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Are There Edge Effects on the Genetic Diversity of the Trap-Jaw Ant *Odontomachus chelifer* (Formicidae: Ponerinae) in a Neotropical Savanna Fragment? A First Assessment

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Abstract

Habitat fragmentation is considered an important threat to biodiversity, increasing species exposure to edge effects. The Brazilian Cerrado savanna is considered a biodiversity hotspot and has been converted to small, isolated fragments due to human activities. Ant communities and colony survivorship are known to be affected by edge effects in Cerrado, but to date there is no information on the genetic diversity of ant colonies at the edge of fragmented areas. Here, we investigate if colony genetic diversity and structure of *Odontomachus chelifer* (Latreille) ants (Hymenoptera: Formicidae) are subject to edge effects in a Cerrado reserve in southeast Brazil. Using microsatellites, we evaluated the number of breeders (queens and males) and the genetic diversity in *O. chelifer* colonies located in the interior versus edge of a Cerrado fragment. All *O. chelifer* nests had multiple queens, which presented a low mating frequency. The number of breeders and most estimates of genetic diversity did not differ between colonies at the edge versus interior of the fragment. Genetic structure was not influenced by nest location as well. However, we detected a small and positive increase in the observed heterozygosity in colonies located at fragment edges. High heterozygosity is thought to be particularly important in fast-changing environments, such as edges, providing an advantage for genetic diversity. Further investigation is needed to assess in greater detail how habitat loss affects *O. chelifer* biology. Our study is a first step toward elucidating edge effects on genetic diversity of ant colonies, a topic still poorly explored in tropical environments.

Key words: Cerrado vegetation, habitat fragmentation, polygyny, polyandry, simple sequence repeat

Environmental limitation promoted by habitat fragmentation is considered one of the main threats to biodiversity (Wilson et al. 2016), leading to the reduction and isolation of native remnants and increasing the proportion of habitat exposed to edge effects (Murcia 1995). The abiotic changes at borders usually include an increase in light and wind incomes in native ecosystems (Ries et al. 2004), which reduce humidity and increase temperature in the transitional areas (Laurance et al. 2011, Christianini and Oliveira 2013). These changes may alter community composition, population distribution, interspecific interactions, and genetic diversity (Hagen et al. 2012 and included references).

Environmental changes and habitat fragmentation can directly impact the genetic makeup of populations by reducing the genetic diversity (e.g., Vanhala et al. 2014, Schlaepfer et al. 2018), gene flow

(e.g., Gaublomme et al. 2013), and the effective size of populations (Frankham et al. 2002, Keller et al. 2005). The processes underlying the edge effects on genetic diversity are not fully understood. The edge of the fragments may act as population sinks, mainly in small areas with a high perimeter:area ratio, which would increase extinction probability (Woodroffe and Ginsberg 1998). Moreover, the differences in habitat conditions between the interior and the edge can lead to an increase in environmental heterogeneity in the whole fragment. Environmental heterogeneity influences genetic diversity mainly through genetic drift and selection (Kahilainen et al. 2014). This heterogeneity may alter population size, depending on the requirements of species, making them susceptible to stochastic events (Vellend and Geber 2005). Finally, disruptive selection can favor different genotypes, although generalist genotypes that explore distinct habitats may also be favored, reducing genetic variability within populations (Kahilainen et al. 2014).

Ants are commonly affected by edge effects. For instance, in the Amazon rainforest the richness and composition of ant communities differ between border and interior of fragments (Carvalho and Vasconcelos 1999). Leafcutter ants in the genera *Atta* and *Acromyrmex* are more abundant at fragment borders in the Atlantic rainforest and Cerrado savanna of Brazil (Meyer et al. 2009, Vieira-Neto et al.2016), and in the Argentine Chaco (Barrera et al. 2015).

