

Characterization and Multiplexing of 21 Microsatellite Markers for the Herb Noccaea caerulescens (Brassicaceae)

Mathilde Mousset, Elodie Flaven, Fabienne Justy, Juliette Pouzadoux, Cécile Gode, Maxime Pauwels, Cédric Gonneau

▶ To cite this version:

Mathilde Mousset, Elodie Flaven, Fabienne Justy, Juliette Pouzadoux, Cécile Gode, et al.. Characterization and Multiplexing of 21 Microsatellite Markers for the Herb Noccaea caerulescens (Brassicaceae). Applications in Plant Sciences, 2015, 3 (12), pp.1500052. 10.3732/apps.1500052. hal-01282559

HAL Id: hal-01282559 https://hal.science/hal-01282559

Submitted on 3 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Characterization and Multiplexing of 21 Microsatellite Markers for the Herb *Noccaea caerulescens* (Brassicaceae)

Author(s): Mathilde Mousset, Elodie Flaven, Fabienne Justy, Juliette Pouzadoux, Cécile Gode, Maxime Pauwels, and Cédric Gonneau Source: Applications in Plant Sciences, 3(12) Published By: Botanical Society of America DOI: <u>http://dx.doi.org/10.3732/apps.1500052</u> URL: http://www.bioone.org/doi/full/10.3732/apps.1500052

BioOne (<u>www.bioone.org</u>) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



PRIMER NOTE

CHARACTERIZATION AND MULTIPLEXING OF 21 MICROSATELLITE MARKERS FOR THE HERB Noccaea CAERULESCENS (BRASSICACEAE)¹

Mathilde Mousset², Elodie Flaven^{2,6}, Fabienne Justy², Juliette Pouzadoux², Cécile Gode³, Maxime Pauwels^{3,6}, and Cédric Gonneau^{4,5}

²Institut des Sciences de l'Évolution, Université de Montpellier, CNRS, IRD, EPHE, CC 065, Place Eugène Bataillon 34095, Montpellier CEDEX 05, France; ³Université Lille, CNRS, UMR 8198, Evo-Eco-Paleo, F-59000 Lille, France; ⁴Université de Lorraine, Laboratoire Sols et Environnement (UMR 1120), F-54518 Vandœuvre-lès-Nancy CEDEX, France; and ⁵INRA, Laboratoire Sols et Environnement (UMR 1120), F-54518 Vandœuvre-lès-Nancy CEDEX, France

- Premise of the study: Multiplexed microsatellite markers were developed for population genetic studies in the pseudometallophyte Noccaea caerulescens (Brassicaceae), a model species to investigate metal tolerance and hyperaccumulation in higher plants.
- *Methods and Results:* Microsatellite loci were isolated through pyrosequencing of an enriched DNA library. Three multiplexes combining four previously published and 17 newly designed markers were developed. The new markers were screened in metallicolous and nonmetallicolous populations from southern France. The total number of alleles per locus ranged from five to 18. The observed heterozygosity per locus and per population ranged from 0 to 0.83, and expected heterozygosity ranged from 0 to 0.89.
- *Conclusions:* The investigated loci showed reasonable to high levels of polymorphism at the regional scale. The multiplex set should be helpful in investigating genetic diversity, population structure, and demographic history in *N. caerulescens* at various spatial scales.

Key words: Brassicaceae; heavy metal tolerance; microsatellite; Noccaea caerulescens; pseudometallophyte.

The Alpine pennycress, *Noccaea caerulescens* (J. Presl & C. Presl) F. K. Mey. (Brassicaceae), occurs over a large range in Europe. The species is particularly known for its capacity to grow on soils with a high concentration of trace elements such as zinc, cadmium, and nickel. Considering its phylogenetic proximity with *Arabidopsis thaliana*, this characteristic makes *N. caerulescens* a favorite model plant for the study of the genetic bases of metal homeostasis, as well as of the ecological and evolutionary processes involved in local adaptation to extreme environments (Assunção et al., 2003). In order to understand the effect of soil metals on the evolution of *N. caerulescens* populations, it is necessary to study the population genetics of the species. So far, however, the low number of available

¹Manuscript received 4 May 2015; revision accepted 4 August 2015.

