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RESEARCH ARTICLE

Neither connectivity nor genetic diversity matter in the conservation of a rare fern and a moss on insular erratic boulders

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Abstract

Erratic boulders provide habitat for rock-dwelling species and contribute to the biodiversity of landscapes. In the calcareous Swiss lowlands, siliceous erratic boulders are exclusive habitat islands for the regionally critically endangered fern *Asplenium septentrionale*, about 20 bryophyte species and numerous lichens. Focusing on island biogeographical processes, we analysed the conservation genomics of *A. septentrionale* and the moss *Hedwigia ciliata* on insular erratic boulders in the Swiss lowlands and the adjacent "mainland" in siliceous mountains. We genotyped both species using double digest restriction associated DNA sequencing (ddRAD). For the tetraploid *A. septentrionale*, abundant identical multilocus genotypes within populations suggested prevalent intragametophytic selfing, and six out of eight boulder populations consisting of a single multilocus genotype each indicated single spore founder events. The genetic structure of *A. septentrionale* mainland populations coincided with Pleistocene glacial refugia. Four genetic lineages of *H. ciliata* were identified, and populations consisting of a single multilocus genotype were less common than in *A. septentrionale*. For both taxa, multilocus genotype diversity on boulders was lower than in mainland populations. The absence of common genetic groups among boulder populations, and the absence of isolation by distance patterns, suggested colonisation of boulders through independent long-distance dispersal events. Successful boulder colonisation of *A. septentrionale* seems to be rare, while colonisation by *H. ciliata* appears to be more frequent. We conclude that pivotal principles of conservation biology, such as connectivity and genetic diversity, are of less importance for the studied cryptogams on insular erratic boulders because of long-distance dispersal, intragametophytic selfing and polyploidy.

Keywords Bryophytes · ddRAD · Ferns · Island biogeography · Long-distance dispersal

Introduction

Pleistocene erratic boulders are rocks that have been translocated by glaciers during the ice ages, often across large geographic distances (Colgan 2009). These boulders serve as terrestrial habitat islands for rock-dwelling species, especially cryptogams (bryophytes, ferns and lichens). In landscapes where no rock habitats other than erratic boulders exist, they contribute significantly to the biodiversity of such landscapes, for example in the American moraine archipelago between Long Island and Cape Cod (Miller and Robinson 2015) and on the European sand plain between Belgium and Estonia (Krawiec 1938; Wächter 1996; Colpa and van Zanten 2006). Further, in situations where the chemical composition of erratic boulders strongly contrasts with the composition of the surrounding bedrock, as with calcareous erratic boulders on siliceous bedrock in central Finland (Virtanen and Oksanen 2007) and siliceous erratic boulders on calcareous bedrock in the Swiss Plateau and Jura Mountains (Meylan 1912; Mazenauer et al. 2014), erratic boulders form habitat islands for rock-dwelling edaphic specialists.

Scientific interest in the vegetation on erratic boulders has a long history (Heer 1865; Milde 1870; Brockmann-Jerosch

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and Brockmann-Jerosch 1926). More recently, the insular properties of erratic boulders have motivated researchers to test predictions based on island biogeography, especially the species–area relationship (MacArthur and Wilson 1963). Kimmerer and Driscoll (2000) found no significant effects of boulder size and connectivity on bryophyte species richness in a dataset of 39 granitic boulders in the US state of New York. In contrast, Weibull and Rydin (2005) studied 218 granitic boulders in Sweden and found a positive correlation between bryophyte species richness and boulder size. In addition, Virtanen and Oksanen (2007) reported a positive effect of connectivity on combined bryophyte and lichen species richness for 288 calcareous erratic boulders in Finland. In the only genetic study on erratic boulders, Holderegger and Schneller (1994) found isozyme variation among three boulder populations of the fern *Asplenium septentrionale* in the SwissPlateau, of which only one showed within-population variation.

The siliceous erratic boulders in the Swiss Plateau and Jura Mountains are exclusive habitat islands for numerous lichen species (Meylan 1926b), about 20 bryophyte species (Meylan 1912), and the fern *A. septentrionale* (Mazenauer et al. 2014; Fig. 1). Over the last centuries, the erratic boulders and their cryptogam communities have declined as a result of multiple factors. From the eighteenth century onwards, boulders of all sizes have been destroyed due to farmland clearance, which is reflected in contemporary geological maps which indicate around five times fewer erratic boulders in open land than in forests (Akçar et al. 2011; Swisstopo 2013). In the nineteenth and early twentieth century, commercial exploitation of countless large erratic boulders for construction material substantially also reduced the number of erratic boulders (Reynard 2004; Lugon et al. 2006). And since about three decades, large erratic boulders have been cleaned from vegetation for bouldering (sport climbing at low height; Blum 2015; Hepenstrick et al. 2016). The scarcity of occurrence data for bryophytes and lichens (Meylan 1926a) does not allow estimating population trends. However, Swiss populations of *A. septentrionale* on erratic boulders have been actively searched for and documented in the literature and in herbaria (Weber 1912). This enabled a recent revisitation study, which confirmed the occurrence of only 5 out of 17 historically documented populations of *A. septentrionale* on erratic boulders in the Swiss Plateau and Jura Mountains (Mazenauer et al. 2014). Consequently, *A. septentrionale* has been classified as critically endangered in the Swiss Plateau and Jura Mountains (Bornand et al. 2019).

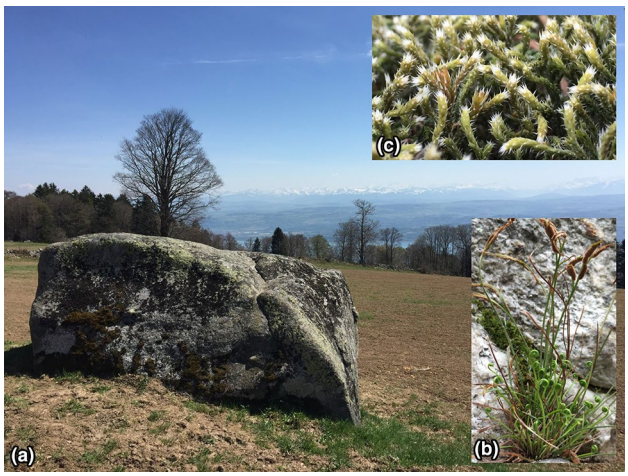


Fig. 1 Pictures of the studied system and species. **a** A siliceous erratic boulder (ID Be as in Fig. 2 and Table 1) in the Jura Mountains (foreground), the Swiss Plateau in the background and the Alps in the far background. **b** Studied fern species *Asplenium septentrionale* with unrolling young fronds and older fronds with sporangia that have released spores. **c** Studied moss species *Hedwigia ciliata* with its typical white hyaline hair points

In order to guide conservation management of *A. septentrionale* and cryptogam communities on erratic boulders, we investigated the genomics of the fern *A. septentrionale* and the moss *Hedwigia ciliata*. Both are emblematic species of the insular cryptogam communities on lowland siliceous erratic boulders (Milde 1870; Weber 1912). Using genome-wide single nucleotide polymorphisms (SNPs) retrieved from double digest restriction associated DNA sequencing (ddRAD), we analysed populations sampled from erratic boulders in the Swiss lowlands (i.e. Swiss Plateau and Jura Mountains) and from adjacent siliceous mountain ranges. We aimed to retrace the island biogeographical processes shaping the genetic structure within and among populations on erratic boulders. We specifically asked whether populations on erratic boulders form genetic groups that are distinct from those growing in "mainland" areas (i.e. siliceous mountains) and whether boulders were primarily colonised from nearby boulders or via long-distance dispersal out of mainlands.

Materials and methods

Study species

Asplenium septentrionale (Fig. 1b) is a rock-dwelling, heliophile and calcifuge fern of holarctic distribution. It typically grows in crevices of sunny siliceous rocks, where its distinctively forked fronds form long-lived tufts (Reichstein 1984). It is homosporous, hence the sporophyte meiotically produces one type of spores that grow into hermaphroditic, short-lived gametophytes. Gametophytes can self- or cross-fertilise and thereby give rise to new sporophytes (Joanne et al. 2010). The European subspecies, *A. septentrionale* ssp. *septentrionale*, is autotetraploid (2n = 144; Reichstein 1984), hence, it

derived from genome doubling without the involvement of hybridisation. For the Southwest Asian diploid subspecies *A. septentrionale* spp. *caucasicum*, Clark et al. (2016) reported a genome size of 2C = 7.1 pg.

