DNA from 238 individuals sampled in the six populations was extracted (CTAB 2%, Tris-HCl 100 mm, EDTA 20 mm, NaCl 1.4 M, β-mercapto-ethanol 0.2% v/v).

The number of alleles per microsatellite locus ranged from 3–15 (Table 1) whereas the five polymorphic allozyme markers had only two alleles; the expected heterozygosity ranged from 0.112 to 0.873 (Table 1) whereas it ranged from 0.030 to 0.070 for allozyme markers (Colas et al. 1997). The increased level of polymorphism detected by microsatellites is encouraging, as it will allow us to investigate the population structure at a very local scale (population level), and to assess gene flow by paternity analysis. At the species level, significant heterozygote deficiency (P < 5%), was detected for each locus except for 17B3 (P = 0.057) (GENEPop 3.1b; Raymond & Rousset 1995). These departures from Hardy–Weinberg expectations are the result of a high differentiation between populations as already shown by allozyme data (Colas et al. 1997).

Using identical PCR conditions, primers were tested in three related species of the genus Centaurea; C. maculosa Lam. ssp. albidia and C. maculosa Lam. ssp. maculosa belong to the section Maculosa as C. corymbosa; C. aspera L. belongs to the section Dumolosae (Tutin et al. 1976). C. m. albidia is an endemic species growing in South of France whereas C. m. maculosa is a widespread species, known from southern France to southern Germany and northern Italy. It has been argued that the endemic species, C. corymbosa and C. m. albidia would have derived from the widespread taxa, C. m. maculosa, following geographical isolation (Fréville et al. 1998).

Successful amplifications were obtained for the seven microsatellite loci, in C. m. albidia and C. m. maculosa. Nevertheless, the band pattern was unclear for 28A7. In contrast, no amplification product was obtained in C. aspera. These results suggest that cross-species amplification could be difficult outside the section level.

Acknowledgements
We acknowledge M.P. Dubois, J. Mavarez and J. Lagnel for technical advice and S. Soulé for help in collecting data. This work was supported by the Bureau des Ressources Génétiques, the Ministère de l’Aménagement du Territoire et de l’Environnement and the Région Languedoc-Roussillon. HF is supported by the CNRS and the Région Languedoc-Roussillon. This publication number 2000–050 of the Institut des Sciences de l’Évolution de Montpellier.

References


Characterization of microsatellite loci from the mountain vizcacha Lagidium viscacia and their use for the plains vizcacha Lagostomus maximus

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The Chinchillidae is a family of hystricomorph rodents indigenous to southern South America. There are three genera, chinchillas (Chinchilla spp.) and mountain vizcachas (Lagidium spp.), which live in crevices in rocky habitat, and plains vizcachas (Lagostomus maximus), which construct underground burrow systems in open grasslands and shrublands. Some species of all three genera have been heavily persecuted and are threatened or endangered. We developed microsatellite markers to study movement among local populations of mountain vizcachas, L. viscacia, in Argentina. We also tested these microsatellite markers on the plains vizcacha.

Genomic DNA was isolated from heart tissue of two mountain vizcachas using standard phenol/chloroform extraction procedures (Sambrook et al. 1989). Twenty μg of pooled DNA were digested with SauIII and fractionated by agarose gel electrophoresis. DNA fragments 300–900 bp long were recovered by electrophoresis onto diethylaminoethyl (DEAE) paper and ligated to SAUL/AUBL oligonucleotide linkers (Armour et al. 1994). SAULA primer was used to amplify ligation products in a 100-μL polymerase chain reaction (PCR) (10× Assay Buffer A (Fisher Scientific), 200 μM dNTPs, 10 μM SAULA primer, 2.5 U Taq DNA polymerase (Fisher Scientific) for 30 cycles (94 °C, 45 s; 57 °C, 45 s; 72 °C, 90 s) in a Biometra

