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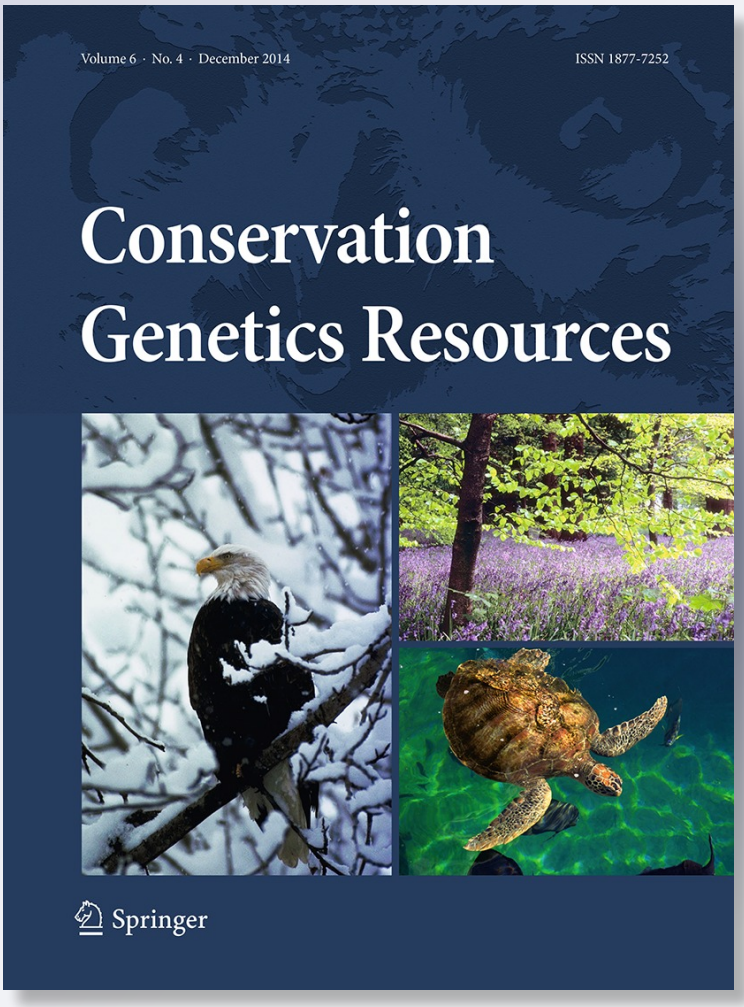

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MICROSATELLITE LETTERS

Characterization of polymorphic microsatellite markers for the fine-leaved water-Dropwort *Oenanthe aquatica* and the Gypsywort *Lycopus europaeus*, two farmland remnant wetland species

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Abstract *Oenanthe aquatica* (Apiaceae) and *Lycopus europaeus* (Lamiaceae) are two hygrophilous plant species found in remnant wetland habitats in agricultural areas. *O. aquatica* is declining and protected and *L. europaeus* more common, while still restricted to remnant wet habitats. To gain insights into their population genetic structure, we developed and characterized 16 (*O. aquatica*) and 15 (*L. europaeus*) novel polymorphic microsatellite markers from next-generation sequencing. The number of alleles ranged from 1 to 14 for *O. aquatica*, and from 1 to 9 for *L. europaeus*. Expected heterozygosity ranged from 0.156 to 0.903 and from 0.333 to 0.749, with a mean multilocus F_{IS} estimate of 0.043 and 0.092 for *O. aquatica* and *L. europaeus*, respectively. Overall, these newly developed microsatellite markers showed high levels of polymorphism that will facilitate fine-scaled population genetic studies.

Keywords *Oenanthe aquatica* · *Lycopus europaeus* · Fragmentation · Wetland remnants · Microsatellites

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Introduction

The fine-leaved water Dropwort *Oenanthe aquatica* (Apiaceae) and the Gypsywort *Lycopus europaeus* (Lamiaceae) are two diploid, mixed-mating, hygrophilous wetland plant species exhibiting hydrochorous dispersal. *O. aquatica* is a declining species, classified as endangered and protected in Northern France, while *L. europaeus* is more common. The development of polymorphic microsatellite markers will enable comparative population genetic structure analyses on both species that have contrasting mating system and local abundances in remnant wetlands found in agricultural areas (Zedler and Kercher 2005).

