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Authors: Bossu, Angèle, Bertaudière-Montès, Valérie, Dubut, Vincent, and Manel, Stéphanie

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Primer Note

Microsatellite primers in *Parietaria judaica* (Urticaceae) to assess genetic diversity and structure in urban landscapes[1]

Angèle Bossu[2,5], Valérie Bertaudière-Montès[2], Vincent Dubut[3], and Stéphanie Manel[2,4]

[2]Aix Marseille Université, Institut de recherche pour le développement (IRD), Laboratoire Population Environnement Développement (LPED) UMR_D151, 13331 Marseille, France; [3]Aix Marseille Université, Centre national de la recherche scientifique (CNRS), IRD, Univ. Avignon, IMBE—UMR7263, 13331 Marseille CEDEX 3, France; and [4]Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), UMR AMAP, TA A51/PS2, 34398 Montpellier CEDEX 5, France

- *Premise of the study:* Urbanization is one of the main factors contributing to loss of genetic diversity, as the resulting landscape fragmentation and habitat loss induce species isolation. However, studies of genetic structure and diversity in urbanized landscapes are still rare. We characterized microsatellite primers for *Parietaria judaica* to study this environment.
- *Methods and Results:* Eleven microsatellite loci from *P. judaica*, an urban plant, were isolated using shotgun pyrosequencing, and the simple sequence repeat (SSR) markers were screened in 20 individuals of *P. judaica*. The loci were tested on 166 individuals from three populations in different cities. The number of alleles ranged from two to 19, and expected and observed heterozygosity values ranged from 0.019 to 0.912 and 0.019 to 0.448, respectively.
- *Conclusions:* The markers amplified well in the species and will be useful for examining genetic diversity and population genetic structure in this urban plant.

Key words: microsatellites; *Parietaria judaica*; urban area; Urticaceae.

Parietaria judaica L. is a perennial herb, with individual plants consisting of many shoots emerging from a common rootstock. The plant is a wind-pollinated species of the Urticaceae family that grows in urban Mediterranean areas (Fotiou et al., 2011) and is very common on urban walls. This wide occurrence will facilitate studies of genetic diversity and genetic structure in urban environments. While urbanization is one of the main factors contributing to loss of genetic biodiversity, genetic patterns in urban areas have been understudied (Manel and Holderegger, 2013). In the current study, we have developed and characterized 11 microsatellite markers for *P. judaica* that will be used for further studies to examine genetic diversity of plants in urban environments.

METHODS AND RESULTS

Twenty individuals of *P. judaica* were collected in Marseilles, France (43.296346°N, 5.369889°E; voucher specimen available at the Herbarium of Muséum d'Histoire Naturelle d'Aix-en-Provence [AIX], barcode number AIX036092). Samples were sent to Genoscreen (Lille, France) to develop and characterize microsatellites. Total genomic DNA was isolated from individuals' leaf tissues using NucleoSpin Plant II (Macherey-Nagel, Duren, Germany). A total of 1 µg of DNA was used for the development of microsatellite libraries through 454 GS FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011). Total DNA was enriched for TG, TC, AAC, AAG, AGG, ACG, ACAT, and ACTC repeat motifs and subsequently amplified. PCR products were purified, quantified, and GS FLX libraries were then constructed following the manufacturer's protocols (Roche Diagnostics, Meylan, France) and sequenced on a GS FLX PicoTiterPlate Kit (Roche Diagnostics). Sequences with a minimum of five repeats were selected. The bioinformatics program QDD version 1 with default parameters (Meglécz et al., 2010) was used to filter for redundancy, resulting in a final set of sequences from which it was possible to design primers. Finally, for a total of 43.9 Mbp read in the run, 10,895 sequences comprised microsatellite motifs with an average length of 304 bp. A total of 1114 primer sets were designed with QDD using BLAST, ClustalW, and Primer3 (Altschul et al., 1990; Larkin et al., 2007; Rozen and Skaletsky, 2000). A subgroup of 47 primer pairs was then tested for amplification. Primer sets were discarded if they failed to amplify or led to multiple fragments.

Each primer pair was assessed in eight individuals of *P. judaica*. Microsatellite loci were amplified in 25-µL reactions containing 20 ng of template DNA, 1 unit of *Taq* polymerase (FastStart *Taq* DNA polymerase, Roche Diagnostics), 0.6 mM of dNTPs, 3.75 mM of $MgCl_2$, and 0.1 µM of each forward and reverse primer. The amplification profiles included initial denaturation at 95°C for 10 min; followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 10 min. For each PCR, a negative control was included. A total of 2 µL of PCR product was separated on 2% agarose gel. Primer pairs that amplified in at least five individuals were validated. Among 47 primer pairs tested, 26 pairs were validated on a subset of eight individuals from Marseilles, France. Polymorphism of 24 microsatellite loci was tested on the same subset of individuals

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[5]Author for correspondence: angele.bossu@etu.univ-amu.fr

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TABLE 1. Characteristics of the microsatellite loci designed for *Parietaria judaica*.

