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Research Article

Evidence of high inbreeding in a population of the endangered giant anteater, *Myrmecophaga tridactyla* (Myrmecophagidae), from Emas National Park, Brazil

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Abstract

We report the genetic structure, relatedness and mating structure of a population of the endangered giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 in the Emas National Park, Brazil, based on variability at five microsatellite loci. Additionally, we addressed the hypothesis that the *M. tridactyla* population studied has low levels of polymorphism and high levels of inbreeding and relatedness and that animals with overlapping home range are highly related. All five microsatellite loci displayed low levels of polymorphism and of expected and observed heterozygosity. The low level of polymorphism and high inbreeding showed by the population studied may be the outcome of high mortality and reduction in population size due to recurrent fire events in the Emas National Park, as reported in 1994. The reduction in population size may have led to a higher frequency of mating between closely related animals, augmented by the isolation of the population in the park because of the expansion of agricultural land and fragmentation of the Cerrado environment. The natural history of *M. tridactyla* and the phylopatric (sex-biased dispersal) behavior of females should increase the effects of isolation and bottlenecking, decreasing gene flow and increasing inbreeding. However, the low levels of polymorphism found in this population may simply be due to the natural history and evolution of *M. tridactyla* as reported for other species. The genetic structure and dynamics of this population needs to be investigated more profoundly in order to provide sound data for the design of conservation strategies for *M. tridactyla* in the Emas National Park.

Key words: Cerrado, conservation, microsatellites, phylopatry, relatedness.

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Introduction

The deleterious effects of inbreeding have been recognized for many years (Darwin, 1876) and the effects of inbreeding in population genetic structure has been extensively discussed by Sewall Wright (Wright, 1917, 1921, 1931, 1937, 1946, 1951 and 1965) who developed theoretical support and statistical methods to measure the extend of inbreeding in populations. From a conservation viewpoint, the effects of inbreeding and the loss of genetic diversity in small and isolated population have been of major concern because of their deleterious effects on population viability(Charlesworth and Charlesworth, 1987; Frankham, 1995, 2003; Frankham *et al*., 2002; Reed *et al*., 2003; Reed and Frankham, 2003; Reed, 2005). Besides theoretical predictions, there is clear evidence that inbreeding and the loss of genetic diversity adversely affects wild and captive populations, reducing reproductive success and survival and increasing the risk of extinction (Ralls *et al*., 1986, 1988; Saccheri *et al*., 1998; Westemeier *et al*., 1998; Coltman *et al.*, 1998; Crnokrak and Roff, 1999; Eldridge *et al*., 1999; Dietz *et al.*, 2000).

Habitat fragmentation is one of the most important threats to long term species viability (Gilpin and Soulé, 1986; Frankham 1995, 2003; Laurance & Bierregaard, 1997). Habitat loss may lead to a decrease in population size and isolation among remnant populations. During such process, genetic variability may be lost through genetic bottlenecks, while subsequently founder effects, genetic drift and restricted gene flow may increase population genetic isolation and divergence (Terborgh and Winter, 1980; Gil-

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pin and Soulé, 1986). These genetic effects, added to the possible enhancement of inbreeding, may lead to fixation of deleterious alleles and endanger species persistence in population fragments and jeopardize their conservation, especially in populations which were originally large and widely distributed (Gilpin and Soulé, 1986; Frankham *et al*., 2002).

Fragmentation of tropical ecosystems because of harvesting for wood products or agricultural expansion has been changing the original landscape to a mosaic of remnant habitats surrounded by unfavorable environments. In Brazil, the Cerrado covers nearly 22% of Brazil (2 million km^2) and is characterized by very heterogeneous vegetation, ranging from tropical broadleaf woodlands to scrublands, containing nearly 160,000 species, including plants, animals and fungi (Ratter *et al*., 1997). The Brazilian Cerrado biome is highly threatened because of the rapid and intensive expansion of agricultural land in Central Brazil (Ratter *et al*., 1997), which results in "islands" of wild habitat surrounded by an "ocean" of crops and jeopardizes species viability.