The Neotropical Cerrado savanna covers nearly 26% of Brazil's surface area and small parts of Bolivia and Paraguay (Oliveira and Marquis 2002, Vieira et al. 2022). The so-called Cerrado domain encompasses a continuum of vegetation physiognomies, ranging from extensive grasslands with scattered shrubs to forest woodlands known as *cerradão*, whose physiognomy consists of 50–90% of trees up to 10–12 m in height (Oliveira-Filho and Ratter 2002). The Cerrado is considered a biodiversity hotspot (Myers et al. 2000), but in the last few decades the once continuous savanna has been converted to small and isolated fragments due to the rapid expansion of agriculture and livestock (Colli et al. 2020). Recent studies have shown that the composition of the ant community differs between the edge and interior of Cerrado fragments (Brandão et al.2011, Bernardes et al. 2020).

Given the pervasive participation of ants in many interspecific interaction systems in Cerrado (e.g., Oliveira and Freitas 2004; Costa et al. 2008, 2017), and the expectation that their role should increase in face of anthropogenic disturbance to natural landscapes (Christianini et al. 2014, Oliveira et al. 2017), it is crucial to evaluate how edge sites can affect ant biology in fragmented Cerrado. Ants are especially susceptible to environmental changes due to their haplodiploid sex-determination system and the small number of reproductive individuals that contribute to offspring (Hedrick and Parker 1997, Seppä 2008). To date there is no information on edge effects on genetic diversity of ants in Cerrado savanna.

The Study Species

The trap-jaw ant *Odontomachus chelifer* (Latreille) (Formicidae: Ponerinae) (Fig. 1) is widely distributed in the Neotropics, from semiarid to rainforest habitats (Brown 2000). *Odontomachus* ants are visually oriented predators that feed primarily on leaflitter and arboreal arthropods (e.g., Oliveira and Hölldobler 1989, Ehmer and Hölldobler 1995, Camargo and Oliveira 2012, Larabee and Suarez 2014, Rodrigues and Oliveira 2014), and that commonly complement their diet with plant-derived food such as extrafloral nectar and fleshy fruits (Blüthgen et al. 2003, Passos and Oliveira 2004). *O. chelifer* nests on the ground and feeds on epigeic arthropods and nutritious fleshy fruits in the Atlantic forest and Cerrado savanna

Fig. 1. Worker of *Odontomachus chelifer* carrying a larva. Photo by Verônica B. Magalhães.

(Raimundo et al. 2009, Christianini and Oliveira 2010, Bottcher and Oliveira 2014). Christianini and Oliveira (2013) showed that residence time of *O. chelifer* nests decreases at the border of fragmented Cerrado compared to moister core sites (but see Salles et al. 2018).

Medeiros et al. (1992) have shown that *O. chelifer* colonies are functionally polygynous, with the reproductive status among coexisting queens linked to their behavioral performances during queen-queen domination contests. Little is known on how edge habitats can affect the genetic structure of organisms in general (Cheptou et al. 2017). Given that the genetic makeup of ant colonies is affected by the number of egg-laying queens (Ross 2001), and that queen number in *Odontomachus* ants can vary with heterogeneous microhabitat conditions (Oliveira et al. 2011), this study investigates if the genetic diversity and structure of *O. chelifer* colonies are subject to edge effects in Cerrado. Specifically, we 1) characterize the genetic diversity of *O. chelifer*, and 2) evaluate if the genetic diversity and functional polygyny of *O. chelifer* are influenced by colony location, i.e., edge versus interior of a Cerrado fragment. Given that habitat fragmentation can reduce animal genetic diversity (Schlaepfer et al. 2018), and populations may respond distinctively to the environmental heterogeneity promoted by fragmentation (Kahilainen et al. 2014), we expect to find differences between the genetic variables estimated for colonies at edge and interior of a Cerrado fragment.

Materials and Methods

Study Site and Sampling Procedure

The study was carried out at 'Fazenda Campininha', a Cerrado savanna reserve located near Mogi-Guaçu (22° 18′ S, 47° 11′ W), state of São Paulo, southeast Brazil. The reserve occupies an area of 470 ha and is surrounded by a matrix of agriculture and *Pinus* plantations. Colonies of *O. chelifer* were sampled in a fragment of *cerradão*, a forest-like physiognomy of Cerrado (Oliveira-Filho and Ratter 2002). The sampling procedure for edge versus interior comparison followed Christianini and Oliveira (2013), who have previously detected edge effects in Cerrado regarding microclimate variables, seed removal, seedling establishment, and survival of *O. chelifer* colonies. Ant samplings were made at two parallel transects of ≈300 m, one at the edge (≤15 m from the fragment edge) and another at the interior of the fragment (≥45 m from the edge; Fig.

