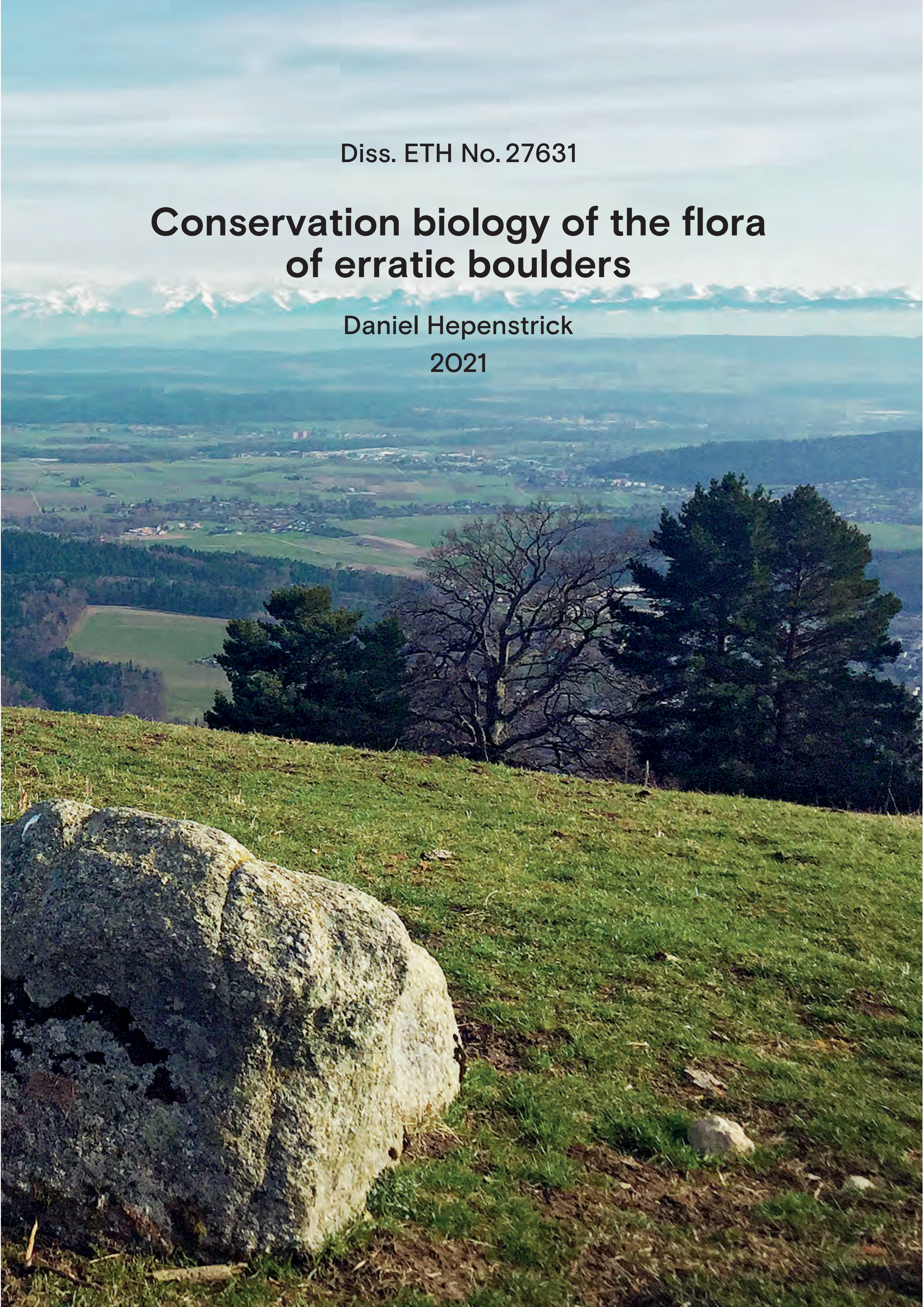


Diss. ETH No. 27631

Conservation biology of the flora of erratic boulders

Daniel Hepenstrick

2021

Diss. ETH No. 27631

CONSERVATION BIOLOGY OF THE FLORA OF ERRATIC BOULDERS

A dissertation submitted to
ETH ZÜRICH

for the degree of
Doctor of Sciences

presented by
DANIEL HEPENSTRICK
MSc Biology, ETH Zürich
born November 17, 1983
citizen of Zürich (ZH)

accepted on the recommendation of
Prof. Dr Rolf Holderegger (examiner)
Prof. Dr Alex Widmer (co-examiner)
Dr Ariel Bergamini (co-examiner)
Prof. Dr Gregor Kozlowski (co-examiner)

2021

“Alles is overal: maar het milieu selecteert.”

Baas Becking (1934, p. 15)

Table of contents

Summary

Pleistocene erratic boulders are rocks that have been relocated, often across hundreds of kilometres, by glaciers during the Ice Ages. When they differ in their geology from the surrounding landscape (e.g. calcareous vs. non-calcareous), erratic boulders form habitat islands for regionally rare rock-dwelling cryptogams (bryophytes, ferns and lichens), which are specialists for the specific rock composition of erratic boulders. In this way, erratic boulders contribute to the biodiversity at the landscape level. The insular bryophyte communities on erratic boulders played an important role in the historical discourse that led to the present-day understanding of the glacial history of species and vegetation. Further, erratic boulders can serve as terrestrial model systems for island biogeography. In many places, however, the cryptogam communities of erratic boulders are threatened because boulders have been destroyed, as a result of land-use changes around boulders, the use of large boulders for sport climbing, and a lack of knowledge on the part of stakeholders regarding the special biodiversity on erratic boulders. Because the conservation biology of erratic boulders had not yet been considered in a comprehensive way, with this thesis I aimed to create an evidence-based foundation for the conservation of insular cryptogam communities on erratic boulders.

In Chapter I, I investigated the factors determining the conservation value of bryophyte communities on insular erratic boulders in Switzerland. I analysed the species richness and the environmental conditions of 160 siliceous erratic boulders in the calcareous Swiss Plateau and Jura Mountains. On these boulders, I identified 138 bryophyte species, 19 of which were specialists of siliceous erratic boulders. The boulder specialists showed a steeper species–area curve than the total species richness, which was in agreement with island biogeographical expectations. Large boulders were more likely to harbour numerous boulder specialists, but at the landscape level the small boulders together contributed more specialist species than large boulders did. Analyses of environmental variables revealed that erratic boulders near buildings were less likely to harbour boulder specialists and that communities in open land host different and more specialist species than boulders within forests.

In Chapter II, I addressed the question of whether populations on erratic boulders are genetically connected. I analysed the regionally critically endangered fern *Asplenium septentrionale* and the moss *Hedwigia ciliata* using double digest restriction associated DNA sequencing (ddRAD). In *A. septentrionale*, frequent identical multilocus genotypes within populations suggested prevalent intragametophytic selfing in this species, and six

out of eight boulder populations consisted of a single multilocus genotype each. This indicated single spore founder events of *A. septentrionale* populations on erratic boulders. In *H. ciliata*, I identified four different genetic lineages, and found that populations consisting of a single multilocus genotype were less common than in *A. septentrionale*. In both taxa, multilocus genotype diversity on boulders was lower than in populations sampled in their “mainland” in siliceous mountains (Alps, Black Forest, Vosges). The absence of a common genetic cluster for boulder populations and the absence of isolation by distance patterns suggested that, for both *A. septentrionale* and *H. ciliata*, populations on erratic boulders are not connected and were colonised through independent long-distance dispersal events out of the species distribution areas. Hence, the survival of a boulder specialist population does not seem to depend on its connectivity to populations on other boulders.

Erratic boulders are increasingly used by rock climbers for bouldering (ropeless climbing at low height), which poses a threat to their special flora. The impact of climbers has mainly been attributed to mechanical disturbances by climbers. However, the chemical impact of widely used climbing chalk powder (magnesium hydroxide carbonate) had never been assessed. Therefore, in Chapter III, I explored the potential effects of climbing chalk by determining its distribution along bouldering routes and assessing its impact on fern and moss species in a climate chamber experiment. The field measurements revealed elevated climbing chalk levels along bouldering routes, even at sampling points without visual traces of climbing chalk. The experiment showed significant negative, though varied, effects of elevated climbing chalk concentrations on the germination and survival of both ferns and mosses. These findings thus suggest that elevated climbing chalk concentrations along climbing routes can occur even where no chalk traces are visible, and that climbing chalk can have negative impacts on rock-dwelling organisms.

Practical conservation of cryptogam communities on insular erratic boulders in Switzerland should prioritise boulders located in the open land outside settlements, because, despite their relative rarity, they contribute many boulder specialist species to the landscape. The flora of both large and small boulders merits protection, but conservation measures for large boulders are logistically more efficient. Conservation measures on individual erratic boulders should have the aims of preventing disturbances on and around boulders and maintaining the light conditions to which the resident specialist species are adapted. In order to raise awareness of the special flora of erratic boulders in the public and amongst conservation practitioners, I complemented this thesis with various outreach activities.

Zusammenfassung

Findlinge sind Felsblöcke, die während den Eiszeiten von Gletschern, oft über hunderte von Kilometern, verfrachtet wurden. Wenn sich das Grundgestein der umgebenden Landschaft vom Gestein der Findlinge unterscheidet (z.B. kalkfrei gegenüber kalkhaltig), dann bilden Findlinge Lebensrauminseln für regional seltene felsbewohnende Kryptogamen (Moose, Farne und Flechten), die auf das Gestein der Findlinge spezialisiert sind. So tragen Findlinge zur Biodiversität in einer Landschaft bei. Die inselartig verbreiteten Moosgesellschaften auf den Findlingen spielten eine wichtige Rolle in der historischen wissenschaftlichen Diskussion, die zum heutigen Verständnis der eiszeitlichen Vegetationsgeschichte führte. Zudem können Findlinge als inselbiogeographisches Modellsystem dienen. Doch vielerorts sind die speziellen Kryptogamengesellschaften der Findlinge bedroht. Dies weil viele Findlinge zerstört wurden, wegen Landnutzungsänderungen in der Umgebung von Findlingen, aufgrund zunehmender Nutzung von grossen Findlingen zum Sportklettern und auch wegen mangelndem Wissen um die Lebensraumfunktion von Findlingen für seltene Arten. Trotzdem ist zur Naturschutzbiologie der Flora von Findlingen bislang kaum etwas bekannt. Das Ziel dieser Dissertation war es deshalb, evidenzbasierte Grundlagen für den Erhalt der speziellen Flora von Findlingen zu schaffen.

Im ersten Kapitel dieser Dissertation untersuchte ich, welche Faktoren den Naturschutzwert der Moosgesellschaften auf silikatischen Findlingen in der Schweiz beeinflussen. Dazu analysierte ich die Moosartenzahl und -zusammensetzung in Abhängigkeit verschiedener Umweltparametern auf 160 silikatischen Findlingen im kalkhaltigen Schweizer Mittelland und im Jura. Dabei fand ich insgesamt 138 Moosarten, von denen 19 auf Silikatfindlinge spezialisierte Arten waren. Diese Findlingsspezialisten zeigten im Vergleich zur Gesamtartenzahl eine steilere Art-Areal-Kurve, was inselbiogeographische Voraussagen bestätigte: je grösser ein Findling war, umso mehr Findlingsspezialisten beherbergte er. Allerdings trugen auf Landschaftsebene die kleinen Findlinge zusammen mehr verschiedene spezialisierte Arten bei als die grossen Blöcke. Die Analyse von Umweltparametern zeigte, dass auf Findlingen in der Nähe von Gebäuden weniger Findlingsspezialisten wachsen und dass Moosgesellschaften von Findlingen im Offenland andere und zahlreichere Findlingsspezialisten beherbergen als Findlinge im Wald.

Im zweiten Kapitel ging ich der Frage nach, ob Populationen auf Findlingen genetisch miteinander vernetzt sind. Dies untersuchte ich für den regional vom Aussterben bedrohten

Farn *Asplenium septentrionale* und für das typische Findlingsmoos *Hedwigia ciliata* mit «double digest restriction associated DNA sequencing» (ddRAD). Innerhalb von Populationen von *A. septentrionale* fand ich häufig Individuen mit identischen Multilocusgenotypen, was zeigt, dass in *A. septentrionale* intragametophytische Selbstbefruchtung häufig vorkommt. Sechs von acht Populationen auf Findlingen enthielten je nur einen einzigen Multilocus-Genotypen, was darauf hinweist, dass diese Populationen je von einer einzelnen Spore gegründet wurden. Innerhalb von *H. ciliata* stellte ich vier separate genetische Linien fest und Populationen, die aus einem einzigen Multilocusgenotypen bestanden, waren weniger häufig als bei *A. septentrionale*. In beiden Taxa war die Diversität der Multilocusgenotypen auf Findlingen geringer als in Vergleichspopulationen aus dem Hauptverbreitungsgebiet der Arten in Silikatgebirgen (Alpen, Schwarzwald, Vogesen). In den genetischen Daten von *A. septentrionale* und *H. ciliata* waren weder gemeinsame genetische Gruppen für die Findlingspopulationen noch ein Zusammenhang zwischen räumlicher und genetischer Distanz festzustellen, was nahelegt, dass die untersuchten Findlingspopulationen nicht miteinander vernetzt sind und dass die Findlinge über weite Distanzen von Sporen aus dem Hauptverbreitungsgebiet der beiden Arten unabhängig besiedelt wurden.

