Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Transferability of microsatellite loci from Cervidae species to the endangered Brazilian marsh deer, *Blastocerus dichotomus*

K.C.E. Leite[1], R.G. Collevatti[1], T.R. Menegasso[2], W.M. Tomas[3] and J.M.B. Duarte[2]

[1]Pós-Graduação em Ciências Genômicas e Biotecnologia, Universidade Católica de Brasília, Brasília, DF, Brasil
[2]Núcleo de Pesquisa e Conservação de Cervídeos, Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brasil
[3]EMBRAPA, Centro de Pesquisa Agropecuária do Pantanal, Corumbá, Mato Grosso do Sul, MS, Brasil
Corresponding author: R.G. Collevatti
E-mail: rosanegc@pos.ucb.br

Genet. Mol. Res. 6 (2): 325-330 (2007)
Received December 22, 2006
Accepted January 22, 2007
Published May 22, 2007

ABSTRACT. *Blastocerus dichotomus*, the marsh deer, is the largest Brazilian Cervidae species. The species is endangered because of hunting and loss of its natural habitat, i.e., flood plain areas, because of hydroelectric power station construction and agricultural land expansion. In the present study, we tested 38 microsatellite loci from four Cervidae species: *Odocoileus virginianus* (7), *Rangifer tarandus* (17), *Capreolus capreolus* (7), and *Mazama bororo* (7). Eleven loci showed clear amplification, opening a new perspective for the generation of fundamental population genetic data for devising conservation strategies for *B. dichotomus*.

Key words: Microsatellites, Cervidae, Transferability, Marsh deer, *Blastocerus dichotomus*

INTRODUCTION

The marsh deer, *Blastocerus dichotomus* (Artiodactyla, Cervidae), is the largest deer of South America, reaching a size of up to 150 kg and 1.20 m high. The species occurs in flood plains from Southwest Peru, Paraguay, Bolivia, Northeast Argentina, and Northwest Uruguay, to the edges of the Amazon forest in Brazil (Mauro et al., 1998). Agricultural expansion and the construction of several hydroelectric power stations have been changing the original landscape of flood plains, reducing and isolating favorable habitats, which jeopardize the species viability (Tomas et al., 1997; Travassos, 2001). Because of that, the species is listed in the World Conservation Union (IUCN) Red List (IUCN, 2004).

Transferability of microsatellite loci among closely related species is a consequence of the homology of the flanking regions of the simple-sequence repeats. Besides the possibility of comparative map construction among related species (Slate et al., 1998), transferability may reduce the cost of genotyping, opening new perspectives for the development of population genetic studies. The high rate of transferability has already been reported for plant species (e.g., Dayanan et al., 1997; White and Powell, 1997; Brondani et al., 1998; Collevatti et al., 1999) and among animal species, such as human and chimpanzee (Deka et al., 1994) and dog and fox (Fredholm and Wintero, 1995).

The rate of transferability across Artiodactyla species is surprisingly high, even between different families, such as Cervidae and Bovidae, indicating a high genome homology (Engel et al., 1996; Talbot et al., 1996; Kühn et al., 1996; Roed and Midthjell, 1998; Roed, 1998; Slate et al., 1998; Broders et al., 1999; Cronin et al., 2003).

We are interested in understanding the population genetic structure and gene flow among remnant populations of the marsh deer, *Blastocerus dichotomus*, to obtain useful information for the development of conservation strategies. In this study, we present the results of the transferability of microsatellite loci from four species of the Cervidae family to *B. dichotomus*: the caribou, *Rangifer tarandus* (Roed and Midthjell, 1998); the white-tailed deer, *Odocoileus virginianus* (DeWoody et al., 1995); the roe deer, *Capreolus capreolus* (Fickel and Reinsch, 2000), and the small red brocket deer, *Mazama bororo* (Duarte JMB, unpublished results).

MATERIAL AND METHODS

For the transferability analysis, a small sample of blood was collected in a vacutainer, from 15 individuals of *B. dichotomus* captured at a remnant area of a flood plain near the hydroelectric plant of Porto Primavera on the Paraná River (São Paulo, Brazil). DNA was extracted with the QIAamp Blood Kit (Quiagen, Hilden, The Netherlands), following the manufacturer's instructions.