Total genomic DNA from *L. europaeus* and *O. aquatica* was isolated using the NucleoSpin 96 plant II kit (Macherey–Nagel, Duren, Germany) following the manufacturer's protocol and sent to GenoScreen, Lille, France (www.genoscreen.fr). By coupling multiplex microsatellite enrichment and next generation sequencing, 1 µg was used for the development of microsatellites libraries through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries as described in Malausa et al. (2011). Of 50,535 (*O. aquatica*) and 42,647 (*L. europaeus*) randomly fragmented sequences, 6,750 (*O. aquatica*) and 10,660 (*L. europaeus*) non-compound sequences containing microsatellite motifs were retained. A total of 564 (*O. aquatica*) and 1,159 (*L. europaeus*) sequences likely to contain suitable markers including microsatellite motif longer than five repeats were then returned, of which 48 loci with the longest repeat sequences (at least eight repeat motif) were initially tested for successful amplification on 7 individuals for both species.

Forward primers of the selected loci were labelled with 6-FAM, PET, NED, or VIC fluorescent dye (Applied Biosystem). PCR reactions were performed in 10 µl

Table 1 Name, species, primer sequence (5′–3′), repeat motif from the original sequence, annealing temperature (*Tm*), allelic size range (bp), multiplex number, Dye used, the total number of allele (A_n), the mean observed (H_o) and expected (H_e) heterozygosity, the mean intra-population fixation index (F_{IS}) estimated over two populations for both species ($N = 30$ individuals) and the accession number for 16 and 15 polymorphic microsatellite loci isolated in *Oenanthe aquatica* (Oa) and *Lycopus europaeus* (Le), respectively

Locus name	Species	Primer sequences (5′–3′)	Repeat motif	*Tm* (°C)	Allelic size range	Multiplex number	Dye	A_n	H_0	H_e	F_{IS}	Accession no.
O_01	Oa	F: AGTCGCAAGTTAAAGGGAAGC R: GGAGCATACTCTTGGGAGAGG	(AGC)6	57	191–195	3	NED	3	0.100	0.098	−0.024	KJ946205
O_02	Oa	F: CCACAACTCTTCACATTCTCCT R : TGGTTGGGGTCTCAGTTCTC	(TA)6	57	128	1	VIC	1	–	–	–	KJ946206
O_03	Oa	F: AATGTCATTCATCCCACCAC R: TGGGTTCCATGCAAAATTATC	(TTG)7	57	185–191	3	VIC	2	0.367	0.360	−0.020	KJ946207
O_10	Oa	F: ACATCGATCTGGGCTGGTAA R: ATTTAATTCAAGGCGTTGCG	(CA)11	57	139–177	2	6-FAM	10	0.867	0.836	−0.037	KJ946208
O_13	Oa	F: ACACAAGATTTATAATCTGGCAAA R: GCAATGACATAGTCCAAGCTG	(AC)11	57	183–189	2	NED	3	0.533	0.540	0.013	KJ946209
O_17	Oa	F:ATCTCAGTGTTATGTGCTGTGTAGA R:TCACCGGGCGTTGAATAATA	(TC)12	57	164–219	2	VIC	14	0.667	0.592	−0.127	KJ946210
O_18	Oa	F: TACACGAAAGCGACGGTGAT R: CATCAGGGTCCGATATGACA	(AG)12	57	239–251	1	VIC	5	0.800	0.723	−0.107	KJ946211
O_20	Oa	F: TTACCGTATTGTTAATTTTACCGGAG R:TTGCTCGAATTCCAACATAAA	(TC)12	57	291–319	1	NED	12	0.867	0.887	0.023	KJ946212
O_21	Oa	F: CACCCAACAAGAAACAGTACTATAAA R: TCAAAGCATTCTTGGCCTTC	(AC)12	57	286–303	1	PET	6	0.648	0.728	0.102	KJ946213
O_23	Oa	F: CTTAAACTCTCCAACTCTTAAATGCC R: ACTTCACCAGCTTCACCCAC	(CT)12	57	179–192	2	PET	2	0.333	0.512	0.349**	KJ946214
O_28	Oa	F: GCACTGTCCCGGTAAGTCTG R: AAGTTTGACTGATAAGGTTTCCA	(GT)13	57	112–123	1	6-FAM	4	0.467	0.402	−0.160	KJ946215
O_32	Oa	F: AGAAAACGGGGACGAAGAAG R: CACCAAGAAGCGACTCCACT	(TG)13	57	255–284	1	6-FAM	9	0.833	0.742	−0.124	KJ946216
O_34	Oa	F: TGTTTAACCTGAAAGCCAACA R: TGTCTCCGTGATGCAACATT	(CA)14	57	181–191	3	6-FAM	4	0.233	0.425	0.451***	KJ946217
O_37	Oa	F: TCGATAGCCACAAGAGCAAA R: TTACAATCATGGCTTCGTGA	(GA)15	57	231–257	3	PET	13	0.826	0.871	0.050	KJ946218
O_38	Oa	F: CAATCCAACACTCTCATTTTCC R: TCCTAAGCAAAGTCATCAATGC	(AC)15	57	121–139	1	NED	8	0.400	0.646	0.381***	KJ946219
O_47	Oa	F: CCATCGATAGCATCCAGGTA R: AATAGTAATTAGGAATCTCACGCAC	(TG)18	57	131–152	1	PET	8	0.833	0.814	−0.023	KJ946220
L_10	Le	F: TCAAGGAAAATCAGCAAGATTC	(TC)11	57	242–256	1	6-FAM	5	0.500	0.630	0.206*	KJ946221

