Molecular Ecology Resources (2008) **8**, 425–427 doi: 10.1111/j.1471-8286.2007.01980.x

PERMANENT GENETIC RESOURCES

Characterization of microsatellite markers for the almendro (*Dipteryx panamensis*), a tetraploid rainforest tree

THOR R. HANSON,* STEVEN J. BRUNSFELD,* BRYAN FINEGAN† and LISETTE P. WAITS*

**Department of Forest Resources, University of Idaho, Moscow, Idaho 83844-1133, USA,†Departamento Recursos Naturales y Ambiente, Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), 7170 Turrialba, Costa Rica*

Abstract

The almendro (*Dipteryx panamensis*, Fabaceae) is a tetraploid tree native to the Atlantic lowland rainforests of Central America. We present nine microsatellite primer pairs amplified in three multiplexed reactions for 549 individuals from four sites in Costa Rica. All loci were polymorphic, ranging from three to 13 alleles per locus. Expected heterozygosity was estimated with the program TETRASAT, and ranged from 0.21 to 0.74 across loci. These markers will be used for estimating pollen dispersal, seed dispersal, genetic structure and genetic diversity in fragmented landscapes.

Keywords: *Dipteryx panamensis*, genetic diversity, microsatellites, SSR, tetraploid, TETRASAT

Received 26 May 2007; revision accepted 27 July 2007

The almendro (*Dipteryx panamensis*, Fabaceae) is a large, canopy-emergent rainforest tree endemic to the Atlantic lowland forests of Central America (Flores 1992). Band patterns in this study indicate that the species is a tetraploid, though the chromosome number remains unknown. Its fruit is an important food source for numerous primates, small mammals and birds, including the endangered Great green macaw (*Ara ambiguus*). Although reduced by logging and habitat loss, *D. panamensis* persists in many forest fragments and as a shade tree in pastures. The effects of habitat alteration on its reproductive ecology and genetic diversity are of interest to conservationists and foresters in the region. Molecular markers are being used increasingly to study tropical tree populations in fragmented landscapes (e.g. Nason & Hamrick 1997; Dick *et al*. 2003). We designed nine microsatellite primer pairs from loci developed for the Amazonian congener *D. odourata* (Vinson 2004) for an investigation of *D. panamensis* pollen dispersal, seed dispersal, genetic diversity and genetic structure.

Samples of leaf tissue or cambium were gathered from 224 adult trees (> 30 cm d.b.h.) and 325 progeny from a fragmented landscape in Canton Sarapiqui, Costa Rica. The population included trees in continuous forest, forest fragments, pastures adjacent to fragments, and pastures isolated from forest by > 1.5 km. Sites were separated by a mean distance of 18.4 km, and all samples were collected from 2004 to 2006. DNA from plant tissues was extracted using QIAGEN DNEasy Plant Mini kits, standardized to a concentration of 25 ng/μL, and stored in QIAGEN AE buffer at –20 °C.

Nine primer pairs were designed from loci developed for the Amazonian congener *Dipteryx odourata* (Vinson 2004) using the program PRIMER 3 (Rozen & Skaletsky 2000). Sequences, repeat motifs and fragment sizes are detailed in Table 1. Primers were triplexed in 25 μL polymerase chain reactions (PCR) containing 10 pmol of each primer, 25 ng DNA template, 10 μmol of each dNTP, 1 μg BSA, 1× Promega PCR buffer (10 mM Tris-HCl pH 9, 50 mM KCl, 0.1% Triton® X-100) and 0.75 U Promega Taq polymerase using the $MgCl_2$ concentrations and thermocycler profiles detailed in Table 1. Each forward primer was labelled with a 5′ fluorescent dye and fragments were sized on an Applied Biosystems 3130xl capillary sequencer using Applied Biosystems' GeneScan 500 LIZ size standard. Fragments were scored using GENEMAPPER version 3.7 software (© 1999–2004 Applied Biosystems). Genotyping error rates were determined by re-extracting and re-running a subset of 90 samples (16.4% of all samples) for all nine loci.

Results from 549 individuals suggest high polymorphism at all nine loci. The number of alleles per locus ranged from three to 13 with a mean of 7.11 (Table 1). Eight of nine loci behaved in a tetraploid fashion with a maximum of four alleles per individual. One locus (Do5) showed

Correspondence: Thor R. Hanson, Fax: 208-885-6226; E-mail: thor@rockisland.com

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

Table 1 Sequences and characteristics of nine *Dipteryx panamensis* primers designed from microsatellite regions developed for *Dipteryx odourata* (Vinson 2004), and utilized on 549 individuals

