

Intrapopulation Chromosomal Polymorphism in *Mazama gouazoubira* (Cetartiodactyla; Cervidae): The Emergence of a New Species?

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Keywords

B chromosome · Centric fusion · G-banding · Gray brocket deer

Abstract

Mazama gouazoubira is a small deer species widely distributed in South America. Previous studies have shown that this species presents intraspecific chromosomal polymorphisms, which could affect fertility due to the effects of chromosomal rearrangements on gamete formation. Important aspects regarding the karyotype evolution of this species and the genus remain undefined due to the lack of information concerning the causes of this chromosomal variation. Nineteen individuals belonging to the *Mazama gouazoubira* population located in the Pantanal were cytogenetically evaluated. Among the individuals analyzed, 9 had B chromosomes and 5 carried a heterozygous centric fusion ($2n = 69$ and $FN = 70$). In 3 individuals, the fusion occurred between chromosomes X and 16, in 1 individual between chromosomes 7 and 21, and in another individual between chromosomes 4 and 16. These striking polymorphisms could be explained by several hypotheses. One is that the chromosome rearrangements in this species are recent and not fixed in the population yet, and another hypothesis is that they represent a balanced polymorphism and that heterozygotes have an adaptive ad-

vantage. On the other hand, these polymorphisms may negatively influence fertility and raise questions about sustainability or reproductive isolation of the population.

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Mazama gouazoubira, the gray or brown brocket deer, is a small deer species found from southern Uruguay to the north of the Mato Grosso state in Brazil and from the Andes to the Atlantic. Its habitat varies from closed Cerrado to agricultural areas. This species can readily adapt to modified areas due to its high ecological plasticity [Duarte, 2007; Rodrigues et al., 2014].

M. gouazoubira ($2n = 70$; $FN = 70$) has 68 acrocentric autosomes, an acrocentric X, and a metacentric Y [Neitzel, 1987; Fontana and Rubini, 1990], similar to the putative Cervidae ancestral karyotype [Dementyeva et al., 2010]. The karyotypes of other species in the *Mazama* genus differ from the proposed ancestral deer karyotype by a high number of chromosomal rearrangements, including tandem fusions, centric fusions, pericentric inversions, and the presence of B chromosomes [Fontana and Rubini, 1990].

Considerable intraspecific variation in the chromosome number exists in *M. gouazoubira* due to Robertsonian translocations and B chromosomes [Duarte, 1992,

1998; Duarte and Jorge, 1996]. Some researchers hypothesize that this chromosome variability is due to chromosomal fragility, which promotes chromosomal breaks and rearrangements [Duarte, 1998; Vargas-Munar et al., 2010]. Robertsonian rearrangements (centromeric fissions and fusions) are the most commonly incorporated rearrangements in mammalian karyotype evolution. It is known that this type of rearrangement can lower fitness due to the production of unbalanced gametes [Baker and Bickham, 1986]. Therefore, it would be important to better understand Robertsonian rearrangements due to their frequent occurrence in evolution and their influence on fertility [Daniel, 1988; Ferlin et al., 2006]. Further, the mechanism about how Robertsonian rearrangements can become fixed in a population is not well understood. *M. gouazoubira* populations are an ideal model for clarifying some of these issues. We know that the free-ranging *Mazama* population located in the Pantanal biome differs in the chromosome number due to Robertsonian rearrangements. In this research, we identified the chromosomes involved in these rearrangements and tested the hypothesis that fusions are the responsible mechanism. We also provide data regarding their potential influence on fertility, fitness, and reproductive isolation of this population.

