

# Optimization of 14 microsatellite loci in a Mediterranean demosponge subjected to population decimation, *Ircinia fasciculata*

Ana Riesgo · Gema Blasco · Patrick M. Erwin ·  
Rocío Pérez-Portela · Susanna López-Legentil

Received: 5 December 2013 / Accepted: 30 December 2013  
© Springer Science+Business Media Dordrecht 2014

**Abstract** The recovery potential of decimated populations of sponges will largely hinge on their populations' size retrieval and their connectivity with conspecifics in unaffected locations. Here, we report on the development of microsatellite markers for estimation of the population connectivity and bottleneck and inbreeding signals in a Mediterranean sponge suffering from disease outbreaks, *Ircinia fasciculata*. From the 220,876 sequences obtained by genomic pyrosequencing, we isolated 14 polymorphic microsatellite loci and assessed the allelic variation of loci in 24 individuals from 2 populations in the Northwestern Mediterranean. The allele number per locus ranged from 3 to 11, observed heterozygosity from 0.68 to 0.73, and expected heterozygosity from 0.667 to 0.68. No significant linkage disequilibrium between pairs of loci was detected. The 14 markers developed here will be valuable tools for conservation strategies across the distributional range of this species allowing the detection of populations with large genetic diversity loss and high levels of inbreeding.

**Keywords** Porifera · Conservation genetics · Pyrosequencing · Simple sequence repeats · Disease outbreaks · Inbreeding

---

A. Riesgo (✉) · G. Blasco · S. López-Legentil  
Department of Animal Biology, University of Barcelona,  
Barcelona, Spain  
e-mail: anariesgogil@gmail.com

P. M. Erwin · S. López-Legentil  
Department of Biology and Marine Biology, Center for Marine  
Science, University of North Carolina Wilmington, Wilmington,  
NC, USA

R. Pérez-Portela  
Center for Advanced Studies of Blanes (CEAB-CSIC), Girona,  
Spain

## Introduction

Sponges play an ecologically important role given their abundance and diversity as well as their contribution to primary production and nitrification through complex symbioses in marine benthic communities (Webster 2007). In the Mediterranean, massive mortalities are drastically reducing population size and creating extensive gaps in the distribution of many species, including sponges. In particular, periodic episodes of massive die-offs have been reported for the genus *Ircinia* (Cebrián et al. 2011) and the environmental stress due to elevated seawater temperatures has been suggested to trigger the disease (Maldonado et al. 2010; Cebrián et al. 2011).

Molecular markers have been widely applied in conservation biology to evaluate the vulnerability of marine species but studies on population genetics using microsatellite markers are available for very few sponges (see Pérez-Portela et al. 2013). Here, we report on the optimization of microsatellite markers further estimation of genetic diversity indexes, populations' connectivity, and for detecting signals of recent bottlenecks in order to evaluate the degree of vulnerability of the sponge *Ircinia fasciculata* from the Mediterranean.

## Methods

Sponge tissue was dissociated prior to DNA extraction and the bacterial symbionts removed by sequential centrifugations. Genomic DNA was extracted using DNeasy Tissue and Blood extraction kit (QIAGEN) to a final DNA concentration of 2 µg and distributed in two lanes of a plate. Pyrosequencing was performed on a Roche Life Science 454 GS-FLX System at the Scientific-Technical Services of

