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SHORT COMMUNICATION

Development and characterization of microsatellite loci in *Ulex parviflorus* Pourr. And its cross-transferability to other Genisteae

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Abstract

Background The genus *Ulex* is composed by 15 species distributed in Europe and Africa, but the majority of them are restricted to the Iberian Peninsula and Northwest Africa. Some of these species are common elements at the landscape level, and others contribute to global biodiversity as narrow endemics. Assayed nuclear and plastid Sanger-sequenced regions do not provide enough resolution to perform evolutionary studies on the genus, neither at the intraspecific population level nor at the interspecific phylogenetic level. Thus, we have developed and characterized a set of nuclear microsatellite loci in *U. parviflorus* to provide new highly polymorphic molecular markers for the genus *Ulex*.

Methods and results Genomic DNA enriched in microsatellite motifs using streptavidin-coated M-280 magnetic beads attached to 5′-biotinylated oligonucleotides was sequenced in a 454GS Junior System. After primer design, fluorescent-dyed amplicons were analyzed through capillary sequencing (ABI3730XL). Here we present twelve new high polimorphic SSRs markers developed in *U. parviflorus* specimens and tested in 120 individuals. The 12 SSR loci amplified a total of 152 alleles, and detected expected heterozygosities that ranged from 0.674 to 0.725 in the genotyped populations. Successful cross-species transferability of the 12 SSR loci to the rest of species included in the genus *Ulex* and three other representative Genisteae was achieved.

Conclusions The 12 novel proposed SSRs loci will contribute to perform evolutionary studies and genetic research on the genus *Ulex* and in other Genisteae.

Keywords Microsatellites · *Ulex* · SSRs · Genisteae

Introduction

Gorses (*Ulex* L., Fabaceae, Genisteae) are thorny flammable shrubs. Although the invasiveness of *Ulex europaeus* L. has expand this genus worldwide [1], the majority of the taxa show narrower distribution ranges in the Iberian Peninsula and northwestern Africa, which are considered the centers of diversification of the genus [2, 3]. *Ulex* includes both diploid and polyploid taxa [4]. The large number of polyploid taxa included in *Ulex*, —12 [4, 5]—, spanning from tetra- to hexaploids, suggests that hybridization and whole genome duplication (WGD) events have played an important role in the evolutionary history of *Ulex*. The most comprehensive taxonomic treatment for *Ulex* recognized 21 taxa within 15 species [4]. Notwithstanding, the overall similar morphology among taxa of *Ulex* together with the occurrence of several polyploid complexes has made it difficult the fulfillment of evolutionary studies of the genus [2, 3, 5, 6]. Phylogenetic relationships within the genus remain unresolved due to poor resolution of sequences from plastid and nuclear DNA regions assayed to date [2, 3, 5, 6] indicating a rapid and recent radiation of *Ulex* [3].

In order to develop microsatellite (SSR) markers for the genus *Ulex*, we chose *Ulex parviflorus* Pourr. as a model species. *Ulex parviflorus* is a diploid ($2n=32$) representative species of the fire-prone, eastern Iberian Peninsula shrublands, where it constitutes a relevant element of the landscape and a driver of the fire activity [7]. Despite *U.*

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parviflorus can recruit in vegetation gaps unrelated to fire, its germination is massively triggered by fire. Because it does not resprout after fire (postfire obligate seeder), fire promotes a high population turnover without overlapping generations thus, this species is a promising target to perform population genetic studies in post-fire scenarios.

Although the current affordability of High-Throughput Sequencing (HTS) technologies has led the researchers to conduct genome-wide studies screening ten of thousands of Single Nucleotide Polymorphism (SNPs), SSR markers still represent a powerful tool to perform studies in the fields of population genetics, systematics and heritability [8–10]. These co-dominant, highly polymorphic, genetically neutral and repeatable markers will be useful to perform population genetic analyses in *U. parviflorus*, a suitable model to study the genetic dynamics of a postfire obligate seeder species.

We further screened cross-species transferability of the characterized SSRs markers in all other species of *Ulex* and three other related taxa of Genisteae in order to evaluate their potential use for population genetic analyses. This is of special relevance given the absence of sufficiently polymorphic markers in this group of closely related taxa which include a high proportion of narrow endemic taxa of conservation concern (i.e., *U. erinaceus* Welw. *ex* Webb, *U. canescens* Lange), invasive plants (*U. europaeus*) and hybridogenous polyploid aggregates [3], and which may enable deciphering their auto/allopolyploid origin [11–13].

