

## Different responses to doxorubicin-induced chromosome aberrations in Brazilian deer species

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Genet. Mol. Res. 9 (3): 1545-1549 (2010)  
Received March 17, 2010  
Accepted June 26, 2010  
Published August 10, 2010  
DOI 10.4238/vol9-3gmr822

**ABSTRACT.** The tendency toward chromosome fragility is one of the theories that may explain chromosome variation in brocket deer species (genus *Mazama*). We tested doxorubicin as an inducer of chromosome aberrations in lymphocytes of three brocket deer species, *Mazama gouazoubira*, *M. americana* and *M. nana*, compared to the marsh deer, *Blastocerus dichotomus*. Doxorubicin, at a concentration of 0.25 µg/mL, induced chromosome aberrations and fragile sites in all four species; the highest frequencies were seen in *M. gouazoubira*; they were lowest in *B. dichotomus* and intermediate in *M. americana* and *M. nana*. These results were expected based on previous karyotypic studies, but they failed to explain the higher sensitivity seen in *M. gouazoubira*. This may be because not all the aberrations and fragile sites are related to chromosome evolution in brocket deer; other factors, such as environmental influences, may be involved in chromosome fragility.

**Key words:** *Blastocerus*; Cervidae; Chromosome evolution; Chromosome fragility; *Mazama*

## INTRODUCTION

Cytogenetics and molecular phylogenetics have contributed greatly to the knowledge of the evolutionary complexity of deer family Cervidae (Neitzel, 1987; Fontana and Rubini, 1990), particularly those of the genus *Mazama* (Mammalia; Cervidae), a group of small to medium-sized forest deer with spike-like antlers from Central and South America, characterized by their phenotypic homogeneity in the presence of a polyphyletic origin (Gilbert et al., 2006; Duarte et al., 2008). Some cytogenetic studies have demonstrated very complex processes of chromosome evolution, being more intense in the reddish brocket group (*Mazama americana*, *M. nana* and *M. bororo*) than in grayish brockets (*M. gouazoubira* and *M. nemorivaga*). Duarte and Merino (1997) found a frequency of 20% single Robertsonian translocations in 77 *M. gouazoubira* analyzed, while other studies demonstrated that *M. americana* and *M. nana* had an unusual karyotypic diversity, varying from  $2n = 42$  to 53 and from  $2n = 34$  to 40, respectively, in both cases as a consequence of extensive and complex chromosome rearrangements (Duarte and Jorge, 1996; Abril and Duarte, 2008). In contrast, the marsh deer *Blastocerus dichotomus* showed karyotypic stability, with no chromosome rearrangements in 35 animals analyzed (Duarte and Giannoni, 1995).

The tendency toward chromosome fragility is one of the theories that may explain the chromosome variation in brocket species (Duarte and Jorge, 1996). Chromosome fragility has been associated with karyotypic evolution in some mammalian species such as the rock wallabies of the genus *Petrogale* (Eldridge and Johnston, 1993) and primates of the family Cebidae (Mudry et al., 1995). It also has been associated with cancer (Miró et al., 1987; Yunis et al., 1987). In order to detect fragile sites, several agents have been tested, such as ionizing radiation, chemical reagents and microorganisms, including viruses (Eldridge and Johnston, 1993). However, very few studies have been carried out for this purpose in deer species. Gripenberg et al. (1991) tested 5-bromo-2-deoxyuridine (BrdU) as an inducer of fragile sites in four deer species, and found that three of them were refractory to the agent and only one, the reindeer *Rangifer tarandus*, consistently expressed a fragile site on the X-chromosome.

Given 1) our interest in testing the expression of fragile sites in some Brazilian deer species to determine their possible relationship with chromosome evolution and 2) the necessity to test other inducers of fragile sites since BrdU was ineffective for this purpose, we tested doxorubicin (DXR), also known as Adriamycin, an anthracycline antibiotic. DXR is a potent chemotherapeutic agent used in the treatment of breast and esophageal carcinoma among other types of cancer (Gülkaç et al., 2004). It is known that DXR induces mutations and chromosomal aberrations in normal and tumor cells (Au and Hsu, 1980; Antunes and Takahashi, 1998), and it has been proven that DXR produces chromosome aberrations in cell cultures (Vig, 1971). The goals of this study were 1) to test the ability of DXR to induce chromosome aberrations in deer and 2) to determine its potential use in future studies of chromosome fragility and evolution in brockets and other deer species.

## MATERIAL AND METHODS

We collected samples of peripheral blood from two individuals each of *M. americana*, *M. nana*, *M. gouazoubira*, and *B. dichotomus*. Lymphocytes were cultured in 8 mL Ham's F10 medium enriched with 20% horse serum, supplemented with 0.4 mL *Phaseolus vulgaris* extract and incubated at 38°C for 72 h. For the induction of chromosome aberrations, the cultures were

supplemented with seven different concentrations of DXR (Adriblastin® Pharmacia) varying from 0.10 to 0.40 µg/mL in increments of 0.05 µg/mL. We opted to use the concentration of 0.25 µg/mL because it was able to induce aberrations in all four Brazilian deer species tested; lower concentrations produced no induction and higher concentrations were highly toxic to cells, impairing culture growth. DXR was added 48 h before the beginning of the incubation. Additional cultures without DXR were grown for each individual, as control cultures.

Harvesting was carried out according to standard protocols (Moorhead et al., 1960), and slides of both DXR-treated and DXR-free cells were stained with 10% Giemsa. A total of 100 metaphases per culture were analyzed, and the chromosome aberrations seen were counted and classified according to Savage (1976). In order to evaluate differences in frequency of chromosomal aberrations and total chromosomal aberrations in DXR-treated metaphases, one-way ANOVA was conducted, and the mean values for these features were analyzed using the Tukey test at  $P < 0.05$ .