Locus	Primer sequences (5′–3′)	T_a (°C)	Repeat motif	Fluorescent dye	Allele size (bp)	GenBank accession no.
ParJ_20	F: AGTTCAGTTGGGTTTGGCAG	56	$(AG)_{11}$	PET	146	KJ747956
	R: TATGGGTGTGAGTGGCTACG	56				
ParJ_26	F: CCTCCAATCGTTGAGAGGAA	54	$(GAA)_8$	PET	157	KJ747957
	R: CCGCCATTGCTGTCTAGC	56				
ParJ_27	F: TCCGATTATCCTCTTCGCAT	53	$(TTC)_{12}$	6-FAM	159	KJ789106
	R: GCAGAGAAATGAAAGAGAGTCG	53				
ParJ_31	F: CTTCACGCCACGACTACAAC	56	$(GTAT)_5$	PET	184	KJ789103
	R: TCAAAATCGAGATTGGCTCA	52				
ParJ_33	F: AGCCGTTAAGGTCCCAAAGT	57	$(TTC)_{18}$	VIC	190	KJ872579
	R: GGGACATGCGAAAGAAAAGA	53				
ParJ_34	F: AACGGAATTCATTTCTCCGA	52	$(TGA)_{10}$	NED	190	KJ872580
	R: TTGATTCTCCAAACACAACG	51				
ParJ_37[a]	F: GAAGCCTCGTCGAACCAGT	57	$(TC)_9$	6-FAM	201	
	R: CGACACCTTCACCAACAAGA	55				
ParJ_42	F: CGTATTTTGCGGGAAAGCTA	53	$(CT)_{10}$	6-FAM	235	KJ789107
	R: CCCATTCGTGAAGTTGGTAGA	54				
ParJ_43	F: GTGGCTCTCGACTCGGAC	58	$(CTT)_{14}$	VIC	240	KJ789105
	R: AACTCGAGAGCTTCATCGTTTC	55				
ParJ_44	F: GACAGTGTTCCCTATTGAGCG	56	$(CTT)_{11}$	NED	243	KJ789108
	R: TCGGGAGTTGGAACATATCA	53				
ParJ_45	F: GTAGGAAAAGGGGTTTGTCG	53	$(CT)_{10}$	PET	245	KJ789104
	R: TTTCTCTGATGTGGAAGCCC	55				

Note: T_a = annealing temperature.

[a]No GenBank accession number is available for locus ParJ_37 because the region between the primer and the repeat region has too many undetermined nucleotides.

that was used for amplification tests. PCR cycles and mixture were the same as those mentioned previously except that we used 10 pmol of each forward and reverse primer (instead of 1 pmol). One microliter of each PCR product was run in an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, Massachusetts, USA). Results were analyzed with GeneMapper 4.0 (Applied Biosystems).

Eleven markers were selected, and amplifications were tested for individuals sampled from three different cities: 85 individuals in Marseilles (43.296346°N, 5.369889°E), 29 individuals in Lauris (43.747778°N, 5.313611°E), and 52 individuals in Aix-en-Provence (43.531127°N, 5.454025°E).

The characteristics of the 11 novel microsatellite loci in *P. judaica* are summarized in Table 1. We calculated the expected heterozygosity using an R package (adegenet version 1.3-9.2; Jombart and Ahmed, 2011). The number of alleles per locus ranged from two to 13 in Lauris, two to 15 in Aix-en-Provence, and three to 16 in Marseilles (Table 2). Expected heterozygosity ranged from 0.128 to 0.874, 0.019 to 0.882, and 0.240 to 0.912 in Lauris, Aix-en-Provence, and Marseilles, respectively. Observed heterozygosity ranged from 0.068 to 0.448, 0.019 to 0.231, and 0.059 to 0.423 in Lauris, Aix-en-Provence, and Marseilles, respectively. All loci showed significant linkage disequilibrium (except loci ParJ_31 in the Lauris population and ParJ_37 in the Lauris and Aix-en-Provence populations).

CONCLUSIONS

The molecular markers described here are the first microsatellite loci isolated for *P. judaica.* Most markers showed median levels of polymorphism, while ParJ_33 and ParJ_43 showed high levels of polymorphism. Most loci were at Hardy–Weinberg disequilibrium potentially due to genetic drift caused by landscape fragmentation. The set of microsatellite markers will be useful for studying population genetics of *P. judaica* in urban environments.

TABLE 2. Polymorphism in 11 microsatellite loci developed for three populations of *Parietaria judaica*.[a]

	Total		Lauris (N = 29)			Aix-en-Provence (N = 52)			Marseilles (N = 84)		
Locus	N	A	A	H_e	H_o	A	H_e	H_o	A	H_e	H_o
ParJ_20	166	8	5	0.610[b]	0.345	6	0.712[b]	0.212	7	0.636[b]	0.294
ParJ_26	166	7	3	0.324[b]	0.069	4	0.627[b]	0.173	7	0.654[b]	0.317
ParJ_27	166	7	4	0.509[b]	0.310	4	0.620[b]	0.180	7	0.636[b]	0.321
ParJ_31	166	4	2	0.328	0.207	2	0.158[b]	0.019	4	0.628[b]	0.282
ParJ_33	166	19	13	0.874[b]	0.448	15	0.868[b]	0.212	16	0.912[b]	0.423
ParJ_34	166	11	6	0.561[b]	0.310	6	0.592[b]	0.173	9	0.551[b]	0.294
ParJ_37	166	3	2	0.128	0.068	2	0.019	0.019	3	0.315[b]	0.072
ParJ_42	166	6	4	0.604[b]	0.138	4	0.580[b]	0.137	6	0.661[b]	0.256
ParJ_43	166	19	9	0.815[b]	0.345	15	0.882[b]	0.231	15	0.695[b]	0.289
ParJ_44	166	10	4	0.329[b]	0.138	7	0.633[b]	0.154	8	0.650[b]	0.376
ParJ_45	166	4	3	0.558[b]	0.172	2	0.464[b]	0.115	4	0.240[b]	0.059

Note: A = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = total number of samples analyzed.

[a]Geographic coordinates of populations used in the study: Lauris (43.747778°N, 5.313611°E), Aix-en-Provence (43.531127°N, 5.454025°E), Marseilles (43.296346°N, 5.369889°E).

[b]Markers deviating from Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.004$).

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