Zunehmend werden Findlinge zum Bouldern (Sportklettern ohne Seil in geringer Höhe) genutzt, was eine Bedrohung für deren spezielle Flora darstellen kann. Der negative Einfluss des Klettersports auf Felsvegetation wurde bisher hauptsächlich mit der mechanischen Störung durch Kletternde erklärt, während mögliche chemische Auswirkungen des im Klettersport viel verwendeten Magnesiapulvers (Magnesiumhydroxidcarbonat) ausser Acht gelassen wurden. Deshalb untersuchte ich im dritten Kapitel, wie Magnesia an Boulderblöcken räumlich verteilt ist und in einem Klimakammerexperiment, wie sich unterschiedliche Magnesiakonzentrationen auf die Keimung und das Überleben von felsbewohnenden Farn- und Moosarten auswirken. Die Messungen an Boulderblöcken zeigten erhöhte Magnesiamengen auch an Stellen, wo keine Magnesiaspuren sichtbar waren. Im Experiment zeigten sich signifikant negative Auswirkungen von erhöhten Magensiakonzentrationen auf die Keimung und das Überleben von verschiedenen Farn- und Moosarten. Diese Ergebnisse zeigen, dass entlang von Kletterrouten erhöhte Magnesiakonzentrationen auch an Stellen vorkommen können, wo kein Magnesiapulver sichtbar ist, und dass Magnesia negative Auswirkungen auf felsbewohnende Organismen haben kann.

Für den Erhalt der speziellen Findlingsflora im Schweizer Mittelland und Jura sollten Findlinge im Offenland ausserhalb von Siedlungen prioritär beachtet werden, weil diese

Findlinge trotz ihrer geringen Anzahl besonders viele seltene Arten beherbergen. Sowohl grosse als auf kleine Findlinge tragen wesentlich zum Artenpool in einer Landschaft bei, aber Massnahmen zugunsten grosser Findlinge sind einfacher umzusetzen. Schutzmassnahmen an einzelnen Findlingen sollten darauf abzielen, Störungen an Findlingen zu vermeiden und die Lichtverhältnisse zu erhalten, an welche die auf den Findlingen vorhandenen schützenswerten Arten angepasst sind. Dabei scheint das Überleben der auf Findlinge spezialisierten Arten nicht von der Vernetzung zu anderen Findlingen abhängig zu sein. Um in der Öffentlichkeit und in der Naturschutzpraxis das Bewusstsein für die besondere Flora von Findlingen zu fördern, habe ich diese Dissertation mit Öffentlichkeitsarbeit begleitet.

Introduction, main results and conclusions

Biology of erratic boulders and science

Erratic boulders are rocks that experienced transportation by glaciers during the Ice Ages. They occur in areas that experienced Pleistocene glaciations, i.e. large parts of Europe and North America and major mountain ranges and their surroundings on all continents except Africa and Australia (Ehlers and Gibbard 2007).

The biological value of erratic boulders lies in their function as habitats for rock-dwelling cryptogam species such as bryophytes, ferns and lichens (Fig. 1). This habitat function is particularly important in landscapes where erratic boulders are the only natural rock habitats present and in areas where the chemical composition of the bedrock contrasts with that of the erratic boulders. In such landscapes, erratic boulders form habitat islands for edaphically specialised species, and numerous floristic studies on erratic boulders have underlined their importance for regional bryophyte and lichen species diversity, e.g. in France (Philippe 2010), Germany (Itzigsohn 1853; Neu 1971; Wächter 1996), the Netherlands (Jansen and Wachter 1928; Masselink and van Zanten 1976; Colpa and van Zanten 2006), Poland (Krawiec 1938), Switzerland (Fischer 1871; Meylan 1912; Hepenstrick et al. 2016) and North America (Cain and Sharp 1938).

Fig. 1 Examples of bryophyte, fern and lichen species whose natural occurrence in calcareous landscapes is restricted to siliceous erratic boulders. (a) *Hedwigia ciliata*, a moss; (b) *Asplenium septentrionale*, a fern; and (c) *Lasallia pustulata*, a lichen.

Scientific interest in insular cryptogam communities on erratic boulders started with the upcoming of the Ice Age theory in the 19th century, which proposes that erratic boulder are evidence of the extent of former Pleistocene glaciers (Agassiz 1840). Inspired by this ground-breaking discovery, many botanists interpreted the rare bryophytes on erratic boulders as evidence of the transportation of plants on rocks by Pleistocene glaciers, because the rare species on erratic boulders grow in abundance in the boulders' Alpine and Scandinavian provenance areas (e.g. Müller 1853; Milde 1870; Hegi 1902). This view, however, received harsh opposition by other botanists (e.g. Walther and Molendo 1868; Amann 1894; Brockmann-Jerosch and Brockmann-Jerosch 1926), who doubted survival of the bryophytes within the Pleistocene ice shields (an idea later coined as "nunatak survival"; Stehlik 2000). Instead, the latter authors suggested post-glacial colonisation of erratic boulders by their special bryophytes and ferns, and interpreted the presence of insular cryptogam communities on erratic boulders as evidence of the long-distance dispersal capabilities of spore plants.

Recently, erratic boulders were used as models for exploring aspects of island biogeography and metapopulation dynamics (MacArthur and Wilson 1967; Hanski and Gilpin 1991), i.e. the relationship between the number of resident species and the area of islands, and the connectivity among islands. In Swedish forests, for example, bryophyte species richness on 216 siliceous erratic boulders was found to be positively related to boulder area and within-boulder habitat diversity (Weibull 2001; Weibull and Rydin 2005). Virtanen and Oksanen (2007) also found a positive link between boulder size and species richness and additionally reported a weak positive effect of connectivity to other boulders in a dataset comprising 288 erratic calcareous boulders in Finnish forests. Kimmerer and Driscoll (2000), however, found that neither boulder size nor connectivity among boulders was related to boulder species richness on 39 granitic erratic boulders in the US state of New York.

Threats to and conservation of Swiss erratic boulders and their flora

Since prehistoric times, erratic boulders have received human attention and have been extensively exploited as building material, e.g. for the construction of Neolithic graves (Masselink and van Zanten 1976) and of houses (Hepenstrick 2014) and as border stones (Lugon 2006). From 1837 onwards, the Swiss scientist Louis Agassiz internationally championed transportation of erratic boulders by glaciers as evidence of the Ice Age theory (Imbrie and Imbrie 1986). Meanwhile, in the Swiss Plateau and Jura Mountains, exploitation of erratic boulders for building material reached almost industrial scales, which triggered scientific and patriotic associations to take action by purchasing and safeguarding erratic boulders as “witnesses” of the Ice Ages and with the aim of conserving them for science (Reynard 2004). Cantons and municipalities were also urged to protect their erratic boulders. Remarkably, the first scientifically motivated protection of a natural monument ever was probably the protection of the erratic boulder “Pierre à Bot” by the canton of Neuchâtel in the year 1838 (Vischer 1946). The patriotic and scientific movement dedicated to the protection of erratic boulders reached high public awareness (Favre and Studer 1867) and set the foundations for nature conservation in Switzerland (Bachmann 1999). The tight historical connection between the conservation of erratic boulders and nature conservation is best exemplified by the work of a group of scientists who originally joined forces to preserve one of the largest Swiss erratic boulders, the “Pierre des Marmettes” in the canton of Valais. Subsequently, these scientists extended their engagement by founding the Swiss society for the protection of nature (today, the NGO Pro Natura) and by initiating the set-up of the Swiss National Park in the canton of Grisons (Bachmann 1999).

Destruction of unprotected Swiss erratic boulders continued well into the 20th century (Lugon et al. 2006), if not for their exploitation as building material, which became unprofitable, then for their removal from agricultural land. Akçar et al. (2011, p. 447) summarised the result of centuries of anthropogenic impact on erratic boulders by stating that most Swiss erratic boulders “are now located either in forests, or along property boundaries, or are of poor stone quality”. Today, erratic boulders are part of cantonal legislation for nature conservation and the large boulders are no longer threatened by destruction. However, there is poor public awareness of the fact that erratic boulders are geosites with geological and historical value (Reynard 2004).

While the geological aspects of Swiss erratic boulders have received conservation attention for many decades, their flora is generally overlooked by conservationists and faces declines and threats. For the critically endangered Swiss populations of the fern *Asplenium septentrionale* on erratic boulders (Bornand 2019), declines in their numbers and population sizes have been documented (Mazenauer et al. 2014). For bryophytes and lichens, the scarcity of occurrence data from erratic boulders hinders estimations of population trends in Switzerland. However, studies from Germany and the Netherlands have indicated declines and regional extinctions of boulder specialist bryophytes (Wächter 1996; Colpa and van Zanten 2006), which may be the case in Switzerland as well. For the Swiss Jura mountains, Meylan (1912) described that siliceous erratic boulders in open land host different specialist species than boulders in forests; hence, light-demanding specialist species may be particularly threatened, because of the rarity of erratic boulders in open land (Akçar et al. 2011). The presumed main threats are changes in habitat properties, e.g. due to overgrowth of boulders by shade-producing trees and anthropogenic disturbances such as the cleaning of erratic boulders (Wächter 1996; Colpa and van Zanten 2006). In recent decades, an additional anthropogenic threat has arisen in the form of rock climbers, who increasingly use large erratic boulders for bouldering (ropeless climbing at low height; Mazenauer et al. 2014; Blum 2015; Antz 2019).

Despite of their significance for regional biodiversity and glacial history and the scientific interest in using erratic boulders as terrestrial model systems in island biogeography, the cryptogam communities on erratic boulders have hardly been considered in scientific conservation biology nor in practical conservation management. In this thesis, I aimed to close this gap.

Goal of this thesis

The main goal of this thesis was to create an evidence-based foundation for the conservation of cryptogam communities on insular erratic boulders. Therefore, I addressed issues that are of practical importance for conservation of the insular cryptogam communities on siliceous erratic boulders in the Swiss Plateau and the Swiss Jura Mountains. Many results of my studies, however, may well apply to the conservation of isolated populations of rock-dwelling cryptogams in general.

Rationale and results of the three main chapters

I identified three issues which are important for the conservation biology of erratic boulders and addressed them in the three main chapters of this thesis. I focused on bryophytes and ferns, not explicitly studying lichens because there are taxonomic problems in species identification of typical species of erratic boulders (Christoph Scheidegger, WSL, pers. comm.) and because collecting crustose lichens with a hammer chisel was not an option for erratic boulders protected as geosites.

Chapter I

For conserving the special bryophyte communities associated with siliceous erratic boulders in the Swiss Plateau and in the Jura Mountains, it is important to know which environmental factors influence the number of boulder specialist species and the community composition present on erratic boulders. This knowledge makes it possible to develop and prioritise conservation measures. Prior to this thesis, little was known about the current boulder specialist species diversity in Switzerland, and previous work mainly consisted of floristic studies on selected large boulders (Vust 2013; Hepenstrick et al. 2016; Epard et al. 2020). However, Meylan (1912) reported that siliceous erratic boulders in the Swiss Jura Mountains harbour different specialist species, depending on their location inside or outside of forests, and Swedish and Finnish studies with an island biogeographical focus (Weibull and Rydin 2005; Virtanen and Oksanen 2007) highlighted that boulder size is an important determinant for species richness. Based on this knowledge, I developed a study with the aim of identifying the environmental factors determining the conservation value of bryophyte communities on erratic boulders. Therefore, I studied a stratified (equal number of forest and non-forest boulders) random sample of 160 siliceous erratic boulders in 8 study areas in the Swiss Plateau and Jura Mountains. I analysed how boulder size and various environmental variables influence bryophyte species richness and community composition. In total, I found 138 bryophyte species, 19 of which were specialists of siliceous erratic boulders. I showed that the rate of change of species richness in response to increasing boulder area was higher for boulder specialist species than for the total number of species on a boulder (i.e. a steeper species–area curve), which is a typical pattern for habitats that function as islands for specialist species (MacArthur and Wilson 1967; Dembicz et al. 2020). Although large boulders were more likely to harbour numerous boulder specialists, I found that the small boulders together contributed more different specialist bryophyte species to the landscape than a few large boulders of the same cumulative surface area. The analysis of the influence of environmental variables revealed that the percent cover of buildings within a 25 m radius around the boulders negatively affected the number of specialist species. An analysis of species composition revealed four different bryophyte communities: a pair of open land communities and a pair of forest communities, with differences within the pairs regarding the presence or absence of boulder specialist species (Table 1).

Table 1 Summary of the characterisation of the four bryophyte communities identified on siliceous erratic boulders in the Swiss Plateau and in the Jura Mountains. The communities (c1 to c4 according to Chapter I; as in Fig. 1) are grouped by their main occurrence, either inside or outside forests, and by whether their floristic composition is characterised by the presence or absence of boulder specialists. For each community, six typical species (highest indicator value for the community; Dufrêne and Legendre 1997) are given (boulder specialists in bold), along with the corresponding syntaxonomical alliance (Mucina et al. 2016) and examples of typical surroundings in which the community was frequently found.