For sampling, the animals were captured with darts and immobilized with the aid of ketamine chloride (9.56 mg/kg) and xylazine chloride (1.6 mg/kg) anesthesia, at doses prescribed to maintain the animal immobilized but conscious for 30-45 min. The individuals were handled following ASM guidelines.

Thirty-eight microsatellite loci were tested (Table 1): 7 from *O. virginianus*; 17 from *R. tarandus*; 7 from *C. capreolus*, and 7 from *M. bororo*. For transferability analysis, each locus was first amplified using four individuals of *B. dichotomus*, randomly chosen from the 15 individuals sampled. PCR amplifications were performed in 13-µL reaction mix containing 0.9 µM

of each primer, 1 unit Taq DNA polymerase (Phoneutria, Belo Horizonte, MG, Brazil), 200 µM of each dNTP, 1X reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 25 µg BSA, and 10.0 ng of template DNA. Amplifications were performed using a PE 9700 thermal controller (Applied Biosystems, MD) with the following conditions: 96°C for 2 min, 94°C for 1 min, 58° to 46°C for 1 min (according to each primer), 72°C for 1 min (30 cycles), and 72°C for 10 min. The amplified products were separated on 4% denaturing polyacrylamide gels stained with silver nitrate (Bassam et al., 1991) and sized by comparison to a 10-bp DNA ladder standard (Invitrogen, MD).

Table 1. Characteristics of the 38 microsatellite loci from four species of Cervidae, tested in 15 individuals of *Blastocerus dichotomus*.

Primer	Motif	Species	Temperature (°C)	Observed size (bp)	Expected size (bp)	Number of alleles
CERVID 1[1]	$(CA)_{17}TA(CA)_5$	*Odocoileus virginianus*	-	-	187	10
CERVID 2[1]	$(AC)_{12}AA(AC)_7$	*Odocoileus virginianus*	-	-	155	7
CERVID 3[1]	$(CA)_{15}N_9(CA)_4$ $(GA)_{12}(CA)_5$	*Odocoileus virginianus*	-	-	190	8
CERVID 4[1]	$(CA)_{17}(N)_9(AC)_2$ $GTT(AC)_4$	*Odocoileus virginianus*	-	-	356	5
CERVID 12[1]	$(CA)_{80}$	*Odocoileus virginianus*	-	-	208	ni
CERVID 13[1]	$(CA)_{100}$	*Odocoileus virginianus*	-	-	198	2
CERVID 14[1]	$(AC)_{16}$	*Odocoileus virginianus*	-	-	215	8
NVHRT 1[2]	$(GT)_7GC(GT)_{12}$	*Rangifer tarandus*	52	220-236	164-200	(4)
NVHRT 3[2]	$(CT)_7TA(CA)_{12}$	*Rangifer tarandus*	58	116-142	112-126	(3)
NVHRT 12[2]	$(CAGA)_3(CA)_2$ $TA(CA)_{15}$	*Rangifer tarandus*	-	-	171-179	2
NVHRT 16[2]	$(CA)_5TA(CA)_5$ $(TG)_2CG(CA)_{19}$	*Rangifer tarandus*	-	-	152-192	9
NVHRT 21[2]	$(GT)_{16}(GC)_4GT$	*Rangifer tarandus*	58	156-186	159-167	(3)
NVHRT 22[2]	$(CA)_{21}$	*Rangifer tarandus*	-	-	142-168	9
NVHRT 24[2]	$(TG)_2(TA)_2(CA)_{16}$ $(TA)_3(CA)_3(TA)_5$	*Rangifer tarandus*	52	136-146	147-155	(2)
NVHRT 30[2]	$(GT)_2AT(GT)_{21}$	*Rangifer tarandus*	-	-	159-177	10
NVHRT 31[2]	$(AC)_{12}$	*Rangifer arandus*	-	-	131-139	5
NVHRT 34[2]	$(AC)_{15}$	*Rangifer tarandus*	-	-	126-134	4
NVHRT 46[2]	$(CA)_{11}$	*Rangifer tarandus*	-	-	114-118	1
NVHRT 48[2]	$(GT)_2ATGTAT$ $(GT)_6AT(GT)_{12}$	*Rangifer tarandus*	48	100-120	105-115	(2)
NVHRT 63[2]	$(GT)_{15}$	*Rangifer tarandus*	-	-	136-146	6
NVHRT 66[2]	$CACG(CA)_{14}$	*Rangifer tarandus*	-	-	163-175	6
NVHRT 71[2]	$(GT)_5(GCGT)_2$ $(GTGC)_2AT(GT)_{18}$	*Rangifer tarandus*	-	-	109-123	4
NVHRT 73[2]	$(CT)_4GT(CT)_3GC$ $CTGT(CT)_{12}CCTT$ $(CT)_3TT(CT)_{13}CA$ $CT(CA)_8TA(CA)_3$	*Rangifer tarandus*	-	-	219-231	2
NVHRT 76[2]	$(AC)_{12}TA(GT)_2$	*Rangifer tarandus*	-	-	91-110	6
ROE 1[3]	$(CA)_4TA(CA)_7$	*Capreolus capreolus*	56	130-130	112-132	(1)
ROE 3[3]	$(CG)_2(CA)_4N_6(CA)_4$ $(CG)_3(CA)_{11}$	*Capreolus capreolus*	-	-	116-138	6