Materials and methods

Total DNA was isolated from plant material of four *U. parviflorus* specimens using the SpeedTools Plant DNA Extraction Kit (Biotools, Madrid, Spain). Digestion of DNAs was carried out in four 50µl independent reactions each containing 5U *Mse*I (New England Biolabs, Ipswich, USA), 1 × rCutSmart™ Buffer (New England Biolabs) and 44µl of crude DNA extract from the isolation step and incubated for 11 hours at 37°C and then 20 min at 65°C for enzyme inactivation. Digested DNAs were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and then ligated to sticky ends *Mse*I adapters (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3') and then PCR amplified. Twenty-four PCRs (six per each digestion reaction from the previous step) were performed in 50 µl volume, each containing 1 × *Taq* Buffer, 2mM $MgCl_2$ (Biotools), 0.4mM of each dNTP, 2.5µM primer *Mse*I-N, 2.5U of *Taq* polymerase (Biotools) and 5 µl of a 1:10 dilution of the purified ligation reaction. The PCR program consisted of a first step of 2 min at 72 °C to allow *Taq* polymerase fill the nicks in the DNA template followed by an initial DNA melting step of 4 min at 94 °C, and then 30 cycles each of 30 s at 94 °C for DNA melting, 1 min at 53 °C for annealing and 1 min at 72 °C for extension, followed by a 7 min at 72 °C for final extension. All PCRs were carried out in a FlexCycler Monoblock 96×0.2ml (Analytik Jena, Jena, Germany). After visual verification of the PCR products in 2% agarose gels, PCR products of each four digestions were pooled together in four independent aliquots of 270 µl. DNA from each aliquot was precipitated with 0.5 v/v of 7.5 M ammonium acetate and 2 v/v of absolute ethanol at -20 °C overnight. After several washing steps with 70% ethanol, each precipitate was resuspended in 45 µl of MQ water.

Microsatellite enrichment was performed using streptavidin-coated M-280 magnetic beads (Dynal) attached to $(CT)_{14}$, $(GT)_{14}$, $(AAC)_9$ and $(AAG)_9$ 5′-biotinylated oligonucleotides. The four bead preparations were washed three times each with 1× B&W buffer to remove unbound oligonucleotides. Then, they were resuspended in 100 µl of 3 × Saline Sodium Citrate (SSC, 20 × SSC: 6 M NaCl, 0.6 M Na-citrate, pH 7), 0.1% SDS and 2% PEG-6000 and kept at hybridization temperature (40 °C). Four 15 µl target DNA aliquots were denatured 4 min at 95 °C and put directly on ice. Hybridization was carried out by adding the denatured target DNAs to the dynabead-biotinylated oligonucleotide preparations at 40ºC for 30 min. Hybridization products were washed 12 times in three SSC decreasing concentration solutions (four times each); 2 × SSC, 1 × SSC and 0.5 × SSC plus 0.1%SDS and 1.6µM of the *Mse*I-N primer. Fragments with microsatellite motifs were released at 95ºC for 5 min in 100 µl of 0.2 × SSC solution and desalted with Qiaex II (Qiagen). PCR amplification of the released fragments were carried out with the same conditions as the described above.

PCR products were sequenced on a GS Junior 454 sequencer at SCSIE (Servei Central de Suport a la Investigació Experimental, University of Valencia). The raw reads were deposited under the BioProject number PRJNA888869 with SRA accession SRR21850415. From 75,066 raw reads generated by the sequencing platform, 65,051 were longer than 80 bp after clipping and of these, 20,809 included microsatellite motifs. These included 1065 unique consensus sequences and 2066 singleton sequences. The screening of those 3131 sequences with QDD3 [14] pipe3 retrieved 825 sequences with primers. Primer design was refined through the online resource PRIMER3 [15] based on the results of the software QDD3.

From 87 PCR tested primer pairs, 22 primers corresponding with the clearest amplicons visualized on 2% agarose gels were selected for fluorescence labeling.

PCRs were carried out in a final volume of 20 µl containing 1 × *Taq* Buffer, 2mM $MgCl_2$, 0.4mM of each dNTP, 100 µg of BSA (Biotools), 2.5 pmol (0.125 µM) or 5 pmol

(0.25 µM) (Table 1) of both primer R and fluorescence-labeled primer F, 1U of *Taq* polymerase and 1.5 µl DNA template. PCR program consisted of an initial DNA melting step of 4 min at 94 °C then 39 cycles each of 1 min at 94 °C for DNA melting, 1 min at annealing temperature (Table 1), 1 min at 72 °C for extension, followed by a final extension step of 7 min at 72 °C. Amplified fragments were electrophoresed on an ABI3730XL (Applied Biosystems, Madrid, Spain) capillary sequencer using LIZ500 as internal size standard. Amplicon fragments were assigned to alleles using GeneMarker v.1.85 (Softgenetics, State College, USA).