Hedwigia ciliata (Fig. 1c) is a rock-dwelling, heliophile and calcifuge moss with a cosmopolitan distribution. It typically grows on sunny siliceous rocks in the form of loose cushions (Nebel and Philippi 2000). It is a monoecious moss; hence, its long-lived gametophytes can self- or cross-fertilise and give rise to short-lived sporophytes, which meiotically produce spores that germinate and form new gametophytes (Glime 2017). Traditionally, *H. ciliata* was considered the only species of the monospecific genus *Hedwigia*, whose morphological diversity (e.g. the extent of the white leaf tip) was recognised as subspecies, varieties and forms (Hedenäs 1994). A distinctive form with reflexed leaf apices and unipapillose median leaf cells was described by Hedenäs (1994) as *H. stellata*, and its monophyly was confirmed by Buchbender et al. (2014) based on nuclear, mitochondrial and plastid sequences. The latter study further highlighted that *H. ciliata* is a species complex with potential cryptic species and hybridisation. In the present study, we refer to *H. ciliata* in its traditional circumscription, excluding *H. stellata* (Nebel and Philippi 2000). For *Hedwigia*, a chromosome number of n = 11 has been reported for specimens from Australia, UK, USA, India and Japan, and polyploid lineages with n = 22 have been reported for specimens from Finland, Russia and Poland (Fritsch 1991; Ramsay 2011). Bainard et al. (2010) reported a genome size of 1C = 0.3 pg for *H. ciliata* collected from Canada.

Asplenium septentrionale and *H. ciliata* both deviate from the standard diploid genetic model in multiple aspects. Both species can conduct intragametophytic selfing, which is self-fertilisation within single hermaphroditic gametophytes. This leads – in diploids – to completely homozygous sporophytes (Klekowski and Lloyd 1968). Consequently, spores of sporophytes that descended from intragametophytic selfing are (in the absence of mutations) genetically uniform. If these spores germinate and gametophytes further propagate with intragametophytic selfing, or if they cross-fertilise among each other, the resulting sporophytes are genetically identical to their parent sporophyte (irrespective of recombination). In *A. septentrionale*, however, sporophytes that result from intragametophytic selfing are expected to maintain heterozygosity as a result of autotetraploidy. In *H. ciliata*, in contrast, gametophytes are expected to be homozygous, except in the case of polyploidy. In the latter case, fixed heterozygosity due to non-segregating paralogous loci is expected to occur in allopolyploids resulting from genome duplication following hybridisation (Wyatt 1994).

Study region and sampling

In this study, we focused on siliceous erratic boulders of Alpine origin that were left, after the last glacial maximum (ca. 21,000 years ago; Ehlers and Gibbard 2004), on the southern slopes of the Jura Mountains (limestone bedrock) and on the Swiss Plateau (molasse bedrock consisting of calcareous conglomerates, sandstones and shales) across an area of about 300 km × 50 km (Fig. 2; Labhart 1992). In this region, the national geological maps show around 10,000 siliceous erratic boulders, whose diameters range from < 1 m up to ~ 15 m (Swisstopo 2013). As mainland in the terminology of island biogeography – i.e. the main distribution areas of the two studied species – we considered the adjacent siliceous parts of the Alps in the South and the siliceous Black Forest and Vosges in the North (Fig. 2).

We sampled all eight extant populations of *A. septentrionale* on erratic boulders in the Swiss Plateau, Jura Mountains and adjacent regions in France, and eleven mainland populations from adjacent siliceous mountains (Table 1; Fig. 2). We sampled the more abundant *Hedwigia ciliata* at the same sites and from 13 additional sites. For *A. septentrionale* we collected 10 individuals (sporophytes) per population. *Hedwigia ciliata* often forms only small populations

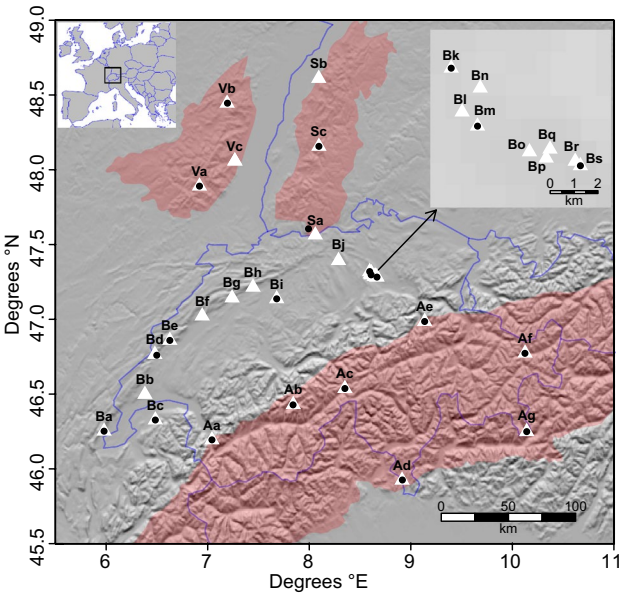


Fig. 2 Study area and sampling locations. Areas with predominantly calcareous bedrock are given in grey, areas with predominantly siliceous bedrock (mainland) in red (Asch 2005), and political borders in blue (Becker et al. 2018). Sampled populations are indicated by white triangles (*Hedwigia ciliata*) or black dots (*Asplenium septentrionale*). The first letter of each population code designates its regional provenance (as in Table 1): siliceous parts of the Alps (A), siliceous erratic boulders on calcareous bedrock (B; a picture of Be is given in Fig. 1), siliceous Black Forest (S) and siliceous Vosges (V). (Background map according to Jarvis et al. 2008)

Table 1 Samples of *Asplenium septentrionale*, *Hedwigia ciliata* and *H. stellata* analysed in the present study