Continued on next page

Table 1. Continued.

Primer	Motif	Species	Temperature (°C)	Observed size (bp)	Expected size (bp)	Number of alleles
ROE 5[3]	$(GT)_2(AT)_2CT(GT)_{20}N_5(AGG)_2(G)_5$	*Capreolus capreolus*	-	-	160-172	6
ROE 6[3]	$(CA)_5(CATG)_2(CA)_2GA(CA)_{13}$	*Capreolus capreolus*	-	-	89-109	7
ROE 8[3]	$(CA)_{10}CG(CA)_6$	*Capreolus capreolus*	-	-	69-89	7
ROE 9[3]	$(CA)_2CGTA(CA)_4TA(CA)_8$	*Capreolus capreolus*	56	180-180	175-179	(1)
ROE 10[3]	$(AC)_8CAGACA(A)_7T(A)_3(AC)_8$	*Capreolus capreolus*	56	176-182	190-192	(2)
BCP 1[4]	-	*Mazama bororo*	-	-	281	
BCP 2[4]	-	*Mazama bororo*	52	160-164	138	(2)
BCP 3[4]	-	*Mazama bororo*	52	162	176	(1)
BCP 4[4]	-	*Mazama bororo*	-	-	241	
BCP 5[4]	-	*Mazama bororro*	-	-	181	
BCP 6[4]	-	*Mazama bororro*	-	-	200	
BCP 7[4]	-	*Mazama bororro*	57	140	151	(1)

[1]DeWoody et al. (1995), [2]Roed and Midthjell (1998), [3]Fickel and Reinsch (2000), [4]Duarte JMB, unpublished results. Temperature (°C), annealing temperature optimized for *B. dichotomus* (indicated only for those primers that transferred to *B. dichotomus*); Number of alleles, expected number of alleles and number observed in the present study (in parentheses); ni, information not indicated in the reference.

For those loci that showed clear and reproducible amplification, 15 individuals were genotyped for locus characterization. PCR amplification and polymorphism detection followed the same protocol and conditions described above. The number of alleles per locus, observed and expected heterozygosities under Hardy-Weinberg (Nei, 1978), and inbreeding coefficient (*f*) were estimated (Weir and Cockerham, 1984). Analyses were performed with FSTAT 2.9.3.2 (Goudet, 2002) and randomization based tests with Bonferroni correction were performed generating the log-likelihood statistic G to test for deviation from Hardy-Weinberg expectations and linkage disequilibrium (Goudet et al., 1996). Additionally, probability of genetic identity (*I*) (Chakravarati and Li, 1983), which corresponds to the probability of two random individuals displaying the same genotype, and paternity exclusion probability (*Q*) (Weir, 1996), which corresponds to the power with which a locus excludes an individual of being the parent of an offspring, were estimated. The combined probability of paternity exclusion, $QC = 1 - [\Pi (1 - Q_i)]$ and the combined probability of genetic identity $IC = \Pi I_i$ were also estimated for the battery of loci.