2 – see also Dodonov et al. 2013, 2016; Salles et al. 2018). To locate *O. chelifer* nests, in each transect we set eight pitfalls ≈40 m apart from one another and left open for 24 h. We tagged 18 *O. chelifer* nests through active searching along the transects; 9 nests at edge, and 9 in the interior of the fragment. Edge and interior sites in Cerrado fragments may differ significantly in air temperature and relative humidity, with the inner portions more likely to be cooler and moister than edges (Christianini and Oliveira 2013, Dodonov et al. 2013). The ants were stored in absolute ethanol and at –20°C until genomic DNA extraction, which were obtained using the entire worker body and followed a modified CTAB protocol (Saghai-Maroof et al. 1984). Ant voucher specimens are deposited in the Hymenoptera section of the Museum of Zoology at the State University of Campinas (Accession Number: ZUECHYM-7675).

Microsatellite Analyses

In total, 270 workers (15 from each nest) were genotyped for nine polymorphic microsatellite markers previously developed for *O. chelifer*: Och47, Och03, Och60, Och34, Och11, Och69, Och54, Och08, and Och72 (Lemos et al. 2020). The amplifications were conducted using touchdown PCR protocols, with hybridization temperatures between 52 and 57°C or between 55 and 60°C, as follows: 1) 94°C for 4 min; 2) 10 cycles of 94°C for 45 s, 60°C or 57°C (–0.5°C/cycle) for 1 min, and 72°C for 1 min and 15 s; 3) 25 cycles of 94°C for 45 s, 50°C for 1 min, and 72°C for 1 min and 15 s; and 4) 72°C for 10 min (temperature range for amplification of each locus is shown in Supp Table S1 [online only]). Amplified fragments were analyzed in the 3500 Genetic Analyzer (Applied Biosystems) sequencer and post-sequencing analysis was conducted on Geneious prime software (v. 2019.2; Biomatters Limited, New Zealand). The genotyping for all workers of *O. chelifer* is available in Supp Table S2 (online only).

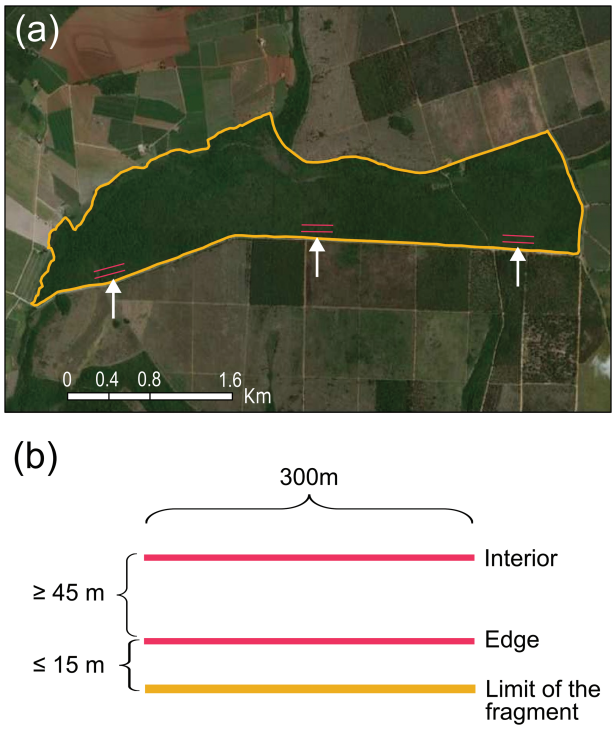

Fig 2. Scheme of the sampling procedure employed at the Cerrado fragment of Mogi-Guaçu, SE Brazil. (a) Location of the paired parallel transects (arrows) where ant nests were tagged at the edge and interior sites (adapted from Salles et al. 2018, with permission). (b) Schematic outline of the parallel transects established in the study area, at the edge and interior of the fragment.