Provenance	Site name (Country)	ID	Coordinates (lat./lon. WGS84)	Taxon sampled	N	n per linage: st/h1/h2/d1/d2	n MLG per linage: st/h1/h2/d1/d2	Simpson index
Alps (mainland)	Collognes (CH)	Aa	46.193941/7.041595	*H. ciliata*	5 (0)	0/2/1/0/2	0/2/1/0/2	-
				A. septentrionale	8 (0)		7	0.84
	Lötschental (CH)	Ab	46.429488/7.84245	*H. ciliata*	5 (0)	0/1/0/0/4	0/1/0/0/1	0
			46.428048/7.840318	*A. septentrionale*	10 (0)		10	0.9
	Oberwald (CH)	Ac	46.539454/8.356024	*H. ciliata*	5 (0)	0/2/0/2/1	0/1/0/1/1	-
			46.537256/8.350998	*A. septentrionale*	10 (0)		8	0.86
	Morcote (CH)	Ad	45.923897/8.913934	*H. ciliata*	5 (0)	0/0/0/0/5	0/0/0/0/5	0.8
			45.925722/8.916832	*A. septentrionale*	10 (0)		8	0.86
	Sernftal (CH)	Ae	46.985533/9.136344	*H. ciliata*	5 (0)	0/0/0/4/1	0/0/0/4/1	0.75
				A. septentrionale	10 (0)		1	0
	Lavin (CH)	Af	46.77376/10.125245	*H. ciliata*	5 (1)	0/5/0/0/0	0/3/0/0/0	0.56
			46.772522/10.123926	*A. septentrionale*	10 (1)		8	0.86
	Viano (CH)	Ag	46.249111/10.141431	*H. ciliata*	5 (1)	0/3/0/0/2	0/3/0/0/2	-
				A. septentrionale	10 (1)		10	0.9
Erratic boulders (islands)	Thoiry (F)	Ba	46.252058/5.979478	*H. ciliata*	5 (2)	1/4/0/0/0	1/1/0/0/0	0
				A. septentrionale	10 (1)		1	0
	Aubonne (CH)	Bb	46.497636/6.381872	*H. ciliata*	5 (1)	0/0/0/0/5	0/0/0/0/2	0.32
				H. stellata	3 (0)	3/0/0/0/0	1/0/0/0/0	-
	Allignes (F)	Bc	46.326596/6.484203	*H. ciliata*	5 (0)	0/4/0/0/1	0/1/0/0/1	0
				H. stellata	3 (0)	3/0/0/0/0	1/0/0/0/0	-
				A. septentrionale	5 (1)		3	0.56
	Vuitebouef (CH)	Bd	46.76478/6.479694	*H. ciliata*	5 (1)	0/0/0/5/0	0/0/0/2/0	0.48
			46.759825/6.498386	*A. septentrionale*	10 (1)		1	0
				H. stellata	3 (0)	3/0/0/0/0	1/0/0/0/0	-
		Be	46.858871/6.627056	*H. ciliata*	5 (0)	0/0/0/5/0	0/0/0/5/0	0.8
				A. septentrionale	9 (1)		1	0
	Corcelles (CH)	Bf	47.02201/6.945709	*H. ciliata*	4 (0)	0/0/0/4/0	0/0/0/1/0	0
	Evilard (CH)	Bg	47.142665/7.240139	*H. ciliata*	5 (0)	0/0/0/3/2	0/0/0/2/2	-
	Langebrugg (CH)	Bh	47.214417/7.446306	*H. ciliata*	5 (0)	0/0/0/0/5	0/0/0/0/2	0.48
	Riedtwil (CH)	Bi	47.137074/7.679436	*H. ciliata*	5 (1)	0/0/0/0/5	0/0/0/0/3	0.64
				A. septentrionale	10 (1)		1	0
	Niederwil (CH)	Bj	47.392768/8.288129	*H. ciliata*	4 (0)	0/0/0/4/0	0/0/0/2/0	0.38
	Herrliberg (CH)	Bk	47.319133/8.595127	*H. ciliata*	1 (0)	0/0/0/0/1	0/0/0/0/1	-
				A. septentrionale	6 (1)		1	0
		Bl	47.302635/8.601195	*H. ciliata*	5 (0)	0/0/4/0/1	0/0/3/0/1	0.63
		Bm	47.297342/8.609718	*H. ciliata*	4 (1)	0/0/0/0/4	0/0/0/0/4	0.75
				A. septentrionale	7 (1)		1	0
		Bn	47.311646/8.611218	*H. ciliata*	5 (0)	0/0/1/4/0	0/0/1/1/0	0
		Bo	47.287653/8.63852	*H. ciliata*	5 (0)	0/3/0/0/2	0/2/0/0/2	-
		Bp	47.285307/8.647801	*H. ciliata*	5 (1)	0/0/0/0/5	0/0/0/0/3	0.56
		Bq	47.288685/8.649913	*H. ciliata*	5 (0)	0/0/5/0/0	0/0/2/0/0	0.32
		Br	47.284112/8.663889	*H. ciliata*	5 (1)	0/0/0/0/5	0/0/0/0/1	0
		Bs	47.28244/8.666758	*H. ciliata*	5 (0)	0/0/0/1/4	0/0/0/1/4	0.75
				A. septentrionale	10 (1)		6	0.76
Black Forest (mainland)	Wieladingen (D)	Sa	47.56398/8.059346	*H. ciliata*	5 (1)	0/0/0/1/4	0/0/0/1/3	0.623
			47.60523/7.992884	*A. septentrionale*	7 (1)		1	0
	Achern (D)	Sb	48.61072/8.093502	*H. ciliata*	5 (0)	0/0/0/3/2	0/0/0/3/2	-
	Yach (D)	Sc	48.156618/8.094974	*H. ciliata*	5 (1)	0/0/0/0/5	0/0/0/0/2	0.32

Table 1 (continued)

Provenance	Site name (Country)	ID	Coordinates (lat./lon. WGS84)	Taxon sampled	N	n per linage: st/h1/h2/d1/d2	n MLG per linage: st/h1/h2/d1/d2	Simpson index
				A. septentrionale	10 (0)		5	0.74
Vosges (mainland)	Taye (F)	Va	47.890457/6.918914	*H. ciliata*	5 (0)	0/0/0/5/0	0/0/0/5/0	0.8
				A. septentrionale	10 (0)		4	0.58
	Rothau (F)	Vb	48.444534/7.191003	*H. ciliata*	5 (0)	0/0/0/5/0	0/0/0/5/0	0.8
			48.445197/7.194594	*A. septentrionale*	10 (0)		1	0
	Hohlandsburg (F)	Vc	48.058607/7.267482	*H. ciliata*	5 (0)	1/0/0/4/0	1/0/0/4/0	0.75

The provenances of the populations (mainland in siliceous mountains or islands consisting of siliceous erratic boulders on calcareous bedrock), the names of the sites (names of nearby settlements; CH Switzerland, F France, D Germany), the identification codes of the populations (ID; as in Fig. 2), the geographic coordinates of the populations (separate coordinates if populations were sampled more than 100 m apart from each other), the number of analysed samples (n; in brackets the number of technical replicates), the number of samples per *Hedwigia* lineage (codes as in Fig. 3), the number of multilocus genotypes (MLG), and the Simpson index for populations with four or more samples per lineage

on erratic boulders, and we therefore sampled five individuals (gametophytes) per population. Where populations were smaller than the target sample size, we sampled all available individuals. For each population, we collected individuals as far apart from each other as possible. As an outgroup in the genetic analysis of *H. ciliata* we included *H. stellata*, which we collected from three erratic boulders (Hepenstrick and Kiebacher 2019) with three samples per population (Table 1).

DNA extraction and sequencing

We extracted DNA from silica dried plant material consisting of 3 mg leaf petiole for *A. septentrionale* and 2 mg shoot tips from one continuous moss shoot per *Hedwigia* sample. DNA extraction was conducted on a KingFisher Flex 96 platform (Thermo Fisher Scientific, Waltham, MA, USA) using the sbeadex mini plant kit (LGC, Teddington, UK). We established technical replicates by extracting 11 samples of *A. septentrionale* and 14 samples of *H. ciliata* twice. We prepared ddRAD-seq libraries with a modified version of the protocol of Peterson et al. (2012) using the restriction enzymes EcoRI and TaqαI and AMPure XP beads (Beckman Coulter, Brea, CA, USA) for size selection, which resulted in fragments of 400–700 bp (Westergaard et al. 2019). In order to estimate the number of expected fragments and to obtain long Illumina reads for reference catalogues we prepared one pooled library containing 24 samples of *A. septentrionale* and 24 samples of *H. ciliata* sourcing from 6 populations (4 samples per population). We sequenced this pooled library at low coverage on an Illumina MiSeq at the Genetic Diversity Centre ETH Zurich (Switzerland) using a 300-bp paired-end reads V3 protocol. For genotyping the samples we prepared a total of eight libraries containing all analysed samples and sequenced them on four lanes of an Illumina HiSeq4000 at Novogene (Hong Kong), with each lane containing one 48-plex *A. septentrionale* and one 48-plex *Hedwigia* library with 48 different indexes for the individual samples and contrasting Illumina adapters per species (p6 and p12), pooled in a 1:2 ratio to account for the different catalogue sizes. We deposited raw data at ENA under accession number PRJEB42827.

Data processing

We used the recommended default settings of the DDOCENT pipeline 2.8.12 to call genotypes (Puritz et al. 2014), except for the settings mentioned below. We demultiplexed raw reads using the default settings of STACKS 1.4.2 (Catchen et al. 2013). For the reference catalogues we used the long MiSeq reads. We inferred best parameters by optimising the re-mapping rate on a couple of parameter combinations. Highest re-mapping rates after removing low quality reads (Q1) were obtained with the following settings: reads had to be covered twice in at least one individual, and we used a similarity parameter of 0.95 for the first clustering. For the second clustering we used similarity parameters of 0.92 and 0.96, resulting in 33,154 and 85,938 fragments for *A. septentrionale* and *Hedwigia,* respectively. We then mapped HiSeq paired-reads of all individuals against the reference catalogues using BWA 0.7.12 (mean coverage in *A. septentrionale* 37X, in *Hedwigia* 36X), and we called single nucleotide polymorphisms (SNPs) using FREEBAYES 1.3.1 (Garrison and Marth 2012). To speed up the variant calling we set use-best-n-alleles to four. We set *A. septentrionale* and *Hedwigia* ploidy levels to two, as doing so produced the lowest genotype error rates among technical replicates. We ended up with 155,182 variants for *A. septentrionale* and 1,716,025 for *Hedwigia*.