RESULTS AND DISCUSSION

From the 38 primers tested in this study, 11 showed a robust amplification, with observed fragment sizes inside the expected range (Table 1). For loci NVHRT1, NVHRT3, NVHRT48, and BCP7, observed heterozygosity was lower than expected under Hardy-Weinberg equilibrium leading to a high and significant inbreeding coefficient (Table 2). The significant inbreeding and the low number of alleles detected in this study may be the outcome of sampling design, since all individuals were sampled in the same population and may be highly related. In the present study, we aimed to transfer a battery of heterologous loci to *B. dichotomus.* Further

Table 2. Characterization of the 11 microsatellite loci transferred to *Blastocerus dichotomus*, based on a sample of 15 individuals.

Locus	*A*	H_e	H_o	*f*	*Q*	*I*
NVHRT 1	4	0.549020	0.235294	0.578947	0.3270	0.2598
NVHRT 3	3	0.451282	0.250000	0.452450	0.2543	0.3500
NVHRT 21	3	0.579323	0.411765	0.295597^{ns}	0.3236	0.2580
NVHRT 24	2	0.513369	0.470588	0.085714^{ns}	0.2181	0.3758
NVHRT 48	2	0.398293	0.526316	-0.333333	0.1750	0.4501
ROE 1	1	-	-	-	0.0000	1.0000
ROE 9	1	-	-	-	0.0000	1.0000
ROE 10	2	0.512195	1.000000	-1.000000^{ns}	0.2188	0.3750
BCP 2	2	0.443812	0.000000	1.000000	0.1928	0.4157
BCP 3	1	-	-	-	0.0000	1.0000
BCP 7	1	-	-	-	0.0000	1.0000
Mean over all loci		0.313390	0.263088	0.163643	0.960309	6.19×10^{-4}

A, number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity; *f*, inbreeding coefficient (*f* significant for all loci, $P < 0.05$, except for values followed by *ns*); *Q*, probability of paternity exclusion; *QC*, combined probability of paternity exclusion (0.960309); *I*, probability of genetic identity; *IC*, combined probability of genetic identity (6.19×10^{-4}). All pairs of loci are in linkage equilibrium ($P > 0.05$).

analysis will be carried out with several populations of *B. dichotomus* to study the genetic structure using the primers selected here. In Brazil, there are eight species of deer: *Mazama americana*, *M. nana*, *M. gouazoubira*, *M. nemorivaga*, *M. bororo*, *Odocoileus virginianus cariacus*, *Blastocerus dichotomus*, and *Ozotoceros bezoarticus* (Duarte, 1996; Rossi, 2000). This first report of the transferability of microsatellite loci to Brazilian Cervidae species opens a new perspective for the generation of fundamental population genetic data for devising conservation strategies for these species and for the construction of a comparative genetic map.

ACKNOWLEDGMENTS

Research supported by CESP (Energetic Corporation of São Paulo, Brazil), whose assistance we gratefully acknowledge.

REFERENCES

Bassam BJ, Caetano-Anolles G and Gresshoff PM (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 196: 80-83.

Broders HG, Mahoney SP, Montevecchi WA and Davidson WS (1999). Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. *Mol. Ecol.* 8: 1309-1315.

Brondani RPV, Brondani C, Tarchini R and Grattapaglia D (1998). Development, characterization and mapping of microsatellite markers in *Eucalyptus grandis* and *E. uruphylla*. *Theor. Appl. Genet.* 97: 816-827.

Chakravarati A and Li CC (1983). The effect of linkage on paternity calculations. In: Inclusion probabilities in parentage testing (Walkera RH, ed.). American Association of Blood Banks, Arlington, 411-422.

Collevatti RG, Brondani RV and Grattapaglia D (1999). Development and characterization of microsatellite markers for genetic analysis of a Brazilian endangered tree species *Caryocar brasiliense*. *Heredity* 83: 748-756.

