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Research article

Adaptive differentiation on serpentine soil in diploid versus autotetraploid populations of *Biscutella laevigata* (Brassicaceae)

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Serpentine soils exhibit extreme properties (e.g. high magnesium content) influencing plant growth and survival, and have been repeatedly documented to promote adaptive edaphic differentiation in plants. Individuals from four pairs of nearby diploid and autotetraploid populations of *Biscutella laevigata* sampled on serpentine versus non-serpentine soils in a factorial design are used to assess the genetic and phenotypic changes associated with edaphic origin and ploidy level. Individual samples from natural populations were subjected to soil elemental analysis and genotyping using restriction site-associated DNA sequences (RAD-seq) to link genetic variation with contrasting soils and ploidy levels. In diploids, genetic variation was consistent with demographic contraction and a pattern of isolation by environment with respect to the ratio of calcium/magnesium concentrations, whereas tetraploids presented evidence of expansion with limited edaphic differentiation. The genetic basis of tolerance and adaptation to serpentine was further assessed experimentally on seed-grown individuals from all populations subjected to high (serpentine-like) versus low (control) concentrations of magnesium in hydropony. Fitness-related phenotypic traits under experimental cultivation were consistent with adaptive differentiation among diploid ecotypes but not among the tetraploids that similarly grow in both habitats and consistently present higher investment in roots. Further work comparing experimentally resynthesized polyploids to natural diploids and polyploids would help to tease the role of whole genome duplication apart from the effects of post-polyploidy evolution.

Keywords: autopolyploidy, ddRAD-seq, hydroponic experiment, local adaptation, mixed-ploidy genetic structure, whole genome duplication

Introduction

The evolutionary consequences of whole genome duplication (WGD) are not well understood (Lynch and Conery 2000, Parisod et al. 2010, van de Peer et al. 2017). In particular, to what extent WGD is a nearly-neutral process or promotes the evolution

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of novel adaptive traits fostering species radiation remains debated (Mayrose et al. 2011, Soltis et al. 2014). Extant polyploids often differ from their diploid progenitors in morphological, physiological, and ecological characteristics (Otto and Whitton 2000, Ramsey and Schemske 2002, Leitch and Leitch 2008, Nei 2013), but to what extent WGD potentially contributes to a widening of the ecological niche and expansion to new environments in the resulting autopolyploid lineage is still poorly known (Parisod and Broennimann 2016, Baduel et al. 2018). In particular, direct evidence of an impact of WGD on adaptation in natural populations or even of the extent to which naturally-occurring autopolyploids are locally adapted is scarce (Ramsey 2011, Martin and Husband 2013). Integration of the molecular underpinnings and ecological ramifications of polyploidy ultimately requires the role of WGD per se to be distinguished from the impact of post WGD evolution through experimentally resynthesized polyploids (Ramsey and Schemske 2002, Tayalé and Parisod 2013). However, the outcome of selection acting separately over thousands of generations on diploids and autotetraploids may be inferred from studies of populations across strong selective gradients, such as toxic serpentine versus non-serpentine soils.

Serpentine (S) soils develop on ultramafic bedrocks and can be characterized by distinctly high concentrations of magnesium (Mg), increased contents of heavy metals (e.g. nickel, chromium, cobalt), and a low calcium (Ca) availability, which altogether hinder the growth or survival of non-adapted plants from non-serpentine (NS) soils (Brady et al. 2005). Such naturally toxic soils represent a clear constraint to which plant species have to be adapted regarding ion tolerance and nutritional homeostasis (Bratteler et al. 2006, Arnold et al. 2016), and are accordingly populated with specialized (often endemic) species (Kazakou et al. 2008, Čertner et al. 2019). Although it remains unclear whether a tenfold increase in Mg is directly toxic to plants (as recently suggested by Tang et al. 2015) or it simply interferes with Ca uptake to induce nutritional deficiencies, the ability to maintain a high ratio of Ca/Mg in leaves is key for adaptation to S soils (O'Dell et al. 2006). It can be predicted to require adaptive changes at only a few loci belonging to specific transcriptional pathways (Brady et al. 2005, Turner et al. 2010). Sets of nearby populations growing on S and NS soils in the face of gene flow represent an ideal system to investigate evolutionary processes potentially supporting local adaptation.

Plant adaptation to S soils has been addressed in many plant species (Turner et al. 2010, O'Dell and Rajakaruna 2011), although the impact of WGD on this process still remains elusive. In most polyploid systems investigated so far, natural populations growing on S were either almost exclusively diploid or tetraploid (Kolář et al. 2012, Konečná et al. 2021), limiting comparisons among cytotypes adapted to S versus NS conditions and thus our understanding of the impact of WGD on local adaptation. The recent demonstration that WGD per se enhances salinity tolerance by promoting ion uptake and accumulation might also suggest a polyploidy-driven adaptation to S (Chao et al. 2013). Such a hypothesis was however poorly supported in polyploids of *Arabidopsis arenosa* shown to have undergone parallel adaptation to S following introgression from its S-adapted diploid relative *Arabidopsis lyrata* (Arnold et al. 2016). Variation at more than 60 candidate genes, including a convincing TWO PORE CHANNEL 1 locus, appeared associated with S soils and thus suggested selection on specific loci rather than an impact of WGD in this species (Konečná et al. 2021). To determine the possible role of WGD and how it interacts with adaptation to S soils, additional studies involving different model species are necessary.

Buckler mustards (*Biscutella laevigata*, Brassicaceae related to *Arabidopsis* species) represent a convenient model system to address how WGD interacts with ecological factors such as S soils. The species is widespread across central Europe, with diploid populations occupying areas having remained ice-free during the last glacial maximum and tetraploid populations (i.e. derived from WGD) extending across a wide range of habitats in previously glaciated areas (Manton 1933, Tremetsberger et al. 2002, Parisod and Besnard 2007, Geiser et al. 2016). Although both cytotypes of the heliophilous *B. laevigata* occur mainly on dolomite or carbonate substrates (i.e. NS soils), natural populations on S soils have been reported in both diploids (Tremetsberger et al. 2002) and tetraploids (Gasser 1986). In this study, we use a factorial design involving replicated pairs of populations of diploids versus tetraploids of *B. laevigata* growing on calcareous NS versus toxic S soils to indirectly assess potential adaptation in the face of gene flow using population genetics and then test corresponding phenotypic responses in a hydroponic experiment mimicking the specific chemistry of NS and S soils. In particular, we ask 1) to what extent diploid and tetraploid populations are genetically differentiated according to their NS versus S edaphic origin, 2) does experimental evidence support genetically-based phenotypic differences indicative of local adaptation to either S or NS soils and, finally, 3) to what extent diploids and tetraploids differ in their response to edaphic heterogeneity.

