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# Isolation and characterization of microsatellite loci in the carnivorous subshrub *Drosophyllum lusitanicum* (Drosophyllaceae)

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## 1. Introduction

*Drosophyllum lusitanicum* (L.) Link. (Drosophyllaceae) is a rare carnivorous plant endemic to Mediterranean heathlands in the SW Iberian Peninsula and NW Morocco (Müller and Deil, 2001). It is a diploid ($2n = 12$), short-lived (5–10 years), subshrub with limited dispersal capacity (Ortega-Olivencia et al., 1995) and tightly attached to nutrient-poor, acid soils (Garrido et al., 2003). Populations of *D. lusitanicum* are strongly associated with early post-fire environments (Müller and Deil, 2001).

Human disturbances such as fire suppression, urban sprawl, or heathland afforestation with *Pinus pinaster* have caused a stark range decline of *D. lusitanicum* (Correia and Freitas, 2002; Garrido et al., 2003). The consequences of natural habitat fragmentation and anthropogenic disturbances on population genetics of this unique species are, however, unknown.

This paper presents the results of microsatellite characterization and tests the polymorphism of 12 microsatellite loci as an initial step towards characterizing allelic variation and population structure in *D. lusitanicum*. The population genetic analysis of this flag species in environments characterized by different degrees of anthropogenic and natural (fires) disturbances will allow us to quantify the role of habitat fragmentation and degradation and wildfire suppression in the viability of populations.

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**Table 1**

Characteristics of 12 polymorphic microsatellite loci developed for *Drosophyllum lusitanicum* in two sampled populations.

| Locus | Primer sequence (5′-3′) | Motif | $T$ (°C) | Size (bp) | $N_A$, allele range (bp) | SP09-Spain: Murta, Los Alcornocales Natural Park, 36°19′16″N, 5°33′03″W, 380 m ($N = 30$) | | | | TA06-Morocco: Gueznaia, 35°41′50″N, 5°54′13″W, 116 m ($N = 30$) | | | | GB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $N_A$ | $H_O$ | $H_E$ | $F_{IS}$ | $N_A$ | $H_O$ | $H_E$ | $F_{IS}$ | |
| Dlu02 | F: 6FAM-TTGGCGATTTGATTGATTTG<br>R: AAAGAGCAACCATCTTGTAATTGT | $(GT)_{24}$ | 56 | 155 | 7, 151–185 | 3 | 0.033 | 0.160 | $+0.791^{***}$ | 4 | 0.033 | 0.577 | $0.942^{***}$ | KJ825810 |
| Dlu03 | F: 6FAM-GGTAGCCTCTTATCTACAAGCAA<br>R: TAAGGATGATGACCTAGTTATGG | $(CA)_{25}$ | 61 | 200 | 11, 194–226 | 4 | 0.067 | 0.191 | $+0.652^{**}$ | 7 | 0.067 | 0.773 | $+0.914^{***}$ | KJ825811 |
| Dlu05 | F: VIC- ACTCTCACGCTTGTGTCAAC<br>R: TGAAGTCACGACCATGTAGG | $(CA)_{22}$ | 61 | 227 | 2, 225–227 | 2 | 0.033 | 0.098 | $+0.659^{ns}$ | 1 | 0 | 0 | – | KJ825812 |
| Dlu06 | F: NED-CAAAGACGCGAGAATGTGTTT<br>R: TGTATTGGATGAGGTGACGATT | $(CA)_{22}$ | 56 | 222 | 9, 208–238 | 4 | 0.100 | 0.667 | $+0.850^{***}$ | 5 | 0.033 | 0.759 | $+0.956^{***}$ | KJ825813 |
| Dlu08 | F: PET-TCATAAACCAAGGAAGGGTAG<br>R: AACTAGAACCTTACCATGAGACA | $(CA)_6(CG)_8(CA)_{19}$ | 56 | 287 | 9, 255–283 | 4 | 0.067 | 0.675 | $+0.901^{***}$ | 5 | 0.100 | 0.737 | $+0.864^{***}$ | KJ825814 |
| Dlu09 | F: VIC-TTACTTAGCAGGGATTTAAGAC<br>R: CATGGGTAGCTTATGTTTCA | $(CA)_{11}$ | 56 | 206 | 2, 200–204 | 2 | 0.067 | 0.459 | $+0.855^{***}$ | 1 | 0 | 0 | – | KJ825815 |
| Dlu10 | F: VIC-CATTAGCCAAGGTTAGGTGATAA<br>R: CGTCATTCATTAGTTCCACTTG | $(CA)_{11}$ | 56 | 132 | 4, 128–136 | 3 | 0.100 | 0.618 | $+0.838^{***}$ | 1 | 0 | 0 | – | KJ825816 |
| Dlu11 | F: NED-ATGATGGATAATGTGCTCTCA<br>R: TTGATATGGGTTTGGTTACTT | $(CA)_{10}$ | 56 | 244 | 4, 242–248 | 3 | 0.067 | 0.515 | $+0.871^{***}$ | 2 | 0 | 0.331 | $+1.00^{***}$ | KJ825817 |
| Dlu12 | F: 6FAM-TTTACATCACCGAAAGAGAAACA<br>R: TCAATCACATGCTACAAACGTAA | $(GT)_{10}$ | 56 | 240 | 2, 240–242 | 2 | 0 | 0.460 | $+1.00^{***}$ | 2 | 0 | 0.434 | $+1.00^{***}$ | KJ825818 |
| Dlu13 | F: NED-AACCCAAAGTGACAATAACAAA<br>R: GGAAGTGTCAAGTTAAACAATGG | $(CA)_9$ | 51 | 146 | 7, 146–168 | 6 | 1.00 | 0.684 | $-0.462^{***}$ | 3 | 1.00 | 0.532 | $-0.879^{***}$ | KJ825819 |
| Dlu14 | F: PET-CATGTCAAACCGCTAAAGGAG<br>R: ATTGAAAGGCATGGAGAAGGT | $(CA)_6CGCG(CA)_{10}(TA)_5$ | 56 | 230 | 6, 228–240 | 4 | 0.100 | 0.744 | $+0.866^{***}$ | 3 | 0.033 | 0.677 | $+0.951^{***}$ | KJ825820 |
| Dlu15 | F: PET-GGGTTGTTTCTCCATTGATTT<br>R: TTCATCCAGGTAGGATCTTCG | $(GT)_8$ | 56 | 188 | 2, 186–188 | 2 | 0 | 0.067 | $+1.00^{*}$ | 1 | 0 | 0 | – | KJ825821 |
| Total | | | | | 65 | 39 | 0.136 | 0.445 | $+0.694^{***}$ | 35 | 0.106 | 0.402 | $+0.737^{***}$ | |

For each locus the primer pair sequences, repeat motif, temperature of annealing, size of the original fragment, number of alleles ($N_A$), and allele size range are noted.

For each population and locus, the total number of different alleles ($N_A$) and allele size range, observed ($H_O$) and expected heterozygosity ($H_E$), inbreeding coefficient ion ($F_{IS}$) and Genbank accession numbers are noted. 6-FAM, NED, PET, and VIC are fluorescent dyes from Applied Biosystems (Madrid, Spain). Significance of deviation from Hardy–Weinberg-equilibrium after 1000 permutations: ns, not significant, *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.