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Development of coral and zooxanthella-specific microsatellites in three species of Pocillopora (Cnidaria, Scleractinia) from French Polynesia

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Abstract
Since the building of coral reefs results from the association of corals and zooxanthellae, their intracellular algal symbionts, genetic markers for both organisms are essential for studying the contribution of their respective dispersal to the resilience of endangered reef ecosystems. Very few microsatellites have been obtained in corals thus far. Here we report the successful cloning of six polymorphic microsatellites (allele number: 5–15) from Pocillopora verrucosa, P. meandrina and P. damicornis. Four of them amplified coral, and two amplified zooxanthella DNA.

The closely related Pocillopora verrucosa and P. meandrina are among the dominant coral species of outer reef slopes in French Polynesia and their colonizing ability could play a critical part in the resilience of coral reef ecosystems after destruction due to bleaching events. In French Polynesia, these species behave as broadcasters (Adjeroud, unpublished results): fertilization is external through emission of gametes into water. Little is known of dispersal ability and genetic structure in these species.

Most population genetic studies in coral have been carried out using allozymes. Microsatellites have been rarely used (Maier et al. 2001) despite their high potential as genetic markers, probably because they are difficult to characterize in coral genomes (Marquez et al. 2003). Moreover, the endosymbiosis of corals with zooxanthellae (symbiotic dinoflagellates in the genus Symbiodinium), makes it difficult to extract coral-specific DNA.

Here we report the isolation and characterization of six polymorphic microsatellite loci (four from the coral, two from zooxanthellae) for P. verrucosa, P. meandrina and P. damicornis. Branch tips of the three species were collected on the reef of Moorea Island (Society islands, French Polynesia) and stored in 70% ethanol until use. A population sample of P. meandrina (25 individuals) was collected on the outer reef slope from 5 m distant individuals taken along a linear transect at 13 m depth. Total DNA was extracted from 300 mg of powder from a ground branch tip, using the DNEasy® Tissue Kit (Qiagen), following the manufacturer’s instructions.

Reference coral DNA from Hawaii was extracted from frozen zooxanthella-free sperm of P. meandrina (kindly provided by F. Cox, University of Hawaii). Reference zooxanthella DNA was extracted from a culture of Symbiodinium type C2 (kindly provided by T. LaJeunesse, University of Georgia; see LaJeunesse 2001), to which the symbionts of our samples were found to belong (Magalon et al. unpublished results). The absence of contamination in reference DNA was checked using polymerase chain reaction (PCR) amplifications based on universal primers for the 28S RNA locus (C1′-for: 5′-ACC CGC TGA ATT TAA GCA T-3′ and D2-rev: 5′-TCC GTG TTT CAA GAC GG-3′, Correlation Helene Magalon. Fax: (+33) 1 44 27 35 16; E-mail: hmagalon@snv.jussieu.fr
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A. Tillier personal communication), that resulted in amplification products of diagnostic molecular weight for coral (600 bp) and zooxanthella (700 bp). Amplification from a reference DNA always gave a single band of the expected size.

Microsatellite isolation and characterization followed a protocol developed by Estoup and Turgeon (see http://www.inapg.inra.fr/dsa/microsat/microsat.htm). Briefly, a genomic library for P. verrucosa from Moorea was constructed using Bsp143I-digested genomic DNA; 400–900 bp fragments were selected and ligated to BamH1-digested pUC 18 vector (Amersham) and cloned in Escherichia coli Solopack Gold super competent cells (Stratagene). Synthetic oligonucleotides (TC)10, (TG)10, CT(ATCT)6 and (TGTA)6TG, labelled with [γ-32P]-dATP were used to screen about 2500 recombinant colonies. Thirty-nine positive clones were sequenced on a CEQ2000XL sequencer (Beckman/Coulter) using dye-terminator chemistry. Primer pairs flanking microsatellites were designed using primer 3 (Rozen & Skaletsky 1998). From six loci (numbered PV1 through PV6), four amplified coral reference DNA and two amplified zooxanthella reference DNA. A seventh coral-specific microsatellite (PV7) was derived from an alignment of ITS sequences of the three species of Pocillopora. PCR amplifications were carried out on a Perkin-Elmer GeneAmp 9700® thermocycler using 0.5 μL DNA template, 500 μm each primer, 200 μm each dNTP, 1 μL Q-Biogen T, Pol incubation mix (10 mm TrisHCl pH 9, 50 mm KCl, 1.5 mm MgCl₂, 0.1% Triton 100x, BSA or gelatin 0.2 mg/mL), 0.25 U of Taq DNA polymerase (Q-Biogen), in a total volume of 10 μL. The cycling protocol was: 1× 94 °C (10 min), 30×(45 s at 94 °C, 45 s at the appropriate annealing temperature Tₐ [see Table 1], 30 s at 72 °C), and 1×72 °C (8 min).

Table 1 Microsatellite variation at polymorphic loci in Pocillopora meandrina from Moorea

<table>
<thead>
<tr>
<th>Locus (size, bp)</th>
<th>Specificity</th>
<th>Primer sequences (5’–3’</th>
<th>Accession number</th>
<th>Reference repeat motif</th>
<th>Tₐ (°C)</th>
<th>N</th>
<th>nₐ</th>
<th>Size range (bp)</th>
<th>Hₒ</th>
<th>Hₑ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV2 (184) coral</td>
<td>GCCAGGGCCCATTTATATCC</td>
<td>AY39777</td>
<td>(GA)₂₀</td>
<td>56</td>
<td>25</td>
<td>7</td>
<td>130–196</td>
<td>0.28</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>PV5 (232) coral</td>
<td>GATCAGTACGAAAGTTCC-NED</td>
<td>AY397780</td>
<td>(CA)₁₁</td>
<td>56</td>
<td>25</td>
<td>12</td>
<td>221–255</td>
<td>0.20</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>PV6 (207) coral</td>
<td>CTTGCCCGGACGTTTAGG-GAM</td>
<td>AY397781</td>
<td>(GT)₁</td>
<td>56</td>
<td>25</td>
<td>14</td>
<td>195–219</td>
<td>0.40</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>PV7 (239) coral</td>
<td>AAGAGTTGGAGCTCCTCT-TAG</td>
<td>AY397782</td>
<td>(GT)₃(CT)₂, (GT)₃</td>
<td>55</td>
<td>25</td>
<td>5</td>
<td>215–233</td>
<td>0.22</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>PV1 (159) zooxanthella</td>
<td>ATGGTGATGGACTAGTTACGC-FAM</td>
<td>AY397776</td>
<td>(TC)₃(TA)₇</td>
<td>54</td>
<td>25</td>
<td>15</td>
<td>91–167</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>PV4 (122) zooxanthella</td>
<td>CTTTGGGTGAAAAATTTCTTCC</td>
<td>AY397779</td>
<td>(GT)₁₀(v)₈</td>
<td>54</td>
<td>25</td>
<td>15</td>
<td>89–114</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Tₐ, annealing temperature; N, sample size; nₐ, number of alleles; Hₒ, observed heterozygosity; Hₑ, expected heterozygosity.
Acknowledgements

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References