Materials and Methods

A major advantage of this research is that all animals studied were wild-caught and the provenience is precisely known. Nineteen (12 males and 7 females) free-ranging *M. gouazoubira* were captured by drive net or darts with transmitters [Duarte et al., 2010] within a 15 km radius in the Nhecolândia Pantanal in the municipality of Corumbá, Mato Grosso do Sul, Brazil (19° 00' 33' S, 57° 39' 12' W). Skin biopsies were collected, frozen in liquid nitrogen [Duarte et al., 1999], and later used to obtain chromosomal preparations using fibroblast cultures [Verma and Babu, 1995] which were maintained until passage 4 [Magalhães et al., 2017]. Twenty metaphases of each specimen were analyzed to determine the diploid and fundamental number. G-banding was performed according to Seabright [1971] to correctly identify the chromosomes involved in the rearrangements. The chromosomes were classified as metacentric, submetacentric, or acrocentric by arm ratio [Levan et al., 1964]. B chromosomes were calculated separately of the diploid and fundamental number due to intraindividual variation.

Results

Among the 19 deer analyzed, 4 different karyomorphs were identified. The most frequent is considered the standard karyotype for the species and presented $2n = 70$,

FN = 70, with 68 acrocentric autosomes, an acrocentric X, and a metacentric Y. The other 3 karyomorphs resulted from Robertsonian rearrangements and presented $2n = 69$ and FN = 70 (Fig. 1). If the Robertsonian rearrangements in this population are fusions, the reduction in the diploid number without altering the fundamental number is the result of a centric fusion between various acrocentric chromosomes forming a metacentric or submetacentric chromosome. These 3 karyomorphs differ from each other because of the different chromosomes involved in the fusion.

Of the 19 deer, 5 were carriers of a heterozygous Robertsonian translocation; the chromosomes involved in the rearrangements were identified based on their G-banding pattern. Among these 5 animals, 3 shared the same karyotype in which the bi-armed chromosome, classified as submetacentric, was the result of Robertsonian translocation between the X chromosome and autosome 16 (Fig. 1A). The other 2 carriers of the Robertsonian translocation showed bi-armed chromosomes, classified as metacentric. In 1 individual the translocation occurred between chromosomes 4 and 16 (Fig. 1B) and in the other between chromosomes 7 and 21 (Fig. 1C).

Another source of chromosomal polymorphism identified in the population was the inter- and intraindividual variation in dot-like B chromosomes, with 0–2 B chromosomes present in addition to the basic complement in 9 individuals of the 19 deer analyzed (Table 1). There was no statistical difference (ANOVA test) in the presence of B chromosomes between the individuals with a standard karyotype and the carriers of the Robertsonian translocations.

Discussion

To our knowledge, this is the first cytogenetic study on a population of *M. gouazoubira* with all analyzed animals caught in the wild in a restricted area. The observed intraspecific chromosomal polymorphisms, such as the presence of B chromosomes and Robertsonian translocations, have also been reported for other species of the genus *Mazama*: *M. americana* [Abril et al., 2010], *M. nana* [Abril and Duarte, 2008], *M. bororo* [Duarte and Jorge, 2003], *M. nemorivaga* [Duarte, 1998], and even for *M. gouazoubira* [Duarte, 1992], though not for such a restricted geographic region. In this work, fibroblast cultures were maintained until passage 4, and according to Magalhães et al. [2017], *M. gouazoubira* fibroblast cell lines remain viable and functional until passage 7. Thus,