The giant anteater *Myrmecophaga tridactyla* Linnaeus, 1758 (Xenarthra: Myrmecophagidae) is the largest species of anteater in the world and widely distributed in Central and South America but despite its wide distribution *M. tridactyla* is now extinct in many areas of its original distribution. In Brazil, *M. tridactyla* populations are restricted to national parks and other reserves, with the largest populations being reported in Emas National Park and Serra da Canastra National Park in the Cerrado biome of Central-Western Brazil (Miranda, 2004). Because of its vulnerability, *M. tridactyla* is in the World Conservation Union (IUCN) Red List as vulnerable (IUCN, 2004). Besides habitat loss, *M. tridactyla* may be vulnerable to extinction due to some characteristics of its natural history, such as slow movement, solitary habit, a long gestation with one offspring and a long gestation interval of nine months (Eisenberg and Redford, 1999; Nowak, 1999).

In this paper we report the genetic structure, relatedness and mating structure of a population of *M. tridactyla* from Emas National Park and discuss the potential consequences for conservation. Our working hypothesis was that the population has low levels of polymorphism and high levels of inbreeding and relatedness, and that individuals with an overlapping home range are highly related. Genetic data was generated using microsatellite markers, one of the most powerful molecular markers for estimating genetic parameters and describing population genetic structure and parentage (Goldstein and Schlotterer, 1999).

Material and Methods

Populations, sampling and DNA extraction

The study was conducted in Emas National Park (ENP; 18°15'50" S, 52°53'33" W) which covers131,868 ha in Central Western Brazil and is one of the largest National Park in the Cerrado biome. The population size of *M. tridactyla* was estimated based on line transect, aerial survey and mark-recapture (Miranda, 2004). Because the overall population density of *M. tridactyla* in ENP was only 0.23 animals per square km we sampled the center and the periphery of the park where the population density was higher. All the *M. tridactyla* specimens (n = 27) sighted in ENP during the investigation were included in the study, some were dispersed at the park periphery and others grouped into two subpopulations (SPI and SPII) as shown in Figure 1. We captured 16 *M. tridactyla* (SPI, Figure 1) in the center of the park and fitted them with radio transmitters. The animals were categorized according to their ages as: adult males (specimens TBC02 to TBC06, TBC12, TBC13, TBC15 and TBC17); adult females (TBC07, TBC09, TBC16 and TBC20); a sub-adult male (TBC08); and a juvenile female (TBC11).These animals were monitored for 15 months during 2002 and 2003 to study the detailed structure of the population and to estimate their home range (Miranda, 2004). Another 11 *M. tridactyla* were captured at the periphery of the park (Figure 1) and their ranges mapped using the global positing system (GPS). Seven specimens (SPII) of this set grouped at the same locality at the periphery and outside the park and were categorized according to their ages as adult males (TBC21, TBC22, TBC26 and TBC28) and adult females (TBC29, TBC30 and TBC31), while a further four adult male *M. tridactyla* (TBC01, TBC23, TBC24 and TBC25) were sparsely distributed at the peripheral area of the park. A single male (TBC01) was fitted with a radio transmitter but the signal was lost after few days and this animal was not included in the home range study but was included in the microsatellite analysis. After sexing, a vacutainer was used to collect a small blood sample from each animal for DNA extraction and genetic analysis. Total DNA was extracted with QIAamp Blood Kit (QUIAGEN, NL), following the manufacture instructions.

For sampling, the animals were capture and immobilized with darts using 9.56 mg kg^{-1} of Ketamin and 1.6 mg kg^{-1} of Xylazine, a sufficient amount (based on estimated body weight) to maintain the animal immobilized but conscious for 30-45 min. The individuals were manipulated following ASM (American Society of Mammalogists)guidelines.