We filtered the variant tables as recommended in O'Leary et al. (2018) using VCFTOOLS 0.1.15 (Danecek et al. 2011) and VCFLIB 1.0.1 (Garrison 2012). We only kept variants

with a minimum quality mapping score of 20, a minimum mean depth of 3, a mean depth of 10, a minor allele count of 3, and a minor allele frequency of 5%. We then filtered for allele balance and mapping quality between the two alleles and removed loci with a coverage that was too high, decomposed complex SNPs into single SNPs, removed indels and kept only biallelic SNPs. We removed individuals with more than 50% missing sites and SNPs with > 5% missing genotypes across the remaining individuals and > 20% missing genotypes in at least one population. We then used RAD HAPLOTYPER 1.1.5 (Willis et al. 2017) using the default settings to remove putative paralogous loci. Among technical replicates in *A. septentrionale* we encountered a high mean allelic error rate (9.3%). We tried to tackle this issue, which may originate in the tetrapolyploidy of the species, with various approaches. Removing the allele balance filter as well as setting ploidy levels to four resulted in higher error rates, rising the mean depth only slightly reduced the error rate. Removing loci that had high error rates among replicates resulted in a set of loci that was not able to distinguish individuals. We yielded best results with an additional Hardy–Weinberg-equilibrium filter in order to remove further putative erroneous variant calls due to potential paralogs (Puritz et al. 2014). This procedure reduced the error rate by about half. Finally, we kept only one randomly chosen SNP per fragment. We ended up with 172 *A. septentrionale* samples genotyped for 404 biallelic SNPs (error rate 4.8% in 11 replicates) and 162 *Hedwigia* samples genotyped for 4926 biallelic SNPs (error rate 0.2% in 12 replicates). As described below, we split the *Hedwigia* samples into presumably independent lineages, for which we created separate SNP datasets using the same methods and criteria as described above.

Genetic analyses

Polyploidy, small population sizes, the possibility of intragametophytic selfing, and the potential occurrence of multiple lineages in *H. ciliata* prohibited genetic analyses based on diploid Mendelian inheritance and large sample sizes. Consequently, we analysed the SNP datasets with more general methods with few assumptions in R (R Core Team 2017). We used the package *vcfR* (Knaus and Grünwald 2017) to import vcf files and conducted most of the subsequent analyses with the package *adegenet* (Jombart 2008). We used the package *tidyverse* for data handling and visualisation (Wickham et al. 2019).

Lineage identification in *Hedwigia*

Due to cryptic species (Buchbender et al. 2014) and polyploidy in *H. ciliata* (Ramsay 2011), the aim of our first analyses of the initial SNP dataset was to identify potentially occurring independent genetic lineages. Therefore, we calculated an UPGMA clustering from a Euclidean distance matrix based on allele presences in the initial SNP dataset (package *cluster*; Maechler et al. 2019). We then classified the resulting well separated clusters (Fig. 3) by their ploidy based on observed heterozygosity within clusters, which is (in gametophytes) expected to be zero in haploid lineages and different from zero in diploid (i.e. polyploid) lineages (Wyatt 1994). Ploidy suggested by heterozygosity patterns within clusters was verified at the level of individual samples with PLOIDYNGS (Dos Santos et al. 2017), which can distinguish between haploid and diploid samples based on the frequency distribution of variants in the mapped reads. Within the cluster containing all diploid samples, the observed heterozygosity per locus showed a bimodal distribution whose maxima corresponded to the fraction of samples contributed by two subclusters. This was due to the large number of loci with fixed heterozygosity in one subcluster and fixed homozygosity in the other, which suggested that two allopolyploid lineages have evolved independently (Wyatt 1994). Finally, we defined lineages by cutting the UPGMA clustering at the height that separated the two diploid subclusters and thereby subdividing the rest of the clustering into the lowest possible number of further lineages. For each lineage we called a separate SNP set (as described above). The two haploid lineages of *H. ciliata* were represented by relatively few samples and populations in the present dataset. Hence, except for the identification of multilocus genotypes, we confined all further analyses to the

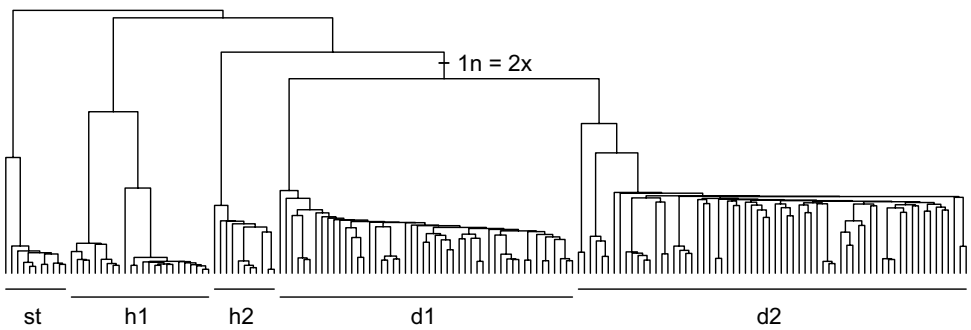


Fig. 3 UPGMA tree based on 4926 SNPs of all 162 *Hedwigia* samples. The cluster containing the polyploid lineages is indicated in the diagram (1n = 2x). Distinguished lineages are indicated below the diagram: the outgroup consists of *H. stellata* (st), *H. ciliata* clusters into two haploid (h1 and h2) and two diploid lineages (d1 and d2)

two diploid lineages (d1 and d2) that were represented by larger sample sizes and more populations (Table 1; Fig. 3).

Multilocus genotype diversity

We used multilocus genotype diversity as a measure of population-level genetic diversity. We assigned samples to multilocus genotypes using the package *polysat* (Clarc and Jasieniuk 2011) based on a simple matching coefficient dissimilarity matrix of allele presence. We visually chose thresholds for distinguishing multilocus genotypes with the help of histograms of dissimilarities, verified the thresholds with the technical replicates, and set the value to 0.085 for *A. septentrionale*, 0.06 for diploid *Hedwigia* lineages and 0.01 for haploid *Hedwigia* lineages. We calculated multilocus genotype diversity within populations with the Simpson diversity index as implemented in the package *vegan* ($1-\sum p_i^2$, where p_i is the proportional abundance of multilocus genotype i in the samples of one population; Oksanen et al. 2019). The chosen Simpson index is indifferent to sample size, but the confidence interval increases with decreasing sample size. Therefore, for *Hedwigia*, we calculated Simpson indices only for populations consisting of four or more samples from the same lineage. We applied one-sided Wilcoxon rank sum tests to determine whether multilocus genotype diversity of *A. septentrionale* and of *H. ciliata* was lower in boulder populations than in mainland populations. For all further analyses we kept only one sample per multilocus genotype per population, to avoid the possibility of inflated similarities within populations masking similarities among populations.

DAPC

We elucidated the general genetic structures present in the SNP datasets via discriminant analysis of principal components (DAPC; Jombart et al. 2010). Similar to STRUCTURE (Pritchard et al. 2000), DAPC assigns individuals to a given numbers of groups (K). However, DAPC does not assume any genetic model, and is therefore applicable to polyploid datasets. DAPC transforms the genetic data into principal components (PCs) and then assigns the samples to K groups by optimising the variance between groups while minimising the variance within groups. We ran DAPC with $K = 2$ to $K = 10$. For each K value, we kept the number of PCs suggested by α-score optimisation to avoid overfitting. And we kept all discriminant functions because the analyses were not limited by computing power. We visualised posterior membership probabilities for groups suggested by DAPC and considered the Bayesian information criterion (BIC) for choosing valid values for K (Jombart and Collins 2015).

Analysis of molecular variance

Complementary to DAPC, which we used to detect any genetic structure present in the datasets, we used analysis of molecular variance models (AMOVA; Excoffier et al. 1992) to specifically explore hierarchical population structures that might arise if boulder populations formed a distinct genetic group. We conducted the calculations based on Euclidean genetic distances with the package *poppr* (Kamvar et al. 2014, 2015). Samples were nested within populations and populations were nested either in boulder sites or in mainland sites. We assessed the significance of the contribution of each stratum to total variance by randomisation tests with 100,000 permutations.

Isolation by distance

Colonisation of boulders by nearby boulder populations would result in an isolation by distance (IBD) pattern (Hutchison and Templeton 1999). Therefore, we compared genetic and geographic distances between populations. We calculated population genetic distances by averaging between individual Euclidean genetic distances. We tested correlations between geographic and genetic distances among populations using overall Mantel tests and with Mantel correlograms implemented in the package *vegan* (Oksanen et al. 2019).

