Table 1 Microsatellite loci of Dinizia excelsa, with number of individuals sampled (n), number of alleles observed (O), observed heterozygosity (H0), expected heterozygosity (H1) and expected exclusion probabilities (P(E)) calculated by genepop (Marshall et al. 1998). All sequences have been deposited in GenBank (AF143976, AF143979, AF143980, AF143982, AF143986, AF143987, AF143988).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat array</th>
<th>Primer sequences (5’–3’</th>
<th>Annealing temp (°C)</th>
<th>Clone size (bp)</th>
<th>n</th>
<th>k</th>
<th>H0</th>
<th>H1</th>
<th>Exclusion probability (PE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE27</td>
<td>(AAT)8</td>
<td>GCATTAAAAAATTTATGAGG</td>
<td>60</td>
<td>118</td>
<td>121</td>
<td>5</td>
<td>0.54</td>
<td>0.49</td>
<td>0.23</td>
</tr>
<tr>
<td>DE37</td>
<td>(ACG)6</td>
<td>GTGACCTTTGACGTTTTC</td>
<td>60</td>
<td>128</td>
<td>115</td>
<td>11</td>
<td>0.72</td>
<td>0.73</td>
<td>0.51</td>
</tr>
<tr>
<td>DE44</td>
<td>(GT)13</td>
<td>ACGGAAAGGCTGTTGACCC</td>
<td>60</td>
<td>144</td>
<td>119</td>
<td>9</td>
<td>0.66</td>
<td>0.64</td>
<td>0.40</td>
</tr>
<tr>
<td>DE48</td>
<td>(GA)27</td>
<td>GAACTGAAAGGCGGGGAGGC</td>
<td>60</td>
<td>143</td>
<td>106</td>
<td>31</td>
<td>0.80</td>
<td>0.94*</td>
<td>0.87</td>
</tr>
<tr>
<td>DE54</td>
<td>(CT)20</td>
<td>GTGCGAGGAGGCAGTCCCT</td>
<td>60</td>
<td>175</td>
<td>84</td>
<td>21</td>
<td>0.62</td>
<td>0.93*</td>
<td>0.85</td>
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<tr>
<td>DE60</td>
<td>(AA)15</td>
<td>CAACGGAAAGGCGGGGAGGC</td>
<td>60</td>
<td>238</td>
<td>23</td>
<td>2</td>
<td>0.35</td>
<td>0.29</td>
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<tr>
<td>DE64</td>
<td>(AAT)6</td>
<td>AATGCTACTCGGGAATGGCG</td>
<td>60</td>
<td>134</td>
<td>36</td>
<td>2</td>
<td>0.03</td>
<td>0.03</td>
<td>—</td>
</tr>
</tbody>
</table>

*Significant excess of homozygotes (P < 0.05) in some of the sample populations.

for paternity analyses. Six loci that did not amplify reliably but may be useful with different primers are (repeat array followed by GenBank Accession no.): (GA)13 (AF143978); (CT)19 (AF143981); (TG)11 (AF143983); (GA)24 (AF143984); and (CT)23 (AT)12 (AF143985).

Acknowledgements

We thank P. Ashton, R. C. Lewontin, S. Palumbi, members of Lewontin laboratory at Harvard and R. Fleischer for fruitful discussions. Laboratory work was funded by a Deland Award from the Arnold Arboretum, Sigma Xi, and the Lewontin Lab.

References


Amplifying dolphin mitochondrial DNA from faecal plumes

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Keywords: DNA extraction, Tursiops truncatus, faeces, PCR, mtDNA, cetaceans

Received 15 April 1999; revision received 2 June 1999; accepted 5 June 1999

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Molecular studies are increasingly used to support cetacean conservation and management (Hoelzel 1994). Biopsy-darting
(e.g. Lambertsen 1987) and collecting sloughed skin (e.g. Amos et al. 1992) have provided DNA from several species, but these methods are often impractical. An alternative is to extract DNA from faeces, as previously applied to terrestrial (e.g. Gerloff et al. 1995) and semiaquatic (Reed et al. 1997) mammals, and to floating dugong faeces (Tikel et al. 1996).