**Table 1** Characteristics of 14 microsatellite markers for *I. fasciculata*

Optimization details				Caials				Blanes							
Locus	F and R primers	Fluor.	Repeat motif	T <sup>n</sup>	Size range	N	Na	Ho/He	FIS	G-W	N	Na	Ho/He	FIS	G-W
3IFAS	CCAACAGAATTGGACATCTT GGAGGGAGTCTCTTTGAAGT	FAM -	(ATG)*6	55	150-190	12	4	0.917/0.685	-0.397	0.364	12	5	0.833/0.722	-0.154	0.356
8IFA	GCTTGGTCTGGAAGTCAGTA TCAAACCATGCATCAAAATTA	FAM -	(TG)*8	55	370-400	12	6	0.667/0.775	0.103	0.08	12	4	0.417/0.642	0.351	0.055
25IFAS	GCAGATAATCTTCCCATGGT GTCCCTATCCTTTTCAGGAG	NED -	(GTA)*6	55	120-160	10	4	0.900/0.658	-0.440	0.210	12	4	1.000/0.642	-0.557	0.364
40IFAS	GCAACCCCTGACAAATAATGT TGACAGGATGACGTTTAAGG	VIC -	(TAA)*9	55	250-280	12	3	0.833/0.648	-0.341	0.103	12	4	<b>0.917/0.688</b>	-0.333*	0.174
44IFAS	GTGTTTTGGGGTATTGCAG GACTGCTTCACCAATTTTGC	VIC -	(TAA)*10	56	160-230	12	4	0.417/0.373	-0.165	0.108	12	3	0.333/0.292	-0.143	0.231
4HIFAS	GAAGGCATTTAAGCTCAGGT GCCCCCTGGTAACTATACAT	FAM -	(CCA)*9	60	268-308	12	3	0.333/0.304	-0.143	0.111	12	3	0.667/0.469	-0.422	0.073
42IFAS	TTTTGGTCACAACTGCACACA GCACACGCATGCACACATA	NED -	(GT)*28	70	200-300	12	3	<b>1.000/0.561</b>	-0.858**	0.049	12	5	0.750/0.542	-0.385	0.238
10IFAS	GTGCAATGGGATAAAACAT CACCACCAATAGTATCATGC	FAM -	(TA)*19	52	97-172	12	8	0.667/0.779	0.107	0.066	12	9	0.583/0.823	0.291	0.113
49IFAS	TCCTTTCCACTTCCCTCCC ACACTTGGCTTAGCTCCCTGT	FAM -	(TA)*9	60	186-240	12	6	<b>0.667/0.721</b>	0.035*	0.353	12	5	0.417/0.361	-0.154	0.217
22IFAS	TGTTGCTTGGCATTTTAGTA CAGGGGCTAATAGTGTATGC	VIC -	(TAG)*18	52	136-361	12	11	1.000/0.917	-0.138	0.229	12	8	<b>0.667/0.819</b>	0.186*	0.32
7IFAS	TACATCTCCGGATATTGGTG CTAAGCTGAGCTGTGTTTCC	VIC -	(TAA)*6	55	170-310	12	6	0.583/0.754	0.192	0.043	12	5	0.582/0.628	0.072	0.046
47IFAS	TGGACCAAACTGTGCTATCG GCCACCTTATCCTTGCAG	NED -	(TACA)*19	58/60	177-229	12	7	<b>0.667/0.833</b>	0.165*	0.079	12	6	0.750/0.726	-0.033	0.352
48IFAS	TAGTTGGCTGGGTATGGCAG CGTTATGTGCCACTACTCGC	FAM -	(ATTG)*6	58/60	136-161	11	5	0.909/0.662	-0.438	0.192	12	7	0.750/0.740	-0.014	0.2
36IFAS	TCCGATTTCAACATCATC TCTTTCATTTTGTGACATGC	FAM -	(TACA)*17	56	190-320	12	9	0.667/0.873	0.203	0.141	12	12	0.917/0.865	-0.06	0.245
Total								0.730/0.682	-0.15	0.15 ± 0.1			0.684/0.667	-0.5	0.21 ± 0.1

N sample size, NA the number of alleles per locus, HE expected heterozygosity, HO observed heterozygosity, FIS inbreeding coefficient, G-W Garza-Williamson index

\*,\*\* Significant deviation from HWE after application of Narum correction (P < 0.05). Locus showing significant excess of heterozygotes are shown in bold letters

the University of Barcelona. Sequences (220,876) were searched for perfect microsatellites (di-, tri-, tetra- and penta-nucleotides) with at least eight repeats and enough priming regions with Phobos ([http://www.rub.de/spezzoo/cm/cm\\_phobos.htm](http://www.rub.de/spezzoo/cm/cm_phobos.htm)). 40,109 sequences contained microsatellites, 61.7 % being dinucleotide, 3.4 % trinucleotide, 3.4 % tetranucleotide, and 0.7 % pentanucleotide. Thirty-six primer sets were designed with the software PRIMER 3 for 14 dinucleotide loci, 14 trinucleotide loci, 7 tetranucleotide loci, and 1 pentanucleotide locus.

