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Characterization of 14 microsatellite DNA markers for the tropical forest tree *Virola surinamensis* (Rol.) Warb. (Myristicaceae)

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Abstract

Fourteen microsatellite DNA markers were developed for studies of gene flow in the Neotropical rain forest tree *Virola surinamensis*. The loci were unlinked and polymorphic in a sample of 21 individuals, with two to 10 alleles per locus and observed heterozygosity ranging from 0.14 to 0.76. The overall exclusion probability (0.997) indicates high resolution for parentage-based analyses of gene flow.

Keywords: Barro Colorado Island, gene flow, Panama, tropical forest, *Virola*

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Virola surinamensis (Neotropical nutmeg) is a dioecious shade-tolerant tree of rain forests in lower Central America to the Amazon basin, with disjunct populations in the Antilles (Croat 1978). In Panama, *V. surinamensis* is strongly associated with moist slopes and streams (Harms *et al.* 2001). The flowers are insect pollinated, although the taxonomic identity of the pollinators remains unknown. Large frugivorous birds (e.g. toucans and guans) and monkeys disperse the arillate seeds (Howe *et al.* 1985).

Virola surinamensis contains alkaloidal compounds that have evolved as defences against pathogens and herbivores, and which are used by Native Americans for medicine and shamanism (Schultes & Raffauf 1990). *Virola surinamensis* is also a model species for studies on how seed dispersal and pathogens impact the demographic dynamics of tropical trees (e.g. Howe *et al.* 1985). Microsatellite DNA markers previously developed for *Virola flexuosa* (Holbrook *et al.* 2006) were not transferable to *V. surinamensis* (C. Dick, unpublished). We developed microsatellite loci for *V. surinamensis* to characterize gene flow and regional genetic structure in this species.

DNA was extracted (DNeasy Plant kit; Qiagen Corporation) from one *V. surinamensis* tree (plot ID # 371) from the forest dynamics plot (FDP) of Barro Colorado Island (BCI), Panama. DNA was twice enriched for simple sequence repeats [$(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$] using the methods of Glenn & Schable (2005). Polymerase chain reaction (PCR) products were ligated to a plasmid vector using the TOPO TA Cloning Kit (Invitrogen Corporation). Plasmid inserts were amplified and sequenced (ABI Model 3730 Sequencer). Twenty-seven of the 62 sequenced clones (43%) contained microsatellites. Primers were designed using the

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software OligoCalc (Kibbe 2007). Polymorphism was screened in 21 mapped individuals from the 50 ha FDP of BCI, Panama (Croat 1978) for 21 primer pairs including seven primer pairs developed for *Virola multiflora* and *Virola sebifera* (Draheim *et al*., unpublished). PCR was carried out in a volume of 10 μL containing ~30 ng of template DNA, 1× PCR buffer (Promega), a primer-specific $MgCl_2$ concentration (Table 1), 0.2 mm of each dNTP, 1 U *Taq* polymerase (*GoTaq*, Promega) and 0.2 μm of each primer (Table 1). PCR for Vsur33, Vsur2-58 and Vsur2-58 included 25 μg/mL of BSA. The thermal cycle began with a 4-min denaturation step at 95 °C, followed by 35 cycles of 30 s at 95 °C, 40 s at a primer-specific annealing temperature (Table 1), and 60 s at 72 °C, and a final extension at 72 °C for 10 min (Table 1). Forward or reverse primers were labelled with FAM, HEX or TAMRA in the 5′ end (see Table 1). Fourteen of the 21 loci were polymorphic in *V. surinamensis* (Table 1). The 14 primer pairs generated easily scored amplification products of the expected size across all tested individuals. Amplified products were genotyped on an ABI 3730 Sequencer and analysed using GeneMaker v. 1.75 (SoftGenetics Corporation).

The program GENEPOP v. 3.4 (Raymond & Rousset 1995) was used to calculate the mean number of alleles (N_A), and observed (H_O) and expected (H_E) heterozygosities for each locus and over all loci. Deviations from Hardy–Weinberg (HW) and tests for linkage disequilibrium were evaluated using Fisher's exact tests and sequential Bonferroni corrections. The probability of null alleles was calculated using the MICRO-CHECKER (Oosterhout *et al*. 2004). Exclusion probability was calculated using GENALEX v. 6 (Peakall & Smouse 2006).

MICRO-CHECKER indicated that locus Vsur45 could exhibit null alleles; however, null allele frequency was not significant ($P > 0.05$). Hardy–Weinberg probability tests revealed no significant deviations from expected genotype proportions ($P > 0.004$). There was no evidence of linkage disequilibrium among loci ($P > 0.001$) after corrections for multiple tests. The multi-locus exclusion probability was 0.997 indicating high information content for parentage-based analyses of gene flow.