Cronin MA, Patton JC, Balmysheva N and MacNeil MD (2003). Genetic variation in caribou and reindeer (*Rangifer tarandus*). *Anim. Genet.* 34: 33-41.

Dayanandan S, Bawa KS and Kesseli R (1997). Conservation of microsatellites among tropical trees (Leguminosae). *Am. J. Bot.* 84: 1658-1663.
Deka R, Shriver MD, Yu LM, Jin L, et al. (1994). Conservation of human chromosome 13 polymorphic microsatellite (CA)n repeats in chimpanzees. *Genomics* 22: 226-230.
DeWoody JA, Honeycutt RL and Skow LC (1995). Microsatellite markers in white-tailed deer. *J. Hered.* 86: 317-319.
Duarte JMB (1996). Guia de identificação de cervídeos brasileiros. Funep, Jaboticabal.
Engel SR, Linn RA, Taylor JF and Davis SK (1996). Conservation of microsatellite loci across species of Artiodactyls: implications for population studies. *J. Mammal.* 77: 504-518.
Fickel J and Reinsch A (2000). Microsatellite markers for the European roe deer (*Capreolus capreolus*). *Mol. Ecol.* 9: 994-995.
Fredholm M and Wintero AK (1995). Variation of short tandem repeats within and between species belonging to the Canidae family. *Mamm. Genome* 6: 11-18.
Goudet J (2002). FSTAT: a program to estimate and test gene diversities and fixation indices.Version 2.9.3.2. Available at http://www.unil.ch/izea/softwares/fstat.html.
Goudet J, Raymond M, de Meeus T and Rousset F (1996). Testing differentiation in diploid populations. *Genetics* 144: 1933-1940.
IUCN (International Union for Conservation of Nature and Natural Resources) (2004). The IUCN mammal red data book. IUCN, Gland.
Kühn R, Anastassiadis C and Pirchner F (1996). Transfer of bovine microsatellites to the cervine (*Cervus elaphus*). *Anim. Genet.* 27: 199-201.
Mauro RA, Mourão GN, Coutinho ME, Silva MP, et al. (1998). Abundance and distribution of marsh deer *Blastocerus dichotomus* (Artiodactyla: Cervidae) in the Pantanal, Brazil. *Rev. Ecol. Latinoam.* 5: 13-20.
Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individual. *Genetics* 89: 583-590.
Roed KH (1998). Microsatellite variation in Scandinavian Cervidae using primers derived from Bovidae. *Hereditas* 129: 19-25.
Roed KH and Midthjell L (1998). Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. *Mol. Ecol.* 7: 1773-1776.
Rossi RV (2000). Taxonomia de Mazama Rafinesque, do Brasil (Artiodactyla, Cervidae). MSc thesis, Instituto de Biociências, USP, São Paulo.
Slate J, Coltman DW, Goodman SJ, MacLean I, et al. (1998). Bovine microsatellite loci are highly conserved in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*) and Soay sheep (*Ovis aries*). *Anim. Genet.* 29: 307-315.
Talbot J, Haigh J and Plante Y (1996). A parentage evaluation test in North American elk (Wapiti) using microsatellites of bovine and bovine origin. *Anim. Genet.* 27: 117-119.
Tomas WM, Beccaceci MD and Pinder L (1997). Cervo-do-Pantanal (*Blastocerus dichotomus*). In: Biologia e conservação de cervídeos sul-americanos: Blastocerus, Ozotocerus e Mazama (Duarte JMB, ed.). FUNEP, Jaboticabal, 24-40.
Travassos LEP (2001). Impactos gerados pela UHE Porto Primavera sobre o meio físico e biótico de Campinal, Presidente Epitácio, SP. Revista de Biologia e Ciências da Terra 1, Campina Grande.
Weir BS (1996). Genetic data analysis II. Sinauer Associates, Sunderland.
Weir BS and Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358-1370.
White G and Powell W (1997). Cross-species amplification of SSR loci in the Meliaceae family. *Mol. Ecol.* 6: 1195-1197.