determined using the BigDye Terminator sequencing kit and an ABI 373 automated sequencer (PE Applied Biosystems). Sequences flanking individual microsatellite repeats were used to design locus-specific polymerase chain reaction (PCR) primers, and one primer per locus was synthesized with a fluorescent dye at the 5′-prime end (Operon Technologies).

Primers were tested on DNA extracted from various collections of *R. vinicolor* as follows: dried tissue was ground in cetyltrimethylammonium bromide (CTAB) buffer (100 mM Tris pH 8–9, 1.4 M NaCl, 20 mM EDTA, 2% CTAB) followed by three cycles of freezing and thawing. The material was subsequently incubated at 65 °C for 1–2 h followed by chloroform extraction. The clear supernatant was transferred to a new tube, and DNA was further purified using the GeneClean III kit (BIO 101). PCR contained 1 × *MasterAmp* premix E (Epicentre Technologies), 0.5 μM each of two locus-specific primers, empirical amounts of genomic DNA, and 50 U/mL *Taq* polymerase (various suppliers) in a 10-μL volume. Thermal cycling was performed on a PTC-100 Programmable Thermal Controller (MJ Research Inc.) under the following conditions: 2 min at 93 °C, 40–45 cycles of (45 s at 93 °C, 30 s at 60 °C, and 30 s at 72 °C), 1 h at 72 °C. Presence of PCR products was verified on agarose gels stained with ethidium bromide, and appropriate dilutions were analysed on an ABI 377 automated sequencer using the 'GS500 Tamra' internal size standard. Band sizes were called using the GENESCAN software (PE Applied Biosystems).

Under the conditions described above, none of the primer pairs given in Table 1 were found to amplify DNA from *Pseudotsuga menziesii* which is the only known EM host of *R. vinicolor* (Massicotte *et al*. 1994). Consequently, both fruit-bodies as well as EM samples were used in the subsequent screening process. The species identity of all fungal samples was confirmed by restriction fragment length polymorphisms of the internal transcribed spacer region of the nuclear ribosomal repeat (ITS-RFLPs) as has been described by Gardes & Bruns (1996). In short, the ITS-region was amplified using fungus-specific PCR primers and subsequently digested with the restriction enzymes *Alu*I and *Hin*fI.

Allelic diversity was assessed across 19 collections which represented 19 distinct genets as evidenced by the microsatellite markers described here ($n = 19$). All collections originated from various locations in Oregon with the exception of *R. vinicolor* T20787 which had been collected in Idaho. Six microsatellite loci given in Table 1 were found to be both polymorphic and fairly unambiguous to score. The observed numbers of alleles ranged from 2 to 6, and expected and observed heterozygosities ranged from 0.05 to 0.76. In summary, polymorphic microsatellite markers presented here should prove useful for studying the distribution of genets as well as population structure at different spatial scales in *R. vinicolor*. Furthermore, since the primers do not amplify any DNA from the plant host *P. menziesii*, they will allow us to investigate fungal population structure from EM roots.

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Highly polymorphic microsatellite markers in the landsnail *Helix aspersa* (Mollusca Gastropoda)

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In an attempt to trace back the history of the landsnail, *Helix aspersa*, spread in the western Mediterranean, anatomical, biochemical and molecular markers have been used to explore genetic variation in native populations of the species (Guiller *et al*. 1994, 1998; Madec & Guiller 1994; Thomaz *et al*. 1996). In this context, two observations have drawn our attention to the population structure of the species: (i) the very high level of mitochondrial diversity found even at this lowest taxonomic rank; (ii) the frequent occurrence of departures from Hardy–Weinberg expectations in almost all samples scored for allozyme variation. Regarding preliminary results on the fine scale genetic structure of allele frequencies at enzyme loci (Arnaud *et al*. 1999), such possible effects of population mixing may indeed be due to the particular population structure of *H. aspersa* and land molluscs in general, where populations are subdivided in numerous demes with limited migration between them. Because allozyme loci might

Table 1 Primer sequences and some characteristics of polymorphic microsatellites in *Helix aspersa*. The number of scored individuals was 47 for Ha12, 48 for Ha9, 50 for Ha7, and 117 for the rest of the markers (#origin of individuals tested)