Material and methods

Sampling

Plant material from eight populations of the *Biscutella laevigata* autopolyploid complex was sampled as four diploid populations (*B. laevigata* subsp. *kerneri*) in the Wachau region of Austria (Tremetsberger et al. 2002); and four tetraploid populations (*B. laevigata* subsp. *laevigata*) in the Swiss Alps around Davos and Arolla (Gasser 1986, Parisod and Besnard 2007). The populations were sampled as pairs (1, 2, 3, 4) of the same ploidy level and growing no more than 20 km apart on either serpentine (S) or non-serpentine (NS) soils (Table 1). At each site, eight randomly selected individuals growing at a minimum distance of 3 m apart were georeferenced using a GPS receiver and sampled for silica gel-dried

Table 1. Diploid (2x) and tetraploid (4x) field populations of *Biscutella laevigata* sampled on serpentine (S) and non-serpentine (NS) soils. At each locality, eight individuals were sampled for genetic analyses and adjacent soil was collected for elemental composition analyses. Average magnesium (Mg) and calcium (Ca) concentrations and their ratio (Ca/Mg; a typical distinguishing feature of S versus NS soils) is shown. Estimates of allelic richness (A_S), expected heterozygosity (H_E), observed heterozygosity (H_O) and Tajima's D are presented for each population based on ddRADseq genotyping.

Population (locality; latitude/longitude [°]; elevation [m a. s. l.])	Origin	Ploidy	Mg (ppm)	Ca (ppm)	Ca/Mg	A_S	H_E	H_O	Tajima's D
S1 (Kottes, Austria; 48°24'6.5"N/15°19'42.6"E; 620 m)	S	2x	170.0	99.7	0.6	1.64	0.30	0.39	1.11
S2 (Aggsbach, Austria; 48°17'35.9"N/15°26'50.3"E; 360 m)	S	2x	221.0	105.0	0.5	1.65	0.31	0.39	1.33
S3 (Maierhoftäli, Switzerland; 46°49'54.4''N/09°49'26.4''E; 260 m)	S	4x	51.9	23.5	0.5	1.12	0.05	0.05	−1.04
S4 (Seppec, Switzerland; 46°04'31.1"N/07°31'55.6"E; 1680 m)	S	4x	80.9	147.3	1.8	1.11	0.04	0.05	−1.10
N1 (Dürnstein, Austria; 48°23'55.7"N/15°32'4.2"E; 340 m)	NS	2x	13.4	202.8	15.1	1.66	0.31	0.41	1.23
N2 (Hollenburg, Austria; 48°22'19.6"N/15°41'6.7"E; 360 m)	NS	2x	37.8	434.5	11.5	1.69	0.32	0.40	0.97
N3 (Strela, Switzerland; 46°48'17.3''N/09°48'55.8''E; 1960 m)	NS	4x	17.8	97.5	5.5	1.12	0.05	0.05	−1.29
N4 (Gouille, Switzerland; 46°03'18.0"N/07°29'27.2"E; 1860 m)	NS	4x	24.4	217.2	8.9	1.1	0.04	0.04	−1.25

leaves, open-pollinated seeds and > 10 g of soil next to the main root.

The *B. laevigata* genome is estimated to be 970 Mbp per 1C (Lysak et al. 2009) and the ploidy level of each sample was confirmed using DAPI flow cytometry on fresh leaf tissue of plants germinated and grown under greenhouse conditions at the University of Bern, Switzerland (16 h light, 20–40 kLux, 22–26°C; 8 h dark, 16–18°C). Samples were prepared and stained using the CyStain UV Precise P kit (Sysmex Corporation) by chopping a piece of a young leaf with a razor blade in a petri dish containing 400 µl of nuclei extraction buffer, filtering with 30 µm CellTrics filter (Sysmex Corporation) after one minute incubation and adding 1600 µl of staining buffer for two minutes and, finally, recording the fluorescence of at least 3000 nuclei using a CyFlow Cube flow cytometer (Sysmex Corporation). Individuals of known ploidy based on previous chromosome counts (i.e. diploid *B. laevigata* subsp. *varia*; Geiser et al. 2016) were used as external standards for calibration. Although such external standardization may introduce some uncertainty in DNA content estimates and should be avoided for precise genome size measurements, careful assessment does allow ploidy level estimation in spite of leaf tissue endopolyploidy possibly producing limited overlap between sample and standard fluorescence peaks (Temsch et al. 2022).

Edaphic characterization

Soil samples were dried at 65°C for 24 h, sieved with a 1 mm-sized mesh, and 10 g of each sample was mixed with 30 ml of 1 M ammonia acetate at pH 7. After 2h of shaking, the suspension was filtered and diluted (between 1:100 and 1:1000) with distilled water prior to analyses. Cation exchange capacity was assessed quantitatively by using inductively coupled plasma – optical emission spectrometry (ICP-OES) at the Platform of Analytical Chemistry of the University of Neuchâtel, Switzerland. Elemental concentration of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{2+}, Al^{3+} was measured for each of the 64 samples. As the approach fails to consistently detect concentrations below 0.1 ppm, the study was mostly blind to possible edaphic differences in Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{2+} or Al^{3+} and subsequent analyses were thus focused on Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} concentrations.

Differences in chemical soil composition between S and NS sites were first visualized using a principal component analysis (PCA) of the four available macronutrient concentrations supplemented with a Ca/Mg ratio, a parameter widely used to describe serpentine soil-induced chemical stress (Brady et al. 2005). Prior to the analysis, the dataset was checked for potential strong correlation of soil variables and, since absolute values of correlation coefficients never exceeded 0.7 (Supporting information), all variables were retained. Differences between S and NS substrates were tested using a redundancy analysis (RDA). Both simple effects of particular variables (i.e. when alone in the model) and their conditional effects in a stepwise selection of the most informative variables were assessed using a Monte Carlo test with 999 permutations (Lepš and Šmilauer 2014), with Bonferroni-adjusted significance. All multivariate analyses were conducted with Canoco ver. 5 (Microcomputer PowerA).

ddRAD-seq library preparation, de novo assembly and SNP calling

For each of the 64 plant samples, DNA was extracted from 20 mg of dried leaf tissue using the DNeasy Plant Mini Kit (Qiagen) following the manufacturer's instructions. DNA quality and concentration were checked using the NanoDrop (ThermoFisher Scientific) as well as the Qubit 2.0 Fluorimeter (Life Technologies Corp.). Each DNA sample was normalized

to 250 ng in 31 µl through either dilution with water or concentration by binding DNA on magnetic NEB AMPure XP beads at a 1:1 ratio and eluting in an appropriate volume of water.

ddRAD libraries were prepared following a protocol adapted from Peterson et al. (2012) as described in Grünig et al. (2021) using restriction enzymes EcoRI and MseI. Subsequent ligation of uniquely indexed EcoRI adapters and library-specific MseI adapters, including four degenerate bases to identify PCR duplicates, enabled each sample to be distinguished. Library pools were size-selected, targeting a mean length of 550 bp with a ratio 0.6 to 1 AMPure XP beads, and then selected for sequences with the biotin tag on the MseI adapter (performed with Dynabeads M-270 Streptavidin). DNA fragments containing both EcoRI and MseI adapters were amplified with 12 PCR cycles, resulting in double digested restriction associated libraries. Quantity and size distribution of final libraries were checked using the Qubit 2.0 Fluorimeter and Bioanalyzer 2100 System (Agilent Technologies).

