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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite markers in *Oligoryzomys longicaudatus* (Muridae, Sigmodontinae, Oryzomini), the natural reservoir of genotype Andes hantavirus

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Abstract

The rodent *Oligoryzomys longicaudatus* or long-tailed pygmy rice rat is the reservoir of the aetiological agent of the hantavirus pulmonary syndrome in southern Argentina and Chile. We characterize 11 polymorphic microsatellite loci which would be useful for studies on microgeographical population structure in the species. Amplification of these loci in 42 individuals from four natural populations revealed four to 21 alleles per locus, and values of observed heterozygosities ranging from 0.371 to 0.896. Cross-species amplifications showed that some of the primers designed may be useful for other species of the genus *Oligoryzomys*.

Keywords: hantavirus pulmonary syndrome, microsatellites, *Oligoryzomys longicaudatus*

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Oligoryzomys longicaudatus (Muridae, Sigmodontinae, Oryzomini) is one of the most common rodents in the patagonian forests of southern Argentina and Chile (Muñoz-Pedreros & Yañez 2000; Pearson 2002). The study of migration and colonization patterns in this species is important because it has been identified as the natural reservoir of the Andes hantavirus (Padula *et al*. 2004), which causes the hantavirus pulmonary syndrome (HPS). As part of an ongoing study on the population genetic structure of this species and on the potential spread of the infection, we present here results of the isolation and characterization of 11 novel polymorphic microsatellite loci.

Genomic DNA was extracted from liver of four individuals of *O. longicaudatus*, using a standard phenol–chloroform procedure. For microsatellite isolation, we followed the protocol of Hamilton *et al*. (1999), modified by Glenn *et al*. (2000). Total DNA was digested with *Hae*III and ligated to the 'linker-oligonucleotides' SNX-F and SNX-R (5′-CTAAGGCCTTGCTAGCAGAAGC-3′; 5′-pGCTTCTGCT-AGCAAGGCCTTAGAAAA-3′). Hybridization at 50 °C for enrichment was performed using biotinylated oligonucleotides [$(AACC)_5$, $(AACG)_5$, $(AAGC)_5$, $(AAGG)_5$, $(ATCC)_5$ and $(AC)_{13}$] and captured on streptavidin beads. The recovered DNA was amplified by polymerase chain reaction (PCR) using SNX-F as a primer and ligated into the pCR2.1-Topo Vector (Invitrogen). The One Shot Top 10 chemically competent *Escherichia coli* cells were transformed with the ligated product. A total of 135 white colonies were screened for inserts using M13 forward and reverse primers, all of which generated PCR products between 500 bp and 1000 bp. Fifty-two clones were purified using a QIAprep Spin Miniprep Kit (QIAGEN) and sequenced using M13 universal primers in an ABI PRISM 3100 Genetic Analyser (PerkinElmer, Applied Biosystems). Fifteen unique inserts containing microsatellites were found. Primers were designed for all these sequences using the software FASTPCR (Kalendar 2004). Loci were named using the prefix Olong followed by a number.

PCR was optimized in a mix of 15 μL containing 1× reaction buffer [75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$,

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Table 1 Characteristics of microsatellite loci isolated from *Oligoryzomys longicaudatus*. Locus name, repeat motif, primer sequences, annealing temperature, $MgCl_2$ concentration, number of PCR cycles, polyacrylamide gel percentage, range of allele size, number of alleles amplified, average observed and expected heterozygosities, *P* values for deviations from Hardy–Weinberg equilibrium and GenBank Accession no. are indicated

Locus	Repeat motif	Primer sequences (5′–3′)	°C	$MgCl_2$	Number of cycles	Gel percentage	Range of allele size	No. of alleles	H_O	H_E	P_{HW}	GenBank Accession no.
Olong1	$(CA)_{21}$	F: TAATACCACAGTCACTGGAC R: CTTGATGAATGAGAAGCCAAGCTAC	55	1.2	20	6	190–224	14	0,731	0,856	0,689	EU380279
Olong2	Imperfect†	F: AGCTTCAGGGGATCTGGTGCCC R: GGTTCAGGAAGACTGCAAC	57	1.2	22	6	218–298	20	0,448	0,768	0,006	EU380280
Olong4	$(TG)_4TT(TG)_{16}$	F: CTCACAAGGAGCACTGCACAGTCC R: TCTCTGTTAGGTACTGAGACACGA	58	1.6	24	6	236–270	17	0.896	0.914	0,564	EU282010
Olong5	$(CA)_2GA(CA)_{28}$	F: AACTGAGTATCTTGGACTGCCAGC R: GCTGTCACGCCCAAGTCCATCAC	63	1.2	25	8	126–164	18	0.465	0.911	0,009	EU282011
Olong6	$(CA)_9$	F: AATGCTGTCCTGATACAGACACTG R: GCAAAGCATTCTTAGCGTGT	55	1.2	24	8	141–151	4	0,371	0,566	0,489	EU380281
Olong7	Imperfect‡	F: GTTATCGAGTACCCAGTGGTCAT R: CAGCACATTACCTCACCTCC	56	1.6	24	6	232–260	10	0,658	0,667	0,446	EU380282
Olong9	$(CA)_7CCTA(CA)_{16}$	F: CCTCACACAGGGGTGCATAAACGC R: CAGAATGTTCTCGAGTATGCGT	58	1.2	29	8	108–130	10	0.748	0.828	0,293	EU282012
Olong10	$(AC)_{15}$	F: ACACCCTTACTTAAGAGCCACAC R: ATGGTAGCAGTTTTCCCAACGTT	56	1.2	22	6	216–252	14	0,715	0,84	0,599	EU380283
Olong12	$(TG)_{25}(GT)_5$	F: TGTGGGCACCTGGGAGA R: CACTTACATATACACCCTCATGGG	57	1.2	28	8	124–158	17	0.835	0.912	0,474	EU282013
Olong13	$(GT)_{17}$	F: AGACAAGAGCTTTGTGGTCTGTTC R: ACATGCACACATGCCTGTG	57	1.6	28	8	104–146	21	0.621	0.907	0,219	EU282014
Olong14	$(CT)_{30}(CA)_{22}$	F: CCACAGCACATGACGGGATGG R: AGCATTCTTCTGTGTCAGGAGT	57	1.2	22	6	251–297	19	0.494	0.922	0,005	EU282015