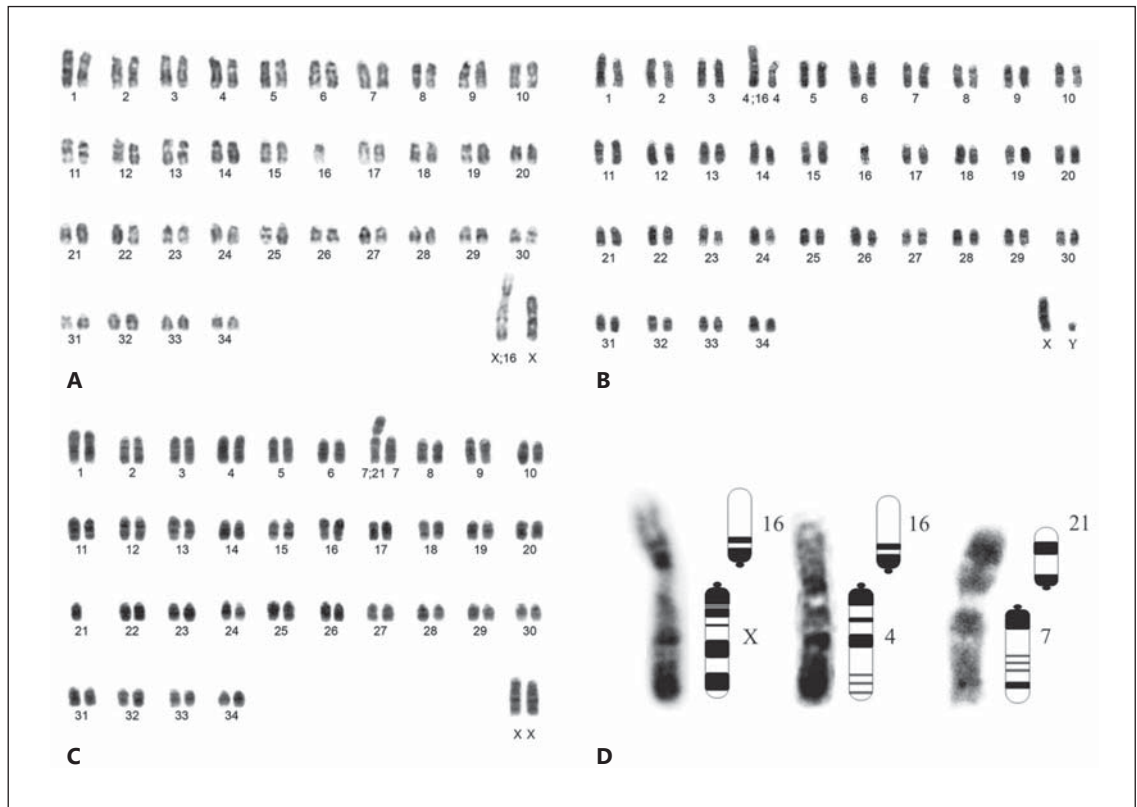


Fig. 1. G-banded karyotypes of *M. gouazoubira*. **A** Representative karyotype of the translocation between the X chromosome and autosome 16. **B** Representative karyotype of the translocation between autosomes 4 and 16. **C** Representative karyotype of the translocation between autosomes 7 and 21. **D** G-banded chromosomes and diagrammatic representation of the respective translocations.

the observed chromosome polymorphism was not related to the number of passages used.

Further, for the first time in *M. gouazoubira*, we determined which Robertsonian translocations were present within the population using G-banding. Since the chromosomes in this species are all acrocentric and of similar size, except for the Y chromosome (a small metacentric chromosome) [Neitzel, 1987], additional FISH experiments should confirm our results.

All the studies that report Robertsonian translocations in *M. gouazoubira* conclude centric fusion and not fission as the mechanism responsible for the chromosomal polymorphism, and we tested this hypothesis. The first point is that *M. gouazoubira* retained the putative ancestral deer karyotype with 70 chromosomes that is standard to the species [Neitzel, 1987; Fontana and Rubini, 1990; Dementyeva et al., 2010]. Five of 19 specimens had $2n = 69$ and maintained $FN = 70$. A mechanism that explains a reduction in the diploid number without changes in the

fundamental number is centric fusion. Also, chromosomal evolution in the Cervidae family is based on centric and tandem fusions, leading to the reduction of the diploid number [Fontana and Rubini, 1990], as observed in the species *Muntiacus* [Yang et al., 1997], *M. americana* [Abril et al., 2010], and *M. nana* [Abril and Duarte, 2008].