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Table 1. Microsatellite loci identified from the genome of *Lagidium viscacia*

<table>
<thead>
<tr>
<th>Locus</th>
<th>GenBank accession no.</th>
<th>Primer sequences (5' - 3')</th>
<th>Repeat Sequence</th>
<th>Anneal °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV1</td>
<td>AF092869</td>
<td>F: 6-FAM-taa gtc cca gtt cca cct c&lt;br&gt;R: ceg ccc tct ctt gc</td>
<td>AC&lt;sub&gt;12&lt;/sub&gt;</td>
<td>52.8</td>
</tr>
<tr>
<td>LV4</td>
<td>AF092870</td>
<td>F: ctg ggg ccc cct cat cc&lt;br&gt;R: 6-FAM-ttg gct gaa tgt gtc aag aag</td>
<td>CA&lt;sub&gt;15&lt;/sub&gt;</td>
<td>56</td>
</tr>
<tr>
<td>LV5</td>
<td>AF092871</td>
<td>F: 6-FAM-gct agg cgt agt gct cca ga&lt;br&gt;R: atg tga ggc ggc ttc cca</td>
<td>CA&lt;sub&gt;12&lt;/sub&gt;</td>
<td>58.3</td>
</tr>
<tr>
<td>LV8</td>
<td>AF092872</td>
<td>F: 6-FAM-cac aga att ggg cat aga at&lt;br&gt;R: gac aag tgt agg ctt cag</td>
<td>CA&lt;sub&gt;19&lt;/sub&gt;</td>
<td>51.2</td>
</tr>
<tr>
<td>LV16</td>
<td>AF092873</td>
<td>F: d-HEX-agc aca cag cca ggg aat g&lt;br&gt;R: gca gaa ctc tcc aca gga ac</td>
<td>AC&lt;sub&gt;10&lt;/sub&gt;(GTG)CA&lt;sub&gt;13&lt;/sub&gt;</td>
<td>54.2</td>
</tr>
<tr>
<td>LV17</td>
<td>AF092874</td>
<td>F: d-HEX-gtg ctt tgt ttc ttc ctt cct&lt;br&gt;R: GCC tgg ata act act cac ct</td>
<td>CA&lt;sub&gt;14&lt;/sub&gt;</td>
<td>52</td>
</tr>
<tr>
<td>LV25</td>
<td>AF092875</td>
<td>F: 6-FAM-cga aga cgc acc ttt acc t&lt;br&gt;R: ttg gca cca tgt att gtt ga</td>
<td>CA&lt;sub&gt;14&lt;/sub&gt;CG&lt;sub&gt;10&lt;/sub&gt;</td>
<td>55.4</td>
</tr>
<tr>
<td>LV33</td>
<td>AF092876</td>
<td>F: d-HEX-tgc cca gca cat tga aaa c&lt;br&gt;R: tta acc aca acc cca acc ac</td>
<td>CA&lt;sub&gt;17&lt;/sub&gt;</td>
<td>52</td>
</tr>
</tbody>
</table>

H<sub>o</sub> observed heterozygosity; H<sub>e</sub> expected heterozygosity; F, forward primer; R, reverse primer.