		Main occurrence of community	
		Inside forests	Outside forests
Floristic composition	Boulder specialists present	Community c3 Typical species: ***Grimmia hartmanii*** *Isothecium alopecuroides* *Plagiothecium nemorale* ***Paraleucobryum longifolium*** *Dicranum scoparium* *Polytrichum formosum* Syntaxonomical alliance: *Grimmio hartmanii–Hypnion cupressiformis* Typical surrounding: Closed forest	Community c2 Typical species: ***Hedwigia ciliata*** *Pterigynandrum filiforme* ***Orthotrichum rupestre*** ***Grimmia trichophylla*** *Hypnum cupressiforme* aggr. * ***Grimmia ovalis*** Syntaxonomical alliance: *Grimmion commutatae* Typical surrounding: Agricultural land, pastures
	Boulder specialists lacking	Community c4 Typical species: *Anomodon attenuatus* *Sciuro-Hypnum populeum* *Brachythecium rutabulum* *Metzgeria furcata* *Porella platyphylla* *Anomodon viticulosus* Syntaxonomical alliance: *Neckerion complanatae* Typical surrounding: Young forest, forest edges, hedges	Community c1 Typical species: *Grimmia pulvinata* *Orthotrichum affine* *Orthotrichum diaphanum* *Schistidium apocarpum* aggr. *Orthotrichum anomalum* *Tortula muralis* Syntaxonomical alliance: *Schistidion apocarpi* Typical surrounding: Settlements

* *Hypnum cupressiforme* aggr. is common in all four communities

Fig. 2 Twelve examples of the 160 erratic boulders studied: a small, a medium and a large boulder (columns) for each of the identified bryophyte communities c1 to c4 (rows) according to Chapter I and Table 1. The white meterstick in the photos is 24 cm long. The boulders' bryophyte community (c1 and c2 are open land communities; c3 and c4 are forest communities), location (municipality), canton and number of boulder specialists and total bryophyte species richness are: (a) Sévery, canton of Vaud, community c1, 0 boulder specialists, 12 species in total; (b) Rüttenen, canton of Solothurn, c1, 0 , 5; (c) Biel, canton of Bern, c1, 0, 21; (d) Heimiswil, canton of Bern, c2, 4, 9; (e) Neuchâtel , canton of Neuchâtel, c2, 6 , 15; (f) Aeschi, canton of Solothurn, c2, 8 , 36; (g) Mont-sur-Rolle, canton of Vaud, c3, 1, 6; (h) Neuchâtel, canton of Neuchâtel, c3, 2, 13; (i) Seeberg, canton of Bern, c3, 5, 18; (j) Grandevent, canton of Vaud, c4, 0, 5; (k) Grandevent, canton of Vaud, c4, 0, 8; and (l) Neuchâtel, canton of Neuchâtel, c4, 0 , 21.

Chapter II

Conservation of boulder specialist populations on insular erratic boulders requires knowledge on whether boulder populations: (i) are connected and therefore form specific gene pools, or (ii) were independently colonised by long-distance dispersal out of the species "mainland", which comprises the species main distribution areas in siliceous mountain ranges. In the first case, conservation measures enhancing connectivity among boulders have great importance, whereas in the second case, effective conservation measures on individual boulders are independent from their distance to other boulders. To my knowledge, the only genetic study on a species occurring on erratic boulders was conducted by Holderegger and Schneller (1994), who found isozyme variation among three boulder populations of *A. septentrionale*, of which only one showed within-population variation. However, the above questions on connectivity and colonisation remained unanswered. Therefore, I addressed these questions in a genetic study using double digest restriction associated DNA sequencing (ddRAD; Peterson et al. 2012) in *Asplenium septentrionale* and in the moss *Hedwigia ciliata*, both of which are typical representatives of the boulder specialist flora (Meylan 1912; Weber 1912; Fig. 1). For both species, I analysed the spatial genetic structure of populations sampled on erratic boulders and in the adjacent mainland (i.e. siliceous Alps, Black-Forest and Vosges). In *A. septentrionale*, abundant identical multilocus genotypes within populations suggested prevalent intragametophytic selfing in this species. The genetic structure of its mainland populations coincided with Pleistocene glacial refugia. And most (six out of eight) boulder populations of *A. septentrionale* consisted of a single multilocus genotype, which indicated that these populations were founded by one single spore. In *H. ciliata*, I identified four genetic lineages, and populations consisting of a single multilocus genotype were less common than for *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 that the studied boulder populations were not connected and that they were colonised by independent long-distance dispersal events out of the species main distribution areas.

Chapter III

In order to mitigate the threat posed by bouldering to the special flora of erratic boulders, it is important to thoroughly understand the reasons for the negative impacts of sport climbing on rock vegetation that have been documented in several studies (reviewed in Holzschuh 2016). In these studies, the negative impact of climbing was mainly attributed to mechanical disturbances such as trampling and removal of soil and vegetation. However, the potential chemical impact of the widely used climbing chalk powder (magnesium hydroxide carbonate) had not been assessed. Because climbing chalk has a high pH that contrasts the acidic conditions on siliceous rocks, climbing chalk may be particularly detrimental to the acidophilic boulder specialist species. Therefore, I explored two fundamental aspects of climbing chalk and its potential impact on rock-dwelling species: (i) I explored the distribution and concentration of climbing chalk along bouldering routes on siliceous boulders in the field. (ii) I investigated the influence of climbing chalk on rock-dwelling plants species in a climate chamber experiment under controlled conditions, where I assessed the germination and survival of four fern and four moss species under different climbing chalk concentrations. The measurements along bouldering routes revealed elevated climbing chalk levels, even on 65% of sampling points without visual traces of climbing chalk. The experiment showed significant negative, though varied, effects of elevated climbing chalk concentrations on the germination and survival of the assessed species. There were no apparent differences between species adapted to siliceous or calcareous rock. Hence, elevated climbing chalk concentrations can occur along climbing routes and climbing chalk may well have negative impacts on rock-dwelling organisms.

Conclusions for the conservation of the flora of erratic boulders

Erratic boulders are isolated islands colonised through long-distance dispersal

The study on species diversity and the genetic study supported the island properties of siliceous erratic boulders in the calcareous Swiss Plateau and Jura Mountains. I confirmed a positive species–area relationship: the larger a boulder is the more species it hosts. As predicted by island biogeography theory (MacArthur and Wilson 1967; Dembicz et al. 2020), the species–area curve was steeper for boulder specialist species than for total species richness. Likewise, I found that populations of *Asplenium septentrionale* and *Hedwigia ciliata* on erratic boulders are genetically less diverse than populations from the mainland (adjacent siliceous mountain ranges). Further, there were no signs of connectivity among boulder populations and no indications that reduced genetic diversity was a problem for survival of the typical boulder specialists *A. septentrionale* and *H. ciliata* on erratic boulders. These results suggest that siliceous erratic boulders colonised by specialist species in the Swiss lowlands do not form an archipelago of connected islands, but rather a group of isolated islands, which were independently colonised by spores from far away source populations in the main distribution areas of the species. Notably, this conclusion implies that common paradigms of conservation biology, such as population connectivity and high genetic diversity, do not play a pivotal role in the conservation of the critically endangered boulder populations of *A. septentrionale* and of *H. ciliata*. This may also hold true for other specialist bryophytes of erratic boulders. Hence, effective conservation of a given population of a rare species on an erratic boulder is independent from its distance to other boulders, and new colonisation of boulders sourcing from boulder populations may only play a role in close proximity (up to ca. 100 m distance; Vanderpoorten et al. 2019).

Both large and small boulders are important

On the one hand, I showed a positive species–area relationship for species richness on boulders. Hence, large boulders are more likely to harbour numerous boulder specialists. On the other hand, I found that the small boulders within a landscape together contribute more specialist bryophyte species than a few large boulders of the same cumulative surface area. For conservation practice, this translates into prioritising large boulders while not neglecting the smaller ones. Prioritising large boulders: (i) maximises the number of specialist species with a minimal number of boulders considered; (ii) favours the boulders that are prone to removal of vegetation for sport climbing (Blum 2015); (iii) accounts for the fact that there are synergies with boulders protected as geosites, where large boulders are usually covered (Reynard et al. 2004; Gonggrijp 2000); and (iv) considers that large boulders can also serve as flagship sites to raise awareness for rare species that also occur on small erratic boulders. In contrast, the boulder specialist diversity contributed by small boulders is more difficult to protect, as a result of the comparably large number of small boulders in a landscape, the general lack of information on their precise locations, and the fact that small boulders are easily destroyed or removed. Additionally, island biogeography theory predicts that populations on small boulders have a higher risk of going extinct (MacArthur and Wilson 1967). Hence, to conserve the rare species on small boulders, which are more difficult to address individually, it is necessary to inform people in forestry, agriculture and nature conservation, as well as the wider public, about the special value of erratic boulders as habitat islands. Such efforts enable these stakeholders to contribute to the conservation of the flora of erratic boulders within their spheres of influence.

Boulders within settlements lack special species

The result that nearby buildings negatively affect the number of specialist species on erratic boulders is in line with the observation of Wächter (1996), that erratic boulders within settlements generally lack specialist species. Also, in my own experience, I never found a specialist bryophyte species on an erratic boulder located within a settlement. Presumably, within settlements the frequency of disturbances is too high for specialist species to successfully colonise a boulder. Such disturbances may be the cleaning or displacement of boulders and frequent changes to their surrounding by building or gardening activities. Hence, erratic boulders within settlements function primarily as geosites with an educational or aesthetic value (Reynard 2004), while these boulders are not of importance for specialist species.

Different communities require different management

I found floristically different bryophyte communities on boulders in forests than on boulders in open land, confirming the results of Meylan (1912). Additionally, I found a partition of each of these two groups into communities with and without boulder specialists, which resulted in a total of four bryophyte communities on siliceous erratic boulders (Table 1; Fig. 2). The communities lacking boulder specialists probably had this characteristic because of disturbances, which impede the survival of specialist species on their habitat islands. The open land bryophyte community lacking boulder specialists was often associated with settlements, which are associated with anthropogenic disturbances (see above). In forests, I suspect that timber harvesting and windthrow may be the main disturbances leading to abrupt changes in the light regime, which may cause the loss of boulder specialists (Meylan 1912). An explorative experiment (conducted in collaboration with Pro Natura; Hepenstrick unpubl. data), in which trees shading boulders were removed, impressively demonstrated the strong impact of an abruptly changed light regime on the boulder's bryophyte community (Fig. 3). In a nutshell, light availability (forest vs. open land boulders) and disturbance frequency (disturbed vs. undisturbed boulders) are presumably the main factors whose combinations lead to the four observed bryophyte communities on siliceous erratic boulders. As conservation should focus on the two communities which are characterised by specialist species, this translates into avoiding disturbances on and around erratic boulders and maintaining the light conditions to which the specialist bryophyte species present on an erratic boulder are adapted. The latter could, for example, involve removing trees surrounding boulders harbouring light-demanding bryophyte communities in order to avoid their gradual disappearance due to encroachment of boulders by vegetation (Fig. 4), but it could also mean avoiding tree harvesting around boulders that harbour specialist bryophyte communities adapted to shade.

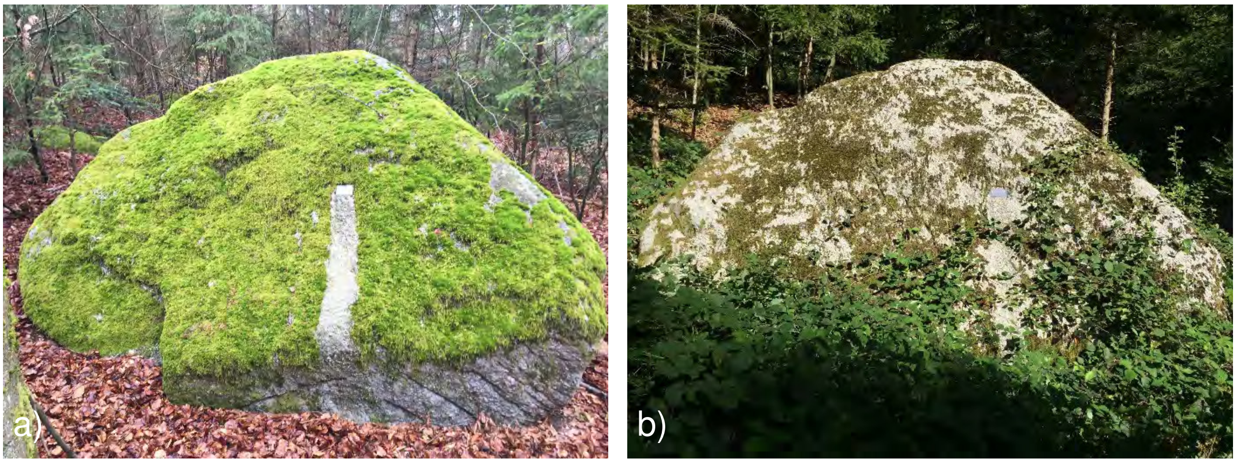


Fig. 3 Example of the effect of abruptly changed light availability. The same view of a siliceous erratic boulder in a forest: before (a; 2016) and three year after (b; 2019) the removal of shading trees. The main change in the bryophyte cover was a decrease of the generalist species *Hypnum cupressiforme*. Neither positive nor negative effects were found for boulder specialists at the time of observation. Further visits in the future may give more information on how bryophyte communities on boulders in forests respond to tree removal. (Note: The conspicuous vertical strip on the boulder in (a) is caused by a metal plaquette, marking the boulder as a protected geosite, whose leachates prevent moss growth)