Diploid samples require at least 8× sequencing coverage to confidently call both alleles at a locus (with 99% confidence), whereas up to 48× coverage is required to accurately genotype autotetraploids 95% of the time (Uitdewilligen et al. 2013). All tetraploid samples were thus included twice, as independent samples from the start of the procedure. All libraries were sequenced as paired-end 2 × 250 bp reads on two Illumina Novaseq 6000 lanes at the Next Generation Sequencing Platform of the University of Bern. After quality control using FASTQC (Andrews 2010), the process_radtag command implemented in Stacks (Catchen et al. 2013) was used to demultiplex raw fastq reads and remove bad quality bases. PCR duplicates were filtered out with clone_filter. Trimmomatic (Bolger et al. 2014) was used to trim the first four low-quality bases and keep only reads with a minimum length of 100 bp.

The de novo reference catalog of RAD tags was generated based on reads from the 32 diploid samples using the dDocent pipeline (parameters: dDocent Cutoff1 = 5, dDocent Cutoff2 = 5, first clustering rate 80%, second clustering rate 80%; Puritz et al. 2014). Then reads from all 64 samples were mapped to the de novo reference catalog using BWA mem (Li 2013). SNPs were called with the Genome Analysis Toolkit ver. 4.1.0.0 (McKenna et al. 2010), which handles mixed-ploidy data, without base recalibration. In a first step, samples where individually genotyped according to their ploidy by local re-assembly of haplotypes with the HaplotypeCaller tool. Single-sample GVCFs were then merged and jointly genotyped using GenomicsDBImport and GenotypeGVCFs tools. The resulting vcf file was first filtered to keep only biallelic SNPs covered in at least 50% of samples with sufficient quality according to GATK best practice (Supporting information). To avoid filters relying on expectations of the Hardy–Weinberg equilibrium that differ among ploidy, positions with an overall depth higher than two standard deviations above the mean were further removed as putative paralogous loci. Individual genotypes were filtered for a minimal depth of three and genotype quality of 20. Filtered genotypes were set to no-call and only SNPs with a maximum of 10% missing data were kept. The dataset was further pruned using PLINK2 (Purcell et al. 2007) based on linkage disequilibrium (–indep-pairwise 1000 100 0.2) and only positions with a minor allele count (MAC) above three and a minor allele frequency (MAF) above 0.05 were finally kept.

Genetic structure

SPAGeDi 1.5 (Hardy and Vekemans 2002) was used to assess the expected number of alleles among k gene copies (i.e. allelic richness, A_S), the expected (H_E) and observed (H_O) heterozygosity in each field population, and estimate genetic differentiation among populations using Rho, a F-statistic allowing comparison between ploidies (Ronfort et al. 1998) with significance assessed through 1000 permutations, as well as pairwise genetic distances among individual samples using Rousset's â (Rousset 2000).

Rho-based genetic differentiation was further visualized as a neighbor network in Splitstree (Huson and Bryant 2006). The partitioning of genetic variation among field populations, ploidy levels, and soil types was tested within the AMOVA hierarchical framework in GenoDive (Meirmans 2020), with significance estimated using 1000 permutations.

Homogeneous genetic groups that best explained the multilocus genetic structure among individuals were investigated using the Bayesian assignment algorithm implemented in STRUCTURE ver. 2.3.4 (Pritchard et al. 2000), which was shown to provide unbiased inferences of genetic clusters in diploid-autotetraploid systems by Stift et al. (2019). Following their recommendations, analyses were conducted on the mixed-ploidy dataset by randomly subsampling two alleles per tetraploid genotype. One SNP per RAD tag was selected to privilege unlinked loci. Following 10 replicate runs with 50 000 burn-in and 500 000 MCMC steps for each K between K = 1 and K = 8 using the admixture model, STRUCTURE harvester (Earl and VonHoldt 2012) was used to plot mean likelihood values for each K and implement the Evanno-method (Evanno et al. 2005). The assignment of samples to particular clusters across replicate runs was assessed using CLUMPP (Jakobsson and Rosenberg 2007).

Genome-wide Tajima's D was estimated with the R package 'PopGenome' ver. 2.7.5 (www.r-project.org, Pfeifer et al. 2014) separately for both ploidy level and for each field population to evaluate demographic contraction or expansion. Significance of the observed Tajima's D was assessed by comparing it to the 95% confidence interval estimated from the distribution of Tajima's D out of 1000 neutral coalescent simulations performed with MS (Hudson 2002).

Partial Mantel tests implemented in the 'phytools' ver. 1.2-0 package (Revell 2012) were used to assess the contribution of spatial distance and edaphic factors in shaping genetic differentiation among individual samples consistent with isolation by distance and isolation by environment (Wang and Bradburd 2014, Huynh et al. 2020). The association between pairwise genetic (Rousset's â), geographic and

edaphic (concentration difference for each element) distances was tested following 10 000 permutations and significance considered after Bonferroni correction.

Hydroponic experiment mimicking S and NS conditions

Each of the eight plants genotyped per population was represented in the hydroponic experiment by six randomly selected seeds. The seeds were germinated on a mixed perlite–vermiculite substrate (1:1) with daily watering and, after 12 days, were included in the hydroponic experiment. As only few seeds from the population S3 were apparently ripe enough to properly germinate, only seven populations were retained in the experimental setting. Half the plants were exposed to low magnesium concentrations (Mg−, 0.5 mM) whereas the other half were exposed to high magnesium concentrations (Mg+, 5 mM). Individuals were first allocated to experimental containers and grown under Mg− conditions for one week to acclimatize. Subsequently, plants were assigned to their respective treatments (Mg− or Mg+) and grown for seven additional weeks in one of the four experimental containers established per treatment (42 plants per container). A final set of 336 plants were grown in a greenhouse of the University of Bern under the following conditions: 16 h of day at 22–26°C and 8h of night at 16–18°C, 65% relative humidity, and an average irradiance of 313 µM photons m^2 s^{-1} at plant level. Seed mass was measured at the family level by averaging the weight of 10 seeds and, showing low correlation with phenotypic traits scored at the end of the experiment, indicated negligible maternal effects (Supporting information).

The hydroponic system followed Conn et al. (2013), using 37-liter RAKO-containers made of polypropylene (Georg Utz Holding) covered with black opaque acrylic glass to avoid algal contamination. The containers supported 50 ml conical tubes (Fisher Scientific) that were modified to hold plants through a 1.3 cm-diameter hole in the lid, whereas the bottom of tubes was cut off to allow roots growing into the nutrient solution. Following Babst-Kostecka et al. (2016), containers were filled with a modified half-strength Hoagland's nutrient solution using chemicals from Sigma-Aldrich unless otherwise specified (i.e. 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 1 mM $NH_4H_2PO_4$, 25 µM H_3BO_3, 2 µM $MnSO_4$, 1 µM KCl, 0.1 µM $CuSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$ (Fisher Scientific), 0.1 µM NaCl, 1 µM $ZnSO_4$ and 20 µM FeEDDHA (6% Fe, Phygenera)) and set to pH 5.5 by using either KOH or HCl. The Mg− and Mg+ treatments were distinguished by 0.5 versus 5 mM $MgSO_4$ (Carl Roth GmbH), respectively. The nutrient solution was aerated by a Hailea ACO-9630 air pump (GarPet) connected to all containers via eight outlets and a 20 cm-long air stone (GarPet) placed in the center of each container. Containers were refilled every fourth day with 1–2 l distilled water to compensate for evaporation; pH, oxygen level, electrical conductivity and light conditions were measured on a weekly basis. The nutrient level was kept constant, with a pH always < 6.2, by a weekly renewal of the nutrient solution coupled with randomizing the position of containers and all plants within each container.

