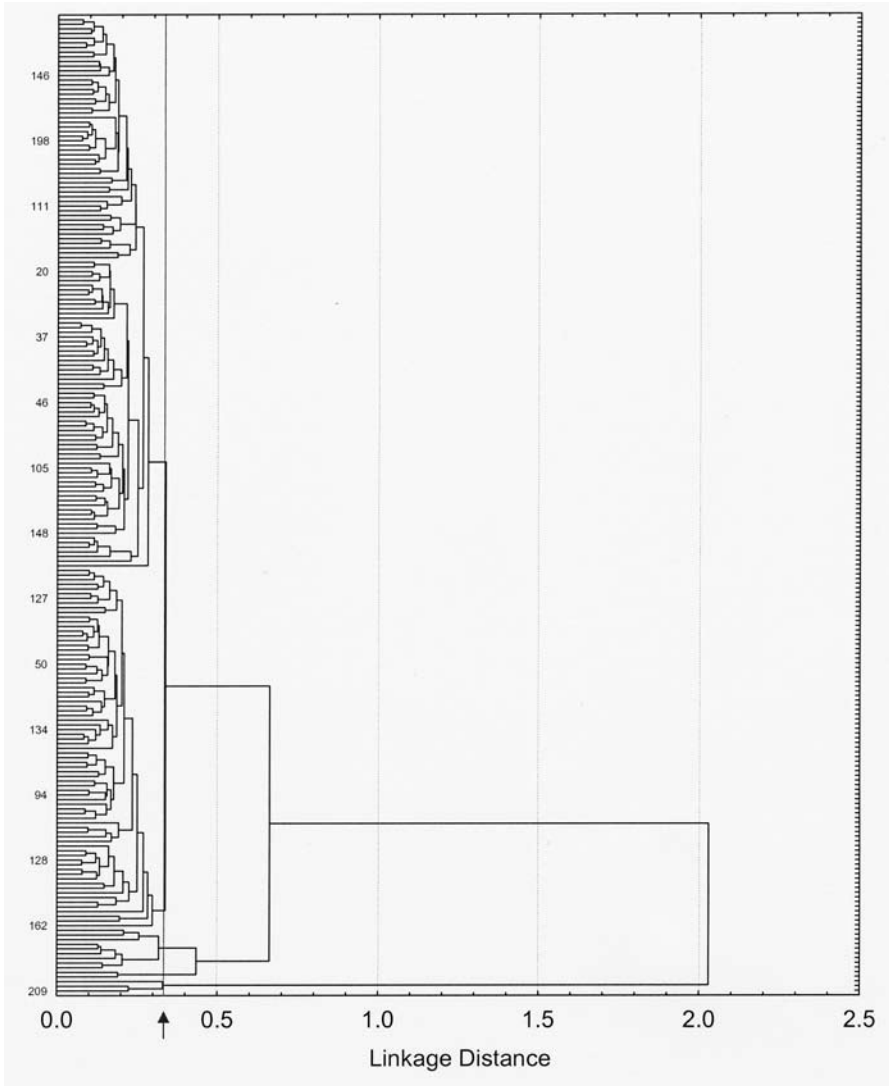

Fig. 5. Mean morphometric distances among *Melipona scutellaris* workers from a polygyne colony with five queens.

Based on conventional morphometry and using PCA, the first four components were required to explain a minimum percentage of 75% variation in the 20 original variables, considering cumulative variance. The first component showed the highest variation of data, explaining 56%, while the second corresponded to 12% variance and the third to 7%, sufficient for the data to be used in the formation of groups generated by UPGMA cluster analysis, making it easy to visualize the distribution of each individual in the groups.

Wing size could be related to a pleiotropic effect, and could also be influenced by several genes (Araújo *et al.* 2000). In *Apis mellifera* wing size-related heritability is usually high, with little variation due to the environment, which suggests that this character has a high genetic component to evaluate population structures (Diniz-Filho & Bini 1994).

Conventional morphometric analysis revealed that all five queens were active and that dominance of one occurred over the others, with 57% of the offspring observed for the dominant queen, 37% for the second queen, 4% for the third queen, and 1% for the fourth and fifth queens. Since the queens probably came from the same mother, wing size variation in their offspring could be the result of paternal source influence on the bees analyzed.