In the genus *Mazama*, chromosomal fragility may facilitate chromosomal breaks and rearrangements and be closely related to intraspecific polymorphisms, chromosomal evolution, and formation of species in the genus following geographic isolation [Vargas-Munar et al., 2010; Tomazella et al., 2017]. The most frequently observed chromosome aberrations in *M. gouazoubira* by Vargas-Munar et al. [2010] and Tomazella et al. [2017] were chromatid breaks and gaps. According to Vargas-Munar et al. [2010], *M. gouazoubira* presents the highest rates of chromosomal aberrations induced by doxorubicin among some deer species (*M. nana*, *M. americana*, and *Blastocercus dichotomus*). Tomazella et al. [2017]

Table 1. Sex, diploid number (2n), rearranged chromosomes, and number of B chromosomes in the specimens analyzed

Specimen	Sex	2n	Robertsonian rearrangement	B chromosomes
T128	m	70	–	–
T155	m	70	–	0–1
T156	f	69	X;16	–
T157	m	70	–	0–1
T298	m	70	–	–
T299	f	70	–	–
T300	m	69	X;16	0–2
T301	f	69	7;21	–
T302	m	69	4;16	0–2
T303	f	70	–	0–1
T307	m	69	X;16	0–1
T313	m	70	–	–
T314	f	70	–	0–1
T315	f	70	–	–
T316	m	70	–	–
T317	f	70	–	–
T318	m	70	–	0–2
T319	m	70	–	0–2
T320	m	70	–	–

identified the chromosomes carrying chromosomal aberrations induced by doxorubicin in *M. gouazoubira*, which could be more prone to chromosomal fragility. The chromosomes with highest frequencies of aberrations were the pairs 1, 2, 4, 5, 6, 7, 15, 16, and X. The chromosomes X, 4, 7 and 16 identified in the polymorphism of the Pantanal population have among the highest rates of chromosomal aberrations; their frequencies were 12.41, 9.65, 15.17, and 9.19%, respectively. The exception was chromosomal pair 21, which was not reported by Tomazella et al. [2017] as having chromosomal aberrations, but this result may be due to difficulty in identifying this small chromosome.

Neitzel [1987], Fontana and Rubini [1990], and Demytyeva et al. [2010] considered the putative ancestral Cervidae X chromosome as acrocentric. However, Fiorillo et al. [2013] suggest the bi-armed morphology of the X as the putative ancestral form. Among *Mazama* species, the X chromosome presents differences in morphology, being metacentric in *M. nana*, submetacentric in *M. bororo*, *M. americana*, and *M. nemorivaga*, and is present as an acrocentric chromosome only in *M. gouazoubira*. These differences are consequences of chromosomal rearrangements such as X-autosomal fusion in *M. americana* and *M. nemorivaga* and a suggested pericentric in-

version in *M. gouazoubira*. The X differences found in brocket deer could be directly related to the chromosomal fragility observed by Vargas-Munar et al. [2010] and Tomazella et al. [2017]. The tendency of rearrangements observed in *Mazama* involving the X chromosome agrees with X-autosomal rearrangements found in this work.

In general, rearrangements involving sex-autosome translocations are rare in mammals. However, additionally to the *Mazama* genus, there are other reports of sex-autosome translocations among Cervidae family, including muntjacs (*Muntiacus muntjak* and *M. crinifrons*) and the tufted deer (*Elaphodus cephalophus*) [Wurster and Benirschke, 1970; Shi et al., 1991; Yang et al., 1995; Cao et al., 2005]. This type of chromosomal rearrangement is also described in bats, rodents, bovids, and primates [Rattonponirina et al., 1986; Dobigny et al., 2004; Solari and Rahn, 2005; Noronha et al., 2010; Veyrunes et al., 2014; Vozdova et al., 2016].

Nine of 19 specimens analyzed had B chromosomes; individuals could have no B chromosomes or up to 2 B chromosomes. One hypothesis of the origin of B chromosomes is that they result from Robertsonian fusions [Camacho et al., 2000]. If this hypothesis is true, we would expect that B chromosomes in *M. gouazoubira* should be more frequent in carriers of the Robertsonian translocations. However, we found no statistical difference in the frequency of B chromosomes between animals with Robertsonian fusions and normal karyotypes. Therefore, our data do not support the hypothesis that Robertsonian fusions generate B chromosomes. B chromosomes in *M. gouazoubira* have an important genetic content, since, although they present sequences patterns similar to the autosomal chromosomes, they are composed of 34 complete and 21 partial genes, including the *RET* and *KIT* protooncogenes [Makunin et al., 2016].