Plant phenotyping and data analysis

After eight weeks of hydroponic cultivation, plants were harvested and the following traits were measured: overall number of leaves, length and width of a largest leaf, chlorophyll content and specific leaf area (SLA). The harvested biomass (root systems and leaf rosettes separately) was dried at 80°C for four days and weighed. Relative chlorophyll content was obtained with SPAD-502 meter (Konica Minolta Sensing Europe B.V.) by averaging three measurements on a fresh largest leaf. A 0.64-cm^2 square punched out of the largest leaf was dried at 80°C and used to estimate the SLA (Pérez-Harguindeguy et al. 2013). Additionally, two derived phenotypic characters were estimated: an approximate largest leaf size (i.e. length × width of the largest leaf) and root/shoot ratio (i.e. belowground biomass/aboveground biomass).

Linear mixed-effects models were used to identify differentiation in magnesium tolerance among individuals of different edaphic origin and/or ploidy level. Ten separate models were fitted with each of the phenotypic traits as a response variable. Although we are well aware that these traits are not independent, this approach was selected to better illustrate the nature of the observed phenotypic differences. The magnesium treatment (Mg+, Mg−), edaphic origin of populations (S, NS), ploidy level (2x, 4x), and all their interactions were used as fixed effects in the models. Seed family (nested within population) and hydroponic container were included as random effects. Linear mixed-effects models of the Gaussian family were fitted with restricted maximum likelihood (REML) method as implemented in the R package 'lme4' ver. 1.1-30 (Bates et al. 2015). Some response variables were log-transformed (Root/shoot ratio, No. of leaves, Specific leaf area) or square-root-transformed (Total biomass, Shoot biomass, Root biomass, Leaf width, Leaf size) to meet the normality assumptions of model residuals. Statistical significance was inferred in a type III analysis of variance with Satterthwaite's method to estimate the denominator degrees of freedom using the R package 'lmerTest' (Kuznetsova et al. 2017). We refitted the models with maximum likelihood (ML) and attempted to simplify them by consecutively removing terms (starting with the three-way interaction). Likelihood-ratio tests however never indicated a significant drop in Akaike's information criterion (AIC) and all predictors and their interactions were thus retained in final models. To assess an amount of variance explained by the fitted models, we used the function *r.squaredGLMM* available within the R package 'MuMIn' ver. 1.47.1 (Bartoń 2022) to estimate pseudo-R^2 coefficients following the method by Nakagawa et al. (2017). Variation explained by fixed effects only was summarized by a marginal R^2, whereas a conditional R^2 was used to estimate the overall amount of variance explained by the model (fixed + random effects). A pairwise matrix of Pearson's correlations among all traits was produced and visualised using the R package 'corrplot' ver. 0.92 (Wei

and Simko 2021). Unless stated otherwise, statistical data analysis was done with R ver. 4.2.1 (www.r-project.org).

Results

Edaphic differentiation among populations

Elemental profiles of individual soil samples (Supporting information) clustered according to their S versus NS origin in PCA, although there was some overlap between groups (Fig. 1). Significant differences in elemental composition of soil samples were detected between S and NS localities in RDA ($p = 0.001$). The most important soil parameter in stepwise model selection in RDA was Ca/Mg ratio, altogether explaining 56.6% of overall variation, followed by the soil concentration of Mg^{2+} (Table 2). On the other hand, K^+ and Na^+ had only minimal contribution to the overall edaphic differentiation of localities. On average, S soils had 13.4 times lower Ca/Mg ratios (generally < 1) and contained 5.6 times more Mg^{2+} (Table 2). Some variation in soil composition could be found among localities, such as the site at Seppec (S4) reported here with the highest Ca/Mg ratio among S localities (1.8), while still retaining a Mg^{2+} concentration 3.5 times higher than NS localities.

ddRAD-seq genotyping and genetic structure

Out of the 438 million raw sequencing reads, our ddRAD-seq approach yielded 426 million paired-end reads after demultiplexing and adapter removal. The de novo reference catalogue contained 17 449 contigs upon which 40% of the total reads could be mapped with high quality. This reference resulted in a total length of ca 5 Mbp, accounting for ca 0.5% of the 970 Mbp genome of *B. laevigata*. A total of 5 050 765 positions were genotyped by the GATK pipeline (Supporting information). Quality and depth filtering on positions and genotypes yielded a dataset including 20 970 biallelic SNPs with less than 10% of missing data. After pruning for linkage disequilibrium and applying MAC and MAF filters, the final dataset that was used for genetic analyses consisted of 1 146 SNPs. The dataset used for STRUCTURE, with one randomly subsampled SNP per RAD tag, included 944 SNPs. One sample was excluded due to a mean sequencing coverage below 8× (Supporting information)

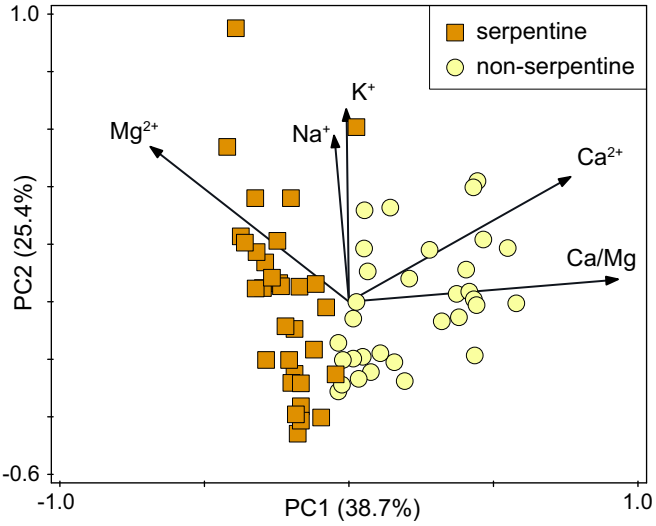


Figure 1. Differences in soil chemical composition between serpentine and non-serpentine localities of *Biscutella laevigata* visualized using a principal component analysis. The relationships among the concentrations of the four elements and the Ca/Mg ratio used in the analysis are shown by arrows.

Among field populations, the mean allelic richness (A_S) ranged from 1.10 to 1.69, whereas the observed (H_O) and the expected heterozygosity (H_E) ranged respectively from 0.04 to 0.41 and from 0.04 to 0.32 (Table 1). These proxies were consistently lower in tetraploid than in diploid populations (Wilcoxon tests $p = 0.03$ for H_E, $p = 0.03$ for H_O, and $p = 0.03$ for A_S), although no statistically significant differences were observed for edaphic origin (S or NS; Wilcoxon tests $p = 0.77$ for H_E, $p = 0.49$ for H_O and $p = 0.77$ for A_S).

