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

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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 In French Polynesia, these species behave as broadcasters (Adjeroud, unpublished results): fertilization is external through emission of gametes into water.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.

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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′,

A. Tillier personal communication), that resulted in amplification products of diagnostic molecular weight for coral (800 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 *Bsp*143I-digested genomic DNA; 400–900 bp fragments were selected and ligated to *Bam*H1-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-^{33}P]-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 *Pocillopora* species.

PCR amplifications were carried out on a Perkin-Elmer GeneAmp 9700® thermocycler using 0.5 µL DNA template, 500 nM 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 $MgCl_2$, 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 T_a [see Table 1], 30 s at 72 °C), and 1 × 72 °C (8 min). Each pair of primers was labelled using fluorescent dyes (FAM, VIC and NED, Applied Biosystems®, Foster City, CA, USA; see Table 1). Allele size was recorded on an ABIPrism 310® Genetic Analyser, Applied Biosystem-Perkin-Elmer, and estimated respective to an internal standard (Genescan-500 ROX). Genotypes were determined using the GENESCAN software. After determining the genotype of population samples, observed (H_O) and expected (H_E) heterozygosities were calculated for coral loci using GENEPOP (Raymond & Rousset 1995). Observed heterozygosity is meaningless in zooxanthellae, which are haploid (Santos & Coffroth 2003), and genetic diversity (analogous to H_E) cannot be inferred from our results, since data suggest that each individual coral is subject to several algal invasions, making it difficult to distinguish individual clones.

For all loci, PCR reactions were successfully carried out on *P. verrucosa*, *P. meandrina* and *P. damicornis*, and also on *Acropora retusa* for the ITS locus. Results for the polymorphic loci are shown in Table 1.

Four of the five coral loci were polymorphic (PV2, PV5, PV6 and PV7), and always showed either one or two bands per individual. Heterozygosity was relatively low for microsatellites (average: 35.5%, range: 26%–42%), and H_O was always close to H_E, suggesting that zygotes are substantially dispersed at the scale of this sampling site.

The two zooxanthella loci (PV1 and PV4) were polymorphic. They gave a unique band using the zooxanthella 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

Locus (size, bp)	Specificity	Primer sequences (5′–3′)	Accession number	Reference repeat motif	T_a (°C)	N	n_a	Size range (bp)	H_O	H_E
PV2 (184)	coral	GCCAGGACCCATTTATACTCC TGCAGTGTTCTACTTGTCAGTGC-VIC	AY397777	$(GA)_{20}$	56	25	7	130–196	0.28	0.26
PV5 (232)	coral	GGTCATCACGCAAAGTTCC-NED GAATAGCCTGCGTTTATTTGG	AY397780	$(CA)_{11}$	56	25	12	221–255	0.20	0.42
PV6 (207)	coral	CTTTCCCGACCAGTTTAGGG-FAM AGCCGTTCAGCTACCTATGG	AY397781	$(GT)_7$	56	25	14	195–219	0.40	0.42
PV7 (239)	coral	GGAGATGGATGGAGACTGC-VIC GGTATCTCTGTGCTCAGTTCTTTG	AY397782	$(GT)_5(CT)_2GT(CT)_3$	55	25	5	215–233	0.22	0.32
PV1 (159)	zooxanthella	TGATTCAGGAAGATGGTAAGTCC-FAM ATGCACATAGGCTTCTTGTCC	AY397776	$(TC)_{33}(TA)_7$	54	25	15	91–167	—	—
PV4 (122)	zooxanthella	CGTGTTTGAAAAGGGTTCTTC CAAACCCAGCCAAAAGTGAC-NED	AY397779	$(GT)_{10}G_{18}$	54	25	15	89–114	—	—

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

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