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Morphological variation of the aedeagus in *Drosophila buzzatii* (Diptera, Drosophilidae).

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Introduction

The insect's aedeagus, which is the intromittent organ of the male genitalia, presents rapid and divergent evolution in relation to other morphological characters and is, therefore, a useful organ for taxonomic studies. Like any other phenotypic character, it shows geographic variation that may be related to environmental factors (Tatsuta and Akimoto, 1998; Kelly *et al.* 2000; Jennions and Kelly, 2002; Franco *et al.*, 2008). In the *Drosophila* genus and particularly in the *D. repleta* species group, the aedeagus morphology of its members has been considered the most important diagnostic character for species identification (Vilela, 1983). The *Drosophila buzzatii* cluster (*D. repleta* group: *D. buzzatii* complex) is a monophyletic group of seven cactophilic species of South America (Manfrin and Sene, 2006), and the male genitalia is a key trait that allows to distinguish its sibling species (Vilela, 1983; Silva and Sene, 1991).

One of these species, *Drosophila buzzatii*, has a wide geographical distribution, being found in Caatinga Domain Brazil until the Chaco Domain (Barker *et al.*, 1985). Although *D. buzzatii* has been extensively studied by cytogenetic and molecular markers (Baimai *et al.*, 1983; Ruiz *et al.*, 1984; Barker *et al.* 1985; Figueiredo and Sene, 1992; DeBrito *et al.* 2002; Khun *et al.*, 2003), there is a lack of information about the morphological diversity of its natural populations.

In this context, the objective was to investigate the population differentiation of the aedeagus morphology collected from nature, from four Brazilian populations of *Drosophila buzzatii* [Irecê-BA, northeastern (n = 6), Furnas-MG, southeastern (n = 7), São Simão-SP, southeastern (n = 6) and Osório-RS, southern (n = 5)].

Material and Methods

The aedeagus (Figure 1) was removed and mounted on slides according to Kaneshiro (1969). The slides were photographed with a digital camera mounted on microscope. In this study, we used elliptical Fourier analysis (Kuhl and Giardina, 1982) to parameterize the coordinates of the of aedeagi

outline. In brief, in this analysis the x and y coordinates of an outline are fit separately as a function of arc length by Fourier analysis, decomposing the contour into a weighted sum of sine and cosine functions, called harmonics. We considered 25 harmonics and, consequently, 100 coefficients (4 per harmonic) were generated. The variance covariance matrix of the 100 estimated elliptic Fourier descriptors (EFD) coefficients was used as input in a principal components analysis (Soto, 2005). The EFDs and the shape PC scores were obtained using the SHAPE package (Iwata and Ukai 2002).

The area of contour was used as a size measurement. The variation of the harmonic components was reduced to eight significant principal components.

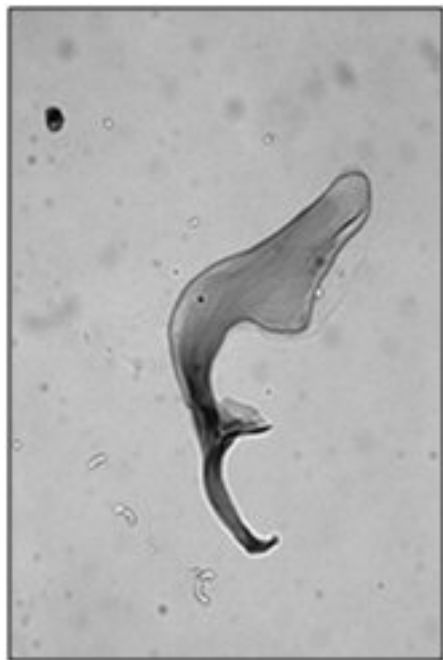


Figure 1. Lateral view of the aedeagus of a *Drosophila buzzatii* specimen (Magnification 100×).

The first two discriminant axes were used to make a scatter plot. The difference between each pair of populations was quantified by Mahalanobis distance (D2), which is a distance measure between two points in multivariate space, defined by two or more correlated variables. From the Mahalanobis distance matrix was constructed a phenogram using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method. A univariate regression was performed with both variables size and latitude. All statistical analyses were performed with Statistica 8.0 (StatSoft, Inc, 2007).