Genetic analysis

Five *M. tridactyla* (Mtri) microsatellite loci (MtriUSP04, MtriUSP07, MtriUSP11, MtriUSP13) MtriUSP17) previously developed by Garcia *et al*. (2005) were used to genotype all the animals sampled. For genotyping, microsatellite amplifications were performed in a 10 µL volume containing 10.0 µM of each primer, 1 unit of Tax DNA polymerase (Finedraw, BR), 200 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3,

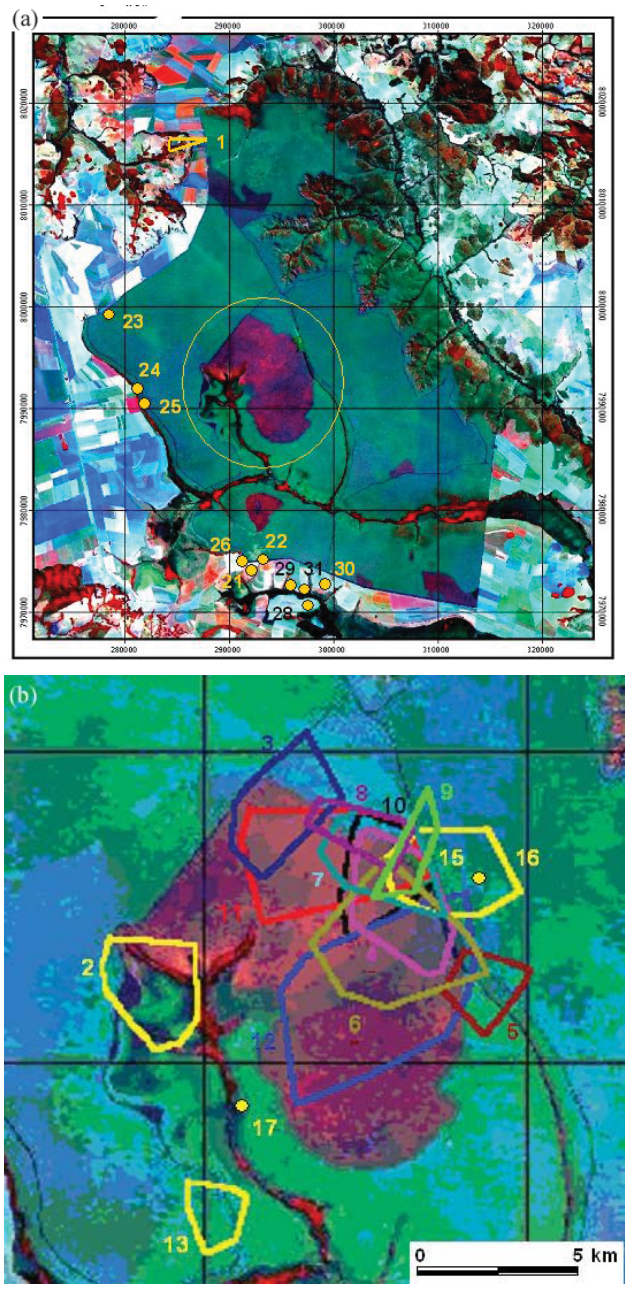

Figure 1 - LANDSAT V satellite image for 2002 of the Emas National Park, Brazil (darker area) showing the locality of the *Myrmecophaga tridactyla* specimens sampled. Figure 1X represents the center of the park circled in Figure 1a. Yellow circles represent the capture localities and polygons represent the home range. Animals in the center of the map (see detail in Figure 1X) belong to subpopulation I (SPI; specimens TBC02 to TBC13, and TBC15, TBC16, TBC17 and TBC20), at the bottom of the map (a) to subpopulation II (SPII; TBC21, TBC22, TBC26, TBC28 to TBC31) and at the left and upper part of the map to the peripheral group (TBC01, TBC23, TBC24, TBC25). The prefix TBC was omitted for clarity.

50 mM KCl, 1.5 mM $MgCl_2$), and 20 ng of template DNA. Amplifications were performed using a PE9700 thermal controller (Applied Biosystems, USA) under the following conditions: 96 ºC for 2 min; 30 cycles of: 94 ºC for 1 min, 55 to 62 ºC for 1 min (according to each locus), 72 ºC for 1 min; and 72 ºC for 10 min. The amplified products were separated on 4% (w/v) denaturing polyacrylamide gels, stained with silver nitrate (Creste *et al*., 2001) and sized by comparison to a 10 bp DNA ladder standard (Invitrogen, USA). All animals were genotyped at least four times using independent PCR amplifications and polyacrylamide gels to avoid genotyping error.

