Pagination not final/Pagination non finale

Genome 52: 1037-1039, 2009

TECHNIQUES / TECHNIQUES

Isolation and characterization of microsatellite loci for *Euphorbia palustris* (Euphorbiaceae)

Walter Durka

Abstract: Swamp spurge (*Euphorbia palustris*, Euphorbiaceae) is a large perennial species of wet grassland and swamps. Its natural habitats are fragmented and isolated both naturally and owing to habitat destruction by human activity. Thus the species is endangered and legally protected in Germany. This report describes seven novel polymorphic microsatellite loci that will be helpful to characterize genetic variation and to analyze the population genetic structure and levels of gene flow within and among populations. All loci were amplified within one multiplex polymerase chain reaction for two populations, yielding between 3 and 13 alleles per locus and high levels of heterozygosity. Trans-species amplification is reported for four *Euphorbia* species.

Key words: swamp spurge, wetlands, microsatellites, *Euphorbia esula*.

Résumé : L'euphorbe des marais (*Euphorbia palustris*, Euphorbiacées) est une espèce pérenne de grande taille qu'on retrouve dans les prairies humides et les marais. Ses habitats naturels sont fragmentés et isolés tant naturellement que suite à la destruction des habitats par les humains. Ainsi, cette espèce est menacée et bénéficie du statut d'espèce protégée en Allemagne. Les auteurs ont développé sept nouveaux marqueurs microsatellites qui seront utiles pour caractériser la variation génétique et pour analyser la structure génétique au sein des populations de même que les flux géniques parmi et entre ces populations. Tous les locus ont été amplifiés lors d'une seule réaction en chaîne à la polymérase multiplexe pour deux populations, produisant entre 3 et 13 allèles par locus et des niveaux élevés d'hétérozygotie. La transportabilité de l'amplification sur d'autres espèces est rapportée pour quatre autres espèces du genre *Euphorbia*.

Mots-clés : euphorbe des marais, tourbières, microsatellites, *Euphorbia esula*.

[Traduit par la Rédaction]

Introduction

Swamp spurge (*Euphorbia palustris* L.) is a diploid (2n = 20) member of the Euphorbiaceae (subgenus *Esula*, section *Helioscopia*) native to Europe. It is a conspicuous perennial herb up to 2 m tall and occurs in wet grassland and swamps. In Central Europe the species shows the peculiar distribution pattern of a "river corridor species" as it is mostly restricted to the floodplains of large rivers (Burkart 2001; Michalski and Durka 2007). Here, the species is typical for habitats that harbor a number of other rare plant species restricted to wet alluvial grasslands. The species is sensitive to mowing and is threatened in Germany mainly because of drainage and agricultural intensification of wet grasslands. It is legally protected and listed as endangered in Germany (Korneck et al. 1996). Although the species has many flowers and sets seed, seedlings and young juvenile plants seem to be rare. Thus, it would be highly desirable to analyze both the breeding system and the spatial genetic structure both within and among river systems using a codominant marker system.

Materials and methods

Leaf samples were collected from 5 individuals in a native population of swamp spurge near Halle, Germany. Total genomic DNA was extracted using DNeasy kits (QIAGEN). A library was made by ecogenics GmbH (Zurich, Switzerland) from size-selected genomic DNA ligated into TSPAD-linker (Tenzer et al. 1999) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GA)_{13}$ oligonucleotide repeats (Gautschi et al. 2000*a*, 2000*b*). Of 384 recombinant colonies screened, 90 gave a positive signal after hybridization and 70 plasmids from positive clones were sequenced. For 17 putative loci that had between 18 and 26 dinucleotide repeats in the sequenced clone, primers were designed using Primer3 (Rozen and Skaletsky 2000) and tested for polymorphism. Sequences of clones for the

Received 18 August 2009. Accepted 7 September 2009. Published on the NRC Research Press Web site at genome.nrc.ca on x November 2009.

Corresponding Editor: B. Golding.

W. Durka. Helmholtz Centre for Environmental Research (UFZ), Department of Community Ecology (BZF), Theodor-Lieser-Strasse 4, D-06110 Halle, Germany (e-mail: walter.durka@ufz.de).

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Table 1. Description of the microsatellite loci in *Euphorbia palustris* and results obtained in two populations, including observed (H_o) and expected (H_e) heterozygosity and inbreeding coefficients (*, $p < 0.05$).

					Halle (n = 96)			Bremen (n = 26)			No. of alleles		
Locus	Repeat motif	Primer sequences (5′–3′)	Dye	Size range (bp)	H_o	H_e	F_{IS}	H_o	H_e	F_{IS}	Halle	Bremen	Total
Ep48a	$(GA)_{18}$	F: CAGACCAGTTTCCGATTTAGAG R: GGGTCCAAGTGAACATTACTCC	FAM	86–96	0.542	0.568	0.046	0.577	0.611	0.055	3	3	3
Ep75	$(TC)_{26}$	F: TGTACGCCTCTCTCTCACTCC R: TGACGTCGAATTTGTAGTTGC	NED	70–116	0.885	0.735	–0.205*	1.000	0.827	–0.209*	8	8	11
Ep88	$(CT)_{17}$	F: TGGTCTTCAAATGATATGTGAGATT R: AGGTGTCGTCTCTGTGTTGTG	PET	76–88	0.583	0.530	–0.101	0.654	0.595	–0.098	4	4	6
Ep05	$(GA)_{25}$	F: AAAGCCCACTACGCAACAAG R: AAAACACTCCGACGGTCAAG	VIC	94–136	0.865	0.791	–0.093*	0.885	0.718	–0.233*	7	6	13
Ep18	$(GA)_{18}$	F: GGAAGAAGAAGAGAGGGATTGG R: TCCGGTCCTAACACAACACTGC	FAM	283–291	0.260	0.460	0.410*	0.038	0.180	0.786*	2	2	4
Ep29	$(TC)_{24}$	F: GGGTTTTGTGATTCTGAAGG R: GAACTGGTGTCAAATTCATCTATCC	NED	279–309	0.521	0.551	0.045	0.692	0.648	–0.069	3	7	8
Ep61	$(GA)_{13}GG(GA)_7$	F: CAGATTCCAGAAAATCAACAGC R: CGTCGTCTTCATTTCTGTCC	PET	158–198	0.646	0.605	–0.067	0.769	0.683	–0.126	5	7	10