Until now, however, the diffuse form of cetacean ‘faecal plumes’ has prevented their collection and genetic analysis. Here, we demonstrate the feasibility of collecting faeces, extracting total DNA, and reliably amplifying mitochondrial DNA (mtDNA) markers from free-ranging dolphins.

Five faecal samples were collected from bottlenose dolphins (Tursiops truncatus) in the Bahamas (26°0' N 77°25' W). Upon observing defaecation, snorkelers collected sinking faeces in 100 mL plastic vials. Samples were immediately preserved in 20% DMSO/5 m NaCl (Amos & Hoelzel 1991), and frozen at –20°C within 10 h. Faeces were thawed immediately preceding extraction (total storage time 157–227 days). Cross-contamination was avoided by using disposable plasticware, and by cleaning metal instruments with ethanol, and flaming. One millilitre of faeces was suspended in 2 mL of 2·CTAB buffer (100 mM Tris-HCl, pH 8, 1.4 mM NaCl, 20 mM EDTA, 2% CTAB) (Milligan 1992), and mixed by rotation. Debris was cleared by centrifugation, and 2 mL of lysate transferred to a 2-mL microcentrifuge tube. Following high-speed centrifugation, 1.5 mL of cleared suspension was extracted twice with 0.5 mL of chloroform, and DNA precipitated by adding 0.67 mL of isopropanol.

DNA pellets were dissolved in 1.8 mL of guanidine thiocyanate (GTC) buffer (5 mM GTC, 0.1 mM Tris-HCl, pH 8.4) at ambient temperature overnight. Three grammes of diatomaceous earth particles (Sigma D-5384) were washed, three times, in 50 mL of molecular-grade water, and centrifuged. Pellet diatoms were then suspended in 12 mL of water, vortexed to create a slurry. One hundred microlitres of diatomaceous slurry was added to the microfuge tube to recover the DNA. Diatoms were pelleted, resuspended in 600 mL of GTC buffer, transferred to the insert of a 10-mL mesh VetcSpin microtube (Whatman, no. 6838 0002) and centrifuged to remove buffer. The diatoms, and DNA, were washed twice with 700 mL of ethanol, then incubated at 55°C for 30 min to evaporate residual traces of ethanol. DNA was eluted in 130 mL of TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), incubated at 55°C for 30 min, centrifuged to pellet diatom traces, and 100 mL was transferred to a new 0.2-mL tube.

The origin and quality of extracted DNA was determined by polymerase chain reaction (PCR) amplification of 558 bp of the 5' section of the mtDNA control region, using primers L15926* and H16498 (Eggert et al. 1998). PCR reactions were carried out in a 50-μL volume containing 10 μL of DNA. Extraction blanks, positive and negative controls were included in all PCR reactions to detect contamination. Products were detected and quantified on ethidium-stained 1% agarose gels. The desired fragment was successfully amplified from the DNA of all five faecal samples.

To confirm that amplified mtDNA fragments originated from T. truncatus, both strands of purified (QIAquick PCR purification kit [Qiagen]) PCR products were sequenced using BigDye sequencing kits (PE Biosystems), and detected on an ABI 377 automated DNA sequencer. Each faecal sample was amplified and sequenced twice. These sequences were aligned to a published T. truncatus mtDNA sequence (GenBank), and to the sequence from a T. truncatus skin sample obtained in the Bahamas (Fig. 1). All five faecal sequences showed a high degree of similarity to the published sequence.
1768 PRIMER NOTES

substitution sites; 14 transitions), and to the sequence from dolphin skin (five substitution sites; five transitions). This indicates that the amplified mtDNA sequences originated from dolphin DNA, and not from contaminating sources of faecal (e.g. prey) DNA.

Low quantities of target DNA can cause complications when using faecal-derived DNA in nuclear genotyping (Taberlet & Waits 1998). However, our mtDNA analyses produced reliable results from the sequence of a single fragment, suggesting that faeces may represent a useful supplement to conventional sources of DNA for future studies of cetacean population genetics.

Acknowledgements

Thanks to the Bahama’s government for permission to conduct field research (permit MAF/FIS 12 A) and to the Centre for Whale Research and Earthwatch for logistic support. K.M.P. was supported by ORS, NSERC, and the British Council.

References


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