From a total of 22 primer pairs, 10 were discarded due to unspecific amplification, no polymorphism or no amplification. The remaining 12 primers (Table 1) were selected and used to genotype a set of 120 individuals, from three populations of *U. parviflorus* (hereafter CAS, SOT and GOD, see Supplementary Table 1) in order to check for levels of polymorphism within and among populations. Basic population genetics statistics (N_A, A_R, H_O, H_E, F_{IS}) were scored for each locus and population and overall loci within populations using the R [16] packages adegenet [17], pegas [18] and PopGenReport [19]. Deviations from Hardy-Weinberg equilibrium were assessed using fisher exact tests based on 10,000 Monte Carlo permutations of alleles [20] as implemented in pegas. Linkage disequilibrium (LD hereafter) across loci was tested through $\bar{r}_d$ index [21] based on a random distribution created via 10,000 permutations of alleles using the R package poppr [22]. Additionally, cross-species amplification of the 12 microsatellite loci was tested in 35 individuals corresponding to 19 taxa including all species of *Ulex* and representative taxa of the related genera *Genista* L., *Pterospartum* (Spach) K. Koch and *Stauracanthus* Link.

Results and discussion

The 12 selected microsatellite loci were polymorphic, revealing a total of 152 alleles in the three analyzed populations of *U. parviflorus* (Table 2); the total number of alleles per population ranged from 97 to 120 in GOD and CAS populations, respectively. The number of alleles per locus ranged from three in Upa54 (SOT and GOD) and Upa44 (GOD), to 25 alleles in Upa78 (CAS). Observed and expected heterozygosities ranged from 0.057 (Upa54 SOT)

Table 1 Characteristics of 12 polymorphic microsatellite loci developed for Ulex parviflorus. For each locus the primer pair sequences, repeat motif, temperature of annealing, number of alleles, allele size range, and Genbank accession numbers are indicated

Locus	Primer sequence (5'- 3')	Repeat Motif	Size (bp)	T (°C)	N_A	Size range (bp)	Genbank number
Upa33	F: NED-CTGCAATCACAGCACTTGGT R: TCGAACATTATCTTAGCCGATCT	$(CT)_{11}$	171	56*	8	155–187	OQ676447
Upa37	F: VIC-ATGAATGTGGCCGGTTCAG R: CAGTTCAACCCTTCCTCCTGA	$(GGT)_7$	149	56*	13	134–155	OQ676451
Upa44	F: PET-GCACCTGCCTTGTAACCATT R: TCCAGTAAAGCTTCATTGCA	$(CA)_7$	171	56*	11	167–177	OQ676452
Upa45	F: PET-GCAGGCACGAGCCTCCATTT R: GAATTCTCGCTTGTGAGCT	$(CT)_9$	143	56*	10	141–159	OQ676450
Upa54	F: VIC-CTGGTATACTGAACACATGGAG R: CATAAGCACATGCATGGGAGC	$(TC)_7(CA)_4$ GA$(CA)_6$	171	56*	14	165–173	OQ676443
Upa55	F: 6FAM-CACTCGTGGTTCAACGCTTA R: GTGGCTTAGGTGCTAGTGTG	$(CT)_{10}$	185	60*	5	137–189	OQ676453
Upa58	F: NED-GTTGAACTCGTAGCCTCTGTC R: TAGGGACCTCGAGAACACAG	$(GT)_7$	149	51*	5	101–153	OQ676449
Upa59	F: PET-ATCACTGCAGTTGCTGGTAC R: ATGTATGTCTGTGCATGTG	$(CT)_{13}$	120	56**	23	104–124	OQ676448
Upa61	F: PET-CCTGCTTCCACAGTCTCCTC R: TCCTAATCTTATGGATCTGCTC	$(CT)_{10}$	153	51*	14	141–157	OQ676446
Upa63	F: NED-AATGCCAAACAACCAAGTCC R: TGGAGGAACAACATCTTCACC	$(CT)_9$	254	63*	27	246–274	OQ676442
Upa64	F: 6FAM-ACCATAACCTGCTCAACCATC R: CAAACAAGGTCAATTAATGTTCTC	$(CAA)_6CAG(CAA)_{10}$	252	48**	16	216–261	OQ676445
Upa78	F: 6FAM-ATCACCCGAGACTCTGAGC R: GAGATTGAGATGAAGAAAGATTGG	$(GA)_{12}$	140	51**	6	126–200	OQ676444

6FAM, NED, PET and VIC are fluorescent dyes from Applied Biosystems (Madrid, Spain).