Descriptive Loci Analyses

For characterization of microsatellite loci, we considered all colonies as a single population, regardless of their location. Microsatellite loci were tested for occurrence of stuttering and reduced amplification of large fragments using Micro-Checker (Van Oosterhout et al. 2004). Expected and observed heterozygosity were estimated using Microsatellite Toolkit supplement in Excel (Park 2001). Allelic richness was estimated using the rarefaction method, implemented in the HP-Rare program (Kalinowski 2004, 2005). Adherence to Hardy–Weinberg equilibrium (HWE) was tested using GENEPOP 4.7 (Rousset 2008), and linkage disequilibrium (LD) was evaluated using the software FSTAT v.2.9.3.2 (Goudet 1995). To overcome biased results due to highly related genotypes in ant colonies, we randomly sampled one worker per colony to conduct HWE and LD analyses. For both analyses, Bonferroni correction for multiple comparisons was employed at the significance level of 0.05. Individuals with more than 50% of genotyping failure were excluded from the upcoming analyses.

Analyses of Edge Effects on Genetic Diversity

Different genetic diversity estimates were calculated at the level of *O. chelifer* colonies. Observed and expected heterozygosity (H_O and H_E, respectively) were calculated using the Microsatellite Toolkit supplement in Excel (Park 2001). The rarefied allele richness (A) and the rarified richness of private alleles (pA) were estimated using the rarefaction method, implemented in the HP-Rare program (Kalinowski 2004, 2005). In ants, the genetic makeup of colonies is primarily influenced by the level of polygyny (multiple queens) and polyandry (multiple mates by a single queen) in colonies (Hölldobler and Wilson 1990, Ross 2001). Thus, to access the number of queens and their mating frequency in *O. chelifer* colonies, we used a method based on maximum likelihood implemented in the COLONY v2.0 program (Jones and Wang 2010). This program enabled us to reconstruct the parental genotypes from the genotypes of the workers. Since the estimated number can be underestimated due to non-sampled genotypes, we calculated the effective number ($M_{e,p}$) of queens ($M_{e,p.q}$) and male mates ($M_{e,p.m}$) using the equation proposed by Nielsen et al. (2003):

$$M_{e,p} = \frac{(n-1)^2}{\sum_{i=1}^{k} Pi^2\,(n+1)\,(n-2)+3-n}$$

Where n is the total number of offspring descending from a queen, k is the total number of males that copulated with this queen, and Pi is the relative contribution of each male to the queen's offspring.

To evaluate possible edge effects on the genetic makeup of *O. chelifer* colonies, the estimates of genetic diversity and number of breeders (queens and males) were compared between nests at the edge versus interior of the Cerrado fragment using Wilcoxon rank sum test in R, with the null hypothesis being the absence of difference. Finally, to evaluate if colony location influences the distribution of genetic diversity in *O. chelifer*, we conducted a Principal Coordinates Analysis (PCoA) using the excel base program GenAlex (Peakall and Smouse 2006, 2012).

Results

Descriptive Loci Analyses

We identified 59 alleles across the 9 loci, with rarified allelic richness ranging from 1.73 to 5.55 alleles per locus. H_E ranged from 0.237 to

0.854, while H_O ranged from 0.204 to 0.832 per locus (Supp Table S1 [online only]). Three loci deviated significantly from HWE. We found no evidence of stuttering and reduced amplification of large fragments for all loci. Only one locus (Och11) showed evidence of LD, and was excluded from the subsequent analyses. Detailed loci characterization is described in Supp Table S1 (online only).

Analyses of Edge Effects on Genetic Diversity

Values of genetic diversity at the interior of the Cerrado fragment (mean ± SE) were as follows: H_O = 0.60 ± 0.01, H_E = 0.59 ± 0.01, A = 3.14 ± 0.06, and pA = 0.04 ± 0.02. At the edge of the fragment, we obtained the following values (mean ± SE): H_O = 0.64 ± 0.01, H_E = 0.61 ± 0.01, A = 3.20 ± 0.08, and pA = 0.02 ± 0.01.