Results

Characterisation of SNP datasets

In spite of the large genome of *A. septentrionale*, its ddRAD catalogue was only 0.39 times the size of the *Hedwigia* catalogue. This may reflect possible high redundancy in large, but still poorly understood fern genomes (Nakazato et al. 2006; Szövényi 2021). It may also have led to the high error rates in this species, which we addressed with rigid filtering resulting in a relatively small number of 404 SNPs. The slightly higher observed than expected heterozygosity (Table 2) was in congruence with the autotetraploidy of *A. septentrionale.*

The UPGMA tree of the 4926 initial *Hedwigia* SNP loci showed five clearly separated clusters, which were defined as separate lineages (Fig. 3). The first cluster contained the samples of the outgroup *H. stellata* and two samples that were collected as *H. ciliata* but were clearly *H. stellata*, as verified in a subsequent morphological identification based on microscopical features (Hedenäs 1994). The *H. stellata* cluster and the two neighbouring clusters were identified as haploid due to a low within-cluster observed heterozygosity of 0.002, while the two other clusters of the UPGMA tree

Table 2 Overview of the analysed taxa and SNP datasets for *Asplenium septentrionale* and *Hedwigia*

	A. septentrionale	*Hedwigia* all samples	*H. ciliata* lineage d1	*H. ciliata* lineage d2	*H. ciliata* lineage h1	*H. ciliata* lineage h2	*H. stellata*
N	172 (11)	162 (12)	50 (2)	66 (7)	24 (3)	11 (0)	11 (0)
N loci	404	4926	4926 / 5030	4926 / 3680	4926 / 3075	4926 / 2831	4926 / 1018
Hobs	0.24	0.41	0.58 / 0.29	0.56 / 0.29	0.002 / 0.008	0.002 / 0.02	0.002 / 0.05
Hexp	0.21	0.37	0.30 / 0.32	0.30 / 0.34	0.08 / 0.45	0.02 / 0.34	0.02 / 0.42
Het fixed	0	0	0.41 / 0.16	0.25 / 0.10	0 / 0.001	0.0004 / 0.003	0.0002 / 0.003
Hom fixed	0	0.04	0.35 / 0.49	0.28 / 0.44	0.98 / 0.96	0.99 / 0.95	0.99 / 0.89
N pops	19	35	14	21	8	4	5
N MLG	78	–	35	45	14	7	5
Ploidy	Tetraploid, autopolyploid	Diploid, haploid	Diploid, allopolyploid	Diploid, allopolyploid	Haploid	Haploid	Haploid

Numbers after slashes refer to separate SNP datasets for the different *Hedwigia* lineages. For each taxon (column) the following information is given: number of samples analysed (N; number of technical replicates in brackets), number of loci in the SNP dataset (N loci), observed heterozygosity (Hobs), expected heterozygosity (Hexp), fraction of loci heterozygous in all samples (Het fixed; fixed heterozygosity), fraction of loci homozygous in all samples (Hom fixed; fixed homozygosity), number of populations that contained the taxon (N pops), number of different multilocus genotypes detected (N MLG), and ploidy of the given taxon (ploidy). The ploidy of *A. septentrionale* is based on Reichstein (1984), and the ploidies of *Hedwigia* lineages were deduced in the present study

were diploid, based on their high observed heterozygosity of > 0.5 (Table 2, Fig. 3). The suggested ploidies were confirmed in the individual samples with PLOIDYNGS (Supplementary Fig. S1). The two diploid clusters were identified as allopolyploid, because of a high frequency of fixed heterozygous loci (0.41%; 0.25%, Table 2). The separate SNP sets for the five lineages contained between 1018 and 5030 SNPs (Table 2, Fig. 3). Compared with the initial SNP set, heterozygosity of the separate SNP sets changed markedly. In the haploid lineages, observed heterozygosity stayed low (< 0.03) but expected heterozygosity increased from < 0.09 to > 0.34, which reflects an increase of variable loci whose alleles only occur in a homozygous configuration, as expected in a haploid organism. In the diploid lineages, a decrease of fixed heterozygous loci (from > 0.25 to < 0.16) occurred, along with an increase of fixed homozygous loci (from < 0.35 to > 0.44).

All four lineages of *H. ciliata* occurred on boulders and in mainland populations (Table 1). In 13 of 19 boulder populations only one *H. ciliata* lineage was detected, and on 6 boulders 2 lineages were found. In 6 of the 13 mainland populations only one lineage was detected, in 6 mainland populations 2 lineages were found, and in one mainland population 3 lineages were detected.

Multilocus genotype diversity

In the 173 samples of *A. septentrionale* 78 multilocus genotypes were identified, and in the 162 *H. ciliata* samples a total of 106 multilocus genotypes were detected (Table 2). Individuals that shared the same multilocus genotype always originated from the same population, except for *H. ciliata* lineage d1, where populations Ae and Vc and populations Va and Vb shared one multilocus genotype each. For *A. septentrionale* and *H. ciliata*, Simpson indices of multilocus genotype diversity of boulder populations were significantly lower than in mainland populations (Fig. 4).

DAPC

DAPC of *A. septentrionale* revealed a distinct spatial population structure, and BIC-values suggested between two and six genetic clusters (K; Fig. 5). At K = 2, the southern Alpine population was separated from all other populations. With K = 3 an additional cluster appeared north of the Alps, in two boulder populations, a Black Forest population and a Vosges

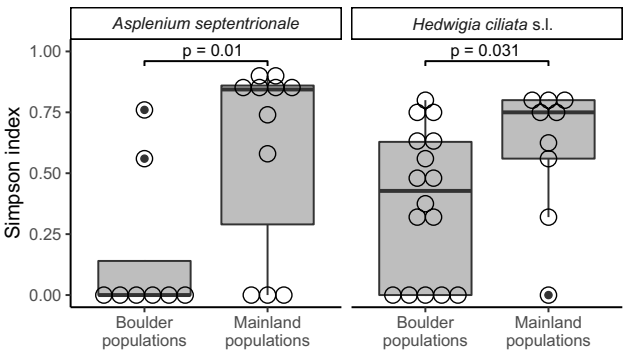


Fig. 4 Multilocus genotype diversity of *Asplenium septentrionale* and *Hedwigia ciliata* in boulder and mainland populations. Above the boxplots of Simpson diversity indices, p-values from one-sided Wilcoxon rank sum tests are given. Data points are given as circles. Populations with a Simpson index of 0 consist of only one multilocus genotype each

population. K = 4 further separated eastern Alpine and western Alpine populations, and the corresponding cluster was also represented on boulders. K = 5 additionally separated the boulder population with the most multilocus genotypes. K = 6 separated the second boulder population with multiple multilocus genotypes. More than six clusters were not supported by BIC values and additional clusters did not contribute to population structure (Fig. 5).

DAPC of *H. ciliata* lineages d1 and d2 did not reveal any spatial genetic structure across populations, neither in BIC values, which continuously increased with increasing K, nor in the posterior assignment probabilities for clusters, where the only pattern detected was a slight tendency of samples from the same population to be assigned to the same cluster (Supplementary Fig. S2).

AMOVA

In the AMOVA, the distinction between boulder and mainland populations did not explain a significant proportion of genetic variance present in *A. septentrionale* or in lineages d1 and d2 of *H. ciliata* (Table 3). Among-population genetic variance was highest in *A. septentrionale* (11.2%, P = 0.001), followed by *H. ciliata* lineage d2 (5.3%, p = 0.002) and lineage d1 (2.6%, p = 0.134). Within-population variance (i.e. variance among samples within populations) explained most of the variance in all three datasets: 88% (p = 0.001) in *A. septentrionale*, 95% (p = 0.001) in *H. ciliata* lineage d2, and 97% (p = 0.058) in *H. ciliata* lineage d1.

Isolation by distance

No correlation between average pairwise Euclidean genetic distance and geographic distance among populations was found in either *A. septentrionale* or *H. ciliata* (Fig. 6). The corresponding Mantel tests were not significant (*A. septentrionale*: R = 0.003, p = 0.48; *H. ciliata* d1: R = -0.24, p = 0.95; *H. ciliata* d2: R = -0.095, p = 0.62) and the Mantel correlograms did not indicate any distance classes with significant correlations between genetic and geographic distance (Supplementary Fig. S3). As expected, averaged pairwise Euclidean genetic distances within populations were smaller than among populations (Fig. 6). In *H. ciliata* lineage d2, a group of conspicuously high distance values in Fig. 6 was caused by two genetically divergent populations (Ab and Bi), which also showed signs of divergence in the UPGMA tree (i.e. clustering at the base of the d2 cluster; Fig. 3).