*: Optimal amplification using 0.125µM concentration of each primer.

**: Optimal amplification using 0.25µM concentration of each primer.

Table 2 Genetic diversity characteristics of 12 Ulex parviflorus microsatellite loci amplified in three populations

	Castell de Castells ($N=50$)					Sot de Ferrer ($N=35$)					Godella ($N=35$)				
Locus	N_A	A_R	H_o	H_e	F_{IS}	N_A	A_R	H_o	H_e	F_{IS}	N_A	A_R	H_o	H_e	F_{IS}
Upa33	10	9.569	0.540	0.817	0.348^{***}	10	9.443	0.714	0.862	0.186^{*}	15	14.22	0.514	0.864	0.417^{***}
Upa37	4	3.939	0.320	0.366	0.136^{*}	4	3.752	0.457	0.622	0.278^{*}	5	4.737	0.629	0.718	0.139^{ns}
Upa44	5	4.691	0.700	0.646	-0.073^{ns}	4	3.752	0.800	0.666	-0.188^{ns}	3	3	0.743	0.573	-0.284^{ns}
Upa45	9	8.628	0.200	0.742	0.735^{***}	7	6.5	0.657	0.734	0.119^{ns}	7	6.67	0.457	0.783	0.428^{***}
Upa54	5	4.675	0.140	0.204	0.323^{**}	3	2.926	0.057	0.135	0.587^{**}	3	2.999	0.314	0.298	-0.039^{ns}
Upa55	16	14.37	0.720	0.850	0.163^{ns}	15	13.638	0.886	0.897	0.027^{ns}	19	17.109	0.771	0.878	0.136^{*}
Upa58	8	7.424	0.440	0.580	0.251^{*}	7	6.686	0.657	0.714	0.094^{*}	7	6.407	0.600	0.670	0.119^{ns}
Upa59	11	9.855	0.600	0.746	0.206^{**}	6	5.503	0.771	0.589	-0.298^{ns}	8	7.585	0.571	0.622	0.095^{**}
Upa61	5	4.925	0.580	0.639	0.103^{ns}	6	5.692	0.657	0.675	0.040^{ns}	6	5.928	0.571	0.674	0.166^{ns}
Upa63	11	10.457	0.680	0.687	0.021^{ns}	10	9.317	0.514	0.601	0.158^{*}	9	8.617	0.706	0.728	0.045^{ns}
Upa64	11	10.481	0.760	0.780	0.036^{ns}	12	11.482	0.914	0.886	-0.017^{ns}	11	10.42	0.857	0.846	0.001^{ns}
Upa78	25	22.643	0.700	0.934	0.260^{***}	13	12.289	0.771	0.805	0.057^{ns}	17	15.723	0.429	0.894	0.531^{***}
Mean	10	9.305	0.532	0.674	0.212^{ns}	8.083	7.582	0.655	0.692	0.055^{ns}	9.167	8.618	0.597	0.725	0.177^{ns}

N: sample size of each population, N_A: number of alleles, A_R: allelic richness, H_o, H_e: observed and expected heterozygosities, F_{IS}: inbreeding coefficient, Hardy-Weinberg equilibrium test: ns, not significant; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

to 0.914 (Upa64 SOT) and 0.135 (Upa54 SOT) to 0.934 (Upa78 CAS), respectively. The high number of alleles, allelic richness and genetic diversity values obtained (see N_A,A_R, and H_e values in Table 2, respectively) in comparison with others SRRs studies on taxa belonging to rosids group [23], could be explained by a combined effect of massive flowering, high fruit set and rapid population turnover in this Mediterranean pyrophyte.

Significant deviation of HWE towards heterozigosity deficiency was found for several loci at different populations (Table 2). These could be interpreted as inbreeding or as the effect of population substructure. *Ulex parviflorus* is self-compatible species, but it relies on bee visits for effective pollination and fruit set due to its papilionoid flowers [24]. The massive blooming of entomophilous hermaphrodite flowers in *U. parviflorus* could increase selfing rate by geitonogamy. Notwithstanding, overall F_{IS} values were not significantly different from zero in any of the three genotyped population (Table 2).