Statistical analysis

Microsatellite loci were characterized for the number of alleles per locus and expected (He) and observed (Ho) heterozygosities under Hardy-Weinberg equilibrium (Nei, 1978). Inbreeding coefficients (*f*), for each *locus* and overall loci, were also estimated (Weir and Cockerham, 1984). Analyses were performed with the FSTAT program version 2.9.3.2 (Goude, 2002) and randomization based tests with Bonferroni correction were performed generating the log-likelihood statistics G to test for deviation from Hardy-Weinberg expectations (Goudet *et al*., 1996).

To assess detailed genetic structure pairwise and mean relatedness were estimated considering the following categories: all animals sampled, all adults males and females, adult males, and adult females. These categories were also considered for relatedness analysis for subpopulations SPI and SPII. Relatedness was estimated based on the unbiased regression estimator of Lynch and Ritland (1999) using the Mark software (Ritland, 2003). Monte Carlo simulation provided estimates of relatedness variance and mean relatedness standard error when sample size was adequate. For some combinations, the Queller and Goodnight (1989) pairwise relatedness estimator was used because of small sample size. An analysis of variance (AMOVA) was performed to test for differences in relatedness between categories followed by the Tukey and Tamhane T2 (considering heterocedasticity) tests (Sokal and Rolf, 2000). The Sperman correlation was used to verify if home range overlapping was correlated to relatedness and gender (Sokal and Rolf 2000).

Genetic differentiation between subpopulations SPI and SPII was assessed by Wright's F-statistics (*F*, θ, and *f*: Wright, 1951) obtained from an analysis of variance of allele frequencies (Cockerham 1969). As most microsatellite mutations involve the addition or subtraction of a small number of repeat units according to a stepwise mutation model (Valdes *et al*. 1993; Slatkin 1995) population differentiation was also assessed by Slatkin's R_{ST} (Slatkin 1995) obtained from an analysis of variance of allele size following Goodman (1997). The analyses were performed using the FSTAT program 2.9.3.2 (Goudet 2002). A significance test of differentiation with Bonferroni correction was performed by randomizing genotypes among samples to obtain the log-likelihood G statistics (Goudet *et al*. 1996). R_{ST} is analogous to θ, and may be interpreted as the correlation between allele size of different individuals in the same population (Cockerham, 1969; Weir & Cockerham, 1984).

To detect evidence of sex-biased dispersal (philopatry) an assignment test was performed, as proposed by Goudet *et al*. (2002) and implemented in the FSTAT program 2.9.3.2 (Goudet *et al.* 2002) under the following assumptions: dispersal occurs at the juvenile stage before reproduction and individual animals were sampled post dispersal. The following parameters were estimated separately for both sexes, considering animals from subpopulations SPI and SPII and SPI compared with the other animals (SPII and peripherally captured animals): the inbreeding coefficient, *f* (Weir and Cockerham, 1984); the co-ancestry coefficient, θ (Weir and Cockerham, 1984); the assignment index, *AIc* (Paetkau *et al*, 1995; Goudet *et al*., 2002); and the variance of the assignment index, *vAIc* (Goudet *et al*., 2002). Statistical differences between the two sexes were verified using 1,000 randomizations to assign a sex randomly to each multilocus genotype.

Maternity and paternity was assessed using a paternity assignment test (Marshall *et al*., 1998), considering that neither parent was known, using CERVUS 2.0 software (Marshall 2001). The analysis was first performed including all adults as candidate parents and then for SPI and SPII separately. A 10,000 cycle simulation was performed considering 100% of candidate parents sampled and loci typed plus a genotyping error of 0.01 to generate the distribution and find the critical values of Δ (difference in the logarithm of the likelihood ratios (LOD scores) between the two most-likely parents). Paternity exclusion probability of the first and second parents were estimated based on estimated allele frequencies and assuming that the first parent was assigned correctly. Paternity exclusion probability corresponds to the power with which a locus excludes an individual from being the parent of an offspring (Chakravarti and Li 1983). The combined probability of paternity exclusion ($P = 1 - [\Pi (1 - P_i)]$) was also estimated for the combined battery of loci using the CERVUS 2 program (Marshall 2001).

