Conservation Genet Resour (2012) 4:175–177
DOI 10.1007/s12686-011-9502-8

TECHNICAL NOTE

Characterization of 12 microsatellite loci in the waterfall damselfly (*Paraphlebia zoe*) for use in population genetic applications

Christopher N. Anderson · Gregory F. Grether · Alex Cordoba-Aguilar

Received: 29 July 2011 / Accepted: 26 August 2011 / Published online: 11 September 2011

Abstract The waterfall damselfly, *Paraphlebia zoe*, is distributed in cloud forest areas in the Mexican states of Veracruz, Hidalgo, and San Luis Potosi. We developed twelve microsatellite loci for *P. zoe* from representative samples from the state of Veracruz. Microsatellites were tested for polymorphism on a panel of 24 individuals. The number of alleles ranged from 3 to 11, observed heterozygosity from 0.083 to 0.875, and the fixation index from 0.021 to 0.563. These loci are the first to be described and characterized for *P. zoe* and should prove useful for population genetics in support of the conservation of this vulnerable species.

Keywords Microsatellite · Waterfall damselfly · *Paraphlebia zoe*

The territorial damselfly, *Paraphlebia zoe* has been used as a model system to investigate alternative reproductive tactics (Romo-Beltran et al. 2009; Munguia-Steyer et al. 2010). *P. zoe* has two male morphs that differ in sexual behavior: wing pigmented males which defend territories and hyaline winged males which, when pigmented males are present, assume a nonterritorial tactic. Published records document *P. zoe* in cloud forest areas in the Mexican states of San Luis Potosi, Hidalgo, and Veracruz (González-Soriano and Novelo-Gutiérrez 2007) Some *P. zoe* populations have gone extinct locally or reduced in size due mainly to habitat loss in the Mexican state of Veracruz (Córdoba-Aguilar, unpub. data). One possible reason for this situation is that the places *P. zoe* larvae inhabit (muddy riparian zones under deep canopy and next to large rivers) may affect larval survival when there is a decrease in humidity due to deforestation. This species is listed as "Vulnerable" in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species. In support of conservation and management efforts for the waterfall damselfly, the Cordoba-Aguilar laboratory has initiated a study to evaluate the degree of population subdivision, migration rates and geneflow between populations, and capability/accuracy of population assignment.

Here we describe the isolation and characterization of 12 novel tetranucleotide microsatellite loci from *P. zoe* as a population genetics characterization resource. Individuals were obtained from riparian sites near 3 towns in central Veracruz: La Gloria (19.353 N 96.996 W), Coscomatepec (19.100 N 97.033 W), and Tlapacoyan (19.925 N 97.218 W). Insects were captured with aerial nets, stored in 95% ethanol, and then refrigerated at −80 C until analysis. DNA was extracted from wing muscle using the Qiagen QIAamp DNA Mini Kit using the manufacturer's protocol. 500 ng of DNA was prepared for whole genome shotgun sequencing on the Roche Genome Sequencer FLX instrument using the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Sciences, Indianapolis, USA) following the manufacturer's protocol. The library was quantified for DNA fragment size distribution and concentration (Agilent 2100 Bioanalyzer) and then processed with the GS FLX emulsion polymerase chain reaction

C. N. Anderson (✉) · A. Cordoba-Aguilar
Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Apdo, Postal 70-275, 04510 Mexico, DF, Mexico
e-mail: cnanderson1980@gmail.com

G. F. Grether
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 621 Charles E. Young Drive South, Los Angeles, CA 90095-1606, USA

Table 1 Characterization of 12 microsatellite loci for *Paraphlebia zoe*

Locus	Forward primer 5′-3′	Reverse Primer 5′-3′	Genbank		Size (bp)	A			
			Motif	Accession #			H_O	H_E	F_{IS}
Marker 1	GGGCTGCAGTCAACCAAATC	AGTGGCTTAAATATACCCTCAATGTC	GAAT	JN653058	165–188	3	0.875	0.609	0.021
Marker 2	CGAAGGAAGAGATGTGCGG	CCAGCAAACGGGCAGATG	CACG	JN653059	174–296	11	0.458	0.839	0.263
Marker 3	GTGCCCGAATGCGAAGTG	CGTCCCGATCTACCCAAGG	GAGG	JN653060	166–180	7	0.250	0.549	0.431
Marker 5	CCTCTCCAATGCGTTGCTC	ACTCGATGACACGCCTTCC	ACGC	JN653061	125–416	5	0.250	0.323	0.151
Marker 7	AGCTTTCTGATGTCCGAGC	GTTCACGGCCAAGAGTTGC	TCCT	JN653062	124–354	7	0.208	0.461	0.289
Marker 11	GCTTCTGTCGCGTTAGTGG	TCTAAACGCACAACATCGGC	GGGT	JN653063	136–204	5	0.083	0.684	0.558
Marker 101	GGAGCGTAGCACATGGTTC	ACCACTCGTCATGGCATCC	GAAG	JN653064	161–344	7	0.250	0.592	0.327
Marker 102	CGGAATTAGAGCCGTGCTTG	AGAATTAGATGGCGACGGG	ACAT	JN653065	197–280	5	0.292	0.576	0.563
Marker 103	GCCTTGTAAGACGGTGTCC	AGTGGGATGTGTTGAAGGCTG	ACAT	JN653066	430–467	4	0.083	0.194	0.134
Marker 106	GCACAGATGATTCAGGCGG	TGCCTCATCCCTAACGGTC	GTAT	JN653067	347–387	10	0.417	0.809	0.464
Marker 107	GAATGCACATCCGTCCTGC	TGTCGAAGCCATCGTGAGG	CATA	JN653068	197–450	3	0.250	0.379	0.127
Marker 110	TTAGCCTGAGCCACACTGC	ATTGTGTTGTCCCAAAGAGC	AGAT	JN653069	312–315	3	0.167	0.531	0.700