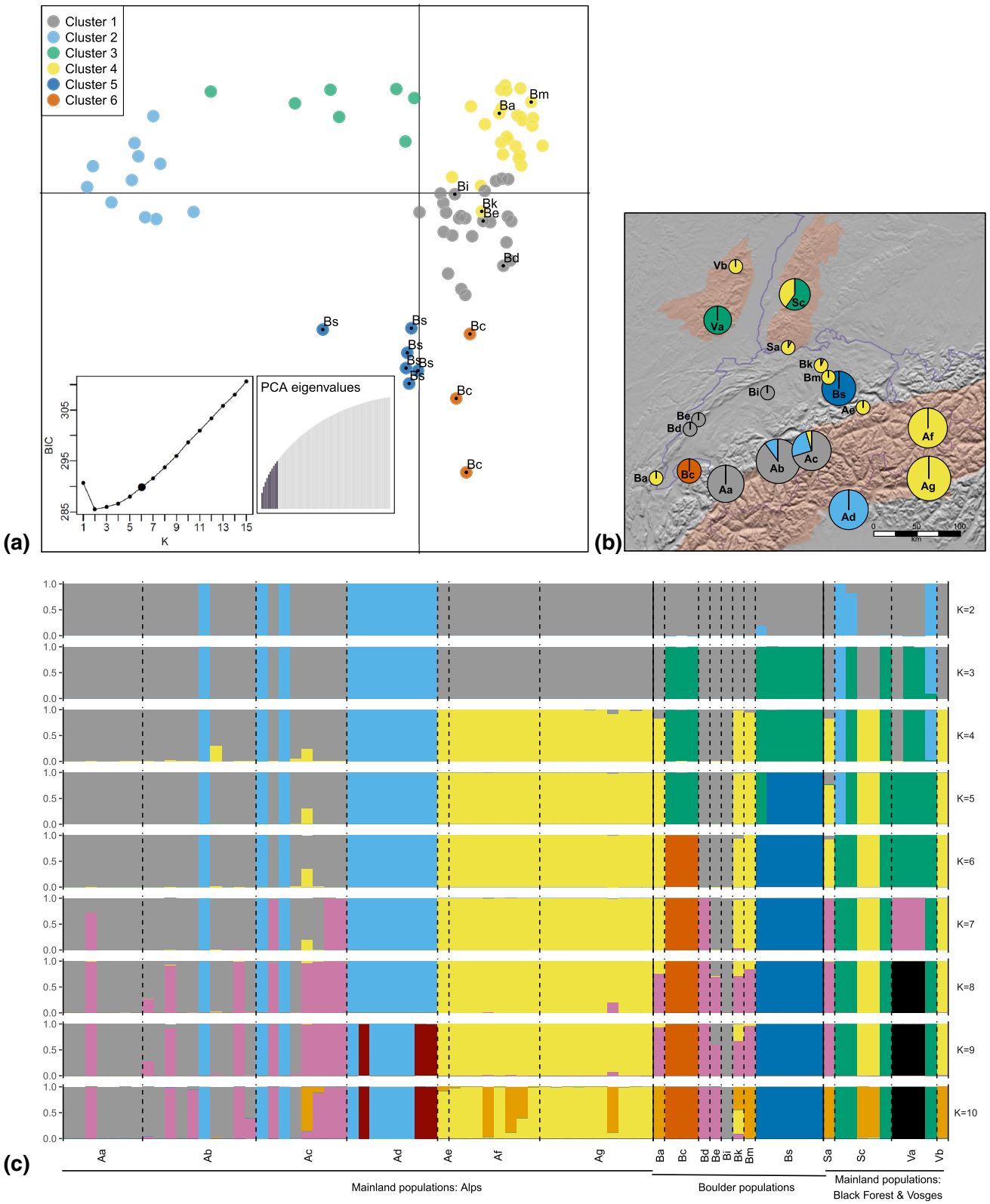
Discussion

On insular siliceous erratic boulders and in adjacent mainland (siliceous mountains) we investigated the population genomics of the fern *A. septentrionale* and the moss *H. ciliata*. In both taxa, multilocus genotype diversity in boulder populations was lower than in mainland populations. For *A. septentrionale* most boulder populations consisted of a single multilocus each. In contrast, the diversity of *H. ciliata* was higher, with four lineages that frequently co-occurred on boulders and less than one third of boulder populations consisted of a single multilocus genotype. In both taxa, no common genetic groups among boulder populations were found, but mainland populations of *A. septentrionale* showed a geographic population structure while in *H. ciliata*, no spatial genetic structure was present.

Spatial genomic patterns in *Asplenium septentrionale*

Abundant shared multilocus genotypes were the most prominent genetic signal detected in the genomic dataset of the fern *A. septentrionale*. We found shared multilocus genotypes among individuals of 17 out of the 19 populations analysed, and 12 populations only showed a single multilocus genotype each (Table 1). Such populations, consisting of few or only one multilocus genotype, have also been documented in isozyme studies of *A. septentrionale* (Holderegger and Schneller 1994) and other tetraploid rock-dwelling *Asplenium* taxa, such as *A. ruta-muraria* (Schneller and Holderegger 1996), *A. csikii* (Vogel et al. 1999) and *A. trichomanes* subsp. *quadrivalens* (Suter et al. 2000). In accordance with the above studies, we conclude that shared multilocus genotypes within populations indicate recurrent intragametophytic selfing and that populations consisting of a single multilocus genotype were most likely founded by a single spore.

The extent of shared genetic structure among the mainland populations of *A. septentrionale* (Fig. 5) was comparable to the genetic structure found in other rock-dwelling *Asplenium* species, such as *A. fontanum*, which shows three genetic clusters along the western Mediterranean coast (Hunt et al. 2009) and *A. ceterach,* which consists of several distinct genetic groups associated with Pleistocene refugia across Europe (Trewick et al. 2002). In fact, the three genetic clusters found in the Alpine populations of *A. septentrionale* (Fig. 5) correspond well to known perialpine Pleistocene refugia (Schönswetter et al. 2005): the three western populations in the Valais (Aa, Ab and Ac) correspond to the southwestern Alpine peripheral



refugium between Nice and the Aoste valley, which was the main source for postglacial colonisation of the Valais (Parisod 2008), and the southern Alpine population (Ad) lies in the refugium of the southern Alps in Ticino, which has been shown to be distinct from more eastern refugia, which correspond to the three easternmost Alpine

◂**Fig. 5** DAPC results for 79 *Asplenium septentrionale* multilocus genotypes. **a** DAPC scatterplot showing the group assignment to six genetic clusters (different colours), with samples from boulder populations labelled. Insets: (left) Bayesian Information Criterion (BIC) as a function of the number of clusters K (enlarged symbol for K = 6); (right) PCA eigenvalues with retained principal components in black. **b** Population pie charts (area proportional to number of multilocus genotypes; Bk and Bs are slightly displaced for better visibility) of posterior assignment probabilities for six clusters. **c** Bar plots of posterior assignment probabilities for an increasing number of clusters (K = 2 to K = 10). Each vertical bar represents one multilocus genotype, and the colours indicate assignment probabilities for the clusters. Vertical dashed lines separate individual populations and solid lines separate boulder populations from mainland populations. Population codes as in Fig. 1 and Table 1

populations of *A. septentrionale* (Ae, Af, Ag; Tribsch and Schönswetter 2003). The presence of a genetic cluster associated with the Black Forest and the Vosges can also be explained by a putative refugial function of these two siliceous low mountain ranges that were partially ice free during the last glacial maximum (Ehlers and Gibbard 2004). Among the eight boulder populations of *A. septentrionale*, DAPC did not detect a common genetic cluster. On the contrary, except for the distinct southern Alpine genetic cluster, all genetic clusters identified for K = 2–6 also appeared in boulder populations (Fig. 5). For the two boulder populations with more than one multilocus genotype (Bc and Bs), multiple colonisation events from genetically distant source populations and subsequent intergametophytic crossing (Klekowski and Lloyd 1968) on boulders may have given rise to the additional genetic clusters which appear with K = 5 and K = 6 (Fig. 5). In accordance, AMOVA did not reveal a significant differentiation of boulder populations from mainland populations (Table 3). Concerning the origin of boulder populations, three of five western erratic boulders (Bd, Be, Bi) clustered with the western Alpine mainland populations and two of three eastern erratic boulders (Bk, Bn) clustered with the eastern Alpine mainland populations. This pattern could reflect spore transport, from the mainland populations in the Alps to the island populations on erratic boulders (Holderegger and Schneller 1994).

