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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 In French Polynesia, these species behave as broadcasters (Adjeroud, unpublished results): fertilization is external through emission of gametes into water.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 GGC GTG TTT CAA GAC GG-3′,...
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 pUC 18 vector (Amersham) and cloned in fragments were selected and ligated to Bsp143I-digested genomic DNA; 400–900 bp digestion products of diagnostic molecular weight for coral (800 bp) and zooxanthella (700 bp). Amplification from a reference DNA (length: 132 bp for PV1 and 98 bp for PV4). Individuals from the population exhibited from one to four bands of varying respective intensity. Since zooxanthellae are haploid, this suggests that several zooxanthella lineages coexist in the same coral host. These markers could be useful for characterizing zooxanthella populations and for contrasting dispersal patterns between the two partners of the symbiosis. These markers are currently being used for investigating reproductive biology, genetic structure and long range dispersal in Central Pacific populations of P. meandrina and of its symbiont.

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>T_a (°C)</th>
<th>N</th>
<th>n_a</th>
<th>Size range (bp)</th>
<th>H_0</th>
<th>H_E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV2 (184)</td>
<td>coral</td>
<td>GCCAGGACCCATTTATCTCC</td>
<td>AY397777</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>
</tr>
<tr>
<td>PV5 (232)</td>
<td>coral</td>
<td>GTCCACCTACCCGAAATGCC-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>
</tr>
<tr>
<td>PV6 (207)</td>
<td>coral</td>
<td>CTTTCCGGACGCCTTTAGG-FAM</td>
<td>AY397781</td>
<td>(GT)_7</td>
<td>56</td>
<td>25</td>
<td>14</td>
<td>195–219</td>
<td>0.40</td>
<td>0.42</td>
</tr>
<tr>
<td>PV7 (239)</td>
<td>coral</td>
<td>GGGACCTAGATCATCTACTAGT-G</td>
<td>AY397782</td>
<td>(GT)<em>(4)(CT)</em>(3)(GT)</td>
<td>55</td>
<td>25</td>
<td>5</td>
<td>215–233</td>
<td>0.22</td>
<td>0.32</td>
</tr>
<tr>
<td>PV1 (159)</td>
<td>zooxanthella</td>
<td>TATTACAGAAGAAGTCTCTCC-FAM</td>
<td>AY397776</td>
<td>(TC)<em>(3)(TA)</em>(7)</td>
<td>54</td>
<td>25</td>
<td>15</td>
<td>91–167</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PV4 (122)</td>
<td>zooxanthella</td>
<td>CTTGTTGGTAAGGGGGTCTCC</td>
<td>AY397779</td>
<td>(GT)<em>(10)(T)</em>(8)</td>
<td>54</td>
<td>25</td>
<td>15</td>
<td>89–114</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

T_a: annealing temperature; N, sample size; n_a: number of alleles; H_0, observed heterozygosity; H_E, expected heterozygosity.
Acknowledgements

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References