Results and Discussion

The discriminant analysis showed that the first principal component of the elliptic Fourier descriptors, which explains 38.7% of shape variation (Wilk's Lambda = 0.301, $p = 0.01$) and the variable size (area) (Wilk's Lambda = 1.00, $p = 0.0002$) were significant. The geral percentage of correct re-classification was 87.5%; all Irecê specimens were correctly classified, and 100% had Irecê and specimen Osorio were not re-classified as Irecê.

The size was positively correlated with latitude ($r = 0.72$), suggesting that the morphological differences in size observed may be explained by environmental variations such as temperature (Andrade *et al.*, 2005), although we cannot rule out the possible influence of host plant (Soto *et al.*, 2007). The phenogram with the Mahalanobis distances (Figure 2) showed that the Irecê population was separated from other populations and also has presented the smallest size (Figure 3).

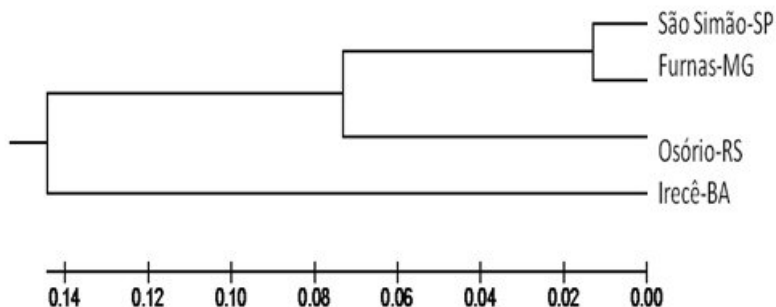


Figure 2. Mahalanobis distance phenogram, based on shape and size variables of the aedeagi, showing the relationship among populations analyzed.

Moreover, the scatter plot of the first two discriminant axes (Figure 4) showed that the first axis

separated the populations Irecê and Osorio, with the other two populations overlap between them. These results suggest a separation between Northeastern and Southern Brazil populations, with the Southeast intermediate between them. The study showed that *Drosophila buzzatii* has low interpopulation morphological variation related to the aedeagus shape.

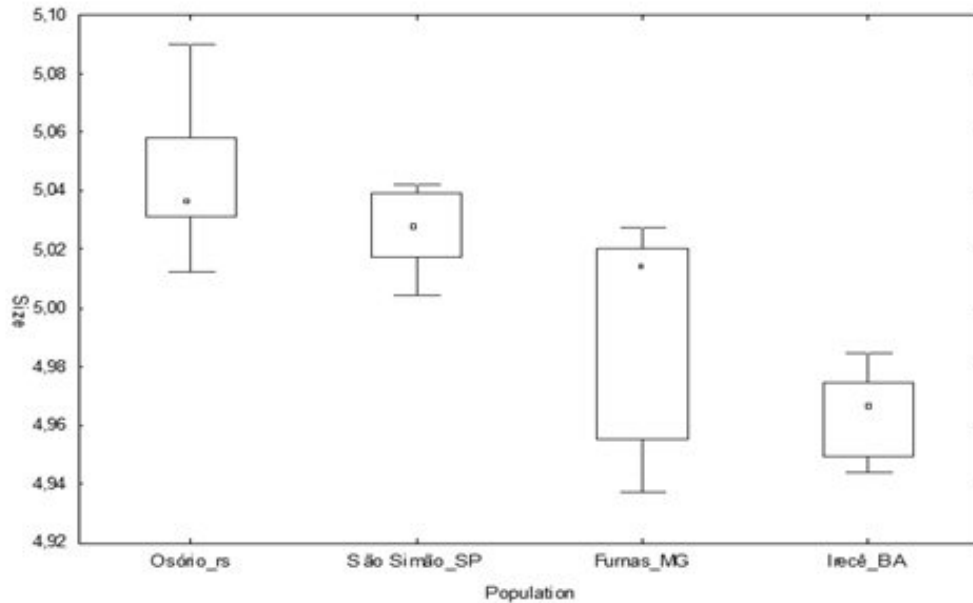


Figure 3. Box plot of the variable size logarithm for the four populations analyzed of *Drosophila buzzatii*.

