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PRIMER NOTE

Development and characterization of microsatellite markers for the poplar rust fungi *Melampsora medusae* and *Melampsora larici-populina*

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Abstract

Melampsora **species and their hybrids are obligate parasites of** ***Populus*** **and** ***Salix*** **species worldwide. The increasing interest in** ***Populus*** **and** ***Salix*** **for biomass and fibre production necessitates genetic markers for population studies of** ***Melampsora*** **spp. Libraries enriched for simple sequence repeats were used to develop five microsatellite markers for** ***Melampsora medusae*** **and** ***Melampsora larici-populina*****. The variation detected by these markers will be valuable for phylogenetic and population genetic studies, substantiate putative hybrids, and deployment of resistant poplar clones.**

Keywords: *Melampsora medusae*, *Melampsora larici-populina*, SSR

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Melampsora larici-populina and *Melampsora medusae* are obligate fungal pathogens that now occur in most parts of the world where *Populus* spp. are grown. *M. larici-populina* is native to Eurasia but has been found in Australia, New Zealand, western United States (Newcombe *et al*. 2000), and eastern Canada (Innes *et al*. 2004). *M. medusae* is endemic to eastern United States on *Populus deltoides* but has been introduced to Argentina, Australia, Africa, and western United States. Although interspecific hybrids of fungi are rare or difficult to document, Spiers *et al*. (1994) and Newcombe *et al*. (2000) have identified putative hybrids involving these *Melampsora* species. However, markers are not available to genetically confirm these hybrids, and studies using population genetics are not possible. These fungi are obligate parasites, so they cannot be grown in culture, and markers are needed that can be resolved with the DNA extracted from single fruiting bodies (uredenia) of 250 μm diameter or less. Though difficult to identify in fungi relative to plants or animals, the polymerase chain reaction (PCR)-based micro-satellites [simple sequence repeats (SSR)] were the rational choice of markers, and we describe here the characterization of five PCR-based SSR markers for these two *Melampsora* species.

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The DNA for microsatellite identification came from urediniospores on inoculated leaves. The isolates were from Ames, Iowa, and Puyallup, Washington. Urediniospores were collected by lightly tapping infected leaves over paper and transferring the collected spores into a microcentrofuge tube. Total genomic DNA of the urediniospores was extracted using a modified protocol of Hamelin *et al.* (1996), as described by Chen (2001).

Three microsatellite-enriched libraries for repeats of CAA, CAG, and CAT were constructed for each species using a modified protocol (Steimel *et al*. 2004) of Edwards *et al*. (1996). The enriched DNA samples were run on a 2% agarose gel, and bands or smears that ranged from 300 to 1000 bases were cut out and purified using a GENECLEAN II kit (Bio 101). The DNA was ligated into a pGEM-T easy vector (Promega) using a 1 : 1 insert : vector molecular weight ratio. Ligated plasmids were then transformed into JM109 high efficiency competent cells (Promega) and plated onto LB AMP medium as per kit instructions. Following incubation overnight at 37 °C, white colonies were transferred and arranged in a 6×7 grid (0.5×0.5 cm) on LB medium and incubated overnight. Colonies were then individually transferred to Hybond-N + nylon membranes (Amersham Biosciences). The prepared membranes were hybridized with a ^{32}P-labelled oligonucleotide of the respective repeat (DeScenzo & Harrington 1994) to screen for colonies containing high

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Table 1 Loci, primers, and GenBank Accession nos for microsatellite markers developed for *Melampsora medusae* and *Melampsora larici-populina*

Locus	Isolation source	Sequence motif*	Primers	Label	Primer sequences	GenBank Accession no.
MmCAG-11	*M. medusae*	$CAG_{(3)}$ + $CAG_{(2)}$ + $CAG_{(5)}$ + $CAG_{(2)}$+ $CAG_{(2)}CAA_{(3)}CAG_{(5)}CAA_{(2)}$	Medu CAG11-F Medu CAG11-R	FAM	5′-CCTTCATACACTTGCGAACTC-3′ 5′-GGCCAGCATGTAATTGTTG-3′	AY787478
MmCAT-30	*M. medusae*	$CAT_{(23)}$	Medu CAT30-F Medu CAT30-R	HEX	5′-AAAGAAGTTCAAATGCCTTAC-3′ 5′-GAAACGAGCTCATCTGTTC-3′	AY787479
MmCAA-57	*M. medusae*	$CAA_{(23)}$ + $CAA_{(2)}$ + $CAT_{(4)}$ + $CAA_{(7)}$	Medu CAA57-F Medu CAA57-R	TET	5′-GCTTACAAGTGAAAATTTG-3′ 5′-TTAATGCAGATTGTAAATTAG-3′	AY787480
MlCAG-30	*M. larici-populina*	$CAG_{(6)}CAA_{(2)}$ + $CAT_{(4)}CAA_{(2)}CAG_{(7)}$	Larici CAG30-F Larici CAG30-R	FAM	5′-ACCATATCCTGCCAGTCTTCTC-3′ 5′-CGTCAGTGAGGGCGTAATG-3′	AY787481
MlCAG101	*M. larici-populina*	CAA or $CAG_{(22)}$	Larici CAG101-F Larici CAG101-R	TET	5′-CTCCGCTGTTCCTTCTGG-3′ 5′-TATCTGTGGTTGCGAGTATTGG-3′	AY787482

†Indicates that the two simple repeats were separated by other bases.