Multiple queens were observed in all *O. chelifer* nests sampled. In total, 86 queens had their genotypes reconstructed by COLONY, with the estimated polygyny overall nests ranging from 3 to 7 queens per nest. The mean effective number of queens per nest (±SE) was 4.75 ± 0.34 at the interior of the fragment, and 4.03 ± 0.30 at the edge.

Queens of *O. chelifer* presented a low mating frequency, consisting of 1.54 ± 0.14 (mean ± SE) effective number of matings per queen at the interior of the fragment. At the edge of the fragment, the effective number of matings per queen was 1.8 ± 0.22 (mean ± SE).

The detailed genetic diversity estimates, and number of breeders are shown in Supp Table S3 (online only). We found a significant difference in H_O estimates between nests at the edge versus interior of the fragment (Fig. 3a). Such a significant difference was not found with the other estimates of genetic diversity, neither for the estimated and effective number of breeders (Fig. 3b–f), suggesting that most of the analyzed parameters of colony genetic diversity do not depend on nest location in Cerrado fragments. Finally, Principal Coordinates Analysis (PCoA) showed no formation of genetic groups in *O. chelifer* based on the locality of the nests, suggesting that population genetic structure of *O. chelifer* in our study area is not influenced by edge effects (Supp Fig. S1 [online only]).

Discussion

Our work using microsatellite markers revealed that all colonies of *O. chelifer* were polygynous, with more than one queen effectively contributing to the offspring. We also provided evidence that coexisting queens mate with a few males. Our findings indicate that nest location did not affect polygyny or polyandry in *O. chelifer* colonies. Although we found no influence of nest location on genetic structure, colonies located at the edge exhibited a higher observed heterozygosity.

The polygynous colony structure of *O. chelifer* observed in Cerrado corroborates previous behavioral data with this species in a forest habitat, in which differential reproduction among coexisting queens results from dominance hierarchies mediated by ritualized queen–queen contests (Medeiros et al. 1992, see also Ito et al. 1996, Oliveira et al. 2011, on other functionally polygynous *Odontomachus* species). In the current study we showed that more than one queen contributes to the offspring, suggesting that simultaneous reproduction by multiple queens (and changes in queen hierarchy through time) may avoid the occurrence of a single maternal lineage within colonies, thus maintaining the genetic diversity in *O. chelifer*. There is evidence that adoption of newly mated queens by mature colonies may occur in *Odontomachus* species (Colombel 1972, Ito et al. 1996, Gibernau et al. 2007, Oliveira et al. 2011). Polygyny is thought to increase the probability of colony survival due to a higher capacity of worker production (Steiner et al. 2010 and included references). Additionally, multiple coexisting queens may occur in response to ecological pressures such as the high cost of independent nesting and/or the low availability of appropriate nest sites in the environment (Hölldobler and Wilson 1977; see Oliveira et al. 2011 on polygynous arboreal *O. hastatus*).

Hughes et al. (2008) found a significant negative relationship between polyandry and polygyny in eusocial Hymenoptera, with a tendency in ant species to invest in only one of these two strategies. Our data corroborate this trend, since the observed number of *O. chelifer* queens in the sampled nests varied from 3 to 7, whereas the average number of copulations per queen was less than 2. Obviously, the factors underlying polygyny and polyandry in *O. chelifer*, and the consequences, await further investigation.