## RESULTS

Compared to human cells, deer are apparently less sensitive to DXR, since the highest concentration tested in humans was 0.15 µg/mL, inducing two to three times more chromosome aberrations (Vig, 1971), probably indicating more genomic stability in deer than humans. When comparing the different deer species, the highest aberration frequencies were seen in *M. gouazoubira* and the lowest in *B. dichotomus*. Aberration frequencies in *M. americana* and *M. nana* were lower than in *M. gouazoubira* but higher than in *B. dichotomus*. Differences in the induction of chromosome aberrations were not statistically significant between *M. americana* and *M. nana*. The aberrations most frequently seen were chromatid breaks and gaps, while other aberrations such as isochromatid breaks, triradials and chromosomal exchanges were rarely seen. All types of aberrations were observed only in *M. gouazoubira*. The results are shown in Table 1.

**Table 1.** Frequency means and types of chromosomal aberrations observed in Brazilian deer lymphocyte cultures: doxorubicin (DXR)-treated group versus controls.

Species	2n	Treatment	Frequency of aberrations*				Total aberrations**	MA**	
			Breaks		Gaps	Triradial			Exchange
			C	IC					
<i>M. gouazoubira</i>	70	Control	3	0	3	0	0	6	6
		DXR	58.5	1.5	46	2	3	108.5 <sup>a</sup>	62.5 <sup>a</sup>
<i>M. americana</i>	52	Control	2	0	3	0	0	5	5
		DXR	26	0	43.5	1	0.5	72 <sup>b</sup>	49.5 <sup>b</sup>
<i>M. nana</i>	36	Control	2	0	1	0	0	3	3
		DXR	31	0	32	1	0	64 <sup>b</sup>	47 <sup>b</sup>
<i>B. dichotomus</i>	66	Control	1	0	1	0	0	2	2
		DXR	23	0	24.5	0	0	49 <sup>c</sup>	32.5 <sup>c</sup>

C = chromatid break; IC = isochromatid (chromosomal) break; MA = mean of the total number of metaphases with chromosomal aberrations. \*A total of 200 metaphases/species analyzed. \*\*Distinct letters indicate statistical difference by the Tukey test ( $P < 0.05$ ).

## DISCUSSION

Among the four species tested, *M. gouazoubira* clearly showed the highest sensitivity to DXR both by frequency and aberration diversity. It could be explained by the hypothesis

that chromosome fragility may be positively correlated to higher  $2n$ , since *M. gouazoubira* has the highest  $2n$  of the four species tested:  $2n = 70$ . However, this notion was not supported because of the fact that *B. dichotomus*, with  $2n = 66$ , showed the lowest sensitivity. Furthermore, there were no statistically significant differences between *M. americana* ( $2n = 52/53$ ) and *M. nana* ( $2n = 36$ ). Another explanation could be a phylogenetic correlation of the genus *Mazama*; however, this hypothesis is also not viable since there is strong molecular evidence demonstrating that *M. americana* and *M. nana* belong to a different clade and that *M. gouazoubira* is phylogenetically related to *B. dichotomus* (Gilbert et al., 2006; Duarte et al., 2008). This hypothesis only partially agrees with results seen in *M. americana* and *M. nana*, but apparently these results are not sufficiently consistent.

Despite the reasons mentioned above, the hypothesis that chromosome fragility is involved in chromosome evolution in brockets cannot be completely rejected. The information presented in the present study partially agrees with the results from cytogenetic studies (Duarte and Giannoni, 1995; Duarte and Jorge, 1996; Duarte and Merino, 1997; Abril and Duarte, 2008), since higher levels of chromosome aberrations were found in *M. gouazoubira*, *M. americana* and *M. nana* and lower levels in *B. dichotomus*; however, they failed to explain why the highest levels were seen in *M. gouazoubira* and not in *M. americana* and *M. nana*, two species with higher chromosome evolution rates. These findings may indicate that not all the aberrations expressed in these species are effectively related to chromosome evolution in brockets, and that factors other than phylogeny such as environmental factors may be involved in chromosome fragility.

The tendency toward fragility is one of the theories that explains the existence of karyotypic change in *M. americana*. Data from different studies suggest that these variants could differentiate species that are geographically isolated, which would result in reproductive isolation. The period to cause this would be inversely proportional to the probability for the occurrence of breaks, inversions, and translocations (Duarte and Jorge, 1996; Duarte and Merino, 1997; Duarte, 1998a). Therefore, the stability of the population depends on chromosomal stability, and when this is deficient, the population could undergo periods of imminent risk of extinction (Duarte and Merino, 1997). In the case of *M. nana*, this event is currently underway due to environmental fragmentation and geographic isolation, which have caused this species to produce large numbers of heterozygotic animals through chromosome rearrangements (Duarte and Merino, 1997). Non-meiotic pairing can cause carriers of rearrangements to be subfertile or even infertile, depending on the number of these rearrangements, which could lead to the extinction of the species (Duarte, 1998b).

Finally, it is necessary to develop further research in order to confirm the exact location of the break points, their molecular composition and their relationship with effective rearrangements documented in these species with high chromosome evolution rates.

## ACKNOWLEDGMENTS

We are grateful to Prof. Dr. Catarina Takahashi of the Faculdade de Medicina de Ribeirão Preto/USP, Brazil, for her assistance and collaboration. We also thank the Brazilian Institute of Forestry Development (IBAMA) for the support given to the Brazilian Cervids Project, to CAPES for the fellowship, and to the Graduate Program in Genetics and Animal Breeding at FCAV/UNESP for the financial support.

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