No consistent significant linkage disequilibrium ($P<0.01$) was found across loci × population comparisons except for loci Upa55 and Upa33 in all populations, and locus pairs Upa64/Upa54 and Upa64/Upa63 in SOT (Supplementary Fig. 1).

Cross-species transferability was achieved for all the studied samples of *Ulex* (Table 3). In addition to the cross-amplified samples corresponding to 14 species of *Ulex*, (which together with *U. parviflorus* constitutes the total of the species previously recognized within the genus [1]), samples of three representative genera from the tribe Genisteae (*Genista triacanthos* Brot. *Pterospartum tridentatum* (L.) Willk and *Stauracanthus boivinii* (Webb) Samp.), were successfully amplified except for locus Upa63 in *G. triacanthos*.

Allelic patterns in the cross-amplified taxa were consistent with ploidy levels (Fig. 1) except for locus Upa63 in *U. micranthus* and *U. minor* where three to four peaks were obtained in all accessions suggesting a locus duplication in this species pair (Table 3).

The proportion of polymorphic loci among the assayed species of *Ulex* was high with an average of 93.3 ± 10.6%, ranging from 75% in *U. eriocladus* to 100% in *U. australis*, *U. baeticus*, *U. borgiae*, *U. erinaceus*, *U. europaeus*, *U. gallii*, *U. jussiaei* and *U. minor* (Table 3). Average proportion of polymorphic loci was slightly higher among the polyploid taxa (97.2 ± 7.9) than among diploid ones (87.5 ± 11.5), this being consistent with the higher probability of different allele accumulation in polyploid genomes. Within the assayed accessions of other Genisteae the proportion of polymorphic loci was on average lower than in *Ulex* (70.7 ± 19.1%). Within this group, the highest proportion of polymorphic loci was obtained for *Stauracanthus bovinii*