Our results revealed that the distribution of genetic diversity in our study site is not influenced by colony location, suggesting that gene flow is occurring between the interior and the edge of the Cerrado fragments. Although we did not observe a significant difference for most of the genetic diversity estimates, the observed heterozygosity was higher in colonies located at the edge of the fragment. Indeed, heterozygosity is regarded as particularly important in fast-changing environments such as edge sites (Sellis et al. 2011). Given that the edge of fragments is more exposed to abiotic changes (Ries et al. 2004, Laurance et al. 2011, Christianini and Oliveira 2013), this genetic variation would confer a diversity advantage during adaptation (Sellis et al. 2011). Additionally, the colonization of edge sites would enable species to occupy new niches, expanding the population and increasing the genetic variability (Cook 1961). There is evidence that *O. chelifer* colonies tend to persist less time at edges compared to moister sites in the interior of the Cerrado (Christianini and Oliveira 2013), although this may vary across years (Salles et al. 2018). Thus, an increment in heterozygosity would likely be beneficial for the survival of *O. chelifer* colonies at the edge sites.

It is known that genetic diversity tends to be higher in fragments surrounded by nonforested matrix (Schlaepfer et al. 2018), and that edge effects become stronger with the age of the fragments (Woodroffe and Ginsberg 1998). Therefore, it would be desirable to evaluate the influence of landscape cover and connectivity in shaping the genetic diversity of *O. chelifer*. Further landscape genetics analyses could be helpful to evaluate how landscape composition and surrounding matrix of our study site affect the genetic variability and microevolutionary processes (such as gene flow, drift, and selection) in *O. chelifer* (Manel et al. 2003).

Finally, edge effect on a single species is just one of the potential ecological consequences of habitat fragmentation. For instance, further studies are needed to assess in greater detail how habitat loss affects *O. chelifer* foraging ecology and its role in relevant interaction systems including nutrient-rich fruits and seeds in Cerrado (Christianini and Oliveira 2010, Magalhães et al. 2018), as well as in Atlantic rainforest (Christianini et al. 2014). Indeed, biodiversity loss associated with habitat fragmentation has been shown to severely disrupt ecological networks and ecosystem services (Saunders et al. 1991, Thompson 1997, Hagen et al. 2012), including ant-based interactions (Bieber et al. 2014).

The current study adds knowledge to the biology and natural history of *O. chelifer* by describing the species' variable mating systems (polygyny and polyandry), and by providing novel information on the genetic diversity in ant colonies in fragmented Cerrado savanna, a topic still poorly understood in tropical environments. Our study is an initial step toward a better understanding of edge effects on genetic variation of ants in Cerrado, a biodiversity hotspot under continuous threat.