Table 2. Polymorphism data for *Lagidium viscacia* and *Lagostomus maximus*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size range (bp)</th>
<th>No. of Alleles</th>
<th>H&lt;sub&gt;O&lt;/sub&gt;</th>
<th>H&lt;sub&gt;E&lt;/sub&gt;</th>
<th>L. viscacia</th>
<th>Size range (bp)</th>
<th>No. of Alleles</th>
<th>H&lt;sub&gt;O&lt;/sub&gt;</th>
<th>H&lt;sub&gt;E&lt;/sub&gt;</th>
<th>L. maximus</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV1</td>
<td>155–161</td>
<td>5</td>
<td>0.61</td>
<td>0.67</td>
<td>157–175</td>
<td>10</td>
<td>0.88</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV4</td>
<td>190–204</td>
<td>4</td>
<td>0.42</td>
<td>0.48</td>
<td>184–204</td>
<td>8</td>
<td>0.68</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV5</td>
<td>181–199</td>
<td>7</td>
<td>0.71</td>
<td>0.80</td>
<td>245–275</td>
<td>11</td>
<td>0.76</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV8</td>
<td>232–236</td>
<td>3</td>
<td>0.44</td>
<td>0.46</td>
<td>178–200</td>
<td>6</td>
<td>0.60</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV6</td>
<td>247–261</td>
<td>8</td>
<td>0.54</td>
<td>0.70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV17</td>
<td>225–243</td>
<td>4</td>
<td>0.71</td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV25</td>
<td>206–214</td>
<td>4</td>
<td>0.86</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV33</td>
<td>177–191</td>
<td>3</td>
<td>0.50</td>
<td>0.51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Unot-Thermoblock thermocycler. Fragments containing (CA)<sub>n</sub> repeats were captured by hybridization of 1 μg of the amplification product to (GT)<sub>10</sub> oligonucleotide bound to nylon membranes, as described by Karaguzov et al. (1993), using only a single round of hybridization. The (CA)<sub>n</sub> repeat enriched DNA was recovered from the filters and ethanol-precipitated overnight at −20 °C using 1 μL SAULA as carrier. The precipitate was amplified in a 100-μL reaction for 25 cycles as described above, with no additional primer, as the pellet contained SAULA primer. One μL of the amplification product was ligated into pCR®2.1 vector (Invitrogen) and transformed into JM110 (Promega). Plasmids were prepared from 81 transformed colonies (WizardPlus Miniprep system, Promega), dotted on a nylon membrane, and screened by hybridization with an alkaline-phosphate linked (CA)<sub>n</sub> probe and detected using the Quick-Light<sup>TM</sup> Hybridization Kit (FMC Bio Products).

Fifty per cent of the colonies (n = 41) screened by hybridization were positive for CA repeats. Twenty-eight of these were DNA-sequenced, and were found to represent 27 distinctive (CA)<sub>n</sub> loci. PCR primers were designed in the flanking region of 10 of the loci using the program Oligo (National Biosciences Inc., version 4.0) and tested on DNA obtained from ear tissue of 24 *Lagidium viscacia* and 45 *Lagostomus maximus* and faeces of three *Chinchilla lanigera* in 12.5 μL PCR reactions [10× Assay Buffer A (Fisher Scientific), 200 μM dNTPs, 0.4 μM primer, 0.5 U Taq DNA polymerase (Fisher Scientific)] using cycling parameters described by Gagneux et al. (1997). Of the 10 primer sets, eight amplified a band of the appropriate size in *L. viscacia* samples (Table 1), and four did so for *L. maximus*. In addition, primers for loci LV1, LV4, LV5, LV17, LV25, and LV33 amplified bands of the appropriate size from the three *Chinchilla* samples.

Alleles were resolved for *Lagidium* and *Lagostomus* samples by electrophoresis using a 4.5% polyacrylamide gel on an Applied Biosystems Inc. (ABI)-Prism 373 automated DNA sequencer. Data were analysed using GENESCAN 2.0.2 and GENOTYPER 2.0 (ABI) software. All loci amplified in both *Lagidium* and *Lagostomus* were polymorphic (Table 2). These microsatellite loci provide useful markers for population studies for two of the three genera of the family Chinchilidae, and their utility in the third genus, *Chinchilla*, warrants further investigation.

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References


Identification of polymorphic autosomal and sex chromosome specific DNA microsatellites in the bushcricket, Poecilimon hoelzlei (Orthoptera, Tettigoniioidea, Phaneropteridae)

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Keywords: DNA microsatellites, paternity testing, Poecilimon hoelzlei, sperm competition, Tettigonioiden

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In many bushcricket species males and females are involved in multiple mating, and because sperm is stored in a storage organ within the female, sperm competition can influence the reproductive success of males (Achmann et al. 1992). We developed a set of polymorphic microsatellite primers for the highly promiscuous bushcricket Poecilimon hoelzlei to examine the outcome of sperm competition in multiple mated females.