The authors thank C. Hatt for her help in the sequencing of some of the individuals used in this study. Data used in this work were partly produced through the technical facilities of the LabEx Centre Méditerranéen de l'Environnement et de la Biodiversité. This work was supported by a grant from the Agence Nationale pour la Recherche (Project BIOADAPT, SEAD: ANR-13-ADAP-0011) and by funding from the Observatoire de Recherche Méditerranéen de l'Environnement (OSU-OREME) program. This is publication ISE-M 2015-208.

 $^6Authors for correspondence: elodie.flaven-noguier@univ-montp2.fr and maxime.pauwels@univ-lille1.fr$

doi:10.3732/apps.1500052

molecular markers (Basic and Besnard, 2006; Jiménez-Ambriz et al., 2007), low polymorphism, presence of null alleles (Basic and Besnard, 2006; Besnard et al., 2009), and low amplification rate (E. Flaven, personal observation), as well as the absence of protocols for high-throughput genotyping, has not allowed the performance of deep population genetic studies. Here, we introduce 17 new microsatellite markers organized in three multiplexes to reduce genotyping time and costs. The multiplexes also include formerly published markers, thus providing a complete resource in this species.

METHODS AND RESULTS

Microsatellite library construction—Genomic DNA of three individuals from the Baraquette population (Appendix 1) was extracted using a cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle, 1990) followed by RNase treatment, and mixed. Development of the microsatellite library was outsourced to Genoscreen (Lille, France). It involved coupling multiplex microsatellite enrichment isolation techniques with 454 GS FLX Titanium pyrosequencing of the enriched DNA, according to the protocol of Malausa et al. (2011). Enrichment was performed using probes containing the following motifs: AG_{10} , AC_{10} , AAC_8 , AGG_8 , AAG_8 , $ACAT_6$, and $ATCT_6$. Sequence data were automatically screened to detect microsatellite motifs, leading to 1852 candidate loci. Primers were designed in silico by Genoscreen, using the QDD pipeline (Meglécz et al., 2010).

Biological validation—Biological validation of a subset of these loci was simultaneously performed at Institut des Sciences de l'Évolution de Montpellier

Applications in Plant Sciences 2015 3(12): 1500052; http://www.bioone.org/loi/apps © 2015 Mousset et al. Published by the Botanical Society of America. This work is licensed under a Creative Commons Attribution License (CC-BY-NC-SA).