For pairwise genetic distances among populations we found no correlations with pairwise geographic distances, neither visually (Fig. 6) nor in Mantel tests or correlograms. In other words, no IBD pattern was detected in *A. septentrionale*. While in animals and seed plants, IBD patterns occur across hundreds or thousands of kilometres (Sharbel et al. 2000; Monsen and Blouin 2004), the few studies on IBD in rock-dwelling ferns failed to detect significant IBD at scales of more than 50 km. Across distances of 20 to 800 km, Luo et al. (2018) found no IBD in *Polystichum glaciale*, while Kang et al. (2008) found significant IBD in *Adiantum reniforme* across distances of 0.8 to 21 km, and Hunt et al. (2009) found IBD in *Asplenium fontanum* in distance classes up to 50 km but not for larger distances up to 1000 km. Our study comprised distances of 2.7 to 303 km, with comparisons among boulder populations involved at all distances of less than 41 km; hence, a lack of IBD indicates that the boulder populations are not connected.

Because DAPC and AMOVA did not find a shared gene pool among boulder populations and because of the lack of an IBD signal, we conclude that colonisation of erratic boulders by *A. septentrionale* is the result of independent long-distance dispersal events. Notably, such repeated wind-mediated long-distance dispersal is a common mechanism in ferns and largely contributed to the fern floras of oceanic islands (Tryon 1970; Geiger et al. 2007). In the DAPC analyses, eastern boulders tended to cluster with eastern Alpine populations and western boulders with western Alpine populations. We therefore hypothesise that the erratic boulders have been colonised from Alpine mainland populations, probably facilitated by intensive, Alpine down-slope winds (i.e. foehn; Brinkmann 1971).

Spatial genomic patterns in *Hedwigia ciliata*

Across all samples, the *Hedwigia* dataset was structured by a phylogenetic signal (Fig. 3). We found haploid and diploid samples, a result in agreement with the two different chromosome numbers published for *H. ciliata* (Ramsay 2011). Based on high levels of fixed heterozygosity, we identified diploid samples as being allopolyploid, which is the prevalent mode of polyploidy in bryophytes (Såstad 2005). Allopolyploidy also explains the results of Buchbender et al. (2014), who assigned a putative hybrid origin to 3 out of 13 analysed *H. ciliata* samples based on incongruent information in nuclear and organellar sequences.

Similar to *A. septentrionale*, shared multilocus genotypes among individuals were frequent in *H. ciliata*: we found shared multilocus genotypes among individuals of the same lineage in 22 of 37 populations that were sampled with more than one sample per lineage (Table 1). Shared multilocus genotypes are commonly found in mosses and can be explained by the fragmentation of continuously growing ramets (Baughman et al. 2017; Grundmann et al. 2007; Szövényi et al. 2012). Hence, it is not clear whether shared multilocus genotypes in *Hedwigia* are caused by recurrent intragametophytic selfing or by vegetative reproduction. Either way, the fact that 13 of 19 boulder populations showed multiple multilocus genotypes in at least one *H. ciliata* lineage suggest that these 13 boulder populations were founded by multiple spores of the same lineage. In *H. ciliata* lineage d1, we found two multilocus genotypes that were shared by geographically distant populations (Ae and Vc, Va and Vb; Fig. 2). We thoroughly checked our protocols and found no indication that these genotypes were erroneous. In

Table 3 AMOVA results for *Asplenium septentrionale* and two lineages (d1 and d2) of *Hedwigia ciliata*

Taxon	Source	DF	%	Phi	P-value
Asplenium septentrionale	Among boulder and mainland populations	1	1.01	0.010	0.134
	Among populations	17	11.24	0.114	0.001
	Within populations	59	87.75	0.122	0.001
Hedwigia ciliata lineage d1	Among boulder and mainland populations	1	0.37	0.004	0.228
	Among populations	12	2.56	0.026	0.134
	Within populations	21	97.07	0.029	0.058
Hedwigia ciliata lineage d2	Among boulder and mainland populations	1	-0.12	-0.001	0.482
	Among populations	19	5.33	0.053	0.002
	Within populations	24	94.79	0.052	0.001

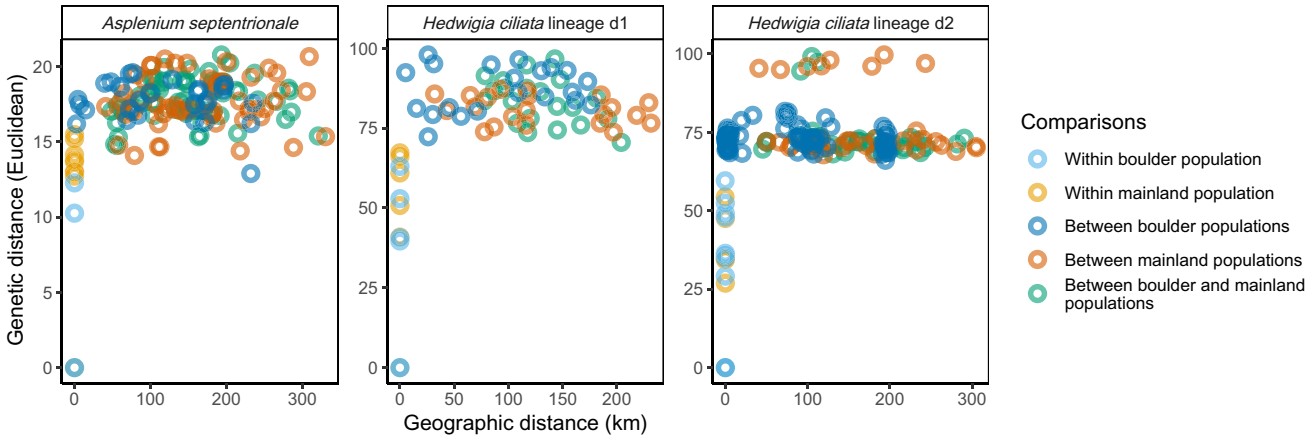


Fig. 6 Geographic distances vs. genetic distances (mean pairwise individual Euclidean distances) for *Asplenium septentrionale* and for *Hedwigia ciliata* lineages d1 and d2

fact, there are reports of shared multilocus genotypes across large distances in mosses (Clarke et al. 2009; Karlin et al. 2011).

The absence of a shared genetic structure among the populations in the two *H. ciliata* lineages that were studied with DAPC fits with the results of Vanderpoorten et al. (2008) for the moss *Grimmia montana,* whose ecology is similar to that of *H. ciliata*. On a worldwide scale, these authors reported no genetic structure within continents but found a transoceanic disjunction, which is a typical phylogeographic pattern in bryophytes (Patiño and Vanderpoorten 2018). Accordingly, AMOVA did not reveal differentiation among boulder and mainland populations of *H. ciliata* in our study.

Among *H. ciliata* populations, Euclidean genetic distances did not correlate with geographic distances (Fig. 6). Isolation by distance patterns have been studied repeatedly for other bryophytes, and in a metanalysis across 28 species Vanderpoorten et al. (2019) found most IBD signals at a range of less than 0.1 km (91% of tests being significant), but no IBD signals at 0.1 to 1 km. For distance classes greater than 1 km, they found that between 30 and 54% of tests were significant. Our study comprised distances of 0.28 to 303 km, with comparisons among boulder populations involved at all distances of less than 32 km; hence, a lack of IBD indicates that the boulder populations are not connected.

The lack of genetic structure of *H. ciliata* in DAPC, of shared variance among boulder populations in AMOVA and of an IBD signal leads us to conclude that colonisation of erratic boulders by *H. ciliata* is the result of independent long-distance dispersal events from diverse source populations of unknown origin.

