PERMANENT GENETIC RESOURCES

Ten polymorphic STR loci in the cosmopolitan reef coral, *Pocillopora damicornis*

CRAIG J. STARGER,* SEAN S. R. YEOH,† CHANG-FENG DAI,† ANDREW C. BAKER‡* and ROB DESALLE§

*Department of Ecology, Evolution, and Environmental Biology, Columbia University, MC-5557, 1200 Amsterdam Avenue, New York, NY 10027, USA, †Division of Marine Biology and Fisheries, Institute of Oceanography, National Taiwan University, no. 1, Sec. 4, Roosevelt Road, Taipei 10617, Taiwan, ‡Division of Marine Biology and Fisheries, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA, §Sackler Institute for Comparative Genomics, American Museum of Natural History, New York, NY 10024, USA

Abstract

We report the development of 10 polymorphic molecular markers containing short tandem repeats in the cosmopolitan reef-building coral, *Pocillopora damicornis*, an important model species for coral health, physiology, ecology, and genetics. The availability of polymorphic DNA markers in *P. damicornis* can act as impetus for investigations into inheritance and population genetics, as well as novel investigations into host-symbiont ecology and evolution. Coral bleaching and gene flow studies performed with these markers can have direct conservation implications.

Keywords: connectivity, coral reef, gene flow, microsatellite, population genetics, Scleractinia

Received 2 July 2007; revision accepted 29 August 2007

The application of DNA markers containing short tandem repeats (STRs), colloquially referred to as 'microsatellites', is the most popular method currently used in molecular population genetics (DeSalle & Amato 2004). STR loci have been discovered in several Indo-Pacific coral species (Maier *et al*. 2001; Miller & Howard 2004; Underwood *et al*. 2005). Here we characterize 10 new STR loci in the cosmopolitan coral, *Pocillopora damicornis*. Common throughout the Indo-Pacific, *P. damicornis* has the widest distribution of any known scleractinian coral genus (the entire Indo-Pacific from the Red Sea to Central America). As such, it has become a model species for investigations into coral health, physiology, ecology, genetics and climate change response.

High molecular weight DNA was isolated from one individual of *P. damicornis* from Indonesia and one from Taiwan using the QIAGEN DNeasy Tissue Kit and digested with *Rsa*I. STR enrichment followed Glenn & Schable (2005) except for our choice of biotinylated oligonucleotide probes: $(AC)_{10}$ $(AG)_{10}$ $(ACC)_6$ $(AGG)_6$ $(AGC)_6$ $(ACG)_6$ $(CCG)_5$ $(AAG)_8$ $(AAT)_{10}$ $(ATC)_8$ $(ACT)_8$ and $(AAC)_8$ (Rob J. Toonen, Hawai'i Institute of Marine Biology, personal communication).

Correspondence: Craig J. Starger, E-mail: cjs52@columbia.edu

Cloning was performed with Invitrogen's TOPO TA cloning kit, One Shot TOP10 cells and imMedia agar plates following manufacturer's protocols. Positive clones were directly sequenced using the BigDye Terminator method (Applied Biosystems) on an ABI 3730xl. Sequences were edited in SEQUENCHER 4.6 (Gene Codes). The Simple Sequence Repeat Identification Tool (Temnykh *et al*. 2001) was used to search for STRs. Primers were designed for 20 putative loci using PRIMER 3 (Rozen & Skaletsky 2000) and checked for applicability to multiplex polymerase chain reaction (PCR) using AUTODIMER with a threshold score of 7 (Vallone & Butler 2004).

Genomic DNA was extracted from 21 individuals from Raja Ampat, West Papua, Indonesia (0°33.384′S, 130°40.681′E) using a modified Chelex protocol (Walsh *et al*. 1991). PCR amplifications for the markers Pd2-001 to Pd2-006 (10 μL total volume) contained 0.5 μL DNA template (*c*. 10 ng), 0.25 U *Taq* polymerase (Promega), 1 μL of 10× PCR buffer, 0.8 mM dNTPs, 2 mM $MgCl_2$ and 0.1 μM of each primer. The cycling protocol was: 1× 94 °C (3 min), 35× [1 min at 94 °C, 1 min 55 °C and 1 min at 74 °C] and 1× 74 °C (7 min). For markers Pd2–007 to Pd3-010, DNA template volumes were reduced to 0.1 μL for each 10 μL-reaction, and the primer concentrations were increased to 0.3 μM each. The cycling protocol was