†$(ACAG)_3(AC)_3GC(AC)_{22}GCAC(GC)_2(AC)_3GCACTC(AC)_2AT(AC)_2(GC)_2ACTC(AC)_2(GC)_5(AC)_{18}$; ‡$(TG)_8TTT(GT)_2(AC)_2A(TG)_3(TA)_3$.

0.01 Tween 20], 130 μM each of dATP, dGTP, dCTP, dTTP; 0.6 μM of each primer, 1.0 U of *Taq* polymerase (Fermentas Life Sciences) and 10 ng of total DNA. $MgCl_2$ varied according to each primer (Table 1). Amplifications were performed using a Biometra *Uno*II thermal cycler (Biometra), with an initial denaturation step of 3 min at 94 °C, followed by cycles (the number of cycles are indicated in Table 1) of 30 s at 94 °C, 30 s at the annealing temperature (Table 1) and 30 s at 72 °C, ending with 5 min at 72 °C. Amplified alleles were separated by electrophoresis using Tris-glycine buffer system (White *et al*. 2002) on 20-cm long polyacrylamide gels (Table 1). Gels were run at 280 V for 3.5 h and stained with silver nitrate (Neilan *et al*. 1994). Allele sizes were determined by comparison with a molecular size standard (10-bp Ladder, Invitrogen). To confirm genotypes and to minimize allele scoring errors, all amplifications were electrophoresed a second or a third time, running together those individuals that appeared to have similar genotypes in the first run.

Primers for eleven loci produced good quality amplification patterns and were tested for polymorphism using 42 individuals from the following populations: El Bolsón (41°58′S, 71°31′W; *N* = 12), Junín de los Andes (39°55′S, 71°05′W; *N* = 10) and Cholila (42°31′S, 71°28′W; *N* = 10) from Argentina and El Prado (36°39′S, 71°49′W; *N* = 10) from Chile.

Primers were also tested for amplification in five individuals of the following species of tribe Oryzomini: *Oligoryzomys flavescens*, *Oligoryzomys chacoensis*, *Oligoryzomys nigripes*, *Oligoryzomys destructor*, *Pseudoryzomys simplex* and *Holochilus chacarius*. Conditions for amplification were, in general, the same as those for *O. longicaudatus*.

The characteristics of the loci are provided in Table 1. Conformance to Hardy–Weinberg equilibrium and linkage disequilibrium were tested using ARLEQUIN version 3.11 (Excoffier *et al*. 2005). In general, the observed heterozygosity values were smaller than those expected. Loci Olong2, Olong5 and Olong14 deviated from Hardy–Weinberg equilibrium significantly in the four populations, and locus Olong13 deviated only in El Bolsón and Cholila populations. These results may indicate some level of population subdivisions (Wahlund effect). In locus Olong2, homozygous individuals for null alleles were detected; in the remaining loci, the presence of heterozygotes for null alleles cannot be discarded. Linkage disequilibrium between loci Olong2 and Olong5, Olong2 and Olong13 and between Olong5 and Olong14 was observed.

Results of cross-species amplifications are shown in Table 2. Amplification of locus Olong9 in *O. chacoensis* was successful using 2.5 mM $MgCl_2$.

The high levels of polymorphism detected indicate that in combination, the loci here characterized would provide

Table 2 Cross-species amplification of six microsatellite loci designed for *Oligoryzomys longicaudatus*. For each locus, the first row shows the number of alleles and the second, the range of allele sizes in base pair found in a sample of five individuals of each species

Species	Olong1	Olong2	Olong4	Olong5	Olong6	Olong7	Olong9	Olong10	Olong12	Olong13	Olong14
Oligoryzomys chacoensis	No amp	6 214–226	7 236–254	6 154–190	4 133–177	3* 234–252	3 122–134	7 214–236	9 110–150	8 96–130	No amp
Oligoryzomys flavescens	6 200–244	4* 210–216	4 230–238	5 134–156	5 151–185	8 232–268	5 98–116	4* 224–232	No amp	6 98–128	9 251–294
Oligoryzomys nigripes	5 190–208	5 218–232	5 236–268	3 105–136	3 137–143	2 248–270	3 108–114	4 212–228	No amp	4 114–122	No amp
Oligoryzomys destructor	No amp	6 220–244	No amp	5 150–172	2 133–157	6 236–258	No amp	9 212–254	No amp	6 88–126	No amp
Pseudoryzomys simplex	No amp	No amp	No amp	No amp	No amp	No amp	No amp	No amp	No amp	2 102–104	No amp
Holochilus chacarius	No amp	No amp	3 266–274	No amp	No amp	No amp	No amp	No amp	No amp	No amp	No amp

*Null alleles were detected.

enough information for genetic structure studies in *O. longicaudatus*. Cross-amplification of these primers in other species of the genus indicates their potential effectiveness for similar studies in those species.

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