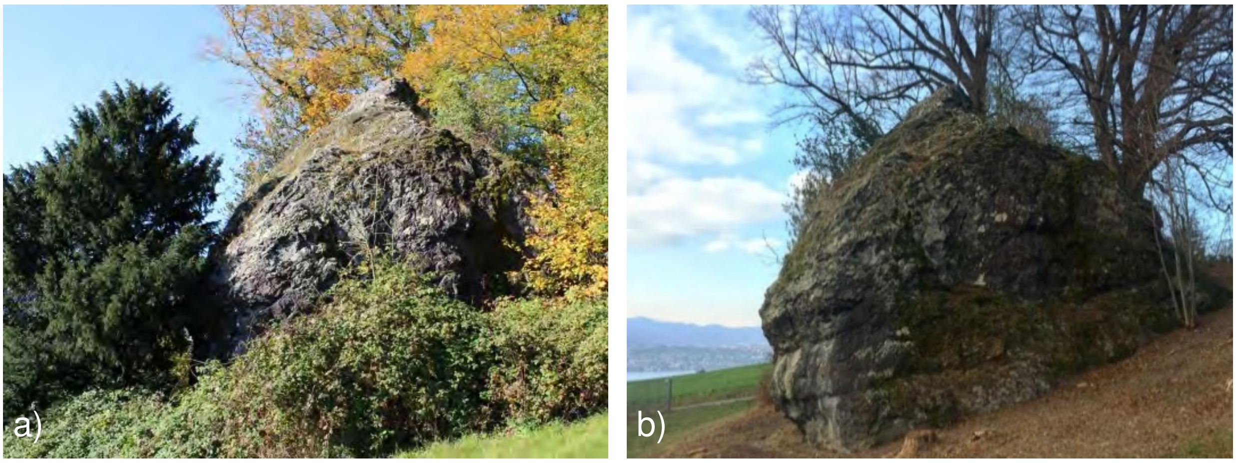


Fig. 4 Example of a conservation measure with the aim of sustaining light conditions on an erratic boulder that harbours numerous light-demanding species such as *Asplenium septentrionale*, *Grimmia laevigata* and *Hedwigia ciliata*. A young evergreen tree on the boulder's south side and encroaching brambles were removed. The photos show the situation before (a; 2014) and right after the measures were implemented (b; 2016).

Priority on open land boulders

I found that the boulder specialist community in open land (c2) harbours different and more specialist species than the boulder specialist community in forests (c3; Table 1). Siliceous erratic boulders in full sun are in fact considered hotspots of specialist lichen diversity (Meylan 1922; Epard et al. 2020). Open land erratic boulders, however, were about five times less abundant than boulders in forests, because they have been destroyed or removed for agricultural land clearance (Akçar et al. 2011). During field work, I found indications of ongoing removal of erratic boulders from agricultural land in the form of boulders that could not be found at the given coordinates and anthropogenically arranged groups of boulders that were removed from fields in the context of agricultural land consolidations. Hence, special conservation attention should be directed at erratic boulders in open land outside of settlements. One way to do so in Switzerland is through the ordinance on direct payments to agriculture (BLW 2013), which offers subsidies for measures enhancing landscape quality and biodiversity on farmland. Therein measures for preserving erratic boulders and their flora can be included.

Climbing chalk may harm rock-dwelling plants on erratic boulders

In the study on climbing chalk I demonstrated that elevated climbing chalk concentrations occur along climbing routes even when no climbing chalk traces are visible. In addition, I found that climbing chalk generally had a negative impact on the germination and early survival of rock-dwelling ferns and mosses in a climate chamber experiment with varying climbing chalk concentrations. These findings suggest that the potential threats imposed by climbing or bouldering not only include the mechanical impacts of climbing, such as trampling or removal of vegetation (Holzschuh 2016), but also encompass the negative chemical impacts of climbing chalk on plants. Because of the rarity of larger erratic boulders in the Swiss lowlands and because of their important function as island habitats for rare and specialised cryptogams, I advise against developing climbing routes on erratic boulders. However, where climbing routes have already been established on erratic boulders (e.g. Blum 2015; Antz 2019), I suggest evaluating the threats posed by climbing activities and climbing chalk on the individual boulders and developing joint solutions in collaboration with climbing stakeholders.

Linking science with practice: outreach is key

Cryptogams rarely appear on conservation agendas (Hallingbäck and Hodgetts 2000). The same holds true for erratic boulders, which are mainly seen as geological objects. However, as shown in this thesis, erratic boulders harbour rich cryptogam communities including many species that are specialists of boulders. In this way, boulders add to the landscape level of biodiversity, with respect to both habitat diversity and species richness.

As conservation practitioners hardly ever consult scientific literature (Fabian et al. 2019), I complemented my thesis with a wide variety of outreach activities (compiled in the

Appendix), with the aim of raising awareness for bryophytes and erratic boulders among stakeholders and the broader public. I reached out to scientists involved in teaching bryology with an article on a tool that helps beginners to conduct cross sections of leaves, which is essential for the identification of bryophyte species (Appendix I). I addressed Swiss bryologists and lichenologists through articles on special species that I found on erratic boulders (Appendix II). For the broader public generally interested in nature, but also for local authorities, I wrote outreach articles and led public excursions (Appendix III). In collaboration with conservation professionals, I initiated and established two information boards near boulders whose flora has been acutely threatened by climbers and boulderers (Appendix IV). Finally, I was fortunate that the subject of my thesis was picked up by mass media, which resulted in reports about the conservation biology of erratic boulders (Appendix V).

From the feedback that I received for all these outreach activities, I can conclude that field botanists, conservation professionals, local authorities and the wider public are receptive to the conservation of the flora of erratic boulders. An example of the effectiveness of outreach activities, is the article by Hepenstrick and Walthard (2017) that reached a broad audience and caused the explicit consideration of the flora of erratic boulders in two ongoing projects, namely in the redesign of a public park next to Zürich Airport (Meier 2019) and in the revision of the inventory of the protected geological objects in the canton of Bern (Stampfli 2020).

Outlook

The various studies I conducted led to new questions and scientific perspectives, which may be addressed in follow-up studies. The dataset generated in Chapter I may serve for studies addressing macroecological questions. For instance, the diversity of different functional groups, e.g. based on diaspore size, could be assessed and analysed in relation to boulder size (Virtanen and Oksanen 2007). For the four lineages of *H. ciliata* detected in the genetic study in Chapter II, it remains open how they relate to each other and to other *Hedwigia* species, which have recently been distinguished and described in Russia (Ignatova et al. 2016). If there are morphological traits that make it possible to distinguish between different *H. ciliata* lineages, *H. ciliata* may be a useful model to further investigate the connectivity amongst boulders at a smaller spatial scale than the one considered in Chapter II (e.g. <100 m; Vanderpoorten et al. 2019). As a follow-up to the study on climbing chalk in Chapter III, it would be worthwhile to study the in-situ impact of climbing chalk using an experimental approach by applying climbing chalk on unclimbed rocks of varying geology and assessing its effect on rock-dwelling plants and lichens. Finally, conservation measures implemented for the flora of erratic boulders, such as felling shading trees or defining areas on boulders that can be used for climbing and areas that are reserved for boulder specialist cryptogams, should be scientifically accompanied and evaluated in order to develop conservation measures in the framework of evidence based conservation (Sutherland et al. 2004; Hofer 2016).

Concerning the conservation of the flora of erratic boulders in the Swiss lowlands, my thesis laid a foundation, upon which a more fully-fledged conservation strategy could be developed. As a next step, I therefore plan a discussion of the results of the thesis with stakeholders from geology, agriculture, conservation and forest management in order to develop sound conservation recommendations and measures that fit with and complement the already existing framework of nature conservation legislation in Switzerland. I also intend to continue and even intensify outreach activities concerning the flora of erratic boulders and the function of these boulders as special habitats that increase biodiversity at the landscape level.

References

Agassiz L (1840) Etudes sur les glaciers. Jent et Gassmann, Neuchâtel.

Akçar N, Ivy-Ochs S, Kubik PW, Schlüchter C (2011) Post-depositional impacts on ‘Findlinge’(erratic boulders) and their implications for surface-exposure dating. Swiss Journal of Geosciences 104: 445-453.

Amann J (1894) Woher stammen die Laubmoose der erratischen Blöcke der schweizerischen Hochebene und des Jura? Berichte der Schweizerischen Botanischen Gesellschaft 4: 19-30.

Antz W, Trachsel P, Schmid R (2019) Bimano, smartphone application. v.2.2. Bimano GmbH, Ittingen.

Bachmann S (1999) Zwischen Patriotismus und Wissenschaft. Die schweizerischen Naturschutzpioniere (1900–1938). Chronos, Zürich.

BLW (2013) Verordnung vom 23. Oktober 2013 über die Direktzahlungen an die Landwirtschaft (Direktzahlungsverordnung, DZV). Bundesamt für Landwirtschaft, Bern.

Becking B (1934) Geobiologie of inleiding tot de milieukunde. Van Stockum & Zoon, Den Haag.

Blum C (2015) Steinhof – Steineberg, Klettern, Familienklettern, Bouldern am grössten Findling im Mittelland und seinen Vasallen. Topo, Basel.

Bornand C, Eggenberg S, Gygax A, Juillerat P, Jutzi M, Marazzi B, Möhl A, Rometsch S, Sager L, Santiago H (2019) Regionale rote Liste der Gefässpflanzen der Schweiz. Info Flora, Bern.

Brockmann-Jerosch H, Brockmann-Jerosch M (1926) Die Geschichte der schweizerischen Alpenflora. In: Schröter C (ed) Das Pflanzenleben der Alpen. Raustein, Zürich, pp 1149-1199.

Cain SA, AJ Sharp (1938) Bryophytic unions of certain forest types of the great smoky mountains. American Midland Naturalist 20: 249-301.

Colpa JG, van Zanten BO (2006) Mossen op de Nederlandse hunebedden in 2004/2005. Buxbaumiella 75: 34-50.

Dembicz I, Moysiyenko II, Kozub Ł, Dengler J, Zakharova M, Sudnik-Wójcikowska B (2020) Steppe islands in a sea of fields: where island biogeography meets the reality of a severely transformed landscape. Journal of Vegetation Science 32: e12930.

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67: 345-366.

Ehlers J, Gibbard PL (2007) The extent and chronology of Cenozoic global glaciation. Quaternary International 164: 6-20.

Epard JL, Gex P, Vust M (2020) Les blocs erratiques propriété de la Société Vaudoise des Sciences Naturelles. Bulletin de la Société Vaudoise des Sciences Naturelles 99: 29-66.

Fabian Y, Bollmann K, Brang P, Heiri C, Olschewski R, Rigling A, Stofer S, Holderegger R (2019) How to close the science-practice gap in nature conservation? Information sources used by practitioners. Biological Conservation 235: 93-101.

Favre A, Studer B (1867) Appel aux Suisses pour les engager à conserver les blocs erratiques. Actes de la Société Helvétique des Sciences Naturelles, Rheinfelden.

Fischer L (1871) Über die an erratischen Blöcken im Kt. Bern vorkommenden Pflanzen. Mitteilungen der Naturforschenden Gesellschaft Bern 711: 85-88.

Gonggrijp GP (2000) Planning and management for geoconservation. In: Barettino D,. Wimbledon WAP, Gallego E (eds) Geological heritage: its conservation and management. Instituto Tecnológico Geominero de España, Madrid, pp 29-45.

Hallingbäck T, Hodgetts N (2000) Status survey and conservation action plan for bryophytes: mosses, liverworts and hornworts. IUCN, Cambridge.

Hanski I, Gilpin M (1991) Metapopulation dynamics: brief history and conceptual domain. Biological Journal of the Linnean Society 42: 3-16.

Hegi G (1902) Das obere Tösstal und die angrenzenden Gebiete, floristisch und pflanzengeographisch dargestellt. Romet, Genève.

Hepenstrick D (2014). Spannend, wertvoll und gesucht: Nordische Streifenfarne (*Asplenium septentrionale*) auf Findlingen. Info Flora plus 1: 24-25.

Hepenstrick D, Urmi E, Meier MK, Bergamini A (2016) Die Moosflora des silikatischen Findlings Alexanderstein in Küsnacht (ZH). Meylania 57: 15-23.

Hepenstrick D, Walthard P (2017). Granitinsel im Kalkmeer / Ilots de granit dans une mer de calcaire / Isole granitiche nel mare calcareo. Die Alpen / Les Alpes / Le Alpi 2017(5): 60-61.

Hofer U (2016) Evidenzbasierter Artenschutz, Begriffe, Konzepte, Methoden. Haupt, Bern.

Holderegger R, Schneller JJ (1994) Are small isolated populations of *Asplenium septentrionale* variable? Biological Journal of the Linnean Society 51: 377-385.

Holzschuh A (2016) Does rock climbing threaten cliff biodiversity? – A critical review. Biological Conservation 204: 153-62.