Microsatellite sequences were cloned following the approach of Rassmann et al. (1991). Partial genomic libraries were constructed with DNA isolated from a female bushcricket. DNA was simultaneously digested with three different restriction enzymes (AluI, HaeIII, Rsal). Following agarose gel electrophoresis, a 200–400 bp gel-purified size fraction was ligated into Smal-linearized, dephosphorylated pBluescript II KS (Stratagene) or M13mp18 (Sigma) DNA. Ligation products were transformed into competent Escherichia coli XL1 cells (Nishimura et al. 1990). Microsatellite positive clones were identified by colony or plaque hybridization with radiolabelled di- and trinucleotide repeat polymers (Rassmann et al. 1991). Hybridization suggested that approximately 0.04% of clones of the plasmid and 0.4% of clones of the phage library contained a dinucleotide microsatellite sequence, whereas 0.03% of clones of the phage library gave a positive signal after probing with trinucleotide repeat polymers. Positive clones were sequenced and primers were designed for 13 loci from unique sequences flanking the microsatellites. Microsatellite variability was investigated in a sample of 10 male and 10 female bushcrickets.

Bushcricket genomic DNA was isolated from about 20 mg hindleg muscle or abdominal gland tissue by using a salt-chloroform extraction protocol (Müllenbach et al. 1989). Polymerase chain reaction (PCR) amplification of microsatellite loci was carried out in a volume of 10 µL on a Hybaid Thermal Reactor TR1. Twenty pmol of lower primer (Table 1) was end-labelled using T4 polynucleotide kinase with [γ32P]-ATP. PCR reactions included approximately 50 ng of DNA, 5 pmol primers, 0.4 µmol labelled primer, 0.25 mm of each dNTP, 1.5 mM MgCl₂ and 0.3 U of AmpliTaq polymerase (Perkin-Elmer Biosa) in 1 × PCR buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl). Microsatellites were amplified for 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 20 s at annealing temperature (Table 1), 30 s at 72 °C and finally 10 min at 72 °C. The amplification product (9 µL) was mixed with 3 µL loading-buffer (95% Formamid, 20 mM EDTA, 0.05% Bromphenol Blue, 0.05% Xylene Cyanol FF) and denatured for 3 min at 90 °C. One to two µL of denatured product was electrophoresed on 53 cm denaturing (5% urea) polyacrylamide gels (6%) for 2–5 hrs at 55 W. Depending on the radiation intensity dried gels were exposed for 3–5 days without intensifier screens at room temperature. Allele sizes were determined by comparison with a M13 sequence ladder and bushcricket reference samples.

Three loci for which only one allele was amplified were not examined further. Ten loci were polymorphic with the number of alleles ranging from 3–10 (Table 1). Locus Phm52 could not be amplified in five out of 20 samples due to the presence of a null allele. All tested males appeared to be homozygous (H₀ = 0) for loci Phm30, Phm33, and Phm53, but for females heterozygous animals were found (H₀ = 0.70, 0.44, 0.80, respectively; see Table 1). Such an amplification pattern is consistent with a X-chromosomal location of Phm30, Phm33 and Phm53. Sex determination in Poecilimon is of the XX/X0 system (e.g. Warchałowska-Sliwa et al. 1992). The female karyotype comprises two X chromosomes (XX), whereas the male shows only one X chromosome (X0), hence appearing homozgyous for X-chromosomal microsatellite loci. Sex chromosomal location was confirmed by segregation analysis in offspring of 10 families (data not shown). A further cytogetic characteristic in Poecilimon species is that X chromosomes are considerably larger than autosomes. The relative length of