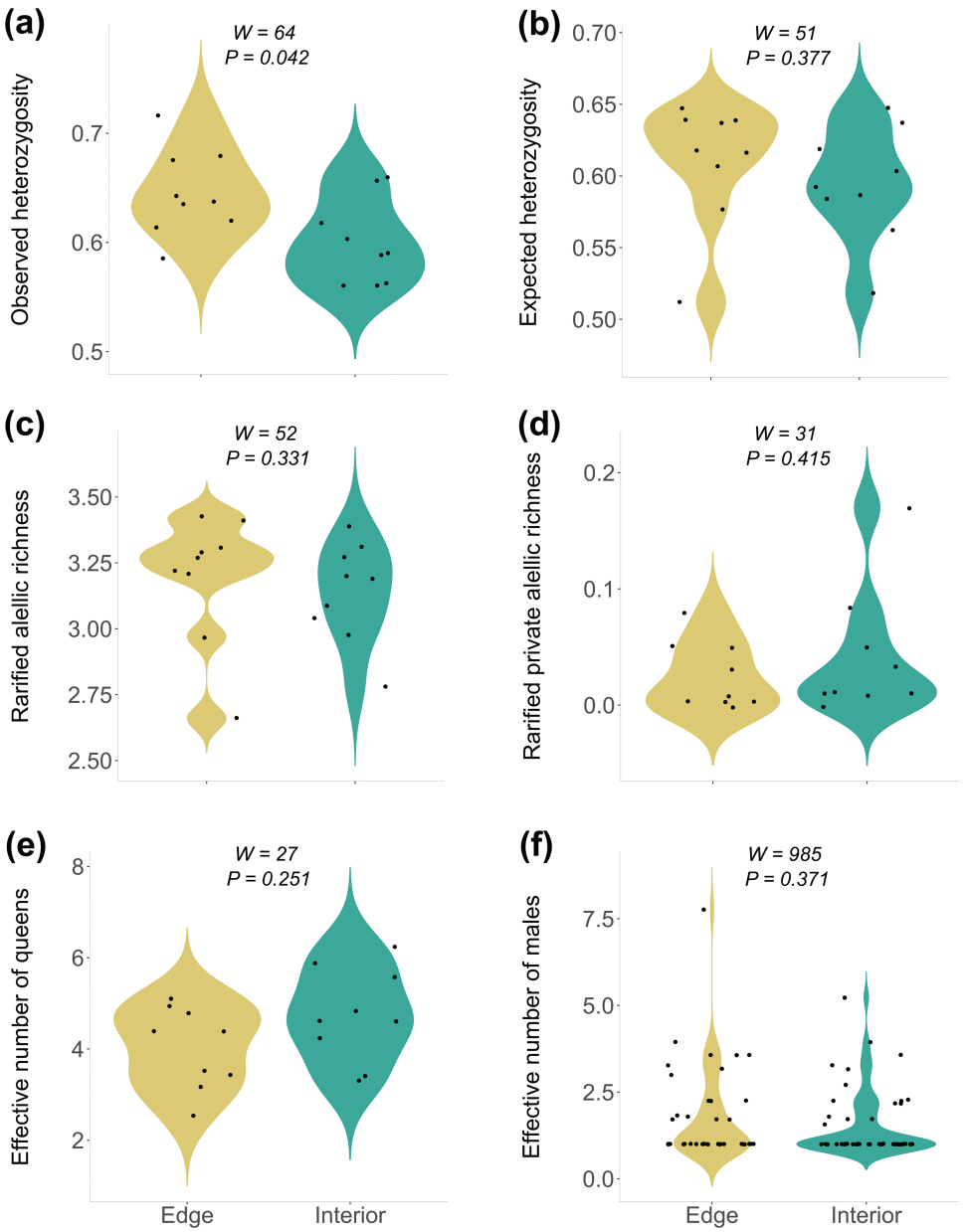

Fig. 3. Comparisons of genetic diversity estimates and number of breeders (queens and males) between nests of *Odontomachus chelifer* located at the edge and in the interior of a cerrado fragment in southeast Brazil. (a) Observed heterozygosity – H_O, (b) Expected heterozygosity – H_E, (c) rarefied allelic richness – A, (d) rarefied private allelic richness – pA, (e) effective number of queens – Me,p.q, and (f) effective number of males per queen – Me,p.m. Values refers to W statistics and corresponding *p*-values from Wilcoxon rank sum test (see Methods).

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Supplemental Data

Supplementary data are available at *Environmental Entomology* online.

Table S1. Characteristics of nine microsatellite loci genotyped for *Odontomachus chelifer*. Locus: identification of microsatellite locus according Lemos et al. 2020; TD: amplification temperature range via touchdown PCR; A: rarified allelic richness; HE: expected heterozygosity; HO: observed heterozygosity; * Significant departures from Hardy–Weinberg equilibrium; e: locus excluded from analyses due to evidence of linkage disequilibrium.

Table S2. Genotyping of workers of *Odontomachus chelifer* for nine microsatellite loci developed by Lemos et al. 2020. Workers are from nests located at the edge and in the interior of a cerrado fragment. Workers with more than 50% of genotyping failure (-) were excluded from the posterior analyses. *Locus excluded from posterior analyses due to evidence of linkage disequilibrium.

Table S3. Genetic diversity estimates, number of queens, and mating frequency per nest of *Odontomachus chelifer*. HO: Observed heterozygosity; HE: expected heterozygosity; A: rarefied allelic richness; pA: rarefied private allelic richness; Nq: number of queens estimated by COLONY v.2.0 (Jones and Wang, 2010); Me,p.q: effective number of queens following Nielsen et al. (2003); Nm: mean number of mates per queen estimated by COLONY v.2.0 (Jones and Wang, 2010); Me,p.m: mean effective mates per queen following Nielsen et al. (2003).

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