Ignatova EA, Kuznetsova OI, Fedosov VE, Ignatov MS (2016) On the genus *Hedwigia* (Hedwigiaceae, Bryophyta) in Russia. Arctoa 25: 241-277.

Imbrie J, Imbrie KP (1986). Ice Ages: solving the mystery. Harvard University Press, Cambridge.

Itzigsohn H (1856) Geologische Bedeutung der Laubmoosflora der erratischen Blöcke Norddeutschlands. Botanische Zeitung 52: 913-920.

Jansen P, Wachter WH (1928). Mossen van de Hunnebedden. Nederlandsch kruidkundig archief 38: 167-171.

Kimmerer RW, Driscoll MJL (2000) Bryophyte species richness on insular boulder habitats: the effect of area, isolation, and microsite diversity. Bryologist 103: 748-756.

Krawiec F (1938) Flora epilityczna glazow narzutowych zachodniej polski (Die epilithische Flora der erratischen Blöcke Westpolens). Jachowskiego, Poznan.

Lugon R, Pralong JP, Reynard E (2006) Patrimoine culturel et géomorphologie: le cas valaisan de quelques blocs erratiques, d'une marmite glaciaire et d'une moraine. Bulletin de la Murithienne 124: 73-87.

MacArthur RH, Wilson E (1967) The theory of island biogeography. Princeton University Press, Princeton.

Masselink AK, van Zanten BO (1976) De Bryofyten-flora van de Drentse hunebedden en zwerfkeien I: De Hunebeddenflora. Lindbergia 3: 323-231.

Mazenauer D, Holderegger R, Krüsi B, Hepenstrick D (2014) Populationsentwicklung und Gefährdung von *Asplenium septentrionale* auf Findlingen im Schweizer Mittelland und Jura. Bauhinia 25: 37-50.

Meier MK (2019). Butzenbüel, Moosflora und -vegetation der Findlinge. Zürich, flora + fauna consult.

Meylan C (1912) La flore bryologique des blocs erratiques du Jura. Bulletin de la Société Vaudoise des Sciences Naturelles 48: 49-70.

Meylan C (1922) Contribution à la connaissance des lichens du Jura. Bulletin de la Société Vaudoise des Sciences Naturelles 45: 287-294.

Milde J (1870) Die erratischen Moose. Botanische Zeitung 28: 129-134.

Mucina L, Bültmann H, Dierßen K, Theurillat JP, Raus T, Čarni A, Šumberová K, Willner W, Dengler J et al. (2016) Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. Applied Vegetation Science 19: 3-264.

Müller K (1853). Die Dilivialperiode. Die Natur 1853(25): 197-200.

Neu F (1971) Moose an Findlingen im westlichen Münsterland. Natur und Heimat 31: 98-101.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PloS ONE 7: e37135.

Philippe M (2010) Bryophytes saxicoles calcarifuges sur substrats exotiques dans l'Ain. Nouvelles Archives de la Flore Jurassienne et du Nord-Est de la France 8: 211-216.

Reynard E (2004) Protecting stones: conservation of erratic blocks in Switzerland. In: Prikryl R (ed) Dimension stone 2004. New perspectives for a traditional building material. Balkema, Leiden, pp 3-7.

Stampfli Y (2020). Geschützte geologische Objekte des Kantons Bern. Amt für Landwirtschaft und Natur des Kantons Bern, Münsingen.

Stehlik I (2000). Nunataks and peripheral refugia for alpine plants during quaternary glaciation in the middle part of the Alps. Botanica Helvetica 110: 25-30.

Sutherland WJ, Pullin AS, Dolman PM, Knight TM (2004). The need for evidence-based conservation. Trends in Ecology & Evolution 19: 305-308.

Virtanen R, Oksanen J (2007) The effects of habitat connectivity on cryptogam richness in boulder metacommunity. Biological Conservation 135: 415-422.

Vischer W (1946) Naturschutz in der Schweiz. Schweizerischer Bund für Naturschutz, Basel.

Vanderpoorten A, Patiño J, Désamoré A, Laenen B, Górski P, Papp B, Holá E, Korpelainen H, Hardy O (2019). To what extent are bryophytes efficient dispersers? Journal of Ecology 107: 2149-2154.

Vust M (2013) Les lichens de la Pierre des Marmettes, Monthey (VS). Meylania 50: 17-20.

Wächter HJ (1996) Zur Moosvegetation von Findlingen zwischen Ems und Weser. Osnabrücker Naturwissenschaftliche Mitteilungen 22: 89-110.

Walther A, Molendo L (1868). Die Laubmoose Oberfrankens. Beiträge zur Pflanzengeographie und Systematik und zur Theorie vom Ursprunge der Arten. Engelmann, Leipzig.

Weber J (1912) Neue Standorte von *Asplenium septentrionale*. Mitteilungen der Naturwissenschaftlichen Gesellschaft Winterthur 9: 140-145.

Weibull H (2001) Influence of tree species on the epilithic bryophyte flora in deciduous forests of Sweden. Journal of Bryology 23: 55-66.

Weibull H, Rydin H (2005) Bryophyte species richness on boulders: relationship to area, habitat diversity and canopy tree species. Biological Conservation 122: 71-79.

Chapter I

Factors determining the conservation value of bryophyte communities on insular siliceous erratic boulders

Daniel Hepenstrick, Ariel Bergamini, Clare Webster, Christian Ginzler, Rolf Holderegger

*Manuscript in press: Journal of Vegetation Science **

** Accepted as: "Factors determining bryophyte species richness and community composition on insular siliceous erratic boulders in calcareous landscapes"*

Abstract

Aim: Pleistocene erratic boulders are rocks that were relocated by glaciers during the Ice Ages. When their geology differs from surrounding landscapes, erratic boulders are habitat islands for regionally rare rock-dwelling cryptogams (bryophytes, ferns and lichens). In many places, the cryptogam communities of erratic boulders are threatened because of land use changes, removal of vegetation from the boulders by sport climbers, and a lack of knowledge about their special biodiversity. In order to create an evidence-based foundation for the conservation of cryptogam communities on insular erratic boulders, we studied the factors driving their bryophyte diversity.

Location: Siliceous erratic boulders in the calcareous Swiss Plateau and Jura Mountains.

Methods: For 160 erratic boulders we recorded all bryophyte species and diverse environmental variables. For all species and specialist species (acidophile rock-dwellers) separately, we analysed species–area relationships and tested the effects of environmental variables. Further, we characterised the bryophyte communities present on the boulders.

Results: We found 138 bryophyte species, 19 of which were specialists of erratic boulders. A steeper species–area curve for boulder specialists than for total species richness underlined the island function of boulders for specialist species. Large boulders were more likely to harbour numerous boulder specialists, but at the landscape level small boulders together contributed more specialist species than large boulders. Erratic boulders near settlements were less likely to harbour boulder specialists. Boulders in open land harboured different and more specialist species than boulders in forests.

Conclusions: Conservation should prioritise undisturbed erratic boulders in open land because the communities on this rare type of boulder harbour the largest number of specialist species. On the landscape level, small and large boulders are of similar importance, however, conserving large boulders is logistically easier, and they may function as flagships for these special island habitats that are not yet adequately appreciated.

Keywords: biodiversity, erratic blocks, geodiversity, island biogeography, mosses, *Racomitrietea heterostichi*, siliceous rock, SLOSS, species–area relationship

Introduction

Geodiversity, the abiotic diversity of the earth's surface, has a pivotal, yet rarely explicitly studied, influence on biodiversity (Tukiainen et al. 2019; Alahuhta et al. 2020). In particular, small geosites such as isolated cliffs or springs can contribute specialist species to the species pool of a larger landscape that otherwise lacks the geosites' special abiotic properties essential for the survival of specialist species (Hjort et al. 2015). Pleistocene erratic boulders – rocks that were relocated by glaciers during the Ice Ages – are small geosites. Erratic boulders contribute greatly to the reconstruction of the earth's history and climate (Imbrie and Imbrie 1986), but they also contribute to biodiversity. Numerous obligate rock-dwelling cryptogams (bryophytes, ferns and lichens) depend on erratic boulders in landscapes where no hard rock is present otherwise, such as on the European sand plain between Belgium and Estonia (Krawiec 1938; Wächter 1996; Colpa and van Zanten 2006). Additionally, in landscapes where the bedrock contrasts the geology of erratic boulders, the boulders harbour regionally rare species, for example in central Finland, where calcareous boulders exist on siliceous bedrock (Virtanen and Oksanen 2007), and in the French and Swiss Jura Mountains, where siliceous boulders occur on calcareous bedrock (Meylan 1912; Philippe 2010; Mazenauer et al. 2014).

Erratic boulders have been used as model for exploring aspects of island biogeography (MacArthur and Wilson 1967). In Swedish forests, bryophyte species richness on 216 siliceous erratic boulders was found to be positively related to boulder area, within-boulder habitat diversity and base-rich litter of the tree species surrounding the boulders (Weibull 2001; Weibull and Rydin 2005). Virtanen and Oksanen (2007) similarly found a positive link between boulder size and species richness and additionally reported a weak positive effect of connectivity to other boulders in a dataset comprising 288 erratic calcareous boulders in Finnish forests. Kimmerer and Driscoll (2000), however, found neither boulder size nor connectivity among boulders to be related to boulder species richness on 39 granitic erratic boulders in the US state of New York. These studies exclusively considered boulders in forests, however, the factors shaping boulder specialist bryophyte diversity on a landscape level have not been studied so far.

Threats and declines of erratic boulders and their special vegetation call for conservation action. In many places, erratic boulders have been vastly exploited as building material and destroyed for agricultural land clearance (Gonggrijp 2000; Akçar et al. 2011), and many boulder specialist bryophytes are classified as threatened in cases where they have been evaluated in red lists (Ulvinen et al. 2002; Siebel et al. 2013; Ingerpuu et al. 2018). Nowadays, large and emblematic erratic boulders are often protected as geosites (Reynard 2004). However, their vegetation is threatened by sport climbing ("bouldering"; Lawyer and Haas 2008; Blum 2015; Antz et al. 2019), and floristic studies on smaller boulders have documented declines in boulder specialist bryophytes (Wächter 1996; Colpa and van Zanten 2006).

In order to conserve the special bryophyte communities on erratic boulders, it is crucial to understand their ecology. In the present study, we thus aimed to determine the factors that influence bryophyte diversity on erratic boulders. We focused on the insular bryophyte communities on siliceous erratic boulders in the calcareous Swiss Plateau (molasse bedrock) and Jura Mountains (limestone), where Pleistocene siliceous erratic boulders originating from siliceous areas in the Alps have been deposited in large numbers (Fig. 1). We addressed the following three questions: (i) How are boulder size and species richness related? (ii) Which ecological factors drive species richness and the occurrence of boulder specialist species? (iii) Which bryophyte communities occur on erratic boulders and how are they ecologically characterised? Based on our findings, we draw conclusions relevant for the conservation of the special bryophyte vegetation on insular erratic boulders, which can serve as an evidence-based scientific foundation for the conservation of the biodiversity on erratic boulders.

Materials and Methods

Study sites and sampling

Our study area was situated on the southern slopes of the Swiss Jura Mountains and in the parts of the Swiss Plateau that were glaciated during the Last Glacial Maximum (Fig. 1). This landscape is characterised by a mosaic of agricultural areas, forests, and residential and industrial areas in a temperate sub-oceanic climate. Prior to this study, little was known about boulder specialist bryophytes in Switzerland: the rare species are sometimes mentioned in local bryophyte floras (e.g. Geheeb 1864; Culmann and Weber 1901; Bergamini 2015), Meylan (1926) and Hepenstrick et al. (2016) each described the species composition of one single boulder, and Meylan (1912) described bryophyte communities specific to erratic boulders in the Swiss Jura Mountains.