The centric fusions observed in *M. gouazoubira* are most likely the result of chromosomal fragility and are usually present in a heterozygous state [Duarte, 1998]. The presence of a heterozygous centric fusion in chromosomes X and 16, 7 and 21, and 4 and 16 identified in the Pantanal population of *M. gouazoubira* may be indicative of a recent karyotype evolution and in which the rearrangements are not fixed yet.

In the case of an individual heterozygous for a centric fusion it is thought that this should result in reduced fertility due to segregation and irregular chromosome pairing during meiosis by producing unbalanced gametes [Nachman and Searle, 2012]. However, heterozygotes for a single centric fusion generally form trivalents in meiosis, causing minimal problems in reproduction [Baker

and Bickham, 1986], as observed in a meiotic study performed on *M. americana* [Aquino et al., 2013]. However, Cursino et al. [2014] and Salviano et al. [2017] verified that hybrids of different *M. americana* cytotypes possess a mechanism of post-zygotic reproductive isolation that involves subfertility or infertility, demonstrating reproductive isolation with the accumulation of chromosomal rearrangements.

When accumulated fixation of several centric fusions occurs, reproductive isolation is increasingly likely due to gamete incompatibility [Dobigny et al., 2002]. In isolated populations, centric fusions for different chromosomes are independently fixed, and fertility problems originating in meiosis are minimal. In the case of 2 isolated populations of the same species coming into contact, it is possible that heterozygous individuals for 2 different fusions are produced, and in this case, serious meiotic problems can result in reproductive isolation [Baker and Bickham, 1986]. The population studied here already has at least 3 different types of rearrangements, and they could be present together in some individuals, which theoretically should have a significant negative impact on reproduction.

The reproductive impact is observed when fixation of an X-autosomal rearrangement occurs that can generate a multiple sex chromosome system, XX/XY₁Y₂, leading to reproductive isolation and perhaps future speciation. Within the genus *Mazama*, the species *M. americana* and *M. nemorivaga* present a multiple XX/XY₁Y₂ sex chromosome system resulting from X-autosomal fusions [Abril et al., 2010; Fiorillo et al., 2013].

The different rearrangements verified in the population of *M. gouazoubira* analyzed could be related to the chromosomal fragility previously observed in the genus *Mazama* [Duarte and Merino, 1997], with the occurrence of frequent mutations that arise and are eliminated within the population due to their effects on fitness and affecting the sustainability of the population. We found centric fusions only in a heterozygous state, probably because of our limited sample, but this could be an indication of a recent occurrence or adaptive advantage of heterozygotes. Banaszek et al. [2009] found higher maximum metabolic rates in heterozygotes for Robertsonian fusions in a common shrew (*Sorex araneus*) population and suggested it as a selective advantage that could outweigh the expected negative effects of Robertsonian heterozygosity upon fertility, thereby maintaining the polymorphism in this species.

Another hypothesis for the presence of the 3 types of translocations in the area under study is that these rear-

rangements exist in other populations and were inserted in the Pantanal population by natural genetic flow. In this case, they might well be eliminated later due to negative effects on fitness.

One interpretation of our results is that the chromosomal data provide evidence of an ongoing process of speciation in *Mazama*. Therefore, a more robust sampling of the population would offer an opportunity to test some of these hypotheses and provide information on the dynamics of chromosome polymorphisms in populations and their role in speciation.

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Statement of Ethics

This study was approved by the institutional ethics committee (CEUA – São Paulo State University [Unesp], Permission Number: 000577/13).

Disclosure Statement

The authors have no conflicts of interest to declare.

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