I ABLE I.		III Noccaea caeru	uescens.										
Locus	Primer sequences $(5'-3')$	Repeat motif	$T_{\rm a}$ (°C)	Ct (µM)	Multiplex	Fluorescent dye	Post-PCR dilution	Position $]$ $A.t.^{a}$	Position C N.c. ^a	lhromosoma blocks ^b	I Allele size range (bp)	GenBank accession no.	Publication
Ncpm09	F: TAGACGCTGCGTTTTGAAGA	CT_{18}	58	0.4	NcM1	6-FAM	1/150	1	7	В	96–130	KR065729	
Ncpm13	R: CCTCTGTTGAGTGAATGGTTCTC F: CCAAAACTATGCCGATCTCA B: CCAACGGAATCTTAGT	AG_{16}	58	0.2	NcM1	VIC	1/150	NA	NA	NA	158-166	KR065730	
Ncpm21	F: GTCACCACTTGCTACGGGAT F: GTCACCACTTGCTACGGGAT F: CATTGGATAGGATGCACAGGAA	CTT_{10}	58	0.2	NcM1	VIC	1/150	ю	٢	Ц	240–277	KR065731	
Ncpm23	F: TTTCATGTCTCGGATCCTCC	TTC ₁₁	58	0.4	NcM1	6-FAM	1/150	NA	NA	NA	197–263	KR065732	
Ncpm31	F: GATTATCGAGCTTACTAAAGCAGC	CTT_{12}	58	0.2	NcM1	NED	1/150	5	4	M	58-101	KR065733	
Tc-up1	R: GTGTTGAAGCGCAATGAAGA F: TGCTCTGTTTCTCTCCACATTC	$(CA)_n(CT)_n$	50	0.2^{d}	NcM1	NED	1/160	NA	NA	NA	132-170	AJ746212	Basic and
Ncpm07	R: TTCCTTGCTTCTTCTCTTCCA F: TGGAATGGTTCTGTGGACAA	TCA_{18}	58	0.4	NcM2	6-FAM	1/150	NA	NA	NA	109-139	KR065734	Besnard, 2006
Ncpm14	R: TTCTGGAATTGGCTGCTTCT F: GACCACATCTCGTCTTGCCT	CTT_{15}	58	0.4	NcM2	PET	1/150	NA	NA	NA	108-123	KR065735	
Ncrim 10	R: GACCCTAACTACAGGCTGTGAAA	(DT) ATOTO	58	70	CMAN		1/150	NA	NA	NA	100-124	XP065736	
6THIDON	F: CCAACAAIGGAIIGGGGGGGGGG R: TCCCCATCACTCCAGCTAAG		00	0.4	INCINIZ		0/1/1	EN .	EVI	Y N	100-124	nc/ convv	
Ncpm29	F: CTCCCTCTTCTCCTACCTACACATA	AC_{17}	58	0.4	NcM2	NED	1/150	NA	NA	NA	78–94	KR065737	
Tc-up4	P. COCAMPERAGE CANELORY P. COCAMPERAGE	CT_{13}	50	0.2^{d}	NcM2	VIC	1/140	NA	NA	NA	253–262	AJ746216.1	Basic and
9C7/Thlc3	F. GTCACGAGTTTCACCATT	AG_{13}	58	0.4	NcM2	VIC	1/150	NA	NA	NA	152-191		Jiménez-Ambriz
Tc-up2	R: ATCTTCCACAATTGTGCC F: TGAGAAGAGGAGACACAGGAAC	$(AG)_5(AG)_5(GA)_6$	58	0.2	NcM2	PET	1/150	NA	NA	NA	232-244	AJ746213.1	et al., 2007 Basic and
Nc02	R: CACTTACCAAATCGAAAACTGCTCC F: GGAGCTGTTGGTTTCTGAAGG	AGG.	68/47°	0.06	NcM3	VIC	None	4	(*	С	170-191	KR065738	Besnard, 2006
	R: AGCATCGTATTCCGATCCAG	0		0.28					1)			
Nc03	F: TGAGCTTCTTCAGTCCCGAT	AC_{12}	68/47°	0.06	NcM3	NED	None	5	4	S	188–194	KR065739	
Nc04	R: IATGAGUTUGIUGUTUAUAG F: ACGGTCGCATACCAAAAAGT	AG_{11}	68/47°	0.06	NcM3	VIC	None	7	5	ſ	123-143	KR065740	
Nc06h	R: AGGATGCACTCCTTGAGACC F: GCGTCTTCTTCCATCCTCA	AG.,	68/47°	0.28	NcM3	PET	None	4	9	11	89–153	KR065741	
	R: GGATTTCCAATTCAATCTTCCC	51) 51		0.37))	2		
Nc07b	F: CCAGTTTCCAACGGCATAGT	AC_{10}	68/47°	0.06	NcM3	NED	None	б	L	Ч	105-112	KR065742	
Nc19	R: TTGGTTTTGGTTTCTTCTTGTGA F: CGGATTGTTGTGAATCCCAT	AGG	68/47°	0.07	NcM3	PET	None	2	Ś	Ĺ	199–235	KR065743	
	R: ACCTCTTCTTTCGCCCTTGT			0.28				I	3	2			
Nc20	F: ACACAACTCCAAAGGCTTCA	AG_{11}	68/47°	0.15	NcM3	6-FAM	None	4	6	0	205–234	KR065744	
Nc22	R: TTTTGTTCTAACCGTTACTCTTT F: TTGCTTTCACATGTCTTGACG D: CAACAACAACAACAACAACAAACAA	AG_{10}	68/47°	0.75 0.11 55 0	NcM3	6-FAM	None	7	5	K	111-121	KR065745	
;	N. GAACAGAACAAGAAGACAI GAAI GA	:	;	00.0									
<i>Note</i> : C ^a Relativ	t = tinal concentration of primers; NA = n $e chromosomal mosition in Arabidonvis the chromosomal position in the chromosomal position in the second sec$	ot available; $T_a = a$	annealin 1ea caer	g temper ulescens	ature. resnective]	lv (Schranz	et al 2006						
^b Genor	ne blocks in the "ancestral karyotype" bloc	ks (Schranz et al.	, 2006).	(m)									:
°Follow	ving Godé et al. (2012), touchdown PCR w	as performed. Du	ring the	first 10 c	ycles, anne	aling tempe	rature was o	lecreased	l from 68°	C to 50°C i	n increments	of 2°C. This	was followed by
d Stands	with an anticating temperature at 47 C. Ilone PCR.												