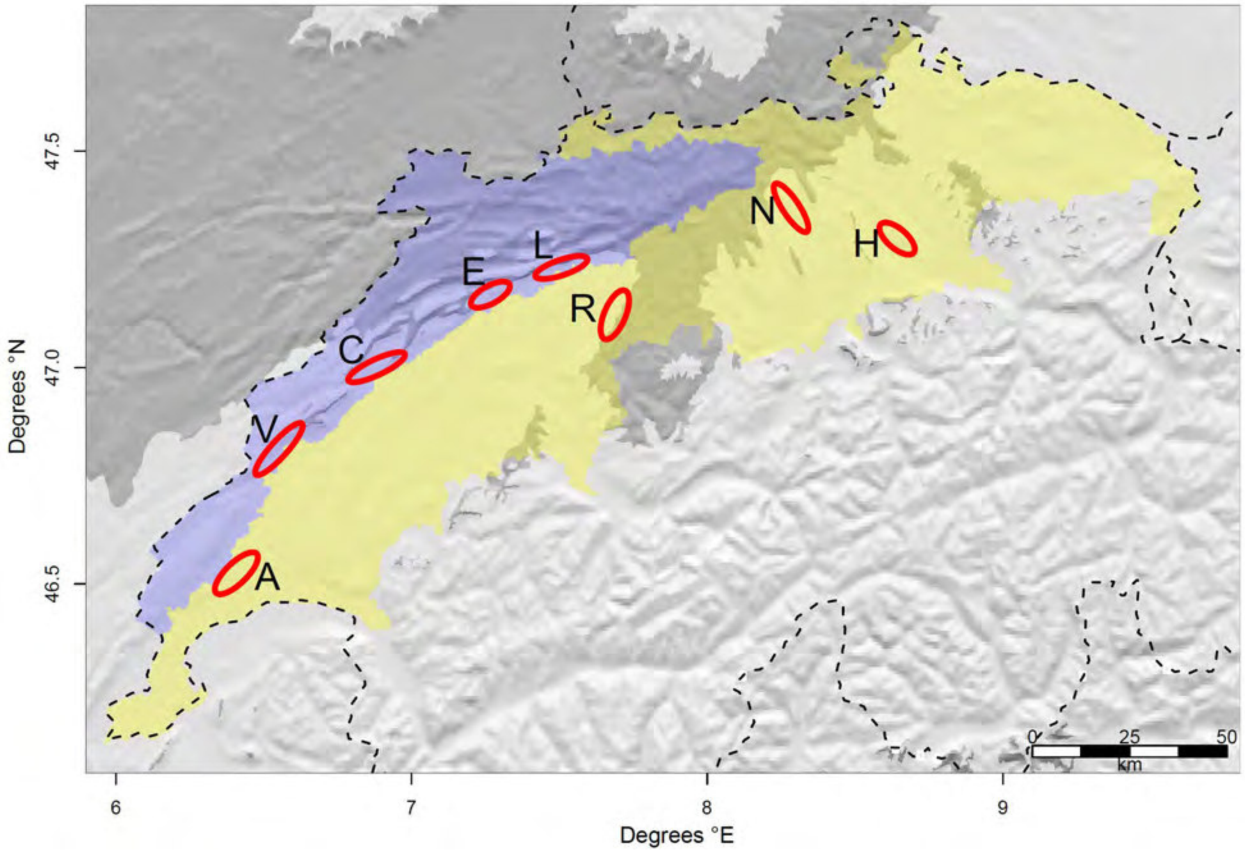


Fig. 1 Location of the eight study areas (red ellipses; identification codes as in Table S1) on the Swiss Plateau (yellow) and in the Swiss Jura Mountains (blue; BAFU 2006). Light grey areas of the background map were glaciated during the Last Glacial Maximum, whereas darker grey areas were ice free (Bini et al. 2009). Dashed lines indicate national borders (Background map: Jarvis et al. 2008).

We selected eight study areas that contain abundant and large siliceous erratic boulders. Four areas were distributed on the southern Jura slopes already explored by Meylan (1912), and four areas were located in glacial landscapes of the Swiss Plateau. Each elliptic study area of 40–62 km^2 enclosed the regional pool of erratic boulders (Fig. 1). We selected a stratified random sample of ten forest and ten non-forest boulders per study area. Stratification was applied because the abundance of erratic boulders was about five times higher in forests than in non-forested land.

The sampling protocol is described in detail in the Supplementary Material (Note S1, Tables S1, S2). In short, we first selected the sample boulders from coordinates of erratic boulders recorded on geological maps. For a selected boulder to be sampled in the field, a series of criteria had to be fulfilled. First, an erratic boulder had to be present at the selected boulder's coordinates. The aboveground dimensions (hereafter, dimensions always refer to aboveground dimensions) had to be at least 0.5 m in height and 0.5 m in length. The boulders had to be non-calcareous (i.e. no reaction with 10% HCl) and not influenced by calcareous surface water. If multiple boulders fulfilled these criteria at a coordinate point, we chose the largest boulder for sampling. If there was no boulder that fulfilled the above criteria, we selected a replacement boulder (see Note S1).

Data

For each sampled boulder, we compiled a complete species list of bryophytes (samples are archived in the Herbarium Z) and a set of numerical variables describing the boulder and its environment (Table 1). The nomenclature followed the Swiss bryophyte checklist of Meier et al. (2013), and taxonomically difficult bryophyte species groups were treated as aggregates (Table S3). We approximated boulder size based on boulder length, height and width, following Virtanen and Oksanen (2007), with a cuboid boulder shape. We described the vegetation structure and substrates on erratic boulders by estimating the percent cover of bryophytes, lichens, tracheophytes, litter, humus and open rock. Meylan (1912) underlined the importance of direct solar radiation for bryophyte species composition on erratic boulders. We considered this point by modelling the maximum potential annual total direct radiation input at the highest point of the boulder (recorded with a differential GNSS device; Geo 7X, Trimble, Sunnyvale, CA, USA) based on synthetic hemispherical images generated from airborne Lidar data and the digital elevation model swissAlti3D from swisstopo (www.swisstopo.admin.ch), using a modified version of the method described by Webster et al. (2020). Weibull and Rydin (2005) showed that the composition of the canopy above a boulder can influence its bryophyte community, which we considered by estimating the percent cover of trees, shrubs, and evergreens above the boulders (shoot presence, in foliate state). Within a radius of 25 m of the boulders, we counted the number of additional siliceous erratic boulders and determined the percent cover of forest and buildings using the digital Topographic Landscape Model (TLM) from swisstopo. We calculated the Euclidean distance to the nearest building (only used for *Paraleucobryum longifolium*). We considered elevation recorded in the field and mean annual precipitation and temperature derived from interpolated maps with 100 m resolution that were generated by Descombes et al. (2020) using data of Karger et al. (2017). As a proxy for air humidity we considered the minimum Euclidean distance to the nearest river, derived from the TLM. Additionally, we characterised the boulders' ecology as their bryophytes' mean unweighted ecological indicator values (Landolt et al. 2010) for moisture (F), light (L), reaction (R), nutrients (N) and hemeroby (EM; hemeroby quantifies the anthropogenic influence on site conditions).

Table 1 Overview of the variables analysed for 160 siliceous erratic boulders in the calcareous Swiss Plateau and Jura Mountains. The last column (GLMM) designates if and how the variable was included in the generalised linear mixed-effects models.

Variable	Range	Mean ± SD	Unit	GLMM
Number of bryophyte species				
All species	0–36	10.61 ± 5.37	Count	1
Boulder specialists	0–8	1.47 ± 1.67	Count	1
Boulder size				
Boulder size	1.511082	31.55 ± 93.76	m^2	Log transformed
Vegetation and substrates on boulder				
Bryophyte cover	0–99.8	49.88 ± 36.64	%	
Lichen cover	0–100	22.62 ± 31.58	%	
Tracheophyte cover	0–80	5.91 ± 13.22	%	
Litter cover	0–95	12.05 ± 18.37	%	
Humus cover	0–95	11.25 ± 20.19	%	
Rock cover	0–100	21.86 ± 26.32	%	
Radiation				
Direct radiation	28–7907	3652.23 ± 2581.26	MJ/m^2/yr	1
Canopy above boulder				
Trees above	0–100	63.65 ± 44.09	%	1
Shrubs above	0–100	12.6 ± 25.19	%	1
Evergreens above	0–100	15.85 ± 32.06	%	1
Surrounding of boulder				
Boulders in 25 m radius	0–26	1.95 ± 3.48	Count	Square-root transformed
Forest in 25 m radius	0–100	55.87 ± 44.86	%	Excluded (highly correlated with radiation)
Buildings in 25 m radius	0–26	1.23 ± 4.29	%	1
Distance to buildings	2–695	159.98 ± 127.23	m	Log transformed (for *Paraleucobryum* models)
Climatic variables				
Elevation	382–1262	635.85 ± 177.12	m	1
Precipitation	880–1669	1277.47 ± 165.11	mm/yr	1
Temperature	5.9–10.6	9.27 ± 0.95	°C	Excluded (highly correlated with elevation)
Distance to river	0.5–2246	468.28 ± 500.93	m	Log transformed
Indicator values				
Moisture F	2–3.13	2.6 ± 0.24		
Light L	1.67–4	2.56 ± 0.51		
Reaction R	1.75–4.33	2.72 ± 0.55		
Nutrients N	1–5	2.1 ± 0.56		
Hemeroby EM	1.33–3.4	2.31 ± 0.49		

Analyses

We conducted data analyses in R 3.6.3 (R Core Team 2017). Unless stated otherwise, we conducted all analyses based on species presence/absence on the 160 sampled boulders once for all bryophytes and once for the boulder specialists separately.

Definition of boulder specialists: We defined boulder specialists based on their substrate preferences and their reaction indicator value R, retrieved from Landolt et al. (2010) and Hill et al. (2007), which together covered all species identified in this study except Sciuro-Hypnum flotowianum. We filtered our species list for species with rock as the primary substrate and acidophile habitat preferences indicated by $R \leq 2$ for the five-level indicator values of Landolt et al. (2010) or $R \leq 4$ for the ten-level indicator values of Hill et al. (2007).

Species–area relationships: We assessed the effect of boulder size (A) on the species richness (S) of an individual boulder by fitting the power function $S = c \times A^z$ with non-linear regression. This power function has been shown to be the most adequate function for describing species–area relationships (Dengler 2009). The fitted parameter c reflects the expected mean number of species per unit area, and the parameter z reflects the rate of change in species richness in response to area. Higher z-values are expected for islands than for mainland because the chances of extinction for a given species are high on a small island and the chances of colonisation are low; while, larger islands approach the high colonisation rates and low extinction rates in a given area of mainland (MacArthur and Wilson 1967). Hence, we expect higher z-values for boulder specialists, for which boulders are actual habitat islands, than for species that also occur in the landscape matrix around boulders (Dembicz et al. 2020). To elucidate the importance of boulder size for total species richness, we calculated cumulative species–area curves, as proposed by Quinn and Harrison (1988), to determine whether single large or several small islands harbour more species (SLOSS; Fahrig 2020), using the package *Lexiguel* (Alvarez 2020). This approach is based on two cumulative species–area curves in which the islands (or any other habitat patches) are ranked by their size, once in ascending and once in descending order. By comparing the two curves, one can determine whether several small islands harbour more species than a few large islands of the same total area. This is the case if the ascending curve lies above the descending curve, which results in a value of > 1 for the SLOSS-index, which is the quotient of the areas under the ascending and the descending curve (Quinn and Harrison 1988).

Ecological drivers: In order to identify variables influencing species richness and species occurrence on erratic boulders, we applied multi-model inference of generalised linear mixed-effects models (GLMMs; Burnham and Anderson 2002; Bolker et al. 2009) as implemented in Kiebacher et al. (2017) for exploring bryophyte species richness on trees. As predictors we used the environmental variables described above, excluding the cover values describing vegetation structure and substrates on erratic boulders, and the ecological indicator values (Table 1). Because the predictor pairs temperature and elevation, and forest cover and direct radiation were highly correlated (|Spearman's rho| > 0.7), we excluded temperature and forest cover. We included the study area as a random effect. In order to improve model convergence, fit and interpretation we transformed selected predictors (Table 1) and standardised all predictors to a mean of 0 and a standard deviation of 0.5 (Schielzeth 2010). We constructed GLMMs using the package *lme4* (Bates et al. 2015). To analyse species richness we conducted Poisson regression by specifying a Poisson error structure and the log link function. To analyse individual boulder specialist species, we conducted logistic regression by specifying a binomial error structure and the logit link function. Thereby, we only analysed boulder specialists that occurred on at least 10% of the sampled boulders, namely *Grimmia hartmanii*, *Grimmia trichophylla*, *Hedwigia ciliata* and *Paraleucobryum longifolium*. For all 27 occurrences of *P. longifolium* the predictor “buildings” had a value zero, which did not allow a meaningful estimate of the regression parameters by the GLMMs. Therefore, for *P. longifolium* we used the minimum Euclidean distance to the nearest building as a predictor for “buildings”. We checked the full models for overdispersion with the package *blmeco* (Korner-Nievergelt et al. 2015) but found no signs of overdispersion. We conducted model simplification, selection and averaging with functions implemented in the package *MuMIn* (Barton and Barton 2015). For each full model, we generated sub-models with all possible predictor combinations and extracted the relative variable importance (RVI) of the predictors. Then, we retained all models with Δ AIC < 2 relative to the best model, averaged the models, and generated average parameter estimates for the predictors.

Characterisation of bryophyte communities: To classify the bryophyte communities on the sampled boulders, we conducted k-means non-hierarchical clustering, as described in Borcard et al. (2018), of the species data filtered for species with more than one occurrence and boulders harbouring more than three species (resulting in a dataset of 101 species and 151 boulders). Based on silhouette plots (package *cluster*; Maechler et al. 2019) for different numbers of clusters, we chose four clusters. For these clusters, we identified significant indicator species using the IndVal method (Dufrêne and Legendre 1997) and the corresponding permutation test, implemented in the package *labdsv* (Roberts 2019). We further characterised clusters with the collected and generated environmental variables (Table 1). To identify differences among the clusters, we conducted Kruskal-Wallis tests and corresponding post-hoc comparisons with the Holm correction, as compiled in custom functions of Borcard et al. (2018), and visualised the significant results as boxplots.

Ordination and radiation gradient: In order to visualise clusters, species and environmental variables in an ordination plot, we conducted a detrended correspondence analysis (DCA) with the same dataset that we used for cluster analysis, using the functions provided in the package *vegan* (Oksanen et al. 2019). We first projected the variables that were significant in the GLMM analyses or among the k-means clusters separately on the ordination plot as trend surfaces and as vectors (Fig. S1). We then selected the variables that showed a uniform linear and significant trend across the ordination plot for visualisation as vectors in the final triplot (Wildi 2017). In the DCA, different boulder specialists resolved along the fitted linear correlation of direct radiation. We explored this relationship further in the form of boxplots of occurrences of all boulder specialists along the radiation gradient.

