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High levels of polymorphism found through cross-amplification of microsatellite loci in a *Ctenomys pearsoni* (Rodentia, Ctenomyidae) population

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ABSTRACT. *Ctenomys pearsoni* (Pearson's tuco-tuco) is a subterranean rodent native to Uruguay. We tested the amplification pattern of 12 microsatellite loci, designed for *C. sociabilis* and *C. haigi* in a *C. pearsoni* population. DNA extractions were made from hair samples, and PCR amplification products were run on an ABI 3100 microcapillary gel. Eight loci were selected to form a highly polymorphic panel that could be used to efficiently screen populations of this species. In

DNA from 35 tuco-tucos, the mean polymorphic information content value was 0.6536 and the mean expected heterozygosity was 0.7166. Paternity non-exclusion probabilities for seven independent loci were NE-1P = 0.0766 and NE-2P = 0.0108, and combined non-exclusion P(ID) was 6.2 x 10^{-7}. This panel of microsatellite loci has sufficient power to make inferences regarding group structure, mating strategies and evolutionary relationships among populations.

Key words: Subterranean rodents; Rodentia: Ctenomyidae; Caviomorpha; Nuclear short tandem repeats

INTRODUCTION

The Ctenomyidae (tuco-tucos) are subterranean rodents that have undergone explosive karyotypic radiation. This group has a large number of species with chromosome numbers ranging from 2n = 10 to 2n = 70 (Reig et al., 1990; Lessa and Cook, 1998; Lacey et al., 2000). *Ctenomys pearsoni* is 1 of 3 species of ctenomyids found in Uruguay (Lessa and Langguth, 1983). It mainly inhabits coastal sandy areas or dunes, although individuals have been found living as far as 500 m from sandy soil (Altuna et al., 1999). Typically, these animals prefer living along the banks or near the mouths of rivers (Altuna, 1983; Lessa and Langguth, 1983; Altuna et al., 1999). Moreover, this species is characterized by having the highest levels of karyotypic variation at intra- and inter-population levels (Reig et al., 1990; Novello et al., 1990, 1996; Villar et al., 2005) that is incongruent with the low levels of morphological and molecular variation observed among populations (D'Anatro and D'Elía, 2011).

Despite the peculiar genetic characteristics of this species, no population genetic studies using hypervariable nuclear loci have been conducted to date. Microsatellites are widely used in studies of population genetic structure because they have high levels of intraspecific variability and high-resolution power in complex evolutionary scenarios (Lacey et al., 1999; Kays et al., 2000; Cosse et al., 2007).

Microsatellite loci previously isolated and characterized for *Ctenomys sociabilis* and *C. haigi* (Lacey et al., 1999; Lacey, 2001) have been used to determine genetic population structure, phylogeography, relatedness and dispersal patterns in several species of this genus (Lacey, 2001; Parada, 2003; Wlasiuk et al., 2003; El Jundi and De Freitas, 2004; Cutrera et al., 2005; Fernandes et al., 2007; Mora et al., 2007). Several studies have taken advantage of the relatively highly conserved microsatellite flanking regions in related species (Wlasiuk et al., 2003; El Jundi and De Freitas, 2004; Cutrera et al., 2005; Cosse et al., 2007). The aim of this study was to test 12 microsatellite loci isolated from *C. sociabilis* and *C. haigi* to characterize a set of highly polymorphic microsatellite loci in *C. pearsoni*.

MATERIAL AND METHODS

Samples

Samples were collected in a *C. pearsoni* population located in Estancia El Relincho, Departamento de San José, in southwestern Uruguay (34° 20' S, 56° 58' W). A clump of ap-

proximately 20 hair samples was taken from each of the 35 animals captured. Captures were carried out using Oneida Victor N° 0 traps located inside the tunnels after opening one of the burrow entrances. Traps were softened with rubber and polyurethane foam to avoid harming the animals and were checked every 10 min to avoid injuries when captured animals tried to escape the trap.

DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from hair samples following a procedure described by González et al. (1998). PCR was conducted in 10-µL reactions containing 10 ng DNA, 0.2 µM of each primer (forward and reverse), 0.2 mM deoxyribonucleotide triphosphate, 1X Taq buffer with KCl, 2.5 mM $MgCl_2$, 0.5 µg/µL bovine serum albumin, and 0.75 U Taq polymerase (Fermentas®, Thermo Scientific Inc., Vilnius, Lithuania). The profile used consisted of 94°C for 5 min, followed by 34 cycles of 94°C for 30 s, annealing temperatures for 30 s, and 72°C for 45 s, with a final extension of 72°C for 5 min. Locus-specific annealing temperatures ranged from 56° to 62°C (Lacey et al., 1999; Lacey, 2001) and fluorochrome labels were FAM, HEX, or TET. To confirm amplification, a volume (4 µL) of each product was electrophoresed on a 2% SbS - Besta™ Agarose gel (SBS Genetech Co. Ltd., Beijing, China) for 180 min. Amplified products were run with a LIZ 500 size standard on an ABI3130® automated sequencer. Migration was performed in a 36-cm capillary array using POP7 polymer (Applied Biosystems, USA) with the following parameters: 50 mA, 15 kV, and 60°C for 1200 s.

Genotyping and microsatellite locus characterization

Genotyping and fragment size analysis were conducted using the GeneMarker® software (Softgenetics® Inc.). We calculated observed and expected values of heterozygosity and tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP® (Raymond and Rousset, 1995). Polymorphic information content (PIC), paternity non-exclusion probability (NE-1P, NE-2P), and combined non-exclusion probability for identity were estimated with Cervus 3.0. (Marshall et al., 1998).

RESULTS

Of the 12 microsatellite loci tested, 4 loci that gave poor amplification success or low polymorphism levels were discarded. The 8 remaining polymorphic loci were then screened for LD between pairs of loci. This test revealed significant results in 2 comparisons - Hai4-Hai9 ($P = 0.0425$) and Hai4-Hai12 ($P = 0.0462$) - and the remaining loci were considered to be independent. For this reason, the Hai4 locus was excluded from the analysis. After Bonferroni's correction for multiple comparisons, none of the loci showed significant deviations from HWE (Table 1).

Allelic diversity of microsatellites across loci ranged from 4 to 8 alleles/locus (mean = 5.8; see Table 1). Mean expected heterozygosity and PIC of the seven loci in linkage equilibrium were 0.7166 and 0.6536, respectively. Paternity non-exclusion probabilities for these loci were NE-1P = 0.0766 and NE-2P = 0.0108. Combined non-exclusion probability for identity was 6.2×10^{-7} (see Table 1).

Table 1. Number of individuals analyzed (N), number of alleles (N_A) and their range of size [S (bp)], polymorphism information content (PIC) values, non-exclusion probabilities for the first (NE-1P) and second (NE-2P) parent, non-exclusion probability for the identity [P(ID)], observed and expected heterozygosity (H_O and H_E, respectively), and P values calculated for the Hardy-Weinberg test for heterozygote deficiency in the 7 loci in linkage equilibrium.

Locus	N	S (bp)	N_A	H_O	H_E	PIC	NE-1P	NE-2P	P(ID)	P
Soc 1[a]	35	269-279	6	0.600	0.709	0.653	0.713	0.539	0.137	0.9101
Soc 2[a]	34	135-161	4	0.618	0.629	0.554	0.803	0.654	0.211	0.9857
Soc 3[a]	30	112-134	6	0.867	0.783	0.735	0.621	0.442	0.088	0.8749
Soc 5[a]	33	260-272	7	0.788	0.686	0.622	0.740	0.574	0.158	0.7044
Soc 6[a]	35	215-221	4	0.543	0.600	0.506	0.818	0.693	0.252	0.0712
Hai 9[b]	20	221-235	6	0.700	0.800	0.745	0.610	0.431	0.083	0.1131
Hai 12[b]	22	112-136	8	0.682	0.809	0.761	0.584	0.406	0.074	0.4109
Hai 4[b]	34	166-182	6	0.706	0.766	0.718	0.641	0.462	0.097	0.2923

[a]Lacey, 2001; [b]Lacey et al., 1999.

DISCUSSION

Although this analysis was performed in a small population of *C. pearsoni*, the 8 loci screened proved to have sufficiently high levels of polymorphism and heterozygosity to conduct a relatedness analysis. Values obtained with this set of loci for combined probabilities of non-exclusion for the parents (NE-1P, NE-2P) and combined probability of non-exclusion for identity proved to be highly reliable when assigning paternity. In addition to enabling relatedness inferences between individuals, this panel of microsatellite loci had enough power to elucidate the levels of genetic structure of the population.

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