Table 1 Forward (F) and reverse (R) primers, repeat motifs, $MgCl_2$ concentrations, annealing temperatures in ° C (T_a), number of alleles (N_a), allelic size range in bp (Size range), observed heterozygosity (H_O), expected heterozygosity (H_E) and GenBank Accession number for 14 *Virola surinamensis* microsatellite loci. Five loci were originally isolated from *Virola multiflora* (Vmul68, Vmul2-65 and Vmul2-66) and *Virola sebifera* (Vseb3 and Vseb21)

Locus	Primer sequence (5′–3′)	Repeat motif	T_a (°C)	$MgCl_2$ (mm)	N_a	Size range (bp)	H_O	H_E	Accession no.
Vsur33	F: HEX-TTT GCT AAT CCC ATT CGC A R: CAC ATC TTT GCT CAC TTT CA	$(GA)_{20}$	48	0.75	5	165–188	0.33	0.38	FJ713439
Vsur34	F: HEX-CTA GTT GAA CTC ATT TCC AC R: TGG TAT TAG ACT AGC ACT CA	$(CT)_{19}$	52	0.75	10	283–305	0.71	0.81	FJ713440
Vsur45	F: GAT ACT GCA TGA TAT AAG GC R: FAM-AAT AGA ATG CTT AGT AAC CTA C	$(TC)_9(TCA)_{10}$	52	1.25	6	289–321	0.48	0.69	FJ713441
Vsur56	F: TAMRA-CTT CCT TTC TCC GTT GCC R: CTG AAT GCA ACT AGG AGT A	$(CT)_{13}$	52	1.25	4	221–237	0.33	0.30	FJ713442
Vsur58	F: TAMRA-TGT AAT TTG ATG CTT TCT TCC R: ATC CAT CCT ACA CAG ATC C	$(CT)_7(GTCT)_2(CTGT)_2$ $(CTCTGT)_2(CT)_2AT(CT)_7$	52	1.25	3	245–271	0.33	0.44	FJ713443
Vsur2-35	F: FAM-CCT CTT TCA TTG GTG CAA C R: ATC GTC TTC ACT CAA ACA ATC	$(GA)_{15}$	52	1.25	5	285–303	0.81	0.62	FJ713444
Vsur2-41	F: GAT TCT GTG TTG TAT AAT GAG C R: TAMRA-TTC TTC GTG ATG TCA GAT TC	$(TC)_{18}$	52	1.25	5	291–318	0.52	0.59	FJ713445
Vsur2-55	F: HEX-AAC TCT GCT GTA GAA GGA TG R: AAT TCA GTG CAA CTC TGT AAG	$(CT)_{20}$	52	1.5	5	264–282	0.57	0.60	FJ713446
Vsur2-58	F: GGA TTC TAT GTG AGT ATT ATC A R: FAM-TTG AAT TCT TTA CAT CTT ATC C	$(GA)_{16}$	52	1.25	6	173–199	0.76	0.68	FJ713447
Vseb3	F: HEX-TAT TCT GAC TTC CAC ATC ATG R: GTA AGA CAA CTT GGC CAT A	$(AG)_{19}$	52	1.5	4	224–230	0.48	0.57	FJ713448
Vseb21	F: FAM-GCA ATA CGT CTC CAT TTA TC R: GTT CAC TTT CTG TTG GAT GA	$(AC)_3G(CA)_2A(AC)_2A$ $(ACACAT)_2(TCACAT)_3$	52	1.25	2	226–227	0.14	0.14	FJ713449
Vmul68	F: GTC TTG GAA CTG CAT GTT C R: TAMRA-TCA AAG ACT CTT TGC CAG C	$(AG)_{16}$	52	0.75	5	271–289	0.71	0.54	FJ713452
Vmul2-65	F: HEX-CTG CTG CAC CAG AGA AAC R: TCG CAT TCA TGA GTT CCC A	$(CT)_8(CA)_{12}$	52	1.5	4	158–192	0.48	0.68	FJ713450
Vmul2-66	F: HEX-TTC GCA TTT CTT TGT CTT TGG R: CTA AGA TTC TCA CTG TAC GA	$(TCC)_3(TTCC)_3$ $(CTC)_2(CTT)_8$	52	1.25	4	239–261	0.57	0.43	FJ713451

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Characterization of microsatellite loci for the pitcher plant midge, *Metriocnemus knabi* Coq. (Diptera: Chironomidae)

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Abstract

As a component of the inquiline community of the purple pitcher plant (*Sarracenia purpurea*), the pitcher plant midge *Metriocneus knabi* has been the subject of various ecological studies. However, very little is known about its characteristics beyond the larval stage, in particular the dispersal ability of adults. This study presents new molecular tools developed for testing of evolutionary and ecological questions in natural populations of this species. We describe a set of 12 microsatellite loci specific to *M. knabi* that are sufficiently polymorphic to provide insight into population genetic structure and dispersal patterns.

Keywords: inquiline, *Metriocnemus*, microsatellite, polymorphic, *Sarracenia purpurea*

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Metriocnemus knabi (Coquillet 1904) is one of several dipteran species that utilizes phytotelmata of the carnivorous purple pitcher plant (*Sarracenia purpurea* L.) as the exclusive habitat for its larval development (Heard 1994). Along with the larvae of the sarcophagid fly (*Fletcherimyia* spp.) and the mosquito (*Wyeomyia smithii*), as well as mites, rotifers and bacteria, *M. knabi* larvae constitute an inquiline community in a complex natural microcosm (Harvey & Miller 1996). This biological system has been recognized as a strong candidate model system in ecology (Srivastava *et al.* 2004) and it has been used in metacommunity (Holyoak *et al.* 2005) and landscape ecological research (Krawchuk & Taylor 2003). Dispersal

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