Technique Notes

Improving sampling protocol for assessing drosophilid diversity: spatial independence and sample size.

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Introduction

The development of monitoring programs is one of the strategic actions set by the Convention of Biological Diversity (CBD, 2010) to achieve the biological conservation targets. However, such monitoring programs often show problems due to the lack of scientific rigor in the sampling design (Lindemayer and Linkens, 2010). Therefore, robust monitoring programs based on a rigorous scientific protocol have emerged as a high priority area in environmental research (Gardner, 2010; McGeoch *et al*., 2011).

Drosophilid assemblages are suitable candidates for monitoring studies in the Brazilian savanna (Mata *et al*., 2008). However, despite the increasing number of studies on drosophilid diversity in Brazil, to date there has been no robust standardized protocol that allows both a reasonable diversity estimate of a particular area and the comparison among different studies. Here we present a first approach in developing a sampling protocol for assessing drosophilid diversity, by investigating two fundamental aspects of sampling designs: the spatial independence between sampling units and sample size adequacy.

Sampling Design

Pilot samples were conducted in the Ecological Reserve of IBGE, located 35 km south of Brasília, the Brazilian capital, in January and May 2013. On each sampling occasion, four habitat types were sampled: conserved *cerrado* (an open vegetation characteristic of the Brazilian savanna), disturbed *cerrado*, conserved forest, and disturbed forest. In all habitats, five sampling units (SU) were established at least 30 m apart. Each SU contained three traps disposed 10 meters apart, totaling 60 traps per sampling occasion.

The flies were captured using baited traps, which retain the attracted specimens (Roque *et al*., 2011). Traps were baited with mashed bananas, fermented with *Saccharomyces cerevisiae* for 24 hours. The traps were left in the field for four consecutive days, and the flies collected were taken to the laboratory and identified, whenever possible, to the species level. Vouchers of the captured species were deposited at the Collection of the *Laboratório de Biologia Evolutiva da Universidade de Brasilia*.

Spatial autocorrelation analysis was used to determine whether the drosophilid assemblages collected in different distance classes were independent of each other. We used a Mantel test between a similarity matrix (based on the Kulczynski quantitative coefficient) and a geographic distance matrix (based on Euclidian distance). This analysis was run in SAM software (Rangel *et al*., 2010).

The statistical power analysis (run in PASS software; Hintzi, 2013) was used to calculate the adequate sample size to detect richness differences between conserved and disturbed *cerrados* and forests (two-sample t-tests allowing unequal variance; $\alpha = 0.05$, $\beta = 0.80$). The power analysis estimated the sampling effort needed for detecting any difference on drosophilid richness between conserved and disturbed sites, to achieve a statistical power around 80% (1- β).

Spatial Independence and Sample Size Adequacy

The spatial autocorrelation analyzes failed to show any spatial structure between sampling units even in the first distance class both in *cerrados* (Distance centroid = 50m; Pearson's r = 0.143; p = 0.367) and in forests (Distance centroid = 30 m; Pearson's r = 0.319; p = 0.211).

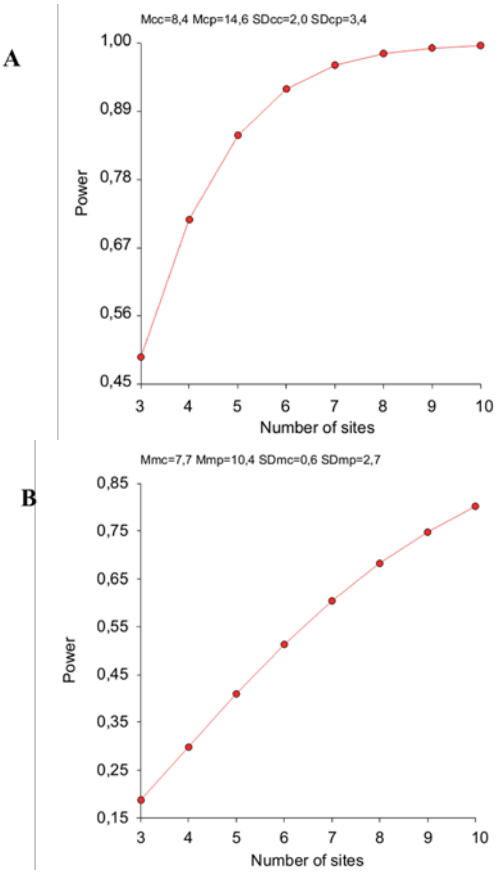

Figure 1. Statistical power increasing as a function of sample size (two-sample T-tests allowing unequal variance; $\alpha = 0.05$, $\beta = 0.80$). A : cerrados; B: forests.

In *cerrados* (Figure 1A), it would require at least five sampling units (composed by three traps each) for each habitat type, while to compare conserved and disturbed forests (Figure 1B), at least ten replicates of each habitat type would be necessary.

From these results we can conclude that – for the Brazilian Savanna – sample independence for drosophilid diversity, ensuring true replicates, can be reached by disposing SUs 50 m apart in *cerrados* and 30 m in forests. Moreover, detecting differences in the drosophilid richness between conserved and disturbed sites requires a sampling effort two times greater in forests than in *cerrados*.

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The Dover wild type strain and four derived isogenic lines.

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Here we report the availability of a wild type *Drosophila melanogaster* strain.

"Dover" and four derived isogenic lines

The Dover strain was founded in 1978 in Iowa City, IA, USA by J. Dawson Mohler, Department of Biology, University of Iowa. Twenty females were captured in Iowa City from a kitchen on Dover Street. Several eggs from each female were de-chorionated, and the offspring were randomly interbred to found the