Significant genetic differentiation among field populations based on Rho estimates appeared higher among diploid (0.32–0.41; mean 0.36) than among tetraploid (0.12–0.31; mean 0.20) populations, and the most pronounced differences were found between ploidy levels (0.62–0.72; Supporting information). The neighbor network based on Rho further highlighted similarly divergent diploid populations, whereas tetraploid populations were grouped into two geographical pairs presenting further reticulation (Fig. 2A). A hierarchical AMOVA model (ploidy/population/individuals) confirmed that the ploidy level explains a considerable and significant proportion of the total genetic variance sampled within *B. laevigata* (48%; Table 3). On the contrary, partitioning the populations according to their edaphic origin (S versus NS; soil/population/individuals) did not result in a significant genetic differentiation. Given the genetic structure driven by ploidy level, the analysis was also conducted separately for diploid and tetraploid populations, and showed that soil type accounted for a very low but significant proportion of the genetic variation within diploids (2.1%), whereas it was non-significant for tetraploid populations (Table 3).

STRUCTURE analyses revealed $K = 2$ as the optimal number of genetic clusters based on both deltaK and the mean likelihood value (Supporting Information Fig. 4) and individual assignment to clusters was concordant with their ploidy level. Clustering at $K = 3$ highlighted further partitioning within diploids, differentiating the non-serpentine population N1 from the serpentine population S2, while making the two remaining populations (N2, S2) admixed between the two 'diploid' clusters.

The genome-wide estimates of Tajima's D were found to be above zero in each of the diploid populations (0.97–1.33; mean 1.16) and below zero in each of the tetraploid populations (−1.04 to −1.29; mean −1.17). Although significantly different between ploidy levels (Wilcoxon rank sum exact test, $W = 16$, p-value $= 0.029$) and thus congruent with an

Table 2. Differences in soil elemental composition between serpentine and non-serpentine localities of *Biscutella laevigata*. Average concentrations of four macronutrients are supplemented with the Ca/Mg ratio, a parameter widely used to describe serpentine soil-induced chemical stress. The independent contribution of these elements to the overall explained variation (i.e. simple effect) were assessed using a Monte Carlo test in a redundancy analysis (RDA). Additionally, unique contributions of these characters (i.e. their conditional effects) were tested during a stepwise selection of best RDA predictors. p-values indicating significant differences after Bonferroni correction are in bold.

Element	Serpentine soils	Non-serpentine soils	RDA: simple effect		RDA: conditional effect	
	mean ± SE	mean ± SE	Contribution (%)	$p_{adj.}$	Contribution (%)	$p_{adj.}$
Ca/Mg ratio	0.82 ± 0.11	10.95 ± 1.12	56.6	**0.005**	56.6	**0.005**
Mg	130.93 ± 14.57 ppm	23.36 ± 2.44 ppm	46.1	**0.005**	10.5	**0.005**
Ca	93.88 ± 13.99 ppm	238.00 ± 25.75 ppm	28.1	**0.005**	1.4	0.46
K	9.16 ± 0.97 ppm	10.42 ± 1.16 ppm	1.1	1	3.7	0.065
Na	1.87 ± 0.54 ppm	1.53 ± 0.38 ppm	0.4	1	0.0	1

excess of rare alleles expected in expanding tetraploids, none of these estimates appeared significantly different from neutral simulations (Supporting information).

Partial Mantel tests revealed a significant association between pairwise genetic and geographic distances among individual samples (Table 4). As expected if spatial isolation contributes strongly to the genetic structure within both ploidy level, such a pattern of isolation by distance remained significant when diploid and tetraploid populations were tested separately ($p < 0.001$ for both datasets). As for isolation by environment, when each edaphic factor was tested while taking spatial distances into consideration, the model explained up to 55% of the genetic variation and magnesium concentration was significantly associated with genetic distances after Bonferroni correction (Table 4). When samples differing by their ploidy level were tested separately, the concentration of calcium among diploids as well as the Ca/Mg ratio were still significantly associated with genetic distances, although it was not significant within tetraploids.

Differences in phenotypic traits between edaphic ecotypes and cytotypes

Altogether, 318 out of 336 individuals (94.6%) survived the eight weeks of hydroponic cultivation and were phenotyped. No significant differences in mortality rates were observed between the treatments (Mg−: 3.6%, Mg+: 7.1%; $\chi^2 = 2$, df = 1, $p = 0.157$). Contrary to our expectations, the experimental Mg treatment only had a weak effect on plant performance. Neither the main effect of the Mg treatment (TR), nor its interaction with edaphic origin of populations (EO; thus EO × TR) were significant in linear mixed-effects models for any investigated phenotypic trait (Table 5). However, there was a general tendency among plants cultivated in Mg+ to have higher root/shoot ratio and lower number of leaves when compared to Mg− conditions (irrespective of their ploidy level and edaphic origin; Fig. 3) and these two phenotypic traits attained highest F-values for TR in models. A significant interaction between the Mg treatment and ploidy (PL) was detected for a single trait, leaf length. While the cytotypes did not differ in leaf length when cultivated under Mg− setting, diploids had leaves significantly longer than tetraploids in the Mg+ setting (Supporting information).

Most observed phenotypic differences could be attributed to the edaphic origin of populations, their ploidy level, and particularly to the interaction of both factors (EO × PL; Table 5). Plants from NS sites produced significantly more aboveground biomass (and the highly correlated total biomass;

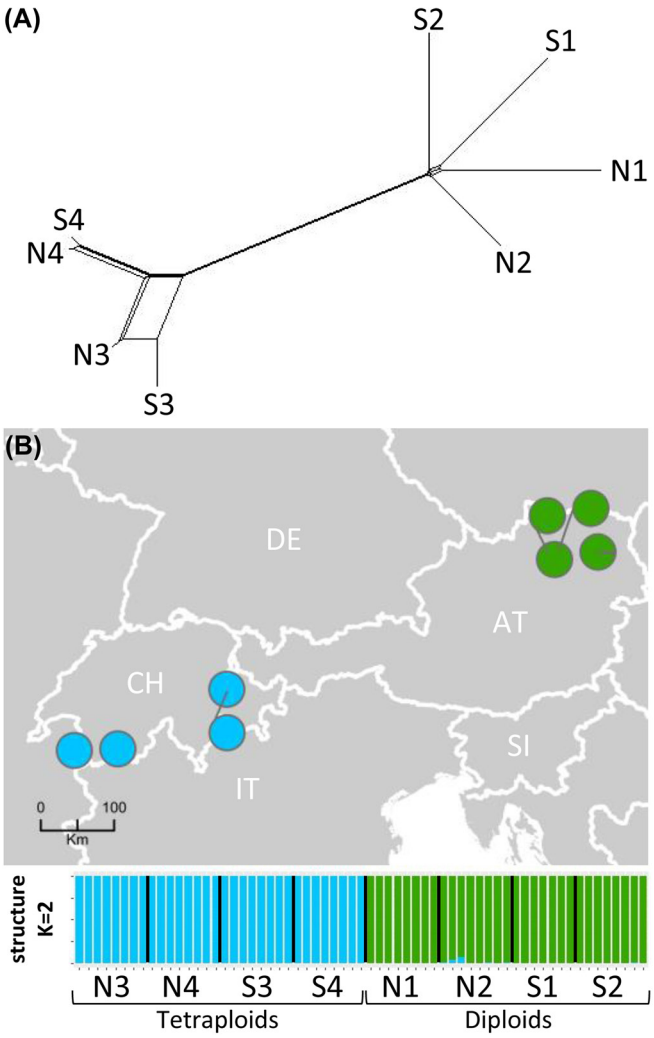


Figure 2. Genetic structure of diploid and tetraploid populations (labeled according to Table 1) of *Biscutella laevigata* assessed by ddRAD-seq. (a) Neighbor network based on Rho among populations. (b) Bar plot of assigned individual samples into K = 2 genetic clusters based on STRUCTURE and their proportion in each population shown as pie chart displayed on a background map.

