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**PRIMER NOTE**

Isolation, characterization and cross-species amplification of microsatellite loci in the cycad genus *Dioon* (Zamiaceae). Potential utilization in population genetics studies of *Dioon edule*

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**Abstract**

*Dioon edule* (Zamiaceae) is an endemic Mexican cycad. Nineteen microsatellite loci were isolated from three enriched genomic libraries of *D. edule* var. *angustifolium*, *D. tomaselli*, and *D. caputoi*. Seven of these loci showed polymorphisms in *D. edule*. Levels of polymorphism were assessed using 16 individuals from each of seven populations throughout the range of this species. The number of alleles per locus ranged from two to five and the observed and expected heterozygosities ranged from 0.0 to 0.9821 and from 0.0088 to 0.6318, respectively. All loci show significant linkage disequilibrium. Three loci depart significantly from Hardy–Weinberg equilibrium.

**Keywords:** cycads, *Dioon*, Mexico, microsatellites, population genetics, SSR

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Cycads are among the oldest of extant seed plants and are geographically restricted to the tropics and subtropics of the New and Old World (Jones 1993). The Neotropical genus *Dioon* Lindl. (Zamiaceae) is composed of 13 taxa (Hill et al. 2004). *Dioon* occurs in restricted pockets throughout Mexico, with one species (*D. mejiae* Standl. & L. O. Williams) from Honduras (Sabato & De-Luca 1985; Jones 1993; Moretti et al. 1993). *Dioon edule* Lindl. is listed as near threatened (*nt* category) in the wild by the World Conservation Union — IUCN. Found along eastern Mexico, it forms a species complex with two varieties *D. edule* var. *edule* and *D. edule* var. *angustifolium* (Miq.) Miq (González-Astorga et al. 2003a). Recent studies suggest that these two varieties be recognized as distinct species (González-Astorga et al. 2003a, b).

Microsatellites or simple sequence repeats (SSRs) have been previously reported for one other cycad genus, *Zamia* L. (Zamiaceae) (Meerow et al., in press). In the present study, SSRs were developed to ascertain the genetic structure of *D. edule* in order to address taxonomic, conservation and biogeographical questions.

Three microsatellite enriched libraries were constructed following a protocol developed at the USDA-ARS-SHRS, Chapman Field, Miami, Florida. The protocol is modified from that of Edwards et al. (1996). Genomic DNA was extracted from fresh leaf material from: *D. edule* var. *angustifolium*, *D. tomaselli* De Luca, Sabato & Vazq. Torres, and *D. caputoi* De Luca, Sabato & Vazq. Torres following a modified protocol by Dellaporta et al. (1983). The DNA was digested, linked with adaptors and amplified. The amplified regions were enriched twice with biotin-labelled oligoprobes and separated with streptavidin-coated magnetic beads. After enrichment, products were separated using a Sepharose CL-4B SizeSepColumn 400 Spun Column (Amersham Pharmacia Biotech), cloned with a TOPO TA Cloning Kit (Invitrogen) and screened via sequencing using ABI PRISM BigDye Terminator version 3.1 (PerkinElmer) and sequenced on an ABI sequencer (Applied Biosystems).

A total of 480 colonies were obtained with 53 of them containing useable repeats (11%). The sequences were imported into Sequencher version 4.1 (Gene Codes) to generate consensus sequences. The preponderance of
Table 1  Primer sequences and related information for seven microsatellite loci from Dioon edule

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5′–3′)</th>
<th>Repeat</th>
<th>N</th>
<th>Size</th>
<th>HO</th>
<th>HE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ed3</td>
<td>F: GCATGAAGAAGCTGTTCCCTT</td>
<td>(CT)_19</td>
<td>2</td>
<td>123–127</td>
<td>0.1476</td>
<td>0.2254</td>
<td>0.5526</td>
</tr>
<tr>
<td>Ed5</td>
<td>R: GCTTTGACTCTGAAAGCATC</td>
<td>(AG)_15</td>
<td>5</td>
<td>136–148</td>
<td>0.9821</td>
<td>0.6318</td>
<td>1.000</td>
</tr>
<tr>
<td>Ed6</td>
<td>F: ATGCAGTGAGACACCC</td>
<td>(TGG)_10</td>
<td>2</td>
<td>239–242</td>
<td>0.0089</td>
<td>0.0086</td>
<td>0.4103†</td>
</tr>
<tr>
<td>Ed9</td>
<td>R: TCTTACACAGATCTGACC</td>
<td>(CA)_{hyp}</td>
<td>5</td>
<td>244–268</td>
<td>0.0805</td>
<td>0.1545</td>
<td>0.0282*</td>
</tr>
<tr>
<td>Cap5</td>
<td>F: GCTCTACCCCCCTTATACCAC</td>
<td>(CT)_23</td>
<td>3</td>
<td>225–241</td>
<td>0.8392</td>
<td>0.6092</td>
<td>1.000</td>
</tr>
<tr>
<td>Tom5</td>
<td>R: GCTTTGACCTGGCTTGGTG</td>
<td>(TC)_10</td>
<td>2</td>
<td>224–226</td>
<td>0.00</td>
<td>0.1317</td>
<td>0.000*</td>
</tr>
<tr>
<td>1660</td>
<td>F: GCTGCTGAGAAGAGAAGAA</td>
<td>(GA)_16</td>
<td>4</td>
<td>194–230</td>
<td>0.0710</td>
<td>0.0863</td>
<td>0.0420*</td>
</tr>
</tbody>
</table>

N, number of alleles; size, allele size range (bp); HO, observed heterozygosity; HE, expected heterozygosity; P, P-values for the HWE tests (HO, heterozygote deficiency) represent global average across seven populations; *significance at P < 0.05; †test was performed on only one population showing allelic polymorphism; the seven loci’s GenBank Accession nos: DQ441409–DQ441415.

Nineteen primers yielded PCR amplifications; however, only seven of those primers captured polymorphisms in Dioon edule (Table 1). The number of alleles per locus ranged from two to five, and both the observed and the expected heterozygosities ranged from 0.0 to 0.9821 and from 0.0088 to 0.6318, respectively. Three loci (Ed9, Tom5, 1660) departed significantly from Hardy–Weinberg equilibrium. Those loci showed significant heterozygote deficiency (Table 1). This may be due to the presence of null alleles or the Wahlund effect. Linkage disequilibrium was detected for all pairs of loci in all populations where the test could be carried out. This linkage disequilibrium is most likely due to high levels of inbreeding (Flint-Garcia et al. 2003).

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References