Applications in Plant Sciences 2015 3(12): 1500052 doi:10.3732/apps.1500052 (ISEM) and at Laboratoire Évolution Écologie et Paléontologie (Evo-Eco-Paleo), with requirements for levels of genetic polymorphism at different spatial scales. At ISEM, amplification trials were performed on seven individuals from five populations from southern France (Appendix 1). DNA extraction followed a classic CTAB protocol (Doyle and Doyle, 1990). Based on type and number of repeat units, repeat structures, and amplicon size, 32 candidate loci from the library were selected and tested separately. The PCR reactions were carried out in a total volume of 10 µL, containing 1 µL of DNA template, forward and reverse primers (0.2 μ M), and 1× QIAGEN Multiplex PCR Master Mix (QIAGEN, Courtaboeuf, France). Cycling conditions were: an initial denaturation step at 95°C for 15 min, then 30 cycles consisting of 30 s at 94°C, 90 s at 58°C, and 1 min at 72°C, followed by a final extension of 30 min at 60°C. PCRs were conducted on Eppendorf Mastercycler pro, Mastercycler nexus gradient (Eppendorf, Hamburg, Germany), and Techne TC-5000 (GMI, Ramsey, Minnesota, USA) machines. Amplification products were visualized with agarose gel (2%) with ethidium bromide stain. Due to inadequate amplification yield, low specificity, or unexpected size, only 15 markers were kept. Forward primers were labeled with one of the FAM, NED, VIC, or PET fluorescent dyes (Applied Biosystems, Waltham, Massachusetts, USA). PCR products were analyzed separately through electrophoresis on an ABI3130 Genetic Analyzer (Applied Biosystems). Nine loci were finally retained based on presence of polymorphism and quality of profiles.

At Evo-Eco-Paleo, 20 primer pairs corresponding to 20 additional loci from the library were selected. They were tested separately on 23 individuals scattered in the European species range (Koch and German, 2013; Appendix 1). Total DNA was extracted using the QIAGEN DNeasy kit (QIAGEN). Extraction and test PCR were performed according to Godé et al. (2012). Each primer pair was tested using FAM labeling. PCR reactions were carried out in a total volume of 10 μ L, containing 1 μ L of 1/20 diluted DNA template, 2 μ M of forward and reverse primers, and 1× QIAGEN Multiplex PCR Master Mix. A final set of eight markers was selected based on the quality of genotyping profiles, compatibility of amplicon sizes for multiplexing, and relative positions on the genome (Table 1). Genomic positions of microsatellite loci were determined by BLASTN searches of microsatellite flanking sequences against the *Arabidopsis thaliana* genome and by using the synteny among chromosomal blocks determined for different Brassicaceae species and the ancestral karyotype (Schranz et al., 2006).

Screening of the new microsatellite markers—All forward primers were labeled with fluorescent dyes, and markers were combined in multiplex PCR based on size compatibility and annealing temperatures (Table 1). Primer dimerization was checked using OligoAnalyzer 1.0.3 (Integrated DNA Technologies, Coralville, Iowa, USA). In addition to newly defined markers, four previously developed markers (Tc-up1, Tc-up2, Tc-up4, Thlc3; Basic and Besnard, 2006; Jiménez-Ambriz et al., 2007) were added to the multiplexes to increase the number of available, multiplexed markers (results not shown, see Table 1 for details). However, due to differing annealing temperatures, Tc-up1 and Tc-up4 were processed in separate PCR and added in the post-PCR steps.