Table 1 continued

Locus name	Species	Primer sequences (5′–3′)	Repeat motif	*Tm* (°C)	Allelic size range	Multiplex number	Dye	A_n	H_0	H_e	F_{IS}	Accession no.
		R: CCAATCTGTGGTATTCGAACTG										
L-11	Le	F: CTCGAGAGCGAAGGCAAA	(CT)12	57	246–250	1	NED	3	0.467	0.485	0.037	KJ946222
		R: CCTGAGAAGAGTTCATTGAGCA										
L-15	Le	F: GATACTGGCGTAGAAGATCGAA	(GA)12	57	154–160	3	PET	4	0.533	0.719	0.258**	KJ946223
		R: TCACGTTTACTGCATGTGGTC										
L-16	Le	F: GATTTTCTGCCGGCTTACAC	(TC)13	57	177–183	2	6-FAM	4	0.333	0.452	0.263*	KJ946224
		R: CAAACTGTGTTGGAATGGCA										
L-17	Le	F: GCCCTTCTTTTTGTGGTCG	(TC)13	57	135–155	3	VIC	7	0.667	0.749	0.110	KJ946225
		R: CGGAGCTTCCTCTCAACAAC										
L-18	Le	F: CAGATCTGGGACACCGCT	(TG)13	57	120–142	1	NED	6	0.567	0.648	0.125	KJ946226
		R: TCCAGCAAAACGTTACATGC										
L-19	Le	F: TTCATATTGCTCGTGATTCATT	(GA)13	57	284–293	1	PET	4	0.500	0.544	0.081	KJ946227
		R: GCATGTATTTTGGTTAGATATCAGG										
L-23	Le	F: GATGCTCTCAAAGAGGTGGG	(TCT)14	57	181–200	2	PET	5	0.588	0.533	−0.098	KJ946228
		R: GAGAAACCTAGACTCCACAACTGA										
L-33	Le	F: GATGATGGGAATAAGCCGTG	(GA)16	57	108–126	1	6-FAM	8	0.533	0.618	0.137	KJ946229
		R: TCATTTTCTTCGCAGCATGA										
L-35	Le	F: CTCGCTCTGCAGAAACACAA	(AC)17	57	103–118	1	VIC	6	0.667	0.574	−0.162	KJ946230
		R : AAGACAGAGTTCTCGTGCCA										
L-38	Le	F: TAGACATGCTTTGTTTGATTGATATT	(CA)18	57	236–242	1	VIC	4	0.633	0.596	−0.062	KJ946231
		R: GACAGCAGCACCTGCAAAT										
L-40	Le	F: GTATAGGAAAAGGGAAGGAAAAA	(GA)19	57	125–146	3	NED	7	0.433	0.454	0.045	KJ946232
		R: CAAGTACACGGTGAGATTCTGC										
L-42	Le	F: TACAAAAGGAGTCGCACCGT	(AG)19	57	122–140	3	6-FAM	5	0.400	0.637	0.372***	KJ946233
		R: GGGAACAAGCTTTTGGCTTT										
L-45	Le	F: ACCATTCTACAATGCAACCG	(GA)19	57	180–202	2	NED	9	0.733	0.679	−0.081	KJ946234
		R: ACAAAACACATCATGGCATATCA										
L-48	Le	F: GGCACTAGTTCCACTTAATTGCC	(CA)10	57	126	1	PET	1	–	–	–	KJ946235
		R: TGCAGAAATGGTAGGATAATGG										