Results

All five microsatellite loci displayed low levels of polymorphism (Table 1). Mean expected (He) and observed (Ho) heterozygosity for all loci was He = 0.073 to 0.672 and Ho = 0.000 to 0.185. For all loci, the observed heterozygosity was significantly lower than that expected under Hardy-Weinberg equilibrium, with the inbreeding coefficient being significantly different from zero (Table 1). Inbreeding was also high and significant for each subpopulation analyzed: 0.833 (p = 0.01) for SPI and 0.999 (p = 0.01) for SPII.

Relatedness between the animals in SPI was not significantly higher than relatedness between those in SPII or between all the *M. tridactyla* specimens sampled. Nevertheless, relatedness between females and males from SPI was significantly different from the other categories but not significantly different from each other (AMOVA, F = 2.515, p = 0.015, Table 2). Considering only the animals in SPI, but excluding TBC15, TBC17 and TBC20

Table 1 - Number of *Myrmecophaga tridactyla* specimens (N), total number of alleles (A), observed (*Ho*) and expected (*He*) heterozygosity, inbreeding coefficient (*f*) and exclusion probability of the first (Exc1) and second (Exc2) parent for the five *M. tridactyla* microsatellite *loci* pooling all specimens sampled at Emas National Park, Brazil. All *f*-values were significantly different from zero at p < 0.01.

Locus	N	A	*He*	*Ho*	*f*	Exc1	Exc2
MtriUSP 04	27	4	0.516	0.185	0.646	0.005	0.045
MtriUSP 07	20	4	0.672	0.000	1.000	0.277	0.455
MtriUSP 11	27	4	0.659	0.111	0.834	0.210	0.380
MtriUSP 13	20	2	0.492	0.000	1.000	0.146	0.273
MtriUSP 17	27	2	0.073	0.000	1.000	0.106	0.237
For all loci			0.482	0.059	0.879	0.566	0.821

Table 2 - Mean relatedness, by category, between *Myrmecophaga tridactyla* specimens sampled at Emas National Park, Brazil.

Category[#] (number of specimens)	Mean relatedness (r)	Standard error of r[&]	Variance of r	Range of r
All specimens (27)	0.095[a]	0.002	0.039	-0.560-0.500
All adults (25)	0.092[a]	0.002	0.040	-0.508-0.500
Adult males (18)	-0.028[a]	0.027	0.052	-0.494-0.500
Adult females (07)[*]	-0.219[a]	-	0.408	-1.429-0.500
SPI (16)	-0.009[a]	0.005	0.016	-0.407-0.500
SPII (07)[*]	-0.103[a]	-	0.190	-0.550-0.500
SPI adult males (12)	0.115[b]	0.002	0.038	-0.313-0.500
SPI adult females (04)[*]	0.111[b]	-	0.092	-0.269-0.500
SPII adult females (03)[*]	-0.167[a]	-	0.056	-0.500-0.000

[#]SPI = subpopulation I; SPII = subpopulation II (see Materials and Methods for details).
[&]The standard error was obtained from a Monte Carlo simulation.
[*]Estimates of the number of specimens obtained using Queller and Goodnigh (1989) because of small sample size.
[a,b]Values in the same column followed by the same letter are not statistically different by the Tukey and Tamhane T2 tests at p > 0.05.

whose home range could not be determined, pairwise relatedness was not correlated with overlapping home range ($p = 0.693$). Although sex was positively correlated with overlapping home range $r = 0.414$, $p = 0.004$) and males had a significantly higher home range overlap with females (AMOVA, $F = 6.389$, $p = 0.001$), males and females with an overlapping home range were not more related than the other animals ($p = 0.110$). We calculated that the mean home range of *M. tridactyla* in ENP was 9.83 ± 6.29 km^2, with no difference between males and females ($t = 0.917$, $p > 0.05$). Home range overlapping ranged from 0 to 90% (detailed data published in Miranda 2004).

