INTRA-POPULATIONAL VARIABILITY OF *Nannotrigona testaceicornis* Lepeletier, 1836 (Hymenoptera, Meliponini) USING RELATIVE WARP ANALYSIS

VARIABILIDADE INTRA-POPULACIONAL EM Nannotrigona testaceicornis *LEPELETIER, 1836 (Hymenoptera, Meliponini) USANDO ANÁLISE DE DEFORMAÇÕES RELATIVAS*

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ABSTRACT: Due to the loss of biodiversity that occurs nowadays, the development of tools that allow us to characterize this biodiversity is of great importance. The recent developed relative warps analysis is very useful at this point. We characterized a population of *Nannotrigona testaceicornis* collected in different areas of Uberlândia using the relative warps analysis of the forewings. The two subpopulations of the urban environment were closer to each other than the one collected in a natural area outside the city. This greater proximity and diversification in relation to the natural area subpopulation is possibly due to inbreeding, that is enhanced by the smaller amount of natural food sources. The geometric morphometric of forewings showed to be a very powerful and informative tool to assess biodiversity within a population.

KEYWORDS: Biodiversity. Geometric morphometrics. Wings. *Nannotrigona testaceicornis.* Populational variability. Urban environment.

INTRODUCTION

The recent alarms about the loss of biodiversity require an international effort in order to develop strategies for conservation and sustainable use of the biodiversity. It is important to develop and to test new methodologies to assess this diversity.

Bees provide an essential ecosystem service – pollination. Besides, since bees are the pollinators responsible for the production of 35% ($23x10^8$Mt) of the global food production, they play a fundamental ecological and economic role what makes this group very important (KLEIN et al., 2007).

Among bees, the stingless bees are composed of about hundreds of species distributed throughout the tropical areas of the world. They are of extreme importance and still very little studied when concerning its biodiversity (MICHENER, 2000).

In studies with *Apis,* morphometry is a very useful tool to assess biodiversity, as well as subspecies definition and evolutionary purposes (RUTTNER et al., 1978; RUTTNER, 1988). It has been shown by several studies that the patterns of wing venation contain important information to discriminate among bee species and subspecies (RUTTNER, 1988; SCHRÖDER et al., 1995, 2006; FRANCOY et al., 2006; DRAUSCHKE et al., 2007).

Among the stingless bees, *Nannotrigona testaceicornis* Lepeletier 1836 are eusocial bees that nests on tree cavities or cavities of man made structures. The nest entrance is short and made of cerumen. During the day, the bee guards stay on the entrance hole, forming a ring. At dusk, the bees close the entrance which remains closed during the night (NOGUEIRA-NETO, 1970). The colonies can have from 2000 to 3000 bees (LINDAUER; KERR, 1960). These bees are black, very small and generally have grey hair (MONTEIRO, 2001). In Brazil, this species is distributed through the States of Bahia, Espírito Santo, Goiás, Minas Gerais, Rio de Janeiro and São Paulo (SILVEIRA et al., 2002). Nevertheless, also occur in Paraná, Santa Catarina and Rio Grande do Sul.

As this species has a large geographic distribution and occupies different biomes, including urban areas (SOUZA et al., 2002), certain variability in the patterns of wing venation is expected among the populations, because of the genetic variability and the selection of different genotypes according to the conditions of the biome (IMPERATRIZ-FONSECA et al., unpublished data).

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When analyzing a population, a great genetic variability within the population can be found and this structure sometimes is organized in subpopulations (WARRIT et al., 2006). As these kinds of evaluations are normally made with genetic markers, our aim is to verify if the population of *Nannotrigona testaceicornis* from Uberlândia, in Minas Gerais State, is composed of subpopulations using techniques of geometric morphometry.

MATERIAL AND METHODS

We collected samples of nine or ten workers per colony from 20 colonies of *Nannotrigona testaceicornis* from the region of Uberlândia – MG (around 18º S; 48º ’W). Six of these colonies were sampled in a natural area between Uberlândia and Araguari (Capim Branco region), eight in a district at the suburbs (Umuarama region), which is about 6 km far from the central region of Uberlândia, and six in the central region of Uberlândia (Centro region).

The right wing of each worker was placed between microscope slides and photographed in a digital camera attached to a stereo microscope.