Table 3. Hierarchical partitioning of genetic variation among individual of *Biscutella laevigata* nested within populations and differing by their ploidy level or their origin on serpentine versus non-serpentine soils, using an analysis of molecular variance (AMOVA). Provided the considerable genetic structure associated with ploidy levels, the partitioning of genetic variance by soil type was further tested within diploids and tetraploids separately. ♯df, degree of freedom; SS, sum of squares; VC, variance component; %Var, percentage of variance explained.

Source of variation	df♯	SS♯	VC♯	%Var♯	F-statistics	p-value
Ploidy						
Within populations	55	65.107	65.107	32.7	Rho_{ST}=0.673	
Among populations	6	365.863	38.421	19.3	Rho_{SC}=0.371	**0.001**
Ploidy	1	3393.394	95.752	48.0	Rho_{CT}=0.480	**0.024**
Soil						
Within populations	55	65.288	65.288	43.1	Rho_{ST}=0.569	
Among populations	6	867.475	101.396	67.0	Rho_{SC}=0.608	**0.001**
Among soil	1	358.455	−15.297	−10.1	Rho_{CT}=−0.101	0.508
Soil within diploids						
Within populations	27	122.754	122.754	62.1	Rho_{ST}=0.379	
Among populations	2	685.239	70.586	35.7	Rho_{SC}=0.365	**0.001**
Among soil	1	702.205	4.198	2.1	Rho_{CT}=0.021	**0.001**
Soil within tetraploids						
Within populations	28	10.095	10.095	80.2	Rho_{ST}=0.198	
Among populations	2	40.371	3.784	30.1	Rho_{SC}=0.273	**0.001**
Among soil	1	19.668	−1.294	−10.3	Rho_{CT}=−0.103	0.675

Supporting information) irrespective of the Mg treatment. Polyploids of *B. laevigata* presented significantly higher root biomass, consequently resulting in an almost two-fold increase in the relative investment into belowground biomass (i.e. the root/shoot ratio) than diploids (Fig. 3). While not differing from diploids in overall amounts of aboveground biomass, tetraploids presented significantly wider and also thicker leaves (i.e. lower SLA) than diploids, although this appeared compensated by a significantly lower overall number of leaves than in diploids (Fig. 3). The average mass of field-collected seeds was significantly greater for tetraploids than diploids (Supporting information, $F_{1, 316}=474.9$, $p < 0.001$).

The EO × PL interaction was significant for most investigated phenotypic traits in statistical models (Table 5), generally indicating differences in the performance of S versus NS diploids and, on the other hand, rather similar performance of S versus NS tetraploids (Figure 3). Specifically, diploid populations from S localities exhibited significantly lower aboveground (and total) biomass, which is linked with the smaller size of their leaves (both leaf length and width being reduced), production of thinner leaves (i.e. higher SLA) and lower relative chlorophyll content.

Discussion

Using serpentine (S) soils as a well-characterized substrate for local adaptation and ecological specialization in plants (Brady et al. 2005, Turner et al. 2010, O'Dell and Rajakaruna 2011), the factorial design implemented here with diploids and autotetraploids of *B. laevigata* can help address how diploids and tetraploids differ in response to environmental heterogeneity following WGD. Soil collected next to individual plant samples showed contrasting elemental compositions, chiefly in terms of magnesium and calcium concentrations, and particularly their respecitve Ca/Mg ratios that is fully consistent with typical S soils as compared to non-serpentine (NS) soils. Elevated Mg^{2+} concentrations in natural S populations are consistent with the edaphic differentiation among samples from NS versus S soils as well as the manipulation of Mg^{2+} concentrations in the hydroponic experiment as a strong potential selective agent.

Genetic structure among diploid and tetraploid populations from NS versus S soils

Using ploidy-dependent sequencing coverage to ensure similarly accurate genotyping among samples and appropriate metrics for mixed-ploidy systems such as Rho

Table 4. Isolation by distance and isolation by environment estimated by partial Mantel tests based on Rousset's â, geographical distances and elemental concentration differences. Analyses were conducted either on a combined dataset of all individuals or for each ploidy level separately (2x, diploids; 4x, tetraploids). Significant associations following 10 000 permutations and Bonferroni correction are highlighted in bold.

Variable	Ploidy	t-value	p-value
Geography	combined	37.94	**< 0.001**
	2x	21.17	**< 0.001**
	4x	15.72	**< 0.001**
Magnesium	combined	19.17	**< 0.001**
	2x	−1.67	0.194
	4x	1.14	0.612
Calcium	combined	6.03	0.046
	2x	−6.09	**< 0.001**
	4x	2.80	0.163
Sodium	combined	7.41	0.025
	2x	3.54	0.033
	4x	4.68	0.062
Potassium	combined	−10.00	**0.001**
	2x	−0.34	0.760
	4x	1.31	0.544
Ca/Mg	combined	6.92	0.033
	2x	4.88	**0.002**
	4x	−6.11	0.016

Table 5. Summary of linear mixed-effects models testing the effect of experimental magnesium treatment (Mg− versus Mg+), edaphic origin of populations (serpentine versus non-serpentine), ploidy level (2x versus 4x) and their interactions on phenotypic traits of *Biscutella laevigata* in hydroponic cultivation. Significant differences ($\alpha = 0.05$) are highlighted in bold. Variation explained by the models is approximated using pseudo-R^2 coefficients, separately for fixed effects (i.e. marginal R^2, R^2_m) and for both random and fixed effects (i.e. conditional R^2, R^2_c). Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001.

Phenotypic trait	Edaphic origin (EO)		Mg treatment (TR)		Ploidy (PL)		EO × TR		EO × PL		TR × PL		EO × TR × PL		Explained variation	
	df	F	df	F	df	F	df	F	df	F	df	F	df	F	R^2_m	R^2_c
Total biomass (sqrt)	51.1	**4.64***	6.2	0.22	51.1	0.37	257.9	2.68	51.1	**4.73***	257.9	2.52	257.9	0.23	0.091	0.335
Aboveground biomass (sqrt)	51.2	**4.62***	6.1	0.09	51.2	0	254.2	2.73	51.2	**4.83***	257.8	2.47	255.5	0.28	0.089	0.336
Belowground biomass (sqrt)	50.5	3.24	6.6	1.79	50.5	**21.05*****	253.1	1.43	50.5	3.3	257	2.32	254.4	0.00	0.179	0.364
Root : shoot ratio (log)	51.3	0.63	6.4	2.74	51.5	**73.63*****	255.2	0.32	51.3	1.61	258.5	0.05	256.2	0.23	0.291	0.38
No. of leaves (log)	51.8	0.99	5.7	2.68	51.9	**12.90*****	256	0.33	51.8	0.00	258.8	1.66	257.1	0.00	0.094	0.318
Leaf length	51.8	1.24	6.4	0.1	51.8	0.8	259.9	0.08	51.8	**8.05****	259.9	**4.12***	259.9	0.92	0.071	0.209
Leaf width (sqrt)	49.6	1.53	6.3	0.12	49.6	**8.70****	257.1	1.84	49.6	**12.00****	257.1	0.19	257.1	2.54	0.128	0.296
Leaf size (sqrt)	50.7	1.6	6.3	0.1	50.7	2.12	257.8	1.21	50.7	**11.87****	257.8	1.55	257.8	2.36	0.108	0.318
Specific leaf area (log)	48.6	2.28	6.7	0.24	49.2	**7.68****	254.2	2.05	48.8	**6.35***	256.9	0.42	255	0.48	0.07	0.129
Chlorophyll content	46.6	0.05	6.5	1.15	47.1	3.93	251.4	1.09	46.7	**11.59****	253.6	1.6	252.1	0.18	0.074	0.177