Both the R_{ST} and θ estimates showed a high and significant level of genetic differentiation between SPI and SPII ($R_{ST} = 0.1517$, $\theta = 0.168$, $p = 0.01$). Additionally, *f* and *F* were also high and significant ($f = 0.88$, $p = 0.002$; $F = 0.900$, $p = 0.002$).

When SPI was compared to SPII or with all animals (*i.e.* SPII plus the peripheral animals, Table 3) the assignment test results indicated that in *M. tridactyla* the female is the more phylopatric sex. Significant differences between male and females were obtained for *f* ($p = 0.025$ for SPI/SPII and $p = 0.036$ for SPI/all animals) and the assignment index ($p = 0.036$ for SPI/SPII and $p = 0.041$ for SPI/all animals), with negative assignment index values for males for both comparisons (Table 3). Although males presented higher assignment index variances they were not significantly different from the values for the females for both comparisons ($p = 0.53$ for SPI/SPII and $p = 0.149$ for SPI/all animals).

The combined probability of exclusion of the first (0.566) and second (0.821) parent were relatively small (Table 1), as a consequence of the low polymorphism displayed by the population studied. The critical LOD scores and critical Δ value for strict and relaxed levels (confidence > 95% and >80%, respectively) were obtained after simulation with all the animals sampled. For the strict level the critical $\Delta = 2.16$, while for the relaxed level it was 1.20. The expected success rate was 3% for strict level, 16% for relaxed level and 84% when unresolved. Considering all the animals sampled, we assigned maternity to four animals with more than 95% confidence and to two animals with at the relaxed level (Table 4). For SPI only one animal could be assign maternity at the 95% confidence level and one at the 80% level (Table 4). For subpopulation SPII maternity and paternity could be assign for four animals. Nevertheless, these cases should be considered unresolved because the same animal was indicated as both an offspring and a father as a consequence of the high relatedness between animals (Table 4).

No difference in genotyping was found when animals were genotyped in at least four independent PCR amplifications and polyacrylamide gels.

Discussion

Our results indicated that the population studied presented low level of polymorphism, with a small number of alleles per locus and that all loci departed from Hardy-Weinberg equilibrium. Additionally, for three loci (MtriUSP11, MtriUSP13 and MtriUSP17) the number of alleles per locus found in our sample was lower than the number found by Garcia *et al*. (2005). This may have been due to differences in the sampling distribution between the two studies, because while our study focused on the genetic structure of one population in Emas National Park (ENP) Garcia *et al*. studied the polymorphism of loci and sampled only seven *M. tridactyla* in ENP and eight from other localities, including animals killed on roads far away from ENP.

Loci with small numbers of alleles or with a skewed frequency distribution (*i.e.* a few high frequency alleles) tend to show low heterozygosity (Nei 1978). Additionally, sample size may be an important factor in the accurate esti-

Table 3 - Sex-biased dispersal analysis, by category, for *Myrmecophaga tridactyla* sampled at Emas National Park, Brazil.

					Heterozygosity	
Category[#] (number of specimens)	Inbreeding coefficient (*f*)	Co-ancestry coefficient (θ)	Assignment index (*Aic*)	*Aic variance (vAIc)*	Observed (*Ho*)	Expected (*He*)
SPI – SPII						
Females (08)	0.530*	0.279	1.126*	3.731	0.075	0.159
Males (15)	0.916*	0.211	-0.600*	11.700	0.040	0.479
Overall (23)	0.880	0.168	–	–	0.050	0.436
SPI – all specimens						
Females (08)	0.530*	0.279	1.313*	2.954	0.075	0.159
Males (19)	0.893*	0.149	-0.553*	11.055	0.053	0.490
Overall (27)	0.868	0.143	–	–	0.059	0.449

[#]SPI = subpopulation I; SPII = subpopulation II (see Materials and Methods for details).
*Female and male values are statistically different at $p < 0.05$.

Table 4 - Maternity and paternity parentage assignment analysis, by category, for *Myrmecophaga tridactyla* sampled at Emas National Park, Brazil. All, includes all categories -SPI, SPII and Periphery.