Amplification success and polymorphism were tested in two populations of the NW Mediterranean (Costa Brava) collected in 2010–2012: Els Caials (42°17'19"N 3°16'40'E) and Blanes (41°41'N 2°48'E). Total DNA was extracted and amplified using the REDEExtract-N-Amp Tissue PCR Kit (Sigma Aldrich). Forward primers were labeled with a fluorescent dye (Table 1). Samples were amplified on a PCR System 9700 (Applied Biosystems) with an initial 2 min denaturation step at 95 °C; followed by 35–40 cycles of 95 °C for 30 s, 52–70 °C for 35 s (depending on each locus; Table 1) and 72 °C for 15 s, followed by a 3 min final extension at 72 °C. Amplification products were analyzed on an Applied Biosystems 3730xl Genetic Analyzer at the Scientific-Technical Services of the University of Barcelona. The length and allele scoring of PCR products was estimated relative to the internal size standard GeneScan 600LIZ using the software PEAKS-CANNER v1.0 (Applied Biosystems).

Linkage disequilibrium, number of alleles per loci and population, observed heterozygosity ( $H_o$ ), and expected heterozygosity ( $H_e$ ) were calculated with GenAlEx (<http://biology.anu.edu.au/GenAlEx/Welcome.html>) and ARLEQUIN c 3.5.1.2 (Excoffier and Lischer 2010). Genetic diversity [Garza–Williamson (G–W) index] and inbreeding coefficients ( $F_{IS}$ ) were calculated using ARLEQUIN c 3.5.1.2 and GenPop (<http://genepop.curtin.edu.au/>). The exact test for departure from Hardy–Weinberg Equilibrium (HWE) was also performed with the same software. Narum ( $P < 0.05$ ) corrections of the  $P$  values for multiple tests were applied.

## Results and discussion

Out of the 36 microsatellite loci attempted, a total of 14 polymorphic microsatellite were optimized, including a selection of different microsatellite types (see Table 1) because different microsatellite types are equally valid to assess genetic diversity and populations structure of marine

invertebrates. No evidence of linkage disequilibrium was detected across all pairwise comparisons. Failed amplifications due to presence of null alleles were detected in 2 loci of the Caials population (Table 1). Five markers showed Hardy–Weinberg disequilibrium after Narum corrections but the overall populations were in HWE (Table 1). Heterozygosity deficit was observed in 5 loci (3 loci in the Caials population and two in the Blanes population; Table 1). The average gene diversity over 14 loci using the Tajima index was  $0.684 \pm 0.367$  in Caials, and  $0.668 \pm 0.354$  in Blanes. Using the genetic G–W index (Garza and Williamson 2001), it seems that all markers were indicating bottleneck events in both populations (Table 1), but more populations should be sequenced for further confirmation. The application of the microsatellite markers developed herein to additional *I. fasciculata* populations will allow to understand how the genetic diversity is distributed in this species, and to know its overall status, identifying hotspots of genetic diversity, populations affected by large genetic diversity loss and high levels of inbreeding. Information on population genetics of the species is crucial for the assessment of ecological threats and recovery potential following disease episodes and population decimation, and the developing of management strategies when necessary.

**Acknowledgments** Dr. Xavier Turon (CEAB-CSIC) helped with some of the sampling. This study was funded by the Spanish Government project SOLID CTM2010-17755, and the Catalan Government Grant 2009SGR-484 for Consolidated Research Groups and Juan de la Cierva contracts to AR and RPP.

## References

- Cebrián E, Uriz MJ, Garrabou J, Ballesteros E (2011) Sponge mass mortalities in a warming Mediterranean Sea: are cyanobacteria-harboring species worse off? *PLoS ONE* 6(6):e20211
- Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Res* 10(3):564–567
- Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Mol Ecol* 10(2):305–318
- Maldonado M, Sánchez-Tocino L, Navarro C (2010) Recurrent disease outbreaks in corneous demosponges of the genus *Ircinia*: epidemic incidence and defense mechanisms. *Mar Biol* 157(7):1577–1590
- Pérez-Portela R, Noyer C, Becerro M (2013) Genetic structure and diversity of the endangered bath sponge *Spongia lamella*. *Aquatic Conserv: Mar Freshw Ecosyst* doi:10.1002/aqc.2423
- Webster NS (2007) Sponge disease: a global threat? *Environ Microbiol* 9(6):1363–1375