Locus	GenBank Accession no.*	Primer sequences (5′–3′)	Repeat motif	Size range (bp)	T_a	$MgCl_2$	*M*‡	E_s (%)	N_a	N_m	N_i	N_G	*G*	H_E§
Do3	AY535410	F: GGGCTTGTAGCTATTGAGTG† R: GTTT AAAAGGGGATTAAGACCTTG	$(CT)_{13}$	223–241	52	1.7 mM	A	2.12	10	4	2.12	10	0.24	0.60 ± 0.02
Do5	AY535411	F: GGCCAAGTAAGCTAGAA R: TTTGAAGTTGAAGCTTGGAT	$(GA)_{18}$	188–214	52	3.4 mM	B	0.63	13	7	3.90	53	0.09	na
Do8	AY535412	F: TCTGTAGCTCTCTGCCTCTC† R: GTT TCGAATGAGTAATGTTGTGC	$(CT)_{14}$	152–170	54	3.4 mM	C	2.50	8	4	2.72	23	0.18	0.74 ± 0.01
Do17	AY535413	F: CTGTCGGTTCTCCATATATTTT R: ATTTAAACCTCTGCGTTGAA	$(GA)_{13}$	141–149	52	3.4 mM	B	0	5	4	2.09	15	0.37	0.61 ± 0.02
Do18	AY535414	F: TCTCTCCCCCTTTGTCTC† R: GTTT AGGTTGGCAGTGAAGGTG	$(CT)_{12}$	99–103	52	3.4 mM	B	1.25	3	3	2.28	7	0.41	0.60 ± 0.01
Do20	AY535416	F: GTCTTCGCCAACAGTAACTC† R: GTT TATAGTGGAAGGGTGGATTG	$(TC)_{17}$	155–175	54	3.4 mM	C	0	6	4	2.74	13	0.14	0.71 ± 0.01
Do24	AY535417	F: CAGGATCTAGCCAAAAGAAA† R: GTTT CAAGCTAACTGGTCTCCCTA	$(GA)_{14}$	132–152	52	1.7 mM	A	2.78	8	4	1.51	11	0.30	0.56 ± 0.02
Do25	AY535418	F: AAAACGGAAGAGGAAGATTT R: GAGACTTCGATGTCAGGGTA	$(GA)_9$	173–195	54	3.4 mM	C	1.41	7	4	2.55	12	0.27	0.58 ± 0.03
Do39	AY535421	F: TAATGGATGCCTGAATGAAT R: GCTTAAAAGCGAGTTGTCAT	$(CT)_{11}$	179–207	52	1.7 mM	A	2.56	4	4	1.65	8	0.47	0.20 ± 0.06

*Original *D. odourata* microsatellite region Accession nos (Vinson 2004); original locus names also retained.
†Tail added to reverse primer to increase adenylation for consistent scoring (Brownstein *et al*. 1996).
‡Thermocycler Profiles for multiplexes: A and B – (45 cycles: 94 °C for 30 s, 52 °C for 30 s, 72 °C for 35 s), C– (45 cycles: 94 °C for 30 s, 54 °C for 30 s, 72 °C for 30 s).
§Mean H_E (± SD) from 10 iterations of TETRASAT analysis (Markwith *et al*. 2006).
T_a, annealing temperature (°C); *M*, multiplex ID; E_s, genotyping error rate; N_a, total alleles; N_m, maximum observed alleles/individual; N_i, mean observed alleles/individual; N_G, unique genotypes/locus; *G*, prevalence of most common genotype.

additional signs of gene doubling, with as many as seven alleles observed per individual. Genotyping error rates averaged 1.47% and ranged from 0.0% to 2.78% per locus (Table 1). We were unable to determine exact allele frequencies or test for linkage disequilibrium due to the unknown allele dosage of partial heterozygotes. The tetraploid genotype ABBC, for example, produces the microsatellite result ABC, and cannot be reliably differentiated from AABC or ABCC. Expected Hardy–Weinberg heterozygosity (H_E) can be calculated using the software TETRASAT, however, which computes all possible allele combinations for partial heterozygotes and reports a mean value for H_E (Markwith *et al*. 2006). Intense computational demands limit the number of partial heterozygotes TETRASAT can process at one time (Markwith *et al*. 2006), so we performed 10 iterations of the analysis on random subsamples of 10 individuals, using the program's default subsampling routines and settings. (The locus exhibiting additional gene doubling (Do5) was excluded from these analyses.) TETRASAT-derived expected heterozygosity ranged from 0.21 to 0.74 across loci (Table 1), generally higher than heterozygosity estimates for *D. panamensis* from an earlier allozyme study (H_E = 0.16) (Hamrick & Loveless 1989).

The levels of polymorphism observed for these markers suggest excellent utility for population-level analysis of genetic diversity, genetic structure, and parentage. These microsatellite regions have now amplified and shown polymorphism for two species within the genus *Dipteryx*, and may hold promise for other related taxa.

Acknowledgements

This work was supported by NSF-IGERT Grant no. 0114304, with additional assistance from the DeVlieg Foundation, the Organization for Tropical Studies, and NSF LTREB Grant no. DEB-0129038. The authors thank Jo Ellen Force, Nilsa Bosque-Perez, Olle Pellmyr, Deborah Clark and David Clark, and the many field assistants, laboratory colleagues and landowners who made the project possible.

References

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *Biotechniques*, **20**, 1006–1010.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd

Dick CW, Etchelecu G, Austerlitz F (2003) Pollen dispersal of tropical trees (*Dinizia excelsa*: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology*, **12**, 753–764.

Flores E (1992) *Dipteryx panamensis*. *Arboles y Semillas Del Neotropico*, **1**, 1–22.

Hamrick JL, Loveless MD (1989) The genetic structure of tropical tree populations: associations with reproductive biology. In: *The Evolutionary Ecology of Plants* (eds Bock JH, Linhart YB), pp. 129–146. Westview Press, Boulder, Colorado.

Markwith SH, Stewart DJ, Dyer JL (2006) TETRASAT: a program for the population analysis of allotetraploid microsatellite data. *Molecular Ecology Notes*, **6**, 586–589.

Nason JD, Hamrick JL (1997) Reproductive and genetic consequences of forest fragmentation: two case studies of Neotropical canopy trees. *Journal of Heredity*, **88**, 264–276.

Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.

Vinson C (2004) Isolamento de microssatélites de espécies madeireiras no contexto da sustentabilidade genética no manejo florestal. MSc Thesis, Federal University of Para, Para, Brazil.

© 2007 The Authors
Journal compilation © 2007 Blackwell Publishing Ltd