Category and offspring	Potential mother	Confidence interval	Pairwise relatedness (r)	Potential father	Confidence interval	Pairwise relatedness (r)
All						
TBC01				TBC02	80*	0.448
TBC02				TBC01	95*	0.448
TBC03	TBC07	95*	0.036			
TBC05	TBC07	80	0.437			
TBC17	TBC07	80*	0.198	TBC05	80*	0.331
TBC21	TBC30	95	0.500			
TBC22	TBC31	80	0.500			
TBC24	TBC30	95	0.500			
TBC25	TBC30	95	0.500			
TBC26	TBC30	95	0.500			
TBC 28	TBC31	80*	0.500	TBC22	80	0.500
SPI						
TBC02				TBC06	80*	0.216
TBC03		95*	0.014			
TBC05	TBC07	95	0.451	TBC04	80*	-0.014
TBC06					80*	0.216
TBC10	TBC07	80	0.200	TBC02		
TBC17		80*	0.198		80*	0.331
SPII						
TBC 21	TBC 30	95	0.500	TBC 26	95	0.500
TBC 22	TBC 31	80	0.500	TBC 28	80	0.500
TBC 26	TBC 30	95	0.500	TBC 21	95	0.500
TBC 28	TBC 31	80	0.500	TBC 22	80	0.500

*Cases where maternity or paternity were assign considering one mismatch.

mation of heterozygosity and *f*-values based on microsatellite loci because of limitations in sampling all possible genotypes at a specific locus (Hedrick 1999). In our study, a limited number (n = 27) of *M. tridactyla* were available to be sampled, however since the number of *M. tridactyla* in the whole park is relatively small at about 300 animals, or 0.23 per km^2, (Miranda, 2004) we sampled almost 10% of the whole *M. tridactyla* population. Based on this, we believe that the low polymorphism and high inbreeding (f = 0.833 for SPI, f = 0.999 for SPII and f = 0.879 for the overall sample) with a departure from Hardy-Weinberg equilibrium at all loci showed by the population studied are not an artifact of sample size. Over the last few decades ENP has suffered recurrent fires and 97% of its area was burned in 1994, including the central area from which the SPI subpopulation was collected. Silveira *et al* (1999) estimated that at least 332 giant anteaters died because of the direct action of the 1994 fire and a population of 43 (0.034 per km^2) remained after the fire. Therefore, our data may be the outcome of a genetic bottleneck suffered by the population in 1994. Thus although a partial recovery of the population may be inferred (Miranda 2004), the current population may display low genetic diversity and high inbreeding because of the genetic bottleneck after the fire. Nevertheless, the low levels of polymorphism may be the outcome of the natural history and evolution of *M. tridactyla*, as reported for other species (*e.g.* Reeve *et al.* 1990, Baumgarten 2001, Caparroz *et al*. 2001).

While high values of pairwise relatedness (r) were presented by many *M. tridactyla* pairs, *e.g.* half r = 0.25) and full sibs r = 0.50) or first r = 0.125) and second cousins r = 0.0625), the mean relatedness among all the animals sampled was low but significantly different from zero r = 0.095 ± 0.002), probably because of many negative values. Queller and Goodnight (1989) have pointed out that negative r-values may occur if the allele frequencies of the two individuals compared differ from the population mean in opposite directions. Although relatedness between all the SPI animals did not differ significantly from the relatedness between all the animals sampled, adult males and females in the central area of the park (SPI) were significantly more related than the other animals sampled. As discussed

above, this result showed that mating between closed related *M. tridactyla* may be very frequent and is playing an important role in the current genetic structure of the total ENP population. Added to the possible genetic bottleneck that occurred about ten years ago, the isolation of the population may favor mating between closed related *M. tridactyla*. Although the ENP is one of the largest cerrado reserves and covers almost 140,000 ha it is surrounded by soybean and maize farms and cattle pasture. The fact that many *M. tridactyla* are run over on the roads near the park and are sometimes illegally hunted outside the park may have resulted in less migration and gene flow between the ENP and the small and rare cerrado fragments in the region, thus increasing mating between closely-related animals.

