Microsatellite Primers in Parietaria judaica (Urticaceae) to Assess Genetic Diversity and Structure in Urban Landscapes
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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 (Mandel 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 Malaua et al. (2011). Total DNA was enriched for TG, TC, AAC, AAG, AGG, AGC, 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) was used to identify 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 MgCl₂, 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 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.

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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 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 (43.531127°N, 5.369889°E), 52 individuals in Lauris (43.747778°N, 5.454025°E), and 51 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; 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 was run in an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, Massachusetts, USA). Results were analyzed with GeneMapper 4.0 (Applied Biosystems).

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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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Total N</th>
<th>A</th>
<th>H_e</th>
<th>H_o</th>
<th>Lauris (N = 29)</th>
<th>A</th>
<th>H_e</th>
<th>H_o</th>
<th>Aix-en-Provence (N = 52)</th>
<th>A</th>
<th>H_e</th>
<th>H_o</th>
<th>Marseilles (N = 84)</th>
<th>A</th>
<th>H_e</th>
<th>H_o</th>
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<tr>
<td>ParJ_20</td>
<td>166</td>
<td>8</td>
<td>5</td>
<td>0.610b</td>
<td>0.345</td>
<td>6</td>
<td>0.712b</td>
<td>0.212</td>
<td>7</td>
<td>0.636b</td>
<td>0.294</td>
<td>0.294</td>
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<tr>
<td>ParJ_26</td>
<td>166</td>
<td>3</td>
<td>7</td>
<td>0.324b</td>
<td>0.069</td>
<td>4</td>
<td>0.627b</td>
<td>0.173</td>
<td>7</td>
<td>0.654b</td>
<td>0.317</td>
<td>0.317</td>
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<tr>
<td>ParJ_27</td>
<td>166</td>
<td>7</td>
<td>4</td>
<td>0.506b</td>
<td>0.310</td>
<td>4</td>
<td>0.620b</td>
<td>0.180</td>
<td>7</td>
<td>0.636b</td>
<td>0.321</td>
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<tr>
<td>ParJ_31</td>
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<td>2</td>
<td>0.328b</td>
<td>0.207</td>
<td>15</td>
<td>0.868b</td>
<td>0.212</td>
<td>16</td>
<td>0.912b</td>
<td>0.423</td>
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<tr>
<td>ParJ_33</td>
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<td>3</td>
<td>13</td>
<td>0.874b</td>
<td>0.448</td>
<td>6</td>
<td>0.592b</td>
<td>0.173</td>
<td>9</td>
<td>0.551b</td>
<td>0.294</td>
<td>0.294</td>
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<tr>
<td>ParJ_34</td>
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<td>3</td>
<td>6</td>
<td>0.561b</td>
<td>0.310</td>
<td>2</td>
<td>0.019</td>
<td>0.019</td>
<td>3</td>
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<td>0.072</td>
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<tr>
<td>ParJ_36</td>
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<td>4</td>
<td>0.604b</td>
<td>0.138</td>
<td>14</td>
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<td>ParJ_39</td>
<td>166</td>
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<td>19</td>
<td>0.815b</td>
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<td>15</td>
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<td>0.326b</td>
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<td>7</td>
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<td>8</td>
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<tr>
<td>ParJ_45</td>
<td>166</td>
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<td>0.558b</td>
<td>0.172</td>
<td>2</td>
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<td>0.115</td>
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<td>0.059</td>
<td>0.059</td>
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</tbody>
</table>

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

*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).

*Markers deviating from Hardy–Weinberg equilibrium after Bonferroni correction (P < 0.004).
LITERATURE CITED