Table 2 Microsatellite alleles, based on approximate band sizes as determined by GENESCAN analysis, found in isolates of *Melampsora medusae* and *Melampsora larici-populina*

Locus	*M. medusae*	*M. larici-populina*
MmCAG-11	265, 268, 274, 277, 280, 283, 286, 289, 295	271, 280, 289, 302
MmCAT-30	190, 196, 199, 202, 205, 208, 211, 214, 217, 220, 223, 226, 229, 232, 247, 289	None
MmCAA-57	280, 283, 286, 289, 292, 298, 301, 304, 307, 310, 313, 316, 319, 325, 340	None
MlCAG-30	286	286
MlCAG101	269, 276, 279, 287, 290, 304, 331, 337, 370, 420, 465	273, 282, 287, 290, 304, 307, 393

copy numbers of the repeat. The most strongly hybridizing clones were sequenced as described in Steimel *et al*. (2004).

Primers were designed from the flanking regions of the repeat and tested with the DNA extracted from single uredinia (*M. medusae*) or from multiple uredinia from an individual *Populus* leaf (*M. larici-populina*). The DNA extraction procedure for single uredina was as described by Hamelin (1996). Amplification was conducted in a 96-well thermal cycler (MJ Research Model PTC-100). For *MmCAT-30*, amplifications were performed in 20.0 µL with 200 µM dNTP mixture, 4.0 mM $MgCl_2$, 1× buffer A, 0.3 units *Taq* DNA polymerase (Promega), 0.2 µM of each primer, and 1 µL of extracted DNA. For *MmCAG-11*, *MmCAA-57*, *MlCAG-30*, and *MlCAG-101*, amplifications were performed in 20.0 µL with 200 µM dNTP mixture, 1× buffer, 0.3 units TaKaRa Ex *Taq*™ (PanVera), 0.2 µM of each primer, and 1 µL of extracted DNA. The thermal cycler was programmed for an initial denaturation step at 95 °C for 2 min, then 35 cycles at 95 °C for 35 s, 55 °C for 1 min, and 72 °C for 2 min. The HEX-, TET-, and FAM-labelled products were combined (0.5 µL each), electrophoresed on a ABI PRISM 377 DNA sequencer, and the alleles scored to the nearest bp as described in Steimel *et al*. (2004).

Four polymorphic and one monomorphic loci were identified (Tables 1 and 2) when tested against samples of *M. medusae* from the United States (Chen 2001) and *M. larici-populina* collected from Quebec, Canada, and Belgium. Two of the three *M. medusae* primer pairs did not produce a PCR product with *M. larici-populina* DNA. Two bands were evident in most samples, indicating heterozygosity in the dikaryotic uredinia. The *M. medusae* markers were tested across a large collection of single-uredinial pustules of *M. medusae* collected from Missouri to Minnesota, and these markers were found to be unlinked and in Hardy–Weinberg equilibrium (Chen 2001). Surprisingly, the primers for *MlCAG-30* amplified a single, identical band with both species of fungi, in spite of the high number of tandem repeats in this region; this highly conserved, homozygous locus may not be a true microsatellite region.

Although limited in number, three of these five markers proved highly polymorphic and are suitable for detailed population studies of *M. medusae* (Chen 2001). A fourth marker was highly polymorphic in both species, and there are now at least two loci for the study of *M. larici-populina* populations. The primers should also be suitable to confirm hybridizations between the two species.

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References

Chen W (2001) Population genetics of *Melampsora medusae* on poplar in Minnesota, Iowa and Missouri. Thesis. Iowa State University, Ames, Iowa.

DeScenzo RA, Harrington TC (1994) Use of $(CAT)_5$ as a DNA fingerprinting probe for fungi. *Phytopathology*, **84**, 534–540.

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequences in plants. *BioTechniques*, **20**, 758–760.

Hamelin RC, Bérubé P, Gignac M, Bourassa M (1996) Identification of root rot fungi in nursery seedlings by nested multiplex PCR. *Applied Environmental Microbiology*, **62**, 4026–4031.

Innes L, Marchand L, Frey P, Bourassa M, Hamelin RC (2004) First report of *Melampsora larici-populina* on *Populus* spp. in eastern North America. *Plant Disease*, **88**, 85.

Newcombe G, Stirling B, McDonald S, Bradshaw HD, Jr (2000) *Melampsora* × *columbiana*, a natural hybrid of *M. medusae* and *M. occidentalis*. *Mycological Research*, **104**, 261–274.

Spiers AG, Hopcroft DH (1994) Comparative studies of the popular rust *Melampsora medusae*, *M. laricis-populina*, and their interspecific hybrid *M. medusae-populina*. *Mycological Research*, **98**, 889–903.

Steimel JP, Engelbrecht CJ, Harrington TC (2004) Development and characterization of microsatellite markers for the fungus *Ceratocystis fimbriata*. *Molecular Ecology Notes*, **4**, 215–218.