Seventy-four individuals from four populations (Appendix 1, identified as "natural populations screening") were analyzed. DNA extraction followed the protocol from Doyle and Doyle (1990). The PCR reactions were carried out following the protocols described above: ISEM section for the first (NcM1) and second (NcM2) multiplexes, Evo-Eco-Paleo section for the third (NcM3), except that forward and reverse primers were mixed (concentrations in Table 1). Three microliters of diluted PCR product (dilutions in Table 1) were transferred in a mix of 15 µL of Hi-Di Formamide (Applied Biosystems) and 0.15 µL of GeneScan 500 LIZ Size Standard (Applied Biosystems). Raw data were analyzed using GeneMapper (version 5.0; Applied Biosystems). Automatic analysis and manual check of all the peaks were performed. Detection of the presence of null alleles in populations was performed with FreeNA (Chapuis and Estoup, 2007). Two new loci (Ncpm31 and Ncpm07) harbor null alleles, with frequencies between 10% and 15%. Expected heterozygosity, intrapopulation fixation index, and linkage equilibrium tests were computed using FSTAT (version 2.9.3; Goudet, 1995), and observed heterozygosity was computed in GENETIX (version 4.05; Belkhir et al., 2004). No linkage disequilibrium was detected between loci (with Bonferroni correction). The number of alleles for each locus ranged from five to 18 (Table 2). The observed heterozygosity per locus and per population ranged from 0 to 0.83, and the expected heterozygosity ranged from 0 to 0.89. Hardy-Weinberg equilibrium was tested in GENEPOP (Rousset, 2008), and most of the loci in the four populations were at equilibrium. This result contrasts with previous studies in N. caerulescens (Dubois et al., 2003; Basic and Besnard, 2006; Jiménez-Ambriz et al., 2007; Besnard et al., 2009) and may be due to sample size.

			All				Aviniè	stes $(n = 18)$			Saint Br	esson (n =	22)	•1	Saint Hipp	olyte (n =	= 17)		Coule	t ($n = 17$)	
Locus	% amp	Α	$H_{\rm o}$	$H_{\rm e}$	$F_{\rm IS}^{\rm b}$	\overline{A}	$H_{\rm o}$	$H_{\rm e}$	$F_{\rm IS}^{\rm \ b}$	A	$H_{\rm o}$	$H_{\rm e}$	$F_{\mathrm{IS}}{}^{\mathrm{b}}$	Α	$H_{\rm o}$	$H_{\rm e}$	$F_{\rm IS}{}^{\rm b}$	A	H_{0}	$H_{\rm e}$	$F_{\rm IS}{}^{\rm b}$
Ncpm09	100	16	0.563	0.699	0.108	~	0.722	0.794	0.091	S	0.545	0.598	0.089	10	0.823	0.893	0.078	9	0.353	0.46	0.232
Ncpm13	100	5	0.260	0.346	0.130	С	0.500	0.606	0.175	З	0.500	0.530	0.057	-	0.000	0.000	NA	4	0.176	0.224	0.213
Ncpm21	100	6	0.505	0.570	0.136	4	0.500	0.556	0.10	S	0.682	0.639	-0.068	С	0.412	0.588	0.300	С	0.294	0.449	0.344
Ncpm23	100	6	0.444	0.557	0.199	4	0.389	0.562	0.308	З	0.409	0.427	0.043	2	0.412	0.566	0.273	4	0.529	0.638	0.170
Ncpm31	98.6	6	0.493	0.694	0.396	9	0.667	0.771	0.136	4	0.111	0.645	0.828*	5	0.412	0.763	0.460	S	0.529	0.658	0.196
Ncpm07	98.6	8	0.485	0.606	0.210	9	0.833	0.725	-0.149	0	0.364	0.359	-0.012	С	0.062	0.383	0.837*	2	0.412	0.717	0.426
Ncpm14	100	5	0.254	0.405	0.357	б	0.500	0.650	0.231	0	0.091	0.089	-0.024	С	0.235	0.318	0.260	0	0.059	0.346	0.830
Ncpm19	100	11	0.544	0.647	0.130	S	0.722	0.730	0.011	4	0.591	0.639	0.075	0	0.187	0.425	0.559	2	0.706	0.774	0.088
Ncpm29	100	6	0.579	0.733	0.174	9	0.611	0.753	0.189	9	0.773	0.696	-0.110	٢	0.529	0.833	0.364	2	0.529	0.746	0.291
Nc02	97.3	9	0.384	0.482	0.081	S	0.750	0.693	-0.042	4	0.273	0.290	0.060		0.000	0.000	NA	S	0.562	0.727	0.226
Nc03	98.6	4	0.296	0.467	0.316	т	0.350	0.292	-0.14	4	0.429	0.699	0.387	0	0.059	0.059	0.000	4	0.375	0.7	0.464
Nc04	100	6	0.528	0.618	0.125	9	0.700	0.778	0.143	4	0.318	0.290	-0.097	4	0.647	0.695	0.069	5	0.529	0.732	0.276
Nc06b	100	18	0.582	0.740	0.233	6	0.700	0.815	0.046	2	0.636	0.812	0.216	2	0.437	0.627	0.302	S	0.412	0.717	0.426
Nc07b	98.6	2	0.501	0.592	0.194	4	0.500	0.672	0.255	4	0.571	0.570	-0.002	4	0.471	0.498	0.055	б	0.294	0.57	0.484
Nc19	97.3	8	0.585	0.628	0.073	5	0.684	0.768	0.158	5	0.762	0.698	-0.092	5	0.529	0.686	0.228	4	0.562	0.575	0.022
Nc20	97.3	6	0.269	0.330	0.305	4	0.600	0.544	-0.021	9	0.619	0.664	0.068	-	0.000	0.000	NA	б	0.125	0.235	0.469
Nc22	100	9	0.347	0.522	0.305	0	0.150	0.157	-0.063	5	0.727	0.778	0.065	0	0.235	0.507	0.536	ю	0.235	0.605	0.611
Note: %	amp = pe	ercenta	ge of suc	cessful ar	mplificati	on; A	= number	r of allele	s; $F_{\rm IS} = int$	trapol	pulation fi	ixation in	dex; $H_{\rm e} = \epsilon$	xpect	ed heteroz	cygosity;	$H_0 = obse$	erved	heterozyg	osity; N/	= not