Results

Diversity of boulders and species

In total we visited 321 coordinates with erratic boulders, 101 of which we replaced because the boulder(s) at the given coordinates did not fulfil our criteria, and 60 of which we replaced because no boulder was present at the given coordinates. The surface area of the sampled boulders covered three orders of magnitude (Table 1). On the 160 boulders studied, we recorded a total of 138 bryophyte species (Table S4), 19 of which we identified as boulder specialists (Table 2) which largely corresponded to the boulder specialists listed for the Jura Mountains by Meylan (1912). Species richness per boulder ranged from 0 to 36 and the number of boulder specialists from 0 to 8 (Table 1). On 61% of the sampled boulders we found at least one boulder specialist.

Table 2 The 19 boulder specialists (acidophilic rock-dwelling bryophyte species) found on 160 siliceous erratic boulders in the calcareous Swiss Plateau and Jura Mountains. Their frequency in this study (number of boulders) and Swiss national red list status (LC: least concern; NE: not evaluated; NT: near threatened; VU: vulnerable; Schnyder et al. 2004) is given in brackets.

Dicranoweisia crispula (1; LC)
Dicranum fulvum (12; LC)
Grimmia decipiens (3; VU)
Grimmia elatior (5; LC)
Grimmia hartmanii (67; LC)
Grimmia laevigata (7; LC)
Grimmia longirostris (3; LC)
Grimmia muehlenbeckii (1; LC)
Grimmia ovalis (11; NE)
Grimmia ramondii (1; LC)
Grimmia trichophylla (25; NT)
Hedwigia ciliata (48; LC)
Hedwigia stellata (2; VU)
Orthotrichum rupestre (14; LC)
Paraleucobryum longifolium (27; LC)
Racomitrium aciculare (1; LC)
Racomitrium heterostichum aggr. (4; NE)
Racomitrium microcarpon (1; VU)
Ulota hutchinsiae (2; VU)

Species richness and boulder area

Z-values retrieved by fitting a power function to the species–area data (Fig. 2) indicated that boulder size had a stronger influence on the number of boulder specialists ($z = 0.40$; 0.34–0.47, 95% confidence interval) than on total species richness ($z = 0.21$; 0.17–0.25). The largest boulder was an outlier in terms of size (Fig. 2). However, when we removed this boulder from analyses the estimated z-values ($z = 0.42$ for boulder specialist richness, $z = 0.18$ for total species richness) remained within the confidence intervals of the estimates for the complete dataset.

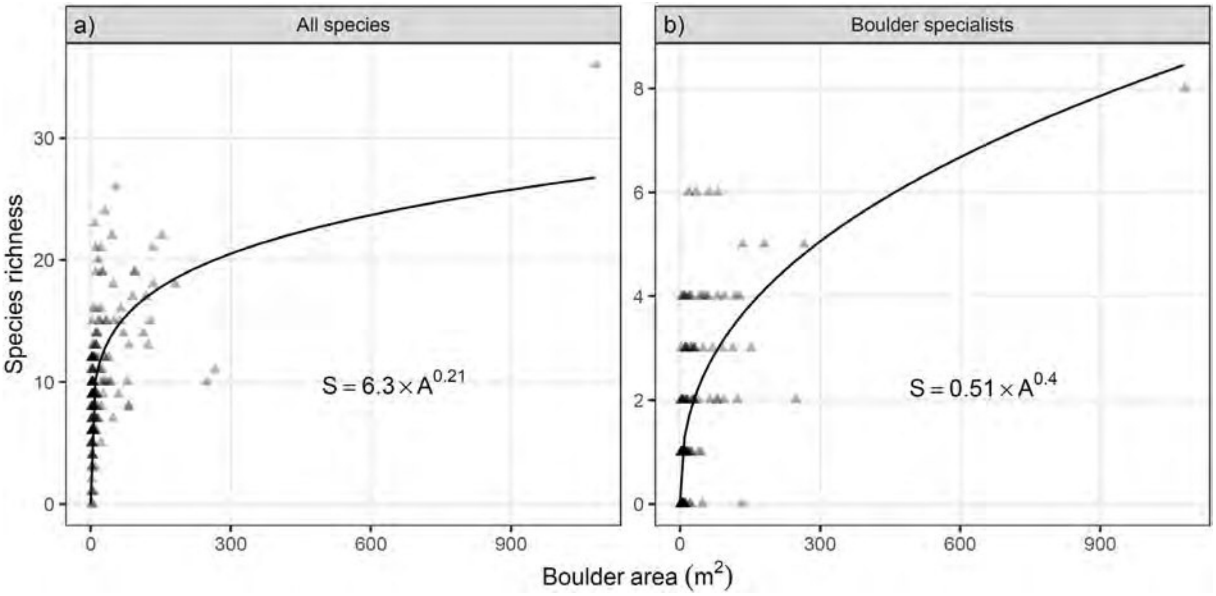


Fig. 2 Species (S) – area (A) relationships of bryophytes on 160 siliceous erratic boulders, (a) for total species richness and (b) for species richness of boulder specialists (note that the y-axis scale differs between panels). The regression lines are power functions fitted with non-linear regression.

SLOSS analyses indicated that several small boulders harboured more species than one or a few large boulders of the same surface area (Fig. 3). This relationship was more accentuated when all species were considered (SLOSS-index 2.03) than for boulder specialists only (SLOSS-index 1.54).

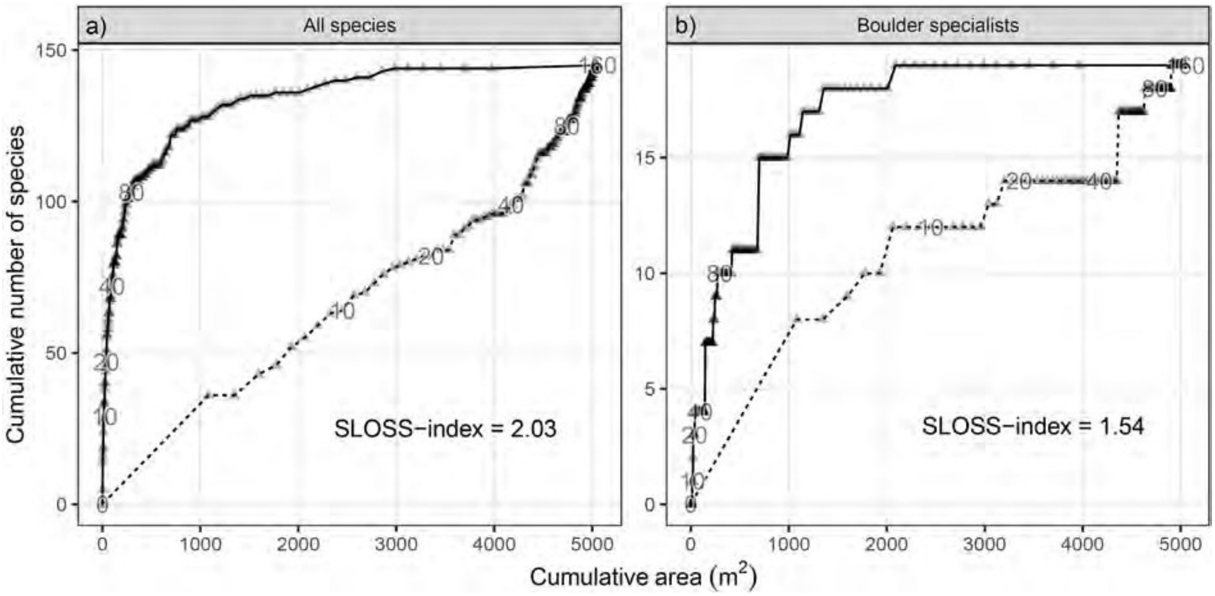


Fig. 3 Cumulative species–area curves of bryophytes on siliceous erratic boulders sorted by surface area in ascending (solid curve) and descending order (dashed curve), (a) for all species and (b) for boulder specialists (note that the y-axis scale differs between panels). Gray numbers indicate the number of boulders involved at given points on the curves. The SLOSS-index is the quotient of the areas below the two curves.

Ecological drivers

Boulder size showed highly significant positive effects in all averaged generalised linear mixed-effects models (Tables 3, S5). Direct radiation was only significant in some single species models, but with opposing directions. The probability of *Hedwigia ciliata* presence on an erratic boulder increased with increasing direct radiation, while the probability of *Paraleucobryum longifolium* and *Grimmia hartmanii* presence decreased. No significant effects were found for the presence of additional erratic boulders in a 25 m radius. The percentage of area covered by buildings in a 25 m radius had a negative effect on the number of boulder specialists and on *G. hartmanii* and *H. ciliata* presence. For *P. longifolium*, distance to the nearest building showed a marginally significant positive effect, which suggests a negative influence of near buildings on this species. The percent cover of tree canopy above a boulder had a significant positive effect on *G. hartmanii* presence. The percent cover of shrubs had a significant positive effect on total species richness. The percent cover of evergreens showed a marginally significant negative effect on *G. trichophylla* presence. Elevation positively affected the number of boulder specialists, as well as *G. hartmanii* and *P. longifolium* presence. The two predictors precipitation and distance to the nearest river were never significant.

Table 3 Results of generalised linear mixed-effects models (GLMMs) fitted to species numbers with Poisson regression and to the occurrence of four boulder specialist species with logistic regression. Estimates are standardised coefficient estimates after model averaging of the best candidate models (in bold: $p < 0.1$; n: predictor not included in the best models). Relative variable importance (RVI) is the sum of Akaike weights over all possible candidate models containing the predictor. Significance levels for parameter estimates: *** *p* <0.001 ,** *p* <0.01, * *p* <0.05, $^{\#}$ *p* <0.1.

	All species		Boulder specialists		*Grimmia hartmanii*		*Grimmia trichophylla*		*Hedwigia ciliata*		*Paraleucobryum longifolium*	
Predictor	Estimate	RVI	Estimate	RVI	Estimate	RVI	Estimate	RVI	Estimate	RVI	Estimate	RVI
Boulder size	**0.64*** **	**1.00**	**1.29*** **	**1.00**	**3.05*** **	**1.00**	**2.15*** **	**1.00**	**2.84*** **	**1.00**	**2.20*** **	**1.00**
Direct radiation	-0.06	0.38	-0.18	0.34	**-1.05$^{\#}$**	**0.73**	n	0.26	**1.57** **	**0.94**	**-2.00** **	**0.95**
Boulders	-0.04	0.31	-0.08	0.29	n	0.26	0.33	0.28	-0.249	0.27	n	0.26
Buildings	-0.09	0.57	**-1.03** **	**1.00**	-1.16	0.68	-0.63	0.36	**-2.57***	**0.99**	**1.37$^{\#}$[1]**	**0.73**
Trees above	0.03	0.30	-0.22	0.52	**1.43***	**0.91**	-0.43	0.36	-0.374	0.32	n	0.29
Shrubs above	**0.18*** **	**1.00**	-0.07	0.28	n	0.27	n	0.25	-0.282	0.28	0.643	0.35
Evergreens above	-0.06	0.36	-0.25	0.50	0.38	0.35	**-3.94$^{\#}$**	**0.96**	-0.776	0.43	n	0.27
Elevation	-0.06	0.28	**0.37** **	**0.91**	**1.99** **	**1.00**	0.41	0.29	-0.454	0.32	**1.99** **	**0.99**
Precipitation	-0.06	0.30	0.15	0.35	-0.26	0.27	-0.23	0.28	n	0.26	n	0.25
Distance to river	-0.09	0.45	-0.20	0.36	0.61	0.41	0.96	0.53	-0.74	0.43	0.382	0.29

[1]Note: For *P. longifolium* the predictor “buildings” corresponds to the minimum Euclidean distance to the next building (see text).

Characterisation of bryophyte communities

The four clusters retrieved by k-means clustering contained 27 to 49 boulders per cluster, and 11 to 14 significant indicator species were identified per cluster (Table 4). Boulder specialists only appeared as indicator species in the two clusters c2 and c3 (six and three specialist species, respectively). Significantly more boulder specialists occurred on boulders in these two clusters (Fig. 4).

Significant ecological differences among the clusters (Fig. 4) led to their circumscription as different bryophyte communities. Clusters c1 and c2 corresponded to open land communities, while clusters c3 and c4 corresponded to forest communities. This bipartition was indicated by higher forest and tree cover canopy values for c3 and c4 and the higher radiation values for c1 and c2. The open land communities had higher lichen cover and lower bryophyte and litter cover than forest communities. Also, the open land communities had higher light and lower moisture indicator values than the forest communities. Among the two open land communities, c1, the community characterised by a lack of boulder specialist indicator species (Table 4), showed more signs of anthropogenic disturbance: the highest cover of nearby buildings, the highest indicator values for nutrients and hemeroby, and the lowest bryophyte cover. Among the two forest communities, c3, the community characterised by boulder specialist indicator species (Table 4), corresponded to closed forest, while c4 rather corresponded to forest edges or hedges. This was indicated by the higher forest cover for c3, while c4 had a higher shrub canopy cover. Further, c3 had a higher humus cover and higher canopy cover of evergreen species above, and the indicator values for light, nutrients, reaction and hemeroby were lower than for c4 while the values for moisture were higher. The number of additional boulders in c3 was higher than in c2 but not significantly different from in c1 and c4. Boulders were largest in c2, followed by cluster c3 and finally c1 and c4. This ranking was the same for the number of boulder specialist species, while for the total number of species only c2 had significantly more species per boulder than the other clusters.