Twelve homologous landmarks were manually plotted at the veins intersections (Figure 1) using the software tpsDig version 2.04 (ROHLF, 2005a). These images were aligned and the relative warps analyses were carried out in the software tpsRelw version 1.42 (ROHLF, 2005b). In these analyses we set $\alpha = 0$, so the same weight was given to all the variables. This procedure is the most suitable for exploratory and taxonomic studies (ROHLF; MARCUS, 1993).

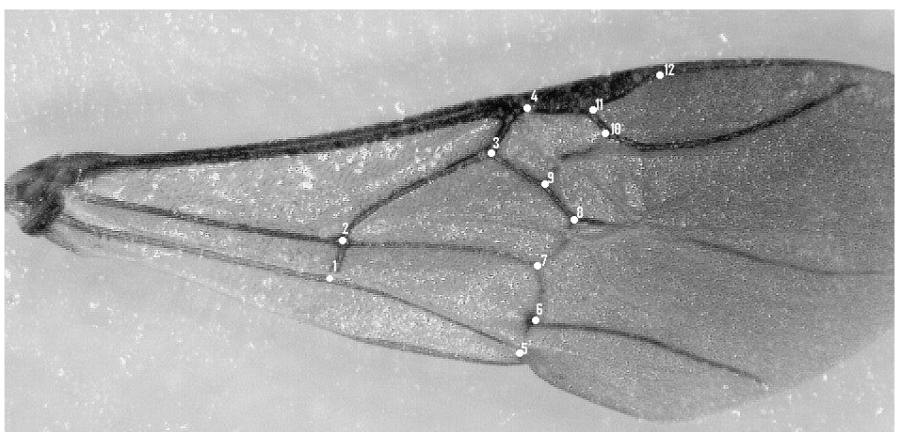

Figure 1. Right fore wing of *Nannotrigona testaceicornis* with the 12 landmarks plotted in the veins intercrosses.

The significant univariate F values ($\alpha = 0.05$) were used to identify the wing parameters that contributed most to the groups’ discrimination and a discriminant analysis was made in order to verify if there is differences among the three groups.

RESULTS

The twelve anatomic landmarks generated 20 relative warps measures for each specimen. The two first relative warps were able to explain 26.29% and 15.92% of the data variability, *i.e.* 42.21%.

From the 20 relative warps generated, 13 significantly contributed ($\alpha = 0.05$) to the separations of the groups. The MANOVA of the measures indicated that the three groups are statistically different (Wilk’s $\lambda = 0.3006$ $P < 0.0001$). A graphical representation of the discriminant analysis (Figure 2) shows the three subpopulations, with the group of Capim Branco, which is the group sampled in the natural area, more distant from the other two, sampled in the urbanized areas. When compared, the two urban groups present more similarities with each other than when compared with the Capim Branco group. The centroids of the distributions are significantly different from each other ($\alpha = 0.05$) (Table 1).

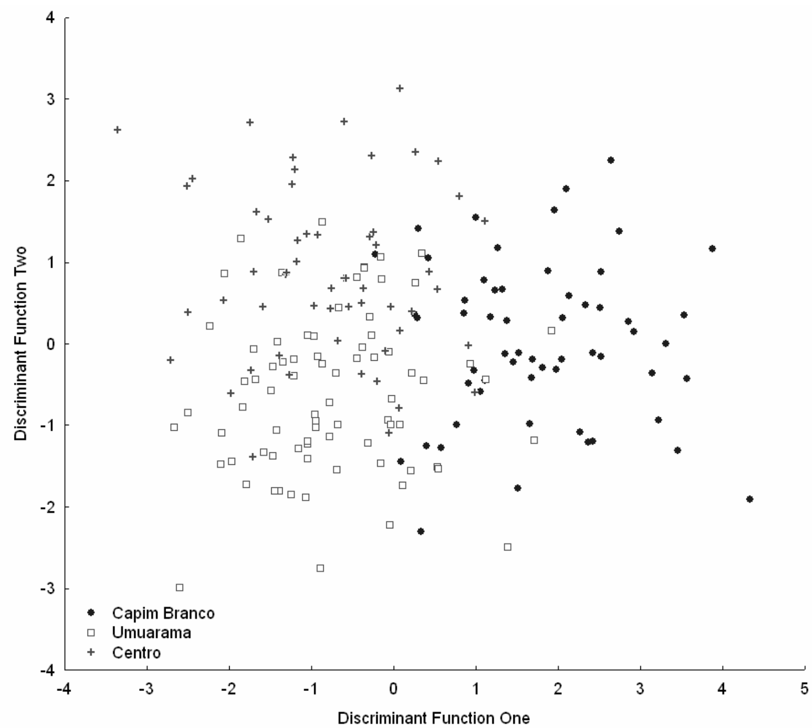

Figure 2. Graphical representation of the discriminant analysis of the three subpopulations of *Nannotrigona testaceicornis* from Uberlândia.