Although the small sample size may have affected the estimated F-statistic (Bailees & Lugon-Moulin, 2002; Koskinen *et al.*, 2004) our results indicated that alleles were not randomly distributed between and within the SPI and SPII subpopulations, since θ and *F* were significantly different from zero (Cockerham, 1969). Because microsatellites are highly variable and subject to high mutation rates ($4N_e u > 1$), they usually display high levels of within-population heterozygosity (Hedrick 1999). However, when alleles are identical by state statistics based on infinite allele models such as θ or F_{ST} tend to underestimate population differentiation (Slatkin 1995; Hedrick 1999). Nevertheless, θ and R_{ST} presented very similar values, indicating that identity-by-descent and by state have not diverged in the subpopulations studied. Additionally, the high and significant inbreeding coefficient showed that mating between close relatives may play an important role in determining the differentiation between SPI and SPII, and ultimately in the genetic structure of the *M. tridactyla* population at Emas National Park.

Our results showed slight evidence that *M. tridactyla* females are more phylopatric than males, with significant differences in *f* and *AIc* between sexes. Members of the dispersal sex may display a higher *f*-value due to the Wahlund effect caused by the admixture of animals from different populations and the genotype of the dispersal sex may be less likely to occur in a sample, hence the dispersal sex tends to have lower *AIc* values than the more phylopatric sex (Goudet *et al*. 2002). We found that SPI and SPII or SPI and all other females presented higher *Aic*-values and lower *f*-values when compared with males. In mammals female phylopatry is more common, although some exceptions have been described (Greenwood 1980; Johnson and Gaines 1990). Female phylopatry may be expected in species where mating system is based on mate defense by males, with females tending to return to, or remain in, the same area while males roam to find other potential mates. The giant anteater is essentially solitary but females may take care of the offspring during the early stages (six to nine months postpartum) carrying the young on her back (Eisenberg and Redford 1999), behavior which may favor female phylopatry.

Parentage assignment can be highly affected by relatedness, and paternity can not be resolved between the true father and his sons when males related as half sibs or more to the offspring r = 0.25) are among the candidate fathers and the mother is unknown (Marshall *et al.*, 1998). Our data clearly showed this effect in that in the SPII subpopulation parentage assignment was unresolved (Table 4) with specimens TBC21 and TBC26 plus TBC22 and TBC28 r = 0.500 for each pairs) being indicated as both offspring and father of each other, probably because of the high relatedness between some animals in this subpopulation (such as the previously cited pairs) which suggests that they are parent-offspring or half-sibs. Nonetheless, maternity could be assigned with 95% confidence, and of the three SPII adult females two (TBC30 and TBC31) were the mothers of many offspring in the SPII subpopulation and the peripheral area of the park. Maternity at SPI could be assigned only to resident females. Nevertheless, only two cases were resolved without invoking mismatches, *i.e.* TBC07 and TBC05 (95% confidence) and TBC09 and TBC10 (80% confidence). The high homozygosity displayed by the population studied indicated a high frequency of null alleles. Nevertheless, a significant overall homozygote excess occurred, indicating that the population might not be panmictic, *i.e.* randomly mated (Wright 1931), and that the mismatch should be interpreted as parentage exclusion and not as null alleles.

In conclusion, our results showed that the population of *M. tridactyla* in Emas National Park has a low level of genetic diversity and a high level of inbreeding. Additionally, a high level of relatedness could be detected between many pairs of animals, and close related and unrelated *M. tridactyla* may have an overlapping home range. The current population genetic structure may be the outcome of the evolution and natural history of the species. The phylopatric behavior of females and their low fecundity, increased by isolation and the recurrent fire events, could have led to high population mortality and may have increased the frequency of mating between closely related animals. Although the low number of young *M. tridactyla* observed in the population studied (one juvenile female and one sub-adult male) may be explained by the low number of adult females (n = 7), this may also indicate inbreeding depression. The genetic structure and dynamics of this population needs to be more profoundly investigated in the future to provide sound information with which to design conservation strategies for *M. tridactyla* in the Emas National Park.

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