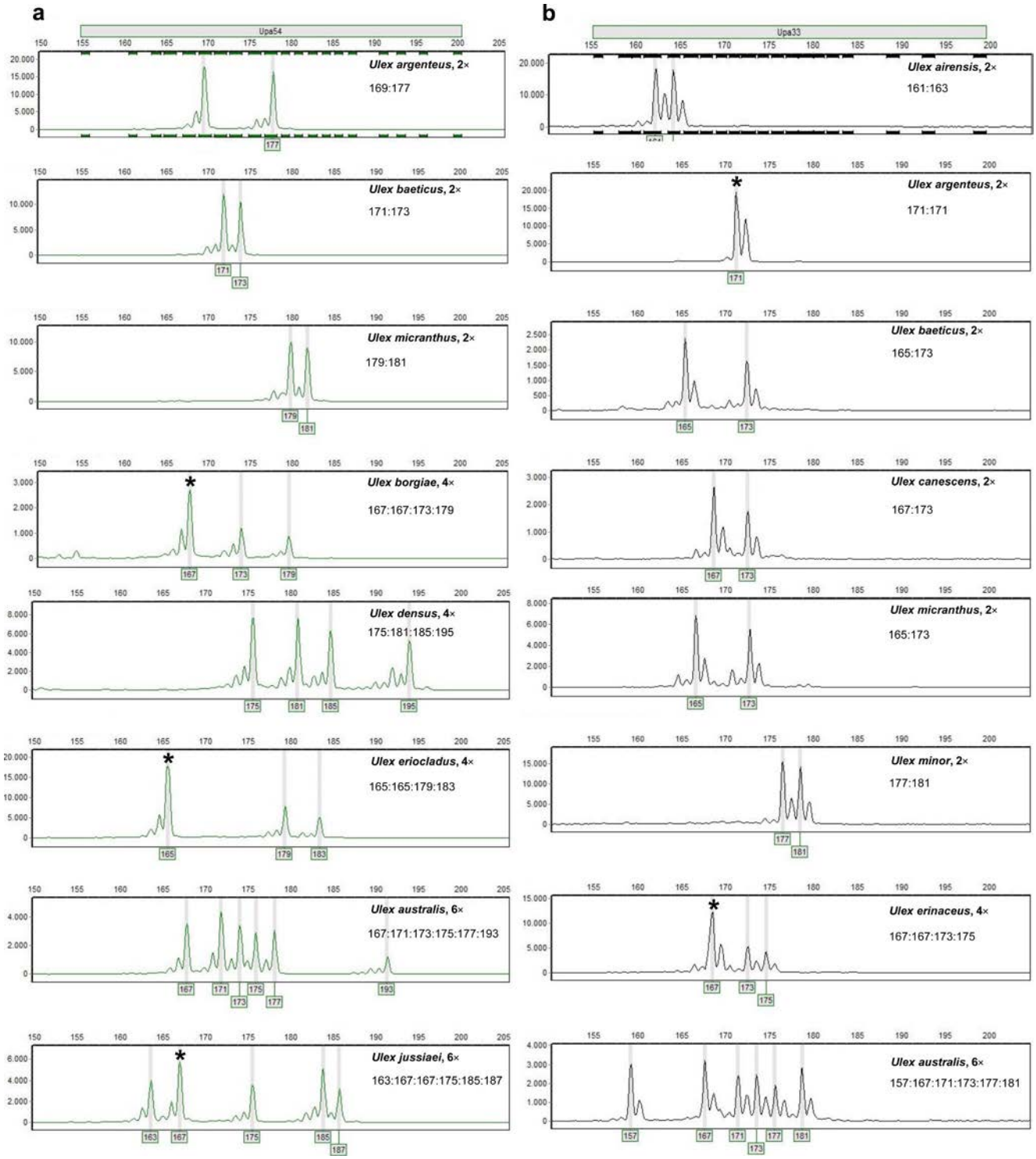

Fig. 1 Sample electropherograms of two of the characterized microsatellite loci cross-amplified in representative species of *Ulex* with different ploidy levels. (**a**) Locus Upa54, (**b**) Locus Upa33. Asterisks denote amplification peaks that indicate alleles present in two copies. For each sample the inferred genotype is indicated with alleles coded in bp

(91.7%), the closest relative to *Ulex* in the most recent published phylogeny [3], and lower in the other two assayed Genisteae species (Table 3). This is consistent with the observed reduction of SSR polymorphism with increasing evolutionary distance of the cross-amplified species to the source species [25]. Notwithstanding, given that availability of accessions for cross-species transferability for some taxa was limited, it is likely that levels of polymorphism

Table 3 Cross-species amplification of the 12 microsatellite loci of Ulex parviflorus in 15 other species of Ulex and related Genisteae. For each sample and locus allelic size ranges and number of alleles, in brackets are indicated. Dashed lines indicate unsuccessful amplification. * Indicates a possible locus duplication event