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

volume containing 20 ng of genomic DNA, 1X multiplex PCR master mix (QIAGEN Hilden, Germany), 0.1 µM of forward and reverse primer. The PCR cycling program had an initial denaturation of 95 °C for 15 min; 30 cycles of 94 °C for 30 s, annealing temperature (see Table 1) for 1 min 30 s, and 72 °C for 1 min; and a final extension at 60 °C for 30 min. PCR was conducted on a Mastercycler ep Gradient S (EPPENDORF France SARL, Le Pecq, France). 1 µl of PCR product were pooled in 9.75 µl of deionized formamide (Applied Biosystems) and 0.25 µl of GeneScan 500 LIZ size standard (Applied Biosystems). PCR products were subsequently electrophoresed and sized using a 3130 XL DNA Sequencer (Applied Biosystems) and the software GeneMapper version 4.0, respectively.

We tested the polymorphism of isolated suitable microsatellite markers on individuals coming from two populations of *L. europaeus* (2°37′5.52″N, 50°36′8.33″E; 2°44′34,91″N, 50°36′0.09″E) and two populations of *O. aquatica* (2°40′42.90″N, 50°35′47.84″E; 2°40′43.23″N, 50°35′21.63″E) located in Northern France (15 individuals per population). 15 markers (*L. europaeus*) and 16 markers (*O. aquatica*) were polymorphic, had easily readable chromatograms with no stutter peaks. Primer pairs were successfully combined into three multiplex per species, ranging from three to eight markers (Table 1). Basic parameters of genetic diversity were estimated using FSTAT, version 2.9.3 (Goudet 1995). For *O. aquatica*, the number of alleles varied from 1 to 14 among loci (mean = 6.5) for a total of 104 alleles observed. For *L. europaeus*, 1–9 alleles were observed among loci (mean = 5.2) for a total of 78 alleles. Mean observed heterozygosity (H_o) values ranged from 0.100 to 0.867 (*O. aquatica*) and from 0.333 to 0.733 (*L. europaeus*). Mean expected heterozygosity (*He*) was comprised between 0.156 and 0.903 for *O. aquatica* and between 0.333 and 0.749 for *L. europaeus*. F_{IS} estimates ranged from -0.160 to 0.451 (*O. aquatica*) and from -0.162 to 0.372 (*L. europaeus*) for a mean multilocus value of 0.043 (±0.045) and 0.092 (±0.042), respectively. No linkage disequilibrium was observed for any pairs of loci for both species.

Overall, these newly developed microsatellite markers will be markers of choice for fine-scaled population genetic studies devoted to study the impact of remnant wetland fragmentation on patterns of gene flow.

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References

Goudet J (1995) FSTAT (version 1.2). A computer program to calculate F-statistics. J Hered 86:485–486

Malausa T, Gilles A, Meglécz E, Blanquart H, Duthoy S, Costedoat C, Dubut V, Pech N, Castagnone-Sereno P, Délye C, Feau N, Frey P, Gauthier P, Guillemaud T, Hazard L, Le Corre V, Lung-Escarmant B, Malé P-J, Ferreira S, Martin J-F (2011) High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNAlibraries. Mol Ecol Res 11:638–644

Zedler JB, Kercher S (2005) Wetland resources: status, trends, ecosystem services, and restorability. Annu Rev Environ Resour 30:39–74