Locus	GenBank Accession no.	Primer Sequences (5′–3′)	Repeat motif	Annealing temp. (°C)	No. of Alleles	Size-range (bp)	Heterozygosity	
							H_O	H_E
Mont-St-Michel's Bay#								
Ha2	AF204939	CGAAGCCTTTGGCACAATGT TCCCTGACACTGGAAGATGGA	$(CA)_7TG(CA)_{11}TA(CA)_6$	54	5	302–310	0.68	0.61
Ha5	AF204931	GTGTGACACACTGCCCTGGA CAATGGCAAACTACTGAAAGCAA	$(TG)_{19}$	54	6	183–195	0.60	0.65
Ha6	AF204932	TTATCCGCTTGATATATCCT ACTCGTACATGGTTGAAAAC	$(GA)_{23}(GGA)_4$	51.4	21	162–232	0.79	0.76
Ha8	AF204937	AGTTTGCTGGTTTGTACACTCG CGTTTTTAGCTCTTGAATACGG	$(CA)_{14}CGTG(CA)_3AGATG(CA)_2$	51.4	18	152–220	0.91	0.85
Ha10	AF204940	GCGTTCAATGTAGTTTATGTGCG GAGAACATGCATACAAACAAACATG	$(CA)_6(CGCA)_3(CA)_4TACACG(CA)_{14}$	54	8	225–241	0.82	0.81
Ha11	AF204938	CGTGTACTACTGGGCAACGT ACGGAAAGAGACAGAAAGTGAG	$(TC)_2ACTGTTCC(TC)_{33}$	54	21	207–309	0.91	0.87
Ha13	AF204930	AAAATAGTTCCTGCATGTTACGTAG CTGGTGTTAACAGCGAAGTTCT	$(TC)_4(AC)_9(GC)_2(ACGC)_7GC(ACGC)_7$	54	25	162–344	0.79	0.79
Southern France#								
Ha7	AF204933	GCCATATGGGATAAAATACCGGTG CGCCCCTTGTTTACACGAGAAA	$(TC)_2G(TC)_{37}$	54	18	216–296	0.66	0.77
Ha9	AF204936	AGCTAACCCACACTCAGATTT AGCCAGCTAATATGTTTGGA	$(TG)_5 \ldots (CA)_{20} \ldots (AT)_6$	49.5	11	138–173	0.52	0.72
Ha12	AF204941	CCATGAAATACGACATATTC TTGAAGTCCATTGAAATCTA	$(CA)_{50}CC(CA)_3CC(CA)_4$	48.5	13	107–147	0.68	0.81

not be sufficiently variable to reveal this underlying model of population level structuring, we have developed microsatellite markers which may help redefine the panmictic unit of the species previously estimated using the marked-recapture method. Here, we describe the first microsatellite loci isolated from the genome of *H. aspersa*. Most snails used to assess the polymorphism of these loci originated from populations living in fragmented habitats (Polders of the Bay of Mt-St-Michel).

Genomic DNA was extracted from foot muscle of one fresh snail using the CTAB extraction protocol (Terret 1992). Approximately 5 μg of DNA extract was digested to completion with *Sau*3AI (Pharmacia). The restriction fragments were separated on a 1.4% agarose gel and a range from 500 to 700 bp were selected with NA45 DEAE membrane. After recovery by phenol–chloroform extraction, DNA fragments were ligated to *Bam*HI-digested pUC18 (Pharmacia) vector and cloned in *Escherichia coli* XL-1 Blue Cells (Stratagene). Over 2000 recombinant clones were transferred onto nylon membranes (Hybond-N^+, Amersham) and hybridized with an equal mix of $(TC)_{10}$, $(TG)_{10}$, $(CCT)_5$, $(CAC)_5$, $(ATCT)_6$, and $(TGTA)_6$ oligonucleotides labelled with digoxygenin, using the DIG oligonucleotide tailing kit (Boehringer Mannheim). Of the 78 positive clones detected (3.7% of the recombinant plaques), inserts from 19 of those harbouring a strong hybridization signal were polymerase chain reaction (PCR) amplified using the M13/pUC18 universal primers, and sequenced using an ABI 377 DNA automated sequencer. Primer sets were designed for 13 microsatellite sequences (the remainder were unsuitable because of incomplete sequences obtained) using OLIGO software version 4.04. One primer of each set was fluorescently labelled with 6-HEX, NED, or 6-FAM dyes (Perkin-Elmer).

DNA for genotyping was extracted by using a 10% Chelex-100 (Sigma) suspension. The PCR reactions were carried out either in 25 μL, when using the amplification 'ready to go' PCR beads (Pharmacia), or in 10 μL which contained ≈ 50 ng of genomic DNA, 1 U of *Taq* polymerase (Appligène), 1.2–1.4 μL of PCR buffer (10 mM TrisHCl, pH 9.0; 50 mM KCl, 0.1% Triton X-100; 0.2 mg/mL BSA or gelatin; 1.5 mM $MgCl_2$), 0.2 μL of 100 mM dNTPmix, 0.20 μM of each primer. Cycling was performed in a Biometra® thermocycler with one cycle of 3.5 min at 95 °C followed by 30 cycles of 30 s at 94 °C, 30 s at the annealing temperature (Table 1), and 30 s at 72 °C, and a final 5 min extension at 72 °C. The PCR products were visualized on a 2% agarose gel and electrophoresed using an ABI Prism 310 Genetic Analyser (Perkin-Elmer).

Initial screening for polymorphic loci was performed with 117 individuals collected in different sample sites from the Mt-St-Michel's Bay. Of the 13 primer pairs designed, 10 reliably amplified a single strong band. Seven loci revealed extensive allelic polymorphism for this fine level of population structuring with the number of alleles per locus ranging between 6 and 25 and the observed heterozygosity between 0.60 and 0.91 (Table 1). Because of two monomorphic loci (Ha9 and Ha12) and a null allele suspected for loci Ha7, new individuals from populations sampled in Southern France were tested. Even with only 50 individuals screened, 11 and 13 alleles were detected for loci Ha9 and Ha12, respectively. For the 10 loci analysed, the observed heterozygosity values were very close to the expected heterozygosity values, meaning that the Wahlund effect due to population substructuring may contribute to the deficit of heterozygotes previously observed with allozyme data. This paper shows that microsatellites provide an ideal tool for studying population structure and estimating gene flow among demes which may function as metapopulations.

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