Taxon	Chromosome number, ploidy	ID	Upa33	Upa37	Upa44	Upa45	Upa54	Upa55	Upa58	Upa59	Upa61	Upa63	Upa64	Upa78
U. airensis Espírito Santo & al.	$2n=32$, 2×	UAIREN1	161–163 (2)	143 (1)	169–171 (2)	149 (1)	171 (1)	145–149 (2)	153–163 (2)	118 (1)	139–147 (2)	252–260 (2)	213–234 (2)	124 (1)
		UAIREN2	173 (1)	143 (1)	171–173 (2)	149–161 (2)	171–175 (2)	165 (1)	143 (1)	124 (1)	147–179 (2)	254 (1)	216–228 (2)	124 (1)
U. argenteus subsp. ***argenteus*** Welw. *ex* Webb	$2n=32$, 2×	UARGEN1	171 (1)	143–149 (2)	171 (1)	149 (1)	169–177 (2)	161 (1)	143–149 (2)	122 (1)	149 (1)	252–264 (2)	219–222 (2)	124–126 (2)
		UARGEN2	171 (1)	134 (1)	169–173 (2)	137 (1)	169 (1)	161 (1)	151 (1)	96–128 (2)	139 (1)	248–252 (2)	216 (2)	122 (1)
U. australis Clemente	$2n=96$, 6×	UAUSTR1	157–181 (6)	131–143 (4)	167–173 (4)	147–155 (2)	167–195 (5)	139–177 (6)	147–177 (3)	100–130 (4)	135–149 (4)	250–264 (3)	213–228 (2)	126–170 (6)
		UAUSTR2	161–177 (3)	137–149 (4)	169–173 (3)	133–157 (3)	167–193 (6)	145–171 (5)	147–149 (2)	100–124 (3)	137–181 (4)	252–262 (4)	213 (1)	128 (1)
U. baeticus Boiss.	$2n=32$, 2×	UBAETI1	177–185 (2)	137 (1)	169–171 (2)	147–149 (2)	171–173 (2)	171–185 (2)	143–159 (2)	120–122 (2)	149 (1)	254 (1)	219–234 (2)	126–128 (2)
		UBAETI2	165–173 (2)	137 (1)	167–171 (2)	149 (1)	171 (1)	151–165 (2)	143 (1)	102–106 (2)	141–149 (2)	256–258 (2)	225 (1)	128 (1)
		UBAETI3	165–177 (2)	137–146 (2)	171–175 (2)	145 (1)	165–169 (2)	151–173 (2)	143 (1)	108–122 (2)	155–165 (2)	252–254 (2)	213–225 (2)	128–148 (2)
U. borgiae Rivas Mart.	$2n=64$, 4×	UBORGI1	171–173 (2)	143–149 (2)	171 (1)	147 (1)	171 (1)	161–165 (2)	143 (1)	106–130 (3)	149 (1)	254–256 (2)	219–225 (2)	122–126 (2)
		UBORGI2	161–183 (4)	128–149 (4)	171–173 (2)	153–155 (2)	167–179 (3)	145–173 (4)	147–153 (2)	96–122 (2)	133–173 (4)	254–262 (3)	216–222 (2)	126–154 (4)
U. canescens Lange	$2n=32$, 2×	UCANES1	167–173 (2)	146–149 (2)	171 (1)	143–157 (2)	171–177 (2)	157–165 (2)	149 (1)	96 (1)	149–151 (2)	254 (1)	210–231 (2)	128–138 (2)
U. densus Welw. *ex* Webb	$2n=64$, 4×	UDENSU1	177–183 (2)	137–143 (2)	169–183 (4)	143–147 (2)	175–195 (4)	171–181 (3)	143 (1)	108–124 (4)	145–151 (2)	250–260 (3)	213–219 (3)	122–142 (4)
U. erinaceus Welw. *ex* Webb	$2n=64$, 4×	UERINA1	167–175 (3)	137–155 (3)	171–173 (2)	143–159 (3)	167–185 (2)	157–169 (3)	143–145 (2)	94–100 (3)	135–157 (4)	248–252 (3)	213–222 (2)	122–134 (2)
U. eriocladus C. Vicioso	$2n=64$, 4×	UERIOC1	159–161 (2)	143–149 (2)	171–175 (2)	141–153 (2)	165–183 (3)	143 (1)	143–151 (2)	118–134 (2)	149 (1)	244–268 (4)	213–222 (3)	170 (1)
U. europaeus L. subsp. ***europaeus***	$2n=96$, 6×	UEUROP1	175–183 (3)	131–143 (4)	169–171 (2)	139–149 (2)	167–175 (2)	169–173 (2)	165–173 (4)	100–118 (3)	135–155 (4)	254–258 (3)	213–228 (4)	122 (1)
		UEUROP2	171–177 (3)	143 (1)	169–175 (3)	143–149 (2)	167–177 (3)	169–173 (2)	165–193 (3)	100–102 (2)	135–155 (4)	250–258 (3)	216–240 (3)	136 (1)
		UEUROP3	171–197 (4)	143 (1)	171–175 (3)	141–149 (2)	161–171 (4)	173–201 (3)	159–175 (2)	100 (1)	135–163 (4)	254–258 (3)	216–231 (3)	150 (1)

Table 3 (continued)