TABLE 2. Statistical analysis of the 17 new microsatellite markers in four populations^a of Noccaea caerulescens in southern France.

 ${}^{b}F_{ls}$ values significantly different from zero after Bonferroni correction (P < 0.0006) are indicated with an asterisk

^a Information on geographic locations and vouchers is provided in Appendix 1.

CONCLUSIONS

Three multiplexes including 17 new and four published microsatellite markers were developed and validated in natural populations. These loci exhibit substantial polymorphism within and between populations. They should provide sufficient power to study population structure and mating system, and to infer demographic history at different spatial scales.

LITERATURE CITED

- ASSUNÇÃO, A. G. L., H. SCHAT, AND M. G. M. AARTS. 2003. Thlaspi caerulescens, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytologist 159: 351–360.
- BASIC, N., AND G. BESNARD. 2006. Gene polymorphisms for elucidating the genetic structure of the heavy-metal hyperaccumulating trait in *Thlaspi caerulescens* and their cross-genera amplification in Brassicaceae. *Journal of Plant Research* 119: 479–487.
- BELKHIR, K., P. BORSA, L. CHIKHI, N. RAUFASTE, AND F. CATCH. 2004. GENETIX 4.0. 5.2, Software under WindowsTM for the genetics of the populations. University of Montpellier, Montpellier, France.
- BESNARD, G., N. BASIC, P.-A. CHRISTIN, D. SAVOVA-BIANCHI AND N. GALLAND. 2009. *Thlaspi caerulescens* (Brassicaceae) population genetics in western Switzerland: Is the genetic structure affected by natural variation of soil heavy metal concentrations? *New Phytologist* 181: 974–984.
- CHAPUIS, M.-P., AND A. ESTOUP. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* 24: 621–631.
- DOYLE, J. J., AND J. L. DOYLE. 1990. DNA extraction from Arabidopsis. Focus (San Francisco, Calif.) 12: 13–15.
- DUBOIS, S., P.-O. CHEPTOU, C. PETIT, P. MEERTS, M. PONCELET, X. VEKEMANS, C. LEFÈBVRE, AND J. ESCARRÉ. 2003. Genetic structure and mating systems of metallicolous and nonmetallicolous populations of *Thlaspi caerulescens*. New Phytologist 157: 633–641.