Island biogeography of *Asplenium septentrionale* and *Hedwigia ciliata*

The theory of island biogeography predicts a higher species richness in larger islands or areas (MacArthur and Wilson 1963). In accordance, studies with large sample sizes (> 150 boulders) that analysed species richness on erratic boulders confirmed a positive species–area relationship for erratic boulders (Weibull and Rydin 2005; Virtanen and Oksanen 2007, Hepenstrick et al. 2021). In the context of our genomic study, this diversity–area relationship was mirrored in a

lower multilocus genotype diversity in boulder populations than in mainland populations (Fig. 4). Also, the mean number of *H. ciliata* lineages was lower in boulder populations than in mainland populations (1.3 vs. 1.6 lineages per population). The immigration rate on boulders appears to be lower for *A. septentrionale* than for *H. ciliata*, as incidences of multiple colonisation events of boulders were rare for *A. septentrionale* (2 out of 8 boulders) but frequent in *H. ciliata* (13 out of 19 boulders). Alternatively, this difference could also be due to a higher extinction rate for *A. septentrionale*. However, persistence of boulder populations of *A. septentrionale* over more than a century (documented in herbaria for seven of the eight boulder populations of this study; Mazenauer et al. 2014) and potentially much longer (Heer 1865) suggests a low immigration rate combined with a low extinction rate for *A. septentrionale* on erratic boulders.

Successful single-spore colonisations of boulders are also in line with Baker's law (Baker 1955), which predicts higher colonisation success for self-compatible than for outcrossing species, because a single propagule suffices to establish a population in selfing species, whereas in outcrossers two compatible individuals must colonise in temporal and spatial vicinity. The absence of inbreeding depression in *A. septentrionale*, as documented for an isolated Estonian population by Rünk et al. (2016), further favours its persistence on boulders. In fact, it is probably the autopolyploidy of *A. septentrionale* that promotes its highly selfing breeding system: Masuyama and Watano (1990) found that autopolyploid lineages of homosporous ferns have overcome signs of inbreeding depression, which are present in diploids, most likely due to deleterious alleles being masked by the extra genome present in polyploids (Soltis and Soltis 2000). Lower resilience to inbreeding in diploids also might explain why the calcifuge diploid *A. trichomanes* subsp. *trichomanes* is missing on erratic boulders in Switzerland, although it frequently co-occurs with *A. septentrionale* in mainland populations.

The island biogeography of *A. septentrionale* and *H. ciliata* may also be circumscribed by dispersal kernels, which describe the probability of successful dispersal at different distances (Nathan 2006). Sundberg (2005) reviewed physical rules and experimental evidence for spore dispersal, and confirmed for *Sphagnum* mosses that the dispersal kernel of spores fits well to an inverse power function $D = a \times r^{-b}$, where D is the number of spores deposited per unit area at radius r from the centre of the spore source, a is the spore's density at the distance of one unit of measurement of r from the spore source, and b is the rate of decline with distance from the spore source. Accordingly, the number of spores landing on an erratic boulder of a given size is the sum of the contribution of all possible spore sources, whose relative contributions depend on their spore production (influencing a), their distance (r) and the dispersion capability of a given type of spore (influencing the rate of decline b). For both taxa studied here, we found that the sampled boulders were not connected. Hence, the contribution of spores from other boulder populations must be negligible despite the comparatively short distances ($r_{boulders} < r_{mainland}$), which we explain with the small population sizes of *A. septentrionale* and *H. ciliata* on boulders producing few spores compared with large mainland populations in mountain ranges ($a_{boulders} << a_{mainland}$). Furthermore, the dispersal kernels of *A. septentrionale* and *H. ciliata* must nevertheless be sufficiently "fat tailed" (small b) such that long-distance dispersal occurs, which is given by effective wind dispersal of spores. The lower immigration rate of *A. septentrionale* may be due to smaller overall mainland spore production ($a_{Asplenium} < a_{Hedwigia}$) because of its patchy distribution (Reichstein 1984), while *H. ciliata* is very common on siliceous rocks, where it covers large surfaces and produces large quantities of spores (Nebel and Philippi 2000). Further, the higher habitat requirements of *A. septentrionale* may lead to fewer successful colonisation events, as successful colonisation requires that a spore lands in a rock crevice, then grows into a gametophyte, and—after a water-dependent fertilisation event—gives rise to the long-lived sporophyte. *Hedwigia ciliata* spores, in contrast, germinate on bare rock and directly give rise to long-lived gametophytes. Finally, the bigger spores of *A. septentrionale* (40–50 µm; Sorsa 1964) are expected to have a somewhat lower dispersal capacity (Norros et al. 2014) than the smaller spores of *H. ciliata* (20–35 µm; Ignatova et al. 2016; $b_{Asplenium} > b_{Hedwigia}$). The lower dispersal capacity of *A. septentrionale* may also explain the presence of a mainland genetic structure in *A. septentrionale* that has arisen by prevalent shorter distance dispersal within the continuous rock habitats in the mountain mainland, while the high dispersal capacity of *H. ciliata* may have prevented the formation of spatial genetic structure among mainland populations.

Conclusions and recommendation for conservations

Our findings suggest that the populations of *A. septentrionale* and *H. ciliata* on siliceous erratic boulders in the Swiss lowlands represent island populations that are not connected with each other and originate from independent long-distance dispersal events, probably from adjacent mountain ranges for *A. septentrionale* and from diverse, unknown and potentially even more distant sources for *H. ciliata*. In fact, a lack of population connectivity, low genetic diversity and high inbreeding do not seem to threaten the critically endangered boulder populations of autotetraploid *A. septentrionale*. In *H. ciliata* we found four presumably independent lineages, which underline the biodiversity contributed by cryptogam communities

exclusively occurring on erratic boulders in the Swiss lowlands (Meylan 1912), but also signal the need for further taxonomic revision of the genus *Hedwigia* (Buchbender et al. 2014; Ignatova et al. 2016). Successful colonisation of erratic boulders by *A. septentrionale* seems to be rare, but established populations persist for long periods if they are not destroyed by human activities, such as the destruction of erratic boulders, changes in their environment and the removal of plants by boulderers to clean climbing routes (Mazenauer et al. 2014). More frequent colonisation was inferred for *H. ciliata*, and we presume that this also holds true for the approximately 20 additional bryophyte species that are specific to siliceous erratic boulders (Meylan 1912).

Conservation measures for boulder populations of *A. septentrionale* should primarily focus on in-situ preservation of existing populations by preventing their destruction by humans, and maintenance of appropriate light conditions for this light-demanding species (e.g. careful removal of trees around boulders within forests; Hepenstrick et al. 2016). Colonisation of new boulders sourcing from boulder populations may only be realistic in close proximity (up to ca. 100 m distance; Vanderpoorten et al. 2019). Re-establishing the habitat quality of boulders where *A. septentrionale* went recently extinct and erratic boulders in general seems worthwhile, although spontaneous recolonisation of *A. septentrionale* is unlikely. However, such conservation measures may well promote the re-establishment of typical light-demanding bryophyte and lichen communities of erratic boulders (Meylan 1912, 1926a, 1926b; Epard et al. 2020). We do not recommend (re)introducing *A. septentrionale* to isolated sites (Mazenauer 2014; Rünk 2016), because only naturally arisen populations genuinely showcase the impressive long-distance dispersal capabilities of spore dispersed species, for which the isolated occurrences of *A. septentrionale* on erratic boulders are a classic example (Brockmann-Jerosch and Brockmann-Jerosch 1926). Finally, our study exemplifies that common paradigms of conservation biology, such as connectivity and high genetic diversity, do not apply to the conservation of the polyploid rock-dwelling species studied here.

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Author contributions The study was designed by DH, RH and AW. Sampling, material preparation and data analyses were conducted by DH. NZ performed the bioinformatic data processing. The first draft of the manuscript was written by DH and all authors commented on the various versions of the manuscript. All authors read and approved the submitted manuscript.

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Availability of data and material Raw data has been deposited at ENA under accession number PRJEB42827. Voucher specimens and extracted DNA can be requested from the corresponding author.

Code availability All analyses were conducted in the free software environment R (R Core Team 2017). The R packages used for the analyses are cited in the text.

Declarations

Conflict of interest The project was financially supported by several foundations, associations, and governmental and non-governmental organisations. None of them influenced the work described in this article in any way. The authors declare no conflict of interest.

Consent for publication The authors are willing to give their consent for Springer to publish the current study.

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