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Table 1 *Pocillopora damicornis* loci were named following the format of Underwood *et al.* (2005): first the prefix Pd for the species name, then 2 or 3 according to repeat motif type (di- or trimer), followed by the number that they appear here. Twenty-one coral colonies were genotyped from Kri Island, Raja Ampat, Indonesia (0°33.384′S, 130°40.681′E). N_a refers to number of alleles. H_E and H_O refer to the expected and observed heterozygosities. GenBank Accession numbers are DQ684672–7 and EF120462–5. Departure from Hardy–Weinberg equilibrium is noted with an asterisk

Locus	Primer sequences (5′–3′)	T_a (°C)	STR motif	Dye	Size range (bp)	N_a	H_E	H_O	HWE (P value)
Pd2-001	CAGACTTGTCGGAATGAAAGC TTTTGTTTATAAGTCGATACAATGCA	55	$(CA)_{11}$	6FAM	158–173	5	0.650	0.600	0.27680
Pd3-002	ATCCGAATACAAGCGAAACG CAAAGCTTCTATCAGAAAATGCAA	55	$(AAC)_{10}$	NED	195–243	10	0.810	0.863	0.40686
Pd2-003	CCTCTTCCTGTTTGGGCTCT TCTGCATTACGTTTGTTTGACA	55	$(CA)_{16}$	VIC	198–202	3	0.333	0.475	0.36116
Pd3-004	ACCAGACAGAAACACGCACA GCAATGTGTAACAGAGGTGGAA	55	$(ATG)_{8}$	6FAM	193–201	4	0.444	0.705	0.05446
Pd3-005	AGAGTGTGGACAGCGAGGAT GTTCCTTCGCCTTCGATTTT	55	$(TGA)_{9}$	6FAM	162–187	3	0.450	0.376	1.00000
Pd2-006	ATCTCCATGTGATCGGCATT GTTCCCCCAGCTGAGAAGTT	55	$(CA)_{8}$	VIC	181–199	7	0.700	0.759	0.03282
Pd2-007	AAGAAGGTGTGGTATTTCAGAGGG GGTGGATAAAGTATTTCTCACTCTTGG	60	(AC) imperfect	HEX	307–489	7	0.762	0.770	0.00112*
Pd3-008	AGTTGAGGTTGTTGAAACATG TCCATGCAGAACCCC	60	$(CTG)_{7}$	6FAM	153–162	4	0.571	0.660	0.08876
Pd3-009	CCAATGCGTCCGTAGCTCTC ATCACCTAAAAATTTCAGTCCCTTACC	47	(CAA)7, [88 bp insert], $(GAG)_{6}$	6FAM	339–358	6	0.810	0.820	0.06195
Pd3-010	CTGATCAACAAACTGGGAGGC TCATTAGAAATCATCTTGATTTGATAAGG	47	$(GTT)_{5}$, $(TGC)_{11}$	6FAM	259–281	7	0.762	0.761	0.24938

modified as: 1× 94 °C (2 min), 30× [1 min at 94 °C, 20 s at the annealing temperature, 15 s at 74 °C] and 1× 72 °C (1 min).

Size fragment analysis was performed with fluorescent-labelled primers on an ABI 3730xl running GENEMAPPER 3.5 software. Results from all 10 markers are presented in Table 1. Hardy–Weinberg equilibrium was assessed using exact tests based on contingency tables in ARLEQUIN 3.11 (Excoffier *et al*. 2005). Only locus Pd2–007 showed a significant departure from equilibrium after Bonferroni correction. Linkage disequilibrium, assessed using contingency tables in ARLEQUIN 3.11, was only significant in two out of 90 pairwise comparisons at the $P < 0.01$ level (locus Pd2–003 vs. Pd3–004, and locus Pd2–006 vs. Pd3–009) indicating virtually no linkage among loci.

With 10 reliable STR loci available for *P. damicornis*, investigators may now address questions of inheritance, population genetics, and reproduction with greater power and precision. These markers will also facilitate investigations into symbiosis ecology, specifically the flexibility of the coral-algal symbiosis in response to climate change. Finally, gene flow estimates may be applied to source-sink population dynamics and the design of marine reserve networks.

Acknowledgements

We thank George Amato, Howard Rosenbaum and Matt Leslie for coordinating the Microsatellite Tutorial at the American Museum of Natural History, and Chien-Hsun Oscar Chen and Shang-Yin Vanson Liu for suggestions during the development. Funding was provided by NSF (Baker, DeSalle), the Sackler Institute for Comparative Genomics, the Wildlife Conservation Society, and National Science Council (Dai), Taiwan. Sample collection and export in Indonesia were facilitated by Paul Barber (Boston University) and the Indonesian Institute of Sciences (LIPI) under research permit # 2712/SU/KS/2005 and CITES export permit number # 07218/IV/SATS-LN/2006.

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