(Ronfort et al. 1998), most of the genetic variation highlighted by ddRAD-seq as segregating within *B. laevigata* is partitioned between diploid and tetraploid samples. Matching taxonomic insights (all diploid samples belong to *B. laevigata* subsp. *kerneri*, whereas tetraploid samples belong to *B. laevigata* subsp. *laevigata*) and the range-wide phylogeography of Trametsberger et al. (2002), the considerable genetic differentiation between the diploid and tetraploid populations is likely a result of the past WGD event followed by limited gene flow between populations differing by ploidy level under scrutiny in the present study. Consistent with early studies (Manton 1933, Trametsberger et al. 2002), genome-wide variation observed here further supports diploid populations as glacial relicts scattered across xeric regions in the landscape that are characterized by high genetic diversity and an excess of frequent variants expected under the contraction of isolated populations, whereas tetraploid populations contrastingly exhibit reduced genetic diversity and an excess of rare variants typical of populations having postglacially expanded across the Alps. Accordingly, populations of *B. laevigata* from both ploidy levels thriving under NS and S soils offer a unique opportunity to examine local adaptation in a mixed-ploidy system (Kolář et al. 2017).

Despite the comparison of pairs of similarly nearby sites on NS and S soils among cytotypes of *B. laevigata*, the contrasting postglacial history and current distribution of corresponding populations across very different landscapes likely contributed together with selection by toxic soils to genetic diversity and differentiation. Diploid remnants trapped in islands of open vegetation on S soils across a matrix of postglacially reforested inhospitable habitats indeed presented relatively large genetic differentiation with more than 35% of the variation being partitioned among populations and hence indicated limited recurrent gene-flow, whereas only up to 2.1% of the total variation was partitioned between populations from NS versus S soils. Together with the significant patterns of isolation by distance and isolation by environment (i.e. soil elemental composition), genetic differentiation appears consistent with a weak role of NS versus S soils in shaping gene flow among diploid populations that had ample time to diverge since the last glacial maximum (Trametsberger et al. 2002). Despite similar partitioning of up to 30% of the genetic variation among populations, continuously-distributed tetraploids of *B. laevigata* having postglacially expanded above the treeline across the Alps contrastingly were not significantly differentiated by either soil type (i.e. NS and S populations) or individual-level soil elemental profiles. Hence, with only significant isolation by distance being detected among tetraploids, genetic differentiation related to NS versus S soils is extremely weak. Even when the ploidy level is accounted for, genetic variation is significantly partitioned among populations of *B. laevigata* that all appear considerably isolated from one another and selective pressures inherent to S may thus ineffectively reach stable edaphic differentiation (Parisod 2022).

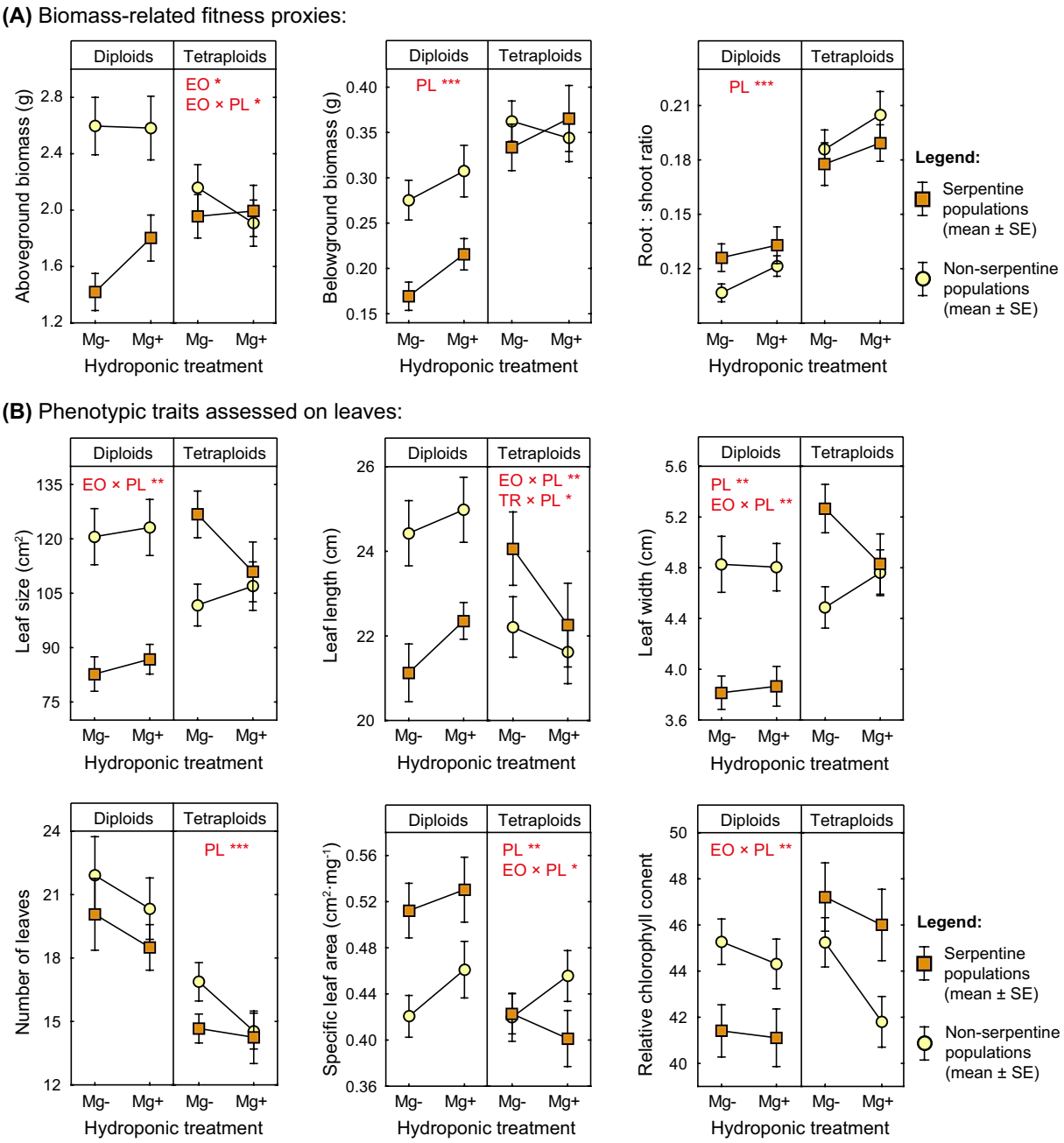


Figure 3. Phenotypes of *Biscutella laevigata* individuals after eight weeks of hydroponic cultivation as described by biomass-related fitness proxies (A) and six additional traits measured on leaves (B). Significant effects of experimental magnesium treatment (TR), edaphic origin of populations (EO), ploidy level (PL) and their interactions in linear mixed-effects models are shown in red (Table 5).