Table 1. Mahalanobis square distances between the centroids of the distribution of the three subpopulations and its statistical significances. The superior part of the table indicates the distances and the inferior indicates the statistical significance.

	Capim Branco	Umuarama	Centro
Capim Branco		6.96	7.48
Umuarama	0.0001		2.29
Centro	0.0001	0.0001	

The discriminant functions were able to distinguish 82.76% of the samples of Capim Branco, 78.20% from Umuarama and 71.60% of the samples of the Centro group. In general, 77.60% of the samples were correctly classified in the respective group and 69.79% of the samples were correctly identified in the cross validation tests.

DISCUSSION

When several features are analyzed at once, the square distances of Mahalanobis can be used as genetic diversity estimates among the groups. This variability is result of morphological, physiological and ecological differences (GHADERI et al., 1984). As our samples were collected in three different places in the region of Uberlândia, we were able to show the differentiation of these groups. In this case, the Mahalanobis square distances show a greater proximity of the two urban groups to each other

(Table 1). It is possible that the number of colonies in the urban areas is smaller than in the natural area, as it might be the resources availability. It can generate certain degree of inbreeding along with a smaller genetic variability. In 1976, Brückner demonstrated the importance of genetic variability in the symmetry of the forewings of *Apis mellifera* by inbreeding colonies for four generations. The workers and drones which resulted from these crossings showed a greater deviation of the measures when compared with the control group. It was also recently demonstrated that a population of *Apis mellifera* isolated in the island of Fernando de Noronha for more than 20 years is also differentiated from the original group introduced (FRANCOY 2007). It is also possible that the selective pressures in the urban areas are different from the ones in the natural area, what might have selected different characteristics in the studied groups.

The greater variability of the group from Capim Branco can be result of the greater number of colonies that can be found in nature. It provides a greater availability of mate choices, consequently increasing the genetic variability of the population. This variability became clear when the clouds of points of the two other groups is analyzed in the discriminant analysis (Figure 2).

It is worthy to mention that the usage of this methodology in stingless bees is just in the beginning but it already shows very good results in discriminating groups of bees. Francisco et al. (unpublished data) were able to show the absence of gene flow between two populations of *Plebeia remota*. Other species of *Plebeia* (SILVA 2006) and *Euglossa* (FRANCOY et al., unpublished results) were also very good discriminated using the relative warps analysis, as well as subspecies of *Melipona bicolor* (FRANCOY et al., unpublished results) and *Apis mellifera* (FRANCOY 2007). In the present work we were able to show that, in stingless bee, the discriminatory power of the technique can be used even to classify groups smaller than populations. As this is a simple and quick technique, with low costs and a very good discriminatory power, it points to a very promising future in the usage of relative warps analysis in identifying bee species through wing morphometrics.

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RESUMO: Devido aos recentes alarmes causados pela perda de biodiversidade, o uso de ferramentas que permitam a caracterização desta biodiversidade é de grande importância. As técnicas de análises de deformações relativas têm se mostrado muito úteis neste ponto. Aqui, nós caracterizamos, por análises de deformações relativas das asas anteriores, uma população de *Nannotrigona testaceicornis* coletada em diferentes áreas de Uberlândia. As duas subpopulações de ambientes urbanos eram mais parecidas entre si do que quando comparadas com a subpopulação coletada em uma área de mata natural. Esta maior proximidade se deve possivelmente ao endocruzamento e à menor disponibilidade de alimento. A morfometria geométrica das asas anteriores se mostra ainda uma ferramenta muito útil e informativa para avaliação de biodiversidade.

PALAVRAS-CHAVE: Biodiversidade. Morfometria geométrica. Asas. *Nannotrigona testaceicornis*. Variabilidade populacional. Ambiente urbano.

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