markers reported were deposited in GenBank with accession Nos. FN386451–FN386457.

Levels of variation were determined among individuals from two German populations of approximately 100 plants each near the cities of Halle (n = 96) and Bremen (n = 26). Multiplex PCR was performed in 10 μL reaction volumes containing 10 ng of genomic DNA, 5 μL of 2× Multiplex PCR Master Mix (QIAGEN), and fluorescently labelled forward and unlabelled reverse primers: Ep48a (labelled with FAM; 2 pmol), Ep18 (FAM; 4 pmol), Ep05 (VIC; 1 pmol), Ep75 (NED; 1 pmol), Ep29 (NED; 4 pmol), Ep88 (PET; 6 pmol), and Ep61 (PET; 4 pmol). A Mastercycler gradient thermocycler (Eppendorf, Germany) was used to run the following hotstart PCR program: 95 °C for 15 min, followed by 25 cycles of 92 °C for 15 s, 60 °C for 90 s, and 72 °C for 60 s, followed by 72 °C for 10 min. Fragments were separated on an ABI 3100 genetic analyzer (Applied Biosystems) with the internal size standard GeneScan 500-LIZ and genotyped with GeneMapper 3.7. Expected and observed heterozygosity was determined using MSA software (Dieringer and Schlötterer 2003). Inbreeding coefficients were calculated and departure from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were tested in FSTAT v. 2.9.3 (Goudet 2001) by randomization procedures.

Results and discussion

Seven of 17 primer pairs gave reproducible PCR products that could be interpreted in a codominant diploid way (Table 1). Several other primers showed multi-banded patterns suggesting duplication of respective loci (not shown). The total number of alleles per locus ranged from 3 (Ep48a) to 13 (Ep05). For population Halle, observed and expected heterozygosity ranged across loci from 0.260 to 0.885 (mean H_o = 0.616) and from 0.460 to 0.791 (mean H_e = 0.606), respectively. For population Bremen, observed and expected heterozygosity ranged from 0.038 to 1.000 (mean H_o = 0.659) and from 0.180 to 0.827 (mean H_e = 0.609), respectively. Significant deviations from HWE ($p <$ 0.05) and an excess of homozygotes were detected for locus Ep18 in both populations. This is likely due to the presence of null alleles, as indicated by the failure of amplification in some individuals at this locus. However, PCR failure for other reasons cannot be excluded. An excess of heterozygosity was found in loci Ep75 and Ep05, which may be due to obligate outcrossing due to the self-incompatible breeding system of *E. palustris* (unpublished data). Linkage disequilibrium was observed between 8 of 21 pairs of loci ($p < 0.05$: Ep05 × Ep48a, Ep05 × Ep61, Ep29 × Ep48a, Ep29 × Ep61, Ep48a × Ep61, Ep61 × Ep75, Ep61 × Ep88, Ep75 × Ep88). These deviations might result from physical linkage, null alleles, nonrandom sampling, or biparental inbreeding.

Trans-species amplification was tested in other congeneric species (Table 2): *E. esula*, a European species invasive in North America, and *E. lucida*, *E. cyparissias* (subgenus *Esula*, section *Esula*), and *E. seguieriana* (subgenus *Esula*, section *Paralias*). While amplification was successful for all markers in *E. esula* and *E. lucida*, only one marker produced bands in both *E. cyparissias* and *E. seguieriana*. However, homozygous null alleles were found across most species and loci.

Table 2. Trans-species amplification of microsatellite loci of *E. palustris* in *E. esula*, *E. lucida*, *E. seguieriana*, and *E. cyparissias*: size range and number of alleles.

	E. esula (*n* = 24)		*E. lucida* (*n* = 28)		*E. seguieriana* (*n* = 5)		*E. cyparissias* (*n* = 5)	
	Size range	Alleles	Size range	Alleles	Size range	Alleles	Size range	Alleles
Ep48a	87–97	3 (N)	87–103	7 (N)	—	0	—	0
Ep75	71–93	7 (N)	71–93	9	—	0	—	0
Ep88	76–92	7 (N)	74–100	6 (N)	—	0	—	0
Ep05	94–126	5	94–126	14	96–116	3 (N)	96–126	4 (N)
Ep18	284–290	4 (N)	282–294	7 (N)	—	0	—	0
Ep29	280–292	6 (N)	276–312	9 (N)	—	0	—	0
Ep61	163–189	9 (N)	159–195	10 (N)	—	0	—	0

Note: N, lack of PCR product, indicating the presence of homozygous null alleles.

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