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Isolation and characterization of 20 microsatellite loci for the saltmarsh plant *Triglochin maritima* L.

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Abstract Twenty microsatellite markers were developed for the polyploid plant *Triglochin maritima* L., an important component of declining saltmarsh ecosystems that are now subject to much restoration effort. All loci were polymorphic when tested across 24 individuals from three populations. The average number of alleles per population was 6, ranging from 2 to 12. Private alleles were identified in each population, demonstrating the utility of these markers for the investigation of the population genetic structure and diversity of this species.

Keywords 454 Sequencing · Polyploid · Saltmarsh · Restoration · Halophyte

The halophytic *Triglochin maritima* L. (Davy and Bishop 1991) is a major component of European saltmarsh communities, which are under significant threat from changes in sea level and land use. In Europe, AFLP molecular marker data have shown that individuals of *T. maritima* are derived from two principal lineages originating from past glacial refugia which have not extensively intermixed until the present day (Lambracht et al. 2007). However, the process by which this phylogeographic structure has been maintained is not well understood, especially since this species is wind pollinated and water dispersed, which should enable extensive gene flow. Given the increasing implementation of saltmarsh restoration programs, the determination of the population structure and diversity of this species will provide important information for conservation and restoration management. Here we describe 20 new microsatellite loci for *T. maritima*, which will enable investigation of the population genetic characteristics of this species throughout its distribution.

Leaves were collected from four different individuals from three populations (Lepe: 50°47′N, 1°21′W; Nigg Bay: 57°44′N, 4°2′W; Brancaster: 52°58′N, 0°37′E). Genomic DNA was extracted using ISOLATE Plant DNA Mini Kit (Bioline) following the manufacturer's instructions. An equimolar DNA solution (total DNA 2 μg) combining DNA from all individuals was used in the preparation of the microsatellite enriched library and sequencing using commercial services provided by Genoscreen (Lille, France) using 454 GS FLX Titanium (Roche Applied Science). 30,817 sequences were obtained and analysed with QDD2, using default parameters to detect microsatellite loci (Meglécz et al. 2010). The obtained candidate loci were then selected following the method presented by Lepais and Bacles (2011), selecting "A" or "B" calibre primers with uninterrupted microsatellite repeats and the lowest penalty score. The sequence data of 48 loci tested for this publication were submitted to Genbank, accession numbers KF147933–KF147980.

Simplex PCR reactions for each selected primer pair were conducted using the "M13 tail" protocol designed by Schuelke (2000). Reactions were carried out in a final volume of 10 μl with 1X of Type-it Multiplex PCR Master Mix (Qiagen), 0.05 μM of M13-forward primer, 1 μM of reverse primer and between 10 and 40 ng of template DNA. The PCR cycle proceeded according to 5 min at 95 °C followed by 32 cycles of (30 s at 95 °C, 90 s at 58 °C, 30 s at 72 °C), 1 μM of M13-sequence oligonucleotide tagged with either

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FAM, HEX, TAMRA or ATTO565 was then added and the PCR continued with 8 cycles of (30 s at 94 °C, 45 s at 53 °C, 45 s at 72 °C), and a final extension step of 10 min at 72 °C. PCR products were analysed by DNA Sequencing and Services, Dundee, UK, using a 3730 DNA Sequencer (Applied Biosystems) with reference to a LIZ 500 size standard. Only primers showing clear and replicable patterns were selected to be included into multiplexes. Twenty loci were then combined into 3 different multiplex PCR combinations (Electronic supplementary material, Table 1).

Multiplex PCR was performed in a final volume of 10 µl with 1X Type-it Multiplex PCR Master Mix (Qiagen), 0.5X Q solution (Qiagen) for multiplex 1 and 3, variable concentration of fluorescently labelled forward and reverse primer (Electronic supplementary material, Table 1) and between 10 and 40 ng of template DNA. The PCR conditions were 5 min at 95 °C followed by 32 cycles of (30 s at 95 °C, 90 s at 62 °C, 30 s at 72 °C) and a final extension of 60 °C for 30 min.

Triglochin maritima is a polyploid species having variable ploidy level but being typically octoploid (Davy and Bishop 1991). Therefore, classic statistics such as deviation from Hardy–Weinberg equilibrium could not be calculated due to uncertainty concerning allelic dosage. Number of alleles per locus, and number of alleles per individual were calculated manually. Number of private alleles was calculated using a custom script using the R programming language and available from the authors on request.

The average number of alleles per locus was 6, ranging from 2 to 12. For all loci, the maximum number of alleles per locus per individual was never more than 4. The number of private alleles within populations across all loci ranged from 10 to 13 indicating that these markers will be useful to discriminate populations in further genetic studies (Electronic supplementary material, Table 2). These 20 microsatellite markers will, therefore, provide a valuable tool to study the population genetics of this species throughout its range.

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