- GODÉ, C., I. DECOMBEIX, A. KOSTECKA, P. WASOWICZ, M. PAUWELS, A. COURSEAUX, AND P. SAUMITOU-LAPRADE. 2012. Nuclear microsatellite loci for Arabidopsis halleri (Brassicaceae), a model species to study plant adaptation to heavy metals. American Journal of Botany 99: e49–e52.
- GOUDET, J. 1995. FSTAT: A computer program to calculate F statistics, version 1.2. Journal of Heredity 86: 485–486.
- JIMÉNEZ-AMBRIZ, G., C. PETIT, I. BOURRIÉ, S. DUBOIS, I. OLIVIERI, AND O. RONCE. 2007. Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France: Role of gene flow, selection and phenotypic plasticity. *New Phytologist* 173: 199–215.
- KIEFER, M., R. SCHMICKL, D. A. GERMAN, T. MANDÁKOVÁ, M. A. LYSAK, I. A. AL-SHEHBAZ, A. FRANZKE, ET AL. 2014. BrassiBase: Introduction to a novel knowledge database on Brassicaceae evolution. *Plant and Cell Physiology* 55: e3–e3.
- KOCH, M. A., AND D. A. GERMAN. 2013. Taxonomy and systematics are key to biological information: Arabidopsis, Eutrema (Thellungiella), Noccaea and Schrenkiella (Brassicaceae) as examples. Frontiers in Plant Science 4: 267.
- MALAUSA, T., A. GILLES, E. MEGLÉCZ, H. BLANQUART, S. DUTHOY, C. COSTEDOAT, V. DUBUT, ET AL. 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. *Molecular Ecology Resources* 11: 638–644.
- MEGLÉCZ, E., C. COSTEDOAT, V. DUBUT, A. GILLES, T. MALAUSA, N. PECH, AND J.-F. MARTIN. 2010. QDD: A user-friendly program to select microsatellite markers and design primers from large sequencing projects. *Bioinformatics (Oxford, England)* 26: 403–404.
- ROUSSET, F. 2008. GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Resources* 8: 103–106.
- SCHRANZ, M. E., M. A. LYSAK, AND T. MITCHELL-OLDS. 2006. The ABC's of comparative genomics in the Brassicaceae: Building blocks of crucifer genomes. *Trends in Plant Science* 11: 535–542.

APPENDIX 1. Vouc	her and location info	rmation for Noccae	a caerulescens populations u	ised in the development and testin	ig of microsatellites.		
Population	Type of population	Collection date	Locality	Geographic coordinates	Sample names and storage location ^a	Voucher no.	Use
Angleur	Metallicolous	2009	Angleur	50°36'44.61"N, 5°36'38.74"E	$B02.01^{b}$		Biological validation of primer
Anjeau	Nonmetallicolous	October 2007	Saint-Laurent-le-Minier	43°55′3.33″N, 3°37′52.52″E	$AN_Z_X_{2007^c}$		Biological validation of primer
Auxelles-Haut	Nonmetallicolous	2009	Auxelles-Haut	47°44'21.52"N, 06°46'35.84"E	$F03.01^{b}$		Biological validation of primer
Auxy	Nonmetallicolous	2009	Auxy	46°57′44.13″N, 04°23′47.05″E	$F05.01^{b,d}$	503854	pairs (EVO-ECO-Faleo) Biological validation of primer
Avinières	Metallicolous	March 2013	Saint-Laurent-le-Minier	43°55'55.67″N, 3°39'46.19″E	$12_AV_ID_X^{\circ}$		pairs (EVO-ECO-Faleo) Natural populations screening
Baraquette	Nonmetallicolous	October 2007	Saint-Laurent-le-Minier	43°55'6.73″N, 3°36'54.82″E	$BQ_Z_A2007^{\circ}$		Library construction
Breinigerberg	Metallicolous	2009	Breining	50°44'12.73"N, 06°14'30.92"E	$G01.01^{b,d}$	501266, 501267	Biological validation of primer
Col du Lautaret	Nonmetallicolous	2007	Lautaret	45°02 ′ 07″N, 06°24′20″E	$F01.01^{b,d}$	501429	Biological validation of primer
Coulet	Nonmetallicolous	September 2013	Saint-Maurice-Navacelles	43°49′44.59″N, 3°33′41.64″E	12_CO_ID_X°		pairs (EVO-ECO-Faleo) Natural populations screening
Coulet	Nonmetallicolous	October 2007	Saint-Maurice-Navacelles	43°49′44.59″N, 3°33′41.64″E	CO_Z_X_2007°		Biological validation of primer
Fellering	Serpentine	2009	Bergenbach	47°54'22.90"N, 06°57'25.50"E	$F04.01^{b}$		Biological validation of primer
Goebelsmühle	Nonmetallicolous	2009	Goebelsmühle	49°55'22.07"N, 06°3'44.08"E	L02.01 ^b		Biological validation of primer
Husavik	Nonmetallicolous	2006	Husavik	66°01'38.89″N, 17°17'21.71″W	101.01 ^{b,d}	911812	Biological validation of primer
Lichtenau	Metallicolous	2006	Blankenrode	51°32′0.34″N, 08°54′17.02″E	G05.01 ^b		pairs (Evo-Eco-Paleo) Biological validation of primer
Lintich	Metallicolous	2010	Bakomi	48°26'05.48"N, 18°55'09.43"E	SK01.01 ^b		pairs (EVO-ECO-Faleo) Biological validation of primer
Mostviertiel	Nonmetallicolous	2006	Tormäuer	47°51'08.70″N, 15°17'13.20″E	$A01.01^{b,d}$	406733	Biological validation of primer
Moyen Age	Metallicolous	October 2007	Saint-Laurent-le-Minier	43°55'52.16″N, 3°38'26.09″E	$MG_Z_X_{2007^{\circ}}$		Biological validation of primer
Oslo	Nonmetallicolous	2009	Hovedoya	59°53'39″N, 10°43'44″E	N01.01 ^{b,d}	501275-501278	Biological validation of primer
Papeterie	Metallicolous	2006	Ganges	43°56'10.98"N, 03°40'19.88"E	$F16.01^{b}$		Biological validation of primer
Prayon	Metallicolous	2009	Prayon	50°35′3.31″N, 5°40′23.69″E	B01.01 ^b		Biological validation of primer
Ramponenche	Metallicolous	2010	Florac	44°20'18.25"N, 03°40'05.45"E	$F02.01^{b}$		Biological validation of primer
Saint Baudille	Nonmetallicolous	October 2007	Montpeyroux	43°44′40.73″N, 3°29'9.96″E	$BD_Z_X_{2007^c}$		Biological validation of primer
Saint Bresson	Metallicolous	October 2007	Pommiers	43°56'35.89″N, 3°37'57.02″E	$SB_Z_X_{2007^c}$		Biological validation of primer
Saint Bresson	Metallicolous	September 2013	Pommiers	43°56'35.89″N, 3°37'57.02″E	$12_SB_ID_X^{c}$		pairs (1354) Natural populations screening
Saint Hippolyte	Metallicolous	April 2014	Saint-Hippolyte-du-Fort	43°58'17.07″N, 3°49'59.98″E	Zi_Qj_Hi_2014_X°		Natural populations screening
Saint Jost	Metallicolous	2009	Virneburg	50°21'08.95″N, 07°06'33.65″E	G02.01 ^{b,d}	501261, 501262	Biological validation of primer pairs (Evo-Eco-Paleo)