Such difference in oviposition among meliponine queens was also observed by Aponte (2003) in a *M. bicolor* polygyne colony with three fertile queens. By means of video recordings, that author observed oviposition distributions of 51%, 38%, and 11% among the queens.

By using a behavioral mechanism or domination by means of pheromones, meliponine queens may inhibit oviposition performed by workers (Kleinert 2005). That author studied reproductive control mechanisms in *Melipona marginata* by conducting assays with the introduction of queens of various ages into colonies that were considered monogynes and observed that a pheromonal action exists that reflects on oviposition. Consequently, something similar might have occurred with the *M. scutellaris* polygyne colony in our study, which would explain the dominance observed for one of the queens on the offspring analyzed (57%).

ACKNOWLEDGMENTS

REFERENCES

Aponte, O.I.C. 2003. Poliginia e monoginia em *Melipona bicolor* (Apidae: Meliponinae): do coletivo para o individual [Ph.D Thesis]. São Paulo (SP): Universidade de São Paulo. 211p.

Araújo, E.D. 2000. Extinção em populações do gênero *Melipona* (Hymenoptera: Meliponinae): efeito do tamanho populacional e da produção de machos por operárias. Naturalia 25: 287-299.

Bego, L.R. 1983. On some aspects of bionomics in *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apidae, Meliponinae). Rev Bras Entomol 27: 211-224.

Carvalho, C.A.L., B.A. Souza, C.S. Dias, R.M.O. Alves, A.F.L. Melo, A.C.F. Soares & G.A. Carvalho-Zilse 2010. Five egg-laying queens in a single colony of Brazilian stingless bees (*Melipona scutellaris* Latreille). Acta Amazon 40: *in press*.

Carvalho-Zilse, GA & W.E. Kerr 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 Amazon 34: 649-652.

Diniz-Filho, J.A.F. & L.M. Bini 1994. Space-free correlation between morphometric and climatic data: a multivariate analysis of Africanized honey bees (*Apis mellifera* L.) in Brazil. Glob Ecol Biogeogr Lett 4: 195-202.

Diniz-Filho, J.A.F. & O. Malaspina 1995. Evolution and population structure of Africanized Honey Bees in Brazil: Evidence from spatial analysis of Morphometric data. Evolution 49: 1172-1179.

Diniz-Filho, J.A.F., R. Balestra, F.M. Rodrigues & E.D. Araújo 1998. Geographic variation of *Tetragonisca angustula angustula* Latreille (Hymenoptera, Meliponinae) in central and southeastern Brazil. Naturalia 3: 193-203.

Kleinert, A.M.P. 2005. Colony strength and queen replacement in *Melipona marginata* (Apidae: Meliponini). Braz J Biol 65: 469-476.

Monteiro, L.R. & S.F. Reis 1999. *Princípios de morfometria geométrica*. Ribeirão Preto: Holos. 188p.

Nunes, L.A., M.F.F. Costa-Pinto, P.L.S Carneiro, D.G. Pereira & A.M. Waldschmidt 2007. Divergência genética em *Melipona scutellaris* Latreille (Hymenoptera: Apidae) com base em caracteres morfológicos. Biosci J 23: 1-9.

Nunes, L.A., E.D. Araujo, C.A.L. Carvalho & A.M. Waldschmidt 2008. Population divergence of *Melipona quadrifasciata anthidioides* (Hymenoptera: Apidae) endemic to the semi-arid region of State of Bahia, Brazil. Sociobiology 52: 81-93.

Rohlf, F.J. 2008. TPSDIG2 for Windows version 2.12. Departament of Ecology and Evolution, State University of New York, Stony Book. On line at: http://life.bio.sunysb.edu/morph/index.html. Acessed in 21 Oct 2008.

Silva, R.B. 2007. Variabilidade genética de *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponina) no Estado de Minas Gerais – Brasil [MSc. Thesis] Viçosa (MG): Universidade Federal de Viçosa. 41p.

Velthuis, H.H.W., H. De Vries & V.L. Imperatriz-Fonseca 2006. The polygyny of *Melipona bicolor*: scramble competition among queens. Apidologie 37: 222-239.

Velthuis, H.H.W., A. Roeling & V.L. Imperatriz-Fonseca 2001. Repartition of reproduction among queens in the polygynous stingless bee *Melipona bicolor*. Proc Exp Appl Entomol 12: 45-49.