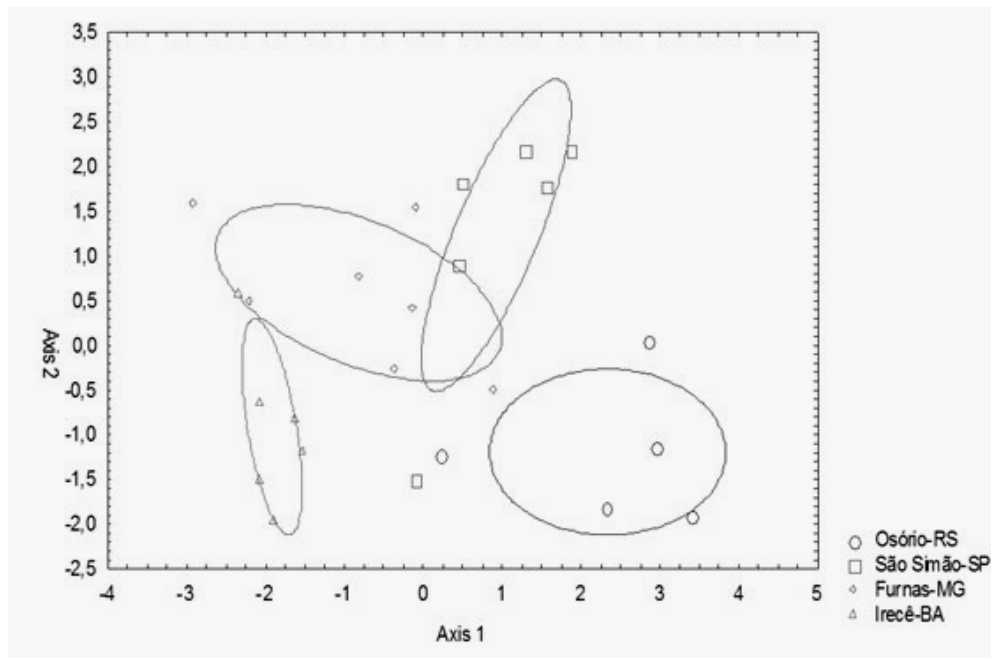


Figure 4. Scatter plot of the first two discriminant axes, Axis 1 and Axis 2, of the four populations analyzed for the shape variables of the *Drosophila buzzatii*.

The groups formed by the aedeagus variation agree with the data obtained for other genetic markers, which also separate *D. buzzatii* populations of the Northeast from those of the Southeast, South in Brazil and Chaco Domain (Baimai *et al.*, 1983; Ruiz *et al.*, 1984; Barker *et al.*, 1985; Figueiredo and Sene, 1992; DeBrito *et al.*, 2002; Khun *et al.*, 2003). Moreover, the aedeagus variable size was a positive correlation with latitude. These data suggest that as well as historical factors, environmental factors also influence the differentiation of aedeagus morphology in *D. buzzatii* populations (Baimai *et al.*, 1983; Ruiz *et al.*, 1984; Barker *et al.*, 1985; Figueiredo and Sene, 1992; DeBrito *et al.*, 2002; Khun *et al.*, 2003). Moreover, the aedeagus variable size presented a positive correlation with latitude. These data suggest that, as well as historical factors, environmental factors also influence the differentiation of *D. buzzatii* populations.

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Distribution of *tom*, a retrotransposon, in natural populations of *D. ananassae*.

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Introduction

Transposable elements (TE) are genomic parasites that maybe inflict detrimental mutations on the fitness of their host. A retrotransposon, *tom*, one of the TEs, is mobilized with high frequency in the germ line of females from the *ca; px* strain; mobilization of *tom* in the *ca; px* strain causes a high incidence of mutations that almost exclusively affect eye morphogenesis (Hinton, 1984; Shrimpton *et al.*, 1986; Matsubayashi *et al.*, 1992; Tanda *et al.*, 1993). Tanda *et al.* (1988) reported that *tom* is a long terminal repeat-containing retrotransposon that encodes three different open reading frames (ORFs). We surveyed the *tom* element in genomes from natural populations of *D. ananassae* by means of *in situ* hybridization on the polytene chromosomes.