Isolation and characterization of microsatellites in iris

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The Louisiana iris species complex consists of three widespread species (*Iris fulva* Ker-Gawler, *I. hexagona* Walter and *I. brevicaulis* Raf.; Viosca 1935) and a rare diploid hybrid species (*I. nelsonii* Rand.). While these species have relatively wide ranges (with the exception of *I. nelsonii*) and distinct ecological preferences, they occur sympatrically in southern Louisiana where interspecific matings have led to the production of hybrid populations (e.g. Viosca 1935; Arnold *et al*. 1990). Although the ecology, taxonomy and evolution of the Louisiana irises have been studied for over 50 years, direct estimates of gene flow, mating patterns and hybrid fitness in natural populations are lacking. To this end, we developed a suite of five microsatellite loci from two species of Louisiana iris, *I. brevicaulis* and *I. fulva*, that will allow us to take a paternity-based approach to the study of these phenomena in natural *Iris* populations.

Cloning and screening procedures followed the methods of Aldrich *et al*. (1998). Total genomic DNA was isolated from fresh leaf tissue of a single *I. brevicaulis* and a single *I. fulva* individual using the DNeasy Plant Mini Kit (QIAGEN). The DNA was digested with *Mbo*I and size selected by excising 300–700 bp fragments from a 2% agarose gel (QIAquick Gel Extraction Kit, QIAGEN). The size-selected DNA fragments were then ligated into the *Bam*HI site of a lambda vector (ZAP Express, Stratagene) and packaged (ZAP Express Gigapack III Gold packaging extract, Stratagene). The packaged libraries were used to infect XL1-Blue *Escherichia coli* cells and plated in top agar. Plaques were lifted with nylon membranes (Hybond N+, Amersham) and screened with a [γ^{32}P]-dATP end-labelled $(GA)_{10}$ probe. Seventeen plates carrying ≈1000 plaques/plate were screened for each species. Candidate plaques were picked and rescreened at a lower density to minimize false positives. A total of 27 *I. brevicaulis* and 26 *I. fulva* clones were amplified using the M13 forward and reverse primers, purified with the High Pure PCR Product Purification Kit (Boehringer Mannheim) and sequenced on an automated sequencer using the ABI Dye Terminator Prism Kit (ABI/Perkin-Elmer). Primers were designed for eight *I. brevicaulis* and seven *I. fulva* loci. Of these, three of the *I. brevicaulis* and two of the *I. fulva* primer pairs produced amplification products that were both interpretable and polymorphic in at least one of the species (Table 1).

Polymerase chain reactions were performed in 20 μL volumes containing 50 ng of genomic DNA, buffer [50 mM Tris-HCl pH 8.0, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol and 1% Triton X-100), 2.0 mM $MgCl_2$, 8 pmol of each primer, 125 μM of each dNTP and 0.8 units of *Taq* DNA polymerase (Promega)]. Amplification conditions consisted of an initial 2 min denaturation at 94 °C, followed by 30 cycles of 94 °C for 1 min, 56–62 °C for 1 min and 72 °C for 1 min. The 30 cycles were followed by 5 min at 72 °C. Reactions were carried out in a Perkin-Elmer Cetus 9810 Thermal Cycler. Polymorphisms were detected by end-labelling one primer with [γ^{32}P]-dATP using T4 polynucleotide kinase (1 μCi per 5 pmol of primer), resolving fragments on 6% polyacrylamide gels and visualizing them by autoradiography.

The five primer pairs produced interpretable, polymorphic amplification products in *I. brevicaulis*. In contrast, IB141 consistently failed to amplify in *I. fulva*, even after multiple attempts to redesign the primers, and IF061 amplified as many as four alleles in each *I. fulva* individual, suggesting that this locus has been duplicated in this species. In order to assess allelic variability at all five loci, we screened 41 *I. brevicaulis* and 19 *I. fulva* individuals collected from natural populations of these two species. The number of alleles ranged from

Table 1 Characteristics of five microsatellite loci cloned from two species of Louisiana iris, including locus name, GenBank Accession no., primer sequences, repeat motif, annealing temperature, size of the sequenced allele and total number of alleles from the two species combined. The number of alleles, observed heterozygosity and expected heterozygosity are reported for each locus in each species separately

Locus (Accession)	Sequence (5′–3′)	Repeat	T_a (°C)	Size (bp)	Alleles (total)	*I. brevicaulis*			*I. fulva*		
						Alleles	H_O	H_E	Alleles	H_O	H_E
IB025 (AF124505)	GATCTCACATCGTTTGGTC CGATAACCCAACTTCACTAC	$(GA)_{27}$	56	104	25	17	0.71	0.91	13	0.94	0.90
IB141 (AF124506)	CTGAACCACCGGTCACAAG GAAGCCATGTTGAAGTTGTCC	$(GA)_{15}$	56	156	12	12	0.90	0.83	—	—	—
IB145 (AF124507)	TGTTGCGGGATTAAGGAGAC CAACGAGAAGAATTATCCGAAAG	$(GA)_{15}$	56	164	22	19	0.83	0.92	10	0.76	0.83
IF061 (AF124508)	TTGGGACAACCATTTGAGGA CGCCGACAAGACCCTGAC	$(GA)_{24}$	62	235	16	16	0.92	0.90	—	—	—
IF073 (AF124509)	TGGCTCTACCTTCACCACAAC CCGAACCCAGAATGGAAGTG	$(GA)_{19}$	58	195	14	8	0.60	0.83	9	0.95	0.85

8 to 17 in *I. brevicaulis* and from 9 to 13 in *I. fulva* (Table 1). Overall, there was a total of 12–25 alleles per locus in the two species combined. In all cases, expected heterozygosities were quite high, ranging from 0.83 to 0.92 in *I. brevicaulis* and from 0.83 to 0.90 in *I. fulva*. Only two of the five loci (IB141 and IF061) conformed to Hardy–Weinberg expectations in *I. brevicaulis* when tested with the probability test of GENEPOP (Raymond & Rousset 1995), whereas all three loci assayed in *I. fulva* were consistent with Hardy–Weinberg expectations. The significant heterozygote deficits ($P < 0.05$) at IB025, IB145 and IF073 in *I. brevicaulis* could be due to the presence of null alleles. Alternatively, these deviations could be an artefact of our sampling strategy. That is, we could have created an apparent heterozygote deficit (i.e. a Wahlund effect) by sampling a substructured *I. brevicaulis* population across which allele frequencies at these three loci were strongly differentiated. The relatively high level of variation described above, combined with the fact that three of our five loci are informative in both *I. brevicaulis* and *I. fulva*, suggests that these markers are well suited for paternity studies in hybrid *Iris* populations.

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