Size includes 16 bp M13F(-20) tag, *A* Number of alleles, H_O Observed heterozygosity, H_E Expected heterozygosity, F_{IS} Fixation indices

(PCR) and sequencing kits. Sequencing was performed using 1/16th of a picotiterplate and yielded 41,217 sequences.

The sequences were screened for potential microsatellite loci by MSATCOMMANDER (Faircloth 2008) under the default settings. Of the 41,217 sequences, 1,891 contained putative microsatellite loci. Primers for tetranucleotide (minimum repeat number = 4) were designed by PRIMER3 software (Rozen and Skaletsky 2000) embedded in MSATCOMMANDER using default settings. In total, 24 tetranucleotide primer pairs were used for amplification trials. The 12 loci that amplified cleanly and were polymorphic in a panel of 8 individuals were used for characterization across a sample set of 24 individuals from three different sampling sites encompassing the distribution of *P. zoe* in central Veracruz State, Mexico.

Fragments were amplified by means of the M13-hybrid primer process. In this process, a 16-bp fragment is added to the 5′ end of the forward primer for binding of the dye-labeled M13-hybrid primer (Boutin-Ganach et al. 2001; Schuelke 2000). Primer mixes were prepared as follows: 2 µl reverse primer (100 µM); 4 µl forward primer (2.5 µM); 4 µl 6FAM dye-labeled M13-hybrid primer (2.5 µM); 90 µl RNAse/DNAse-free water. Amplification was carried out in 10 µl reactions containing 5 µl Qiagen Multiplex Mastermix, 0.5 µl BSA (10 mg/ml), 1 µl primer mix, 2 µl RNAse/DNAse-free water, and 1.5 µl template DNA (30–100 ng in total). The following cycling conditions were used: an initial step of 95°C for 15 min; 25 cycles of: 30 s at 94°C, 90 s at 59°C, 60 s at 72°C; 20 cycles of: 30 s at 94°C, 90 s at 53°C, 60 s at 72°C; and 30 min at 60°C. PCR products were run on an ABI 3730XL capillary sequencer, and allele sizes were scored manually using GS 500-LIZ size standard in GeneMapper v3.7 genotyping software (ABI).

Tests for deviations from Hardy–Weinberg equilibrium (HWE) were carried out in GENALEX v6.4 (Peakall and Smouse 2006) and tests for linkage disequilibrium (LD) were performed in GENEPOP 4.0 (Rousset 2008) for each of the 12 loci and three populations (24 individuals), with Bonferroni correction applied for multiple comparisons. After Bonferroni correction for multiple comparisons, no consistent departures from HWE were detected across populations ($P > 0.05$). LD was suggested for loci 1 and 101 in Population 2071 ($P = 0.033$) and for loci 102 and 107 in population 2091 ($P = 0.040$). The number of alleles for the sample set of all 24 samples ranged from 3 to 11 (Table 1). Calculations of observed (H_O) and expected heterozygosity (H_E) and fixation indices (F_{IS}) were carried out in GENALEX v6.4 and results are shown in Table 1. Observed and expected heterozygosities ranged from 0.241 to 0.933 and from 0.596 to 0.930, respectively. Finally, F_{IS} ranged between −0.097 and 0.730. The microsatellites

developed here should prove a useful resource in support of management and conservation of *P. zoe*.

Acknowledgments John Pollinger and the UCLA Conservation Genetic Resources center provided logistical support and helpful comments on the manuscript. John McCormack also provided helpful comments on the analysis and manuscript. CNA was supported by a CONACyT postdoctoral fellowship and a UCMEXUS-CONACyT postdoctoral fellowship during this project. This project was supported by UCMEXUS-CONACyT collaborative grant CN-10-402 to GFG and ACA.

References

Boutin-Ganach I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improved the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechnic 31:24–28

E González-Soriano and R Novelo-Gutiérrez (2007) Odonata of Mexico: revisited. In: Tyagi BK (ed) Proceedings of Odonata: biology of Dragonflies. Sci Publ. (India) pp 105–136

Faircloth BC (2008) MSATCOMMANDER: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94

Munguia-Steyer R, Cordoba-Aguilar A, Romo-Beltran A (2010) Do individuals in better condition survive for longer? Field survival estimates according to male alternative reproductive tactics and sex. J Evol Biol 23:175–184

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Romo-Beltran A, Macias-Ordonez R, Cordoba-Aguilar A (2009) Male dimorphism, territoriality and mating success in the tropical damselfly, *Paraphlebia zoe* Selys (Odonata: Megapodagrionidae). Evol Ecol 23:699–709

Rousset F (2008) GENEPOP '007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Res 8:103–106

Rozen S, Skaletsky HJ (2000) PRIMER3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics methods and protocols: methods in molecular biology. Humana Press, Totowa, pp 365–386

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nature Biotech 18:233–234