Population	Type of population	Collection date	Locality	Geographic coordinates	storage location ^a	Voucher no.	Use
Silberberg	Metallicolous	2009	Silberberg	52°12′38.72″N, 07°56′46.13″E	G03.01 ^{b,d}	504547	Biological validation of primer
Somerset	Metallicolous	2010	Priddy	51°15′39.06″N, 02°39′02.12″W	$\mathbf{UK1.06^{b,d}}$	501343	pairs (EVO-Eco-Paleo) Biological validation of primer
Son	Nonmetallicolous	2000	Esterri d'Aneu	42°35′51.50″N, 01°04′23.82″E	SP01.02 ^b		Biological validation of primer
Špania Dolina	Nonmetallicolous	2010	Bakomi	48°48'29.87"N, 19°08'14.65"E	SK02.01 ^b		pairs (EVO-Eco-Paleo) Biological validation of primer
Stauffenbergallee	Nonmetallicolous	2009	Dresden	51°06′04.48″N, 13°47′17.61″E	$G04.01^{b,d}$	280040	pairs (EVO-ECO-Faleo) Biological validation of primer
Štiavnické	Nonmetallicolous	2010	Bakomi	48°26′0.48″N, 18°51′29.86″E	$SK03.01^{b}$		pairs (EVO-ECO-Faleo) Biological validation of primer
Uppsala	Nonmetallicolous	2010	Uppsala	59°50'40.68"N, 17°44'54.18"E	S01.01 ^{b,d}	501430	pairs (Evo-Eco-Paleo) Biological validation of primer nairs (Evo-Eco-Daleo)

which seeds were collected. Several specimens were collected in each population. ^bVouchers of leaves were deposited at Laboratoire Évolution Écologie et Paléontologie (Evo-Eco-Paleo), Villeneuve d'Ascq, France. ^cVouchers of leaves were deposited at Institut des Sciences de l'Évolution (ISEM), Montpellier, France. ^dHerbarium specimens of the corresponding population were collected and deposited by M. Koch at the Centre for Organismal Studies (COS), Biodiversity and Plant Systematics, Universität Heidelberg, Heidelberg, Germany. They are available from the BrassiBase database (Kiefer et al., 2014).