Performance of diploids and tetraploids from NS versus S soils under NS and S conditions

Contrary to our expectations, the experimental Mg+ treatment only had a limited effect on plant performance, and most phenotypic differences could be attributed to natural variation in edaphic conditions of origin (NS versus S) and the ploidy level of investigated plants. The interaction between the edaphic origin and the treatment (i.e. EO × TR) expected under local adaptation (i.e. when plants of S origin are favoured under high Mg^{2+} concentrations, whereas plants of NS origin are favoured under control conditions; Kawecki and Ebert 2004) was not significant for any phenotypic trait in *B. laevigata*. Although possibly indicating that the Mg+ treatment ineffectively mimicked main selective agents inherent to S soils, the manipulated Ca/Mg ratio reached four in the Mg− treatment and 0.4 in the Mg+ treatments (i.e. more inhospitable than found on the natural population sites) and is widely considered to be the most important predictor of serpentine soil edaphic differentiation (Proctor and Woodell

1975, O'Dell and Rajakaruna 2011), as also demonstrated through hydroponic culture in other species (Palm et al. 2012). Of course, this does not rule out a selective impact of other features typically associated with S soils that were not accounted for in our study design (e.g. soil physical properties, heavy metals, water limitation, interspecific interactions), but indicates that individuals of *B. laevigata* constitutively tolerate high Mg concentrations typical of S soils and may thus be (pre-)adapted for successful colonization of such sites presenting a challenging chemistry and therein benefit from reduced interspecific competition and open vegetation to survive (Brady et al. 2005).

Within *B. laevigata*, mostly diploids presented experimental evidence of phenotypic differentiation according to their edaphic origin, with plants from S soils constitutively producing less aboveground biomass linked to smaller and thinner leaves with a lower chlorophyll content than their counterparts from NS origin. Given that ddRAD-seq supported equal levels of genetic differentiation among diploid populations, phylogenetic constraints seem unlikely to explain this phenotypic difference that thus appears consistent with possible adaptive changes in resource allocation and/or slower overall growth rates expected under low nutrient availability in S soils or any other specifics of this inhospitable substrate.

Contrary to diploids, no clear pattern of NS versus S phenotypic differentiation was detected in tetraploids of *B. laevigata*. This is consistent with evidence from the reciprocal transplant experiment of Gasser (1986) highlighting limited fitness differences among tetraploid plants originating from NS and S soils on S soils, and even plants performing slightly better on S than NS soils, likely in relation to reduced interspecific competition under such toxic conditions. In fact, several traits appearing here as constitutive properties of tetraploids' phenotypes could support survival under the typical conditions of S soils. In particular, tetraploid individuals produced more belowground biomass on average than diploids, resulting in an almost two-fold increase in their relative investment in root production, seen here as a higher root/shoot ratio that is commonly described as an adaptation among S-tolerant plant species (Proctor and Woodell 1975, Brady et al. 2005). The general tendency of experimental plants to here exhibit higher root/shoot ratios under the hydroponic Mg+ treatment, irrespective of their edaphic origin and ploidy (Fig. 3A), is suggestive of phenotypic plasticity unrelated to drought that may prove adaptive in the context of S soils. Although the extent to which the proportionally higher investment of polyploids into belowground biomass is a direct effect of WGD per se remains elusive, the constitutively higher root/shoot ratio of tetraploids as compared to diploids could represent a convenient trait for tetraploids of *B. laevigata* to cope with S soils and could relax the corresponding selective pressures (i.e. preadaptation). These results show interesting parallels with findings in *Knautia serpentinicola*, another mixed-ploidy system of surmised recent origin (Kolář et al. 2012) that occurs on S soils, in which tetraploids also showed constitutively higher investment in belowground biomass when compared with their direct diploid progenitors (Čertner et al. 2019). Available evidence from other diploid-polyploid systems is however scarce, supporting both higher and lower investment of long-established polyploids in belowground biomass relative to diploids (Sudová et al. 2010, Collins et al. 2011) or lack of ploidy-specific differences (Baack and Stanton 2005, Sudová et al. 2010) and thus calls for the relative impact of WGD per se versus post-WGD evolution to be further assessed.

Although S soils were demonstrated to be conducive of some adaptive convergence in tetraploids of *Arabidopsis arenosa* (Konečná et al. 2021), *B. laevigata* presents mixed support for the rise of stable edaphic ecotypes (Babst-Kostecka et al. 2016 in the context of zinc tolerance on mine sites) and it remains to be clarified to what extent and how WGD may generally promote tolerance to S soils. Additional studies including independent diploid populations on S soils (e.g. in *B. laevigata* subsp. *austriaca*; Tremetsberger et al. 2002) would prove useful to reach firm conclusions, but further work must go beyond the investigation of established tetraploids, having diverged for thousands of generations from their ancestors in order to conclude on the role of WGD per se (Ramsey and Schemske 2002). As a constitutive increase in belowground biomass has commonly been reported as a direct consequence of WGD (i.e. the so called 'gigas effect'; Stebbins 1971) and given that WGD per se has already been conclusively implicated in increased tolerance to key stresses related to S soils; namely salt in *Arabidopsis thaliana* (Chao et al. 2013) and drought in *Chamerion angustifolium* (Maherali et al. 2009), future comparisons of experimentally resynthesized and naturally established polyploids are likely to shed light on the consequences of WGD. Evidence reported here supports the *B. laevigata* autopolyploid complex as a promising mixed-ploidy system for further characterization of candidate loci underlying adaptation to S soils (in diploids) and the consequences of WGD in promoting survival under such challenging environments.

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Author contributions

Tala Bürki and **Valentin** share first authorship. **Martin Čertner** and **Christian Parisod** share last authorship. **Tala Bürki**: Conceptualization (equal); Data curation (equal); Formal analysis (supporting); Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal). **Valentin Pulver**: Conceptualization (equal); Data curation (equal); Formal analysis (supporting);

Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal). **Sandra Grünig**: Formal analysis (equal); Investigation (supporting); Methodology (supporting); Validation (equal); Writing – original draft (supporting); Writing – review and editing (supporting). **Martin Čertner**: Formal analysis (equal); Methodology (supporting); Validation (equal); Writing – review and editing (lead). **Christian Parisod**: Conceptualization (lead); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Project administration (lead); Supervision (lead); Validation (supporting); Writing – original draft (supporting); Writing – review and editing (lead).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.hx3ffbgkj (Bürki et al. 2023).

Raw sequencing reads have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB48869 (https://www.ebi.ac.uk/ena/browser/view/ PRJEB48869).

Supporting information

The Supporting information associated with this article is available with the online version.

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