Taxon	Chromosome number, ploidy	ID	Upa33	Upa37	Upa44	Upa45	Upa54	Upa55	Upa58	Upa59	Upa61	Upa63	Upa64	Upa78
U. europaeus subsp. ***latebracteatus*** (Mariz) Rothm.	2*n* = 64, 4×	ULATEB1	163–171 (2)	140–143 (2)	167–175 (3)	149–159 (3)	183–189 (3)	149–163 (3)	145 (1)	100–118 (4)	135–151 (3)	248–254 (3)	216–225 (3)	122 (1)
		ULATEB2	167–201 (4)	143 (1)	169–171 (2)	149 (1)	167–193 (4)	157–213 (4)	145–167 (3)	100–110 (3)	135–151 (3)	250–260 (4)	216–231 (3)	156 (1)
		ULATEB3	179 (1)	140–143 (2)	169–171 (2)	149 (1)	171–187 (3)	173–185 (2)	165 (1)	104–118 (3)	133–153 (3)	246–254 (2)	213–222 (3)	126 (1)
U. gallii Planch.	2*n* = 96, 6×	UGALII1	171 (1)	131–143 (3)	169–171 (2)	147–149 (2)	169–201 (3)	173–181 (2)	161–195 (4)	100–128 (4)	135–155 (4)	252–262 (3)	216–228 (3)	128–144 (2)
		UGALII2	179 (1)	137 (1)	169–175 (4)	141–151 (3)	167–197 (3)	173–181 (2)	181–191 (2)	106–132 (4)	135–161 (5)	248–260 (4)	216–231 (3)	128 (1)
U. jussiaei Webb	2*n* = 96, 6×	UJUSSI1	163–165 (2)	131–143 (4)	169–173 (3)	133–153 (3)	163–187 (5)	147–163 (2)	149–157 (2)	106–130 (6)	139–155 (3)	252–256 (3)	213–231 (3)	124–126 (2)
		UJUSSI2	169–177 (2)	137–146 (4)	171–173 (2)	139–153 (3)	167–183 (4)	159–173 (3)	143–149 (2)	118–132 (6)	135–155 (4)	252–262 (4)	213–231 (5)	122–158 (2)
U. micranthus Lange	2*n* = 32, 2×	UMICRA1	165–173 (2)	131–149 (2)	173–181 (2)	149 (1)	193 (1)	153–165 (2)	191–195 (2)	141 (1)	133–159 (3)*	268–270 (2)	228–231 (2)	122 (1)
		UMICRA2	179–181 (2)	131–149 (2)	175–181 (2)	149–157 (2)	179–181 (2)	153–165 (2)	151–231 (2)	135 (1)	137–153 (3)*	252 (1)	210–222 (2)	122 (1)
		UMICRA3	179–181 (2)	131–149 (2)	173–175 (2)	149–151 (2)	185 (1)	153–165 (2)	163–211 (2)	135 (1)	135–153 (4)*	252 (1)	210–216 (2)	122 (1)
U. minor Roth	2*n* = 32, 2×	UMINOR1	185 (1)	143 (1)	169–171 (2)	139–149 (2)	167 (1)	185 (1)	143–149 (2)	100–116 (2)	135–151 (3)*	244–256 (2)	222–228 (2)	130–140 (2)
		UMINOR2	177–181 (2)	143–149 (2)	169–171 (2)	139 (1)	167–173 (2)	173–177 (2)	139–151 (2)	126–128 (2)	135–151 (3)*	252–258 (2)	216–228 (2)	130 (1)
		UMINOR3	181–183 (2)	143–149 (2)	177–183 (2)	139–141 (2)	165 (1)	177 (1)	143–151 (2)	139 (1)	135–151 (3)*	244–254 (2)	219–240 (2)	128–148 (2)
Genista triacanthos Brot.	2*n* = 32	GTRIAC1	167 (1)	149 (1)	175 (1)	149–157 (2)	167–171 (2)	153–165 (2)	151 (1)	100–118 (3)	149–167 (2)	-	213 (1)	126–142 (2)
Pterospartum tridentatum (L.) Willk.	2*n* = 56, 4×	PTRIDE1	165–175 (4)	137–143 (2)	169–173 (3)	147–165 (2)	16-1671 (2)	153–165 (2)	151–231 (2)	100 (1)	147–155 (3)	244 (1)	216–228 (2)	122 (1)
Stauracanthus boivinii (Webb) Samp.	2*n* = 48, 4×	SBOVIN1	165–173 (4)	143–149 (2)	165–175 (4)	143–157 (4)	163–177 (3)	153–165 (2)	151–211 (2)	100 (1)	139–151 (2)	254–264 (3)	213–243 (2)	138–142 (3)
		SBOVIN2	157–187 (4)	143 (1)	171–177 (3)	139–151 (2)	161–165 (3)	153–165 (2)	149–151 (2)	100 (1)	149–159 (4)	234–266 (3)	219–228 (2)	130–160 (3)

would increase with the addition of further accessions supporting a broader use of this set of SSR loci in *Ulex* and other Genisteae.

Our study reports a panel of highly polymorphic SSR loci that broadens the perspectives of studying the fields of population genetics, reproductive biology, heritability and polyploid origin in recently diverged taxa of *Ulex* and of other Genisteae.

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Data availability Raw reads from the pyrosequencing run were deposited in the NCBI database under the BioProject number PRJNA888869 with SRA accession SRR21850415, and are available at the following URL: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA888869. Microsatellite primer sequences are provided in Table 2, along with NCBI BankIt vouchers of the microsatellite loci sequences.

Declarations

Competing interests The authors have no relevant financial or non-financial interest to disclose.

Ethics approval This study does not involve animal experimentation.

Consent to participate This study does not involve human subjects.

Consent to publish This study does not involve human subjects.

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