Table 4 Characterisation of the four k-means clusters with indicator species. c1–c4: clusters; n: number of boulders. Significant indicator species (boulder specialists in bold) are listed, sorted by the indicator value (IndVal) for the corresponding cluster (c1–c4), along with the significance level of the permutation test (sign.): *** *p* <0.001 ,** *p* <0.01, * *p* <0.05.

		IndVal for clusters				
Cluster	Indicator species	c1	c2	c3	c4	sign.
c1: n = 27						
	Grimmia pulvinata	0.58	0.12	0	0	***
	Orthotrichum affine	0.53	0.07	<0.01	<0.01	***
	Orthotrichum diaphanum	0.37	<0.01	0	<0.01	***
	Schistidium apocarpum aggr.	0.34	0.14	<0.01	0.24	***
	Orthotrichum anomalum	0.30	0.08	0	0.01	***
	Tortula muralis	0.25	<0.01	0	<0.01	***
	Orthotrichum pallens	0.23	<0.01	0	<0.01	***
	Rhynchostegium confertum	0.19	0	0	0	***
	Ceratodon purpureus	0.18	0.10	<0.01	0	**
	Bryum argenteum	0.18	0.04	0	0	**
	Syntrichia papillosa	0.16	0.03	0	0	**
	Syntrichia virescens	0.11	0	0	0	**
	Orthotrichum speciosum	0.11	0.02	0	0	*
	Orthotrichum obtusifolium	0.09	0.01	0	0	*
c2: n = 33						
	Hedwigia ciliata	<0.01	0.56	0.02	0.05	***
	Pterigynandrum filiforme	<0.01	0.39	0.07	<0.01	***
	Orthotrichum rupestre	<0.01	0.36	0	0	***
	Grimmia trichophylla	<0.01	0.31	0.03	<0.01	***
	Hypnum cupressiforme aggr.	0.14	0.29	0.28	0.18	**
	Grimmia ovalis	0	0.28	0	<0.01	***
	Bryum capillare aggr.	0.13	0.28	<0.01	0.01	***
	Orthotrichum lyellii	0.03	0.21	0	<0.01	***
	Homalothecium sericeum	0.03	0.15	<0.01	0.10	*
	Leucodon sciuroides	0.06	0.14	0	<0.01	**
	Syntrichia ruralis aggr.	0.04	0.13	0	0.04	*
	Grimmia laevigata	0.02	0.10	0	0	*
	Orthotrichum stramineum	0.02	0.10	0	0	*
	Grimmia longirostris	0	0.09	0	0	*
c3: n = 49						
	Grimmia hartmanii	0	0.28	0.40	<0.01	***
	Isothecium alopecuroides	<0.01	0.04	0.36	0.21	***
	Plagiothecium nemorale	0	0	0.33	0.01	***
	Paraleucobryum longifolium	0	0.04	0.30	<0.01	***
	Dicranum scoparium	0	<0.01	0.28	0.02	***
	Polytrichum formosum	0	0.03	0.28	<0.01	***
	Thuidium tamariscinum	0	0	0.27	0.01	***
	Plagiothecium laetum	0	0	0.24	0	***
	Rhizomnium punctatum	0	0	0.22	0	***
	Dicranum fulvum	0	<0.01	0.20	0	***
	Eurhynchium striatum	0	0	0.17	<0.01	**
	Plagiochila asplenioides aggr.	0	0	0.16	0.04	**
	Eurhynchium angustirete	0	0	0.08	0	*
c4: n = 42						
	Anomodon attenuatus	0	0	0	0.31	***
	Sciuro-Hypnum populeum	0.01	0.20	0.02	0.27	**
	Brachythecium rutabulum	0.03	0.17	0.10	0.26	**
	Metzgeria furcata	0.01	0.08	0.12	0.25	**
	Porella platyphylla	0.01	0.07	0.01	0.17	*
	Anomodon viticulosus	0	0	<0.01	0.17	**
	Bryum moravicum	0.02	0.12	0	0.17	*
	Homalia trichomanoides	0	0	<0.01	0.13	**
	Homalothecium lutescens	0	<0.01	0	0.09	*
	Ctenidium molluscum	<0.01	0	<0.01	0.08	*
	Taxiphyllum wissgrillii	0	0	0	0.07	*

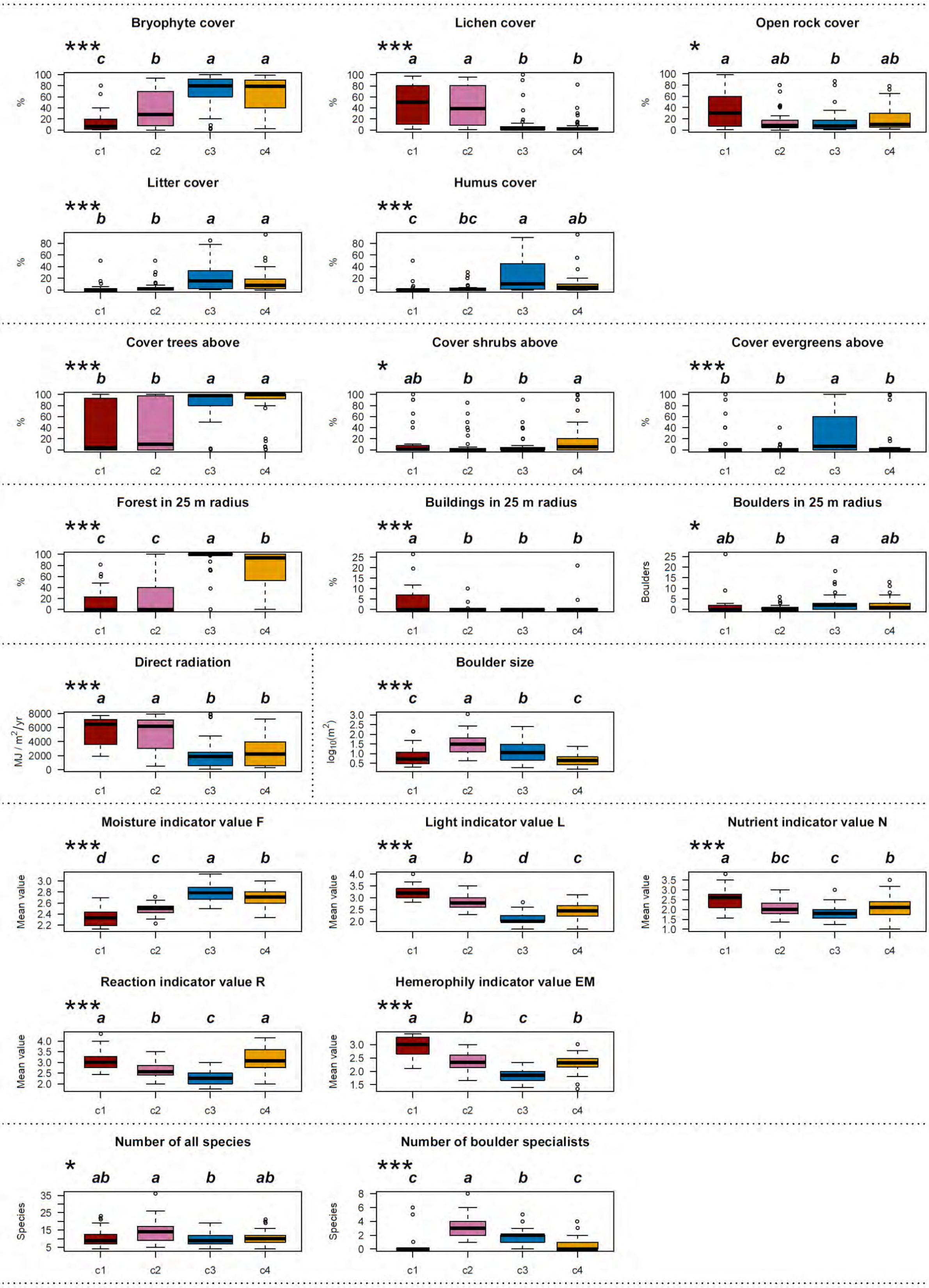


Fig. 4 Characterisation of the four k-means clusters (c1–c4; codes as in Table 4) by variables that showed significant differences among clusters. Significance level of Kruskal-Wallis tests: *** p <0.001 ,** p <0.01, * p <0.05; letters denote which groups are significantly different according to post-hoc comparisons.

Ordination and radiation gradient

The patterns in the DCA (Fig. 5) generally reflected the differences and affinities of clusters, species, and ecological variables found in the other analyses. The overlaid cluster assignments resolved in spatial vicinity, and cluster indicator species (Table 4) resolved in their cluster's direction. When projected as a trend surface (Fig. S1), the three variables that showed the most uniform linear trends across the plot area were the moisture indicator value and direct radiation, pointing in opposite directions and thus separating forest from open land communities, and the hemeroby indicator value, pointing towards the direction separating communities lacking boulder specialists.

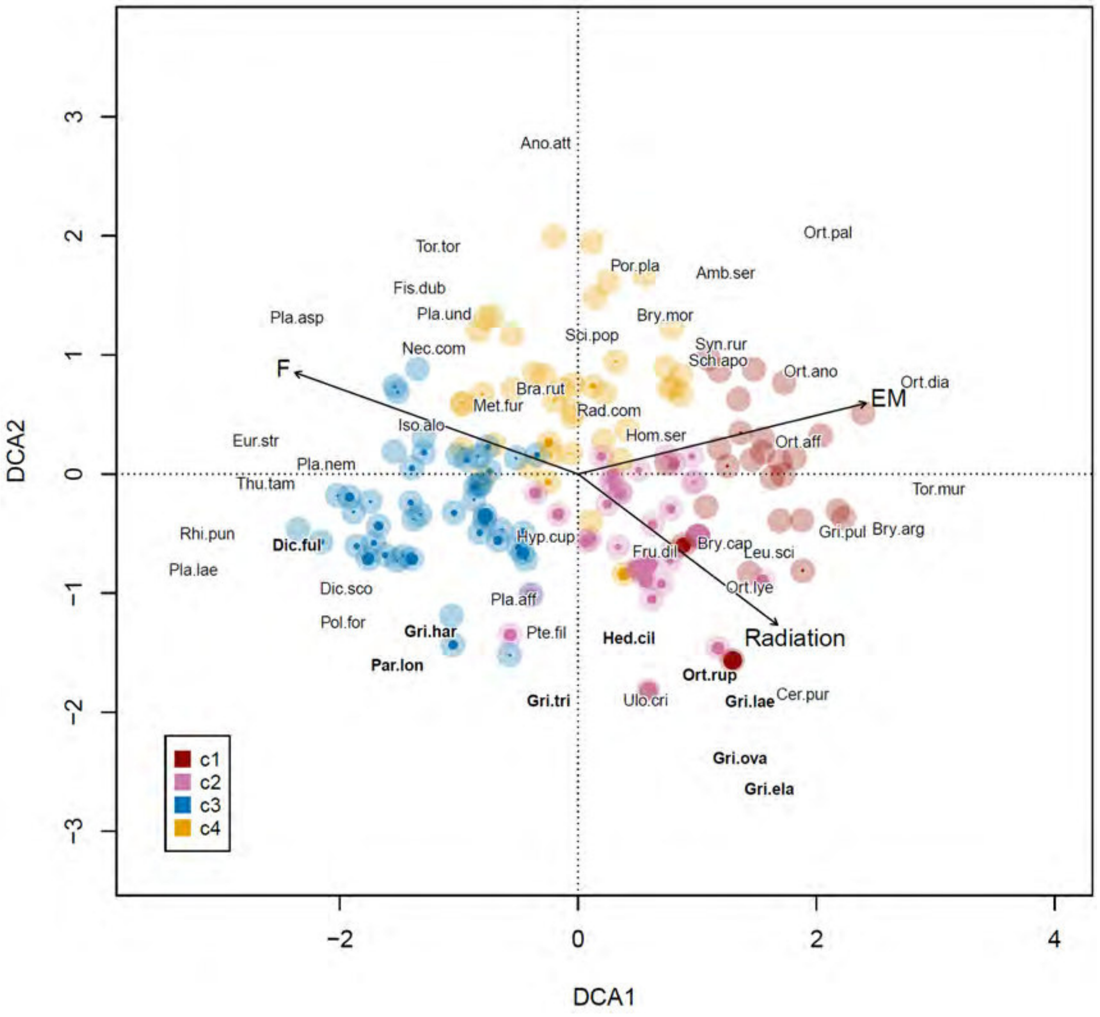


Fig. 5 Detrended correspondence analysis (DCA) of bryophyte species composition on 151 siliceous erratic boulders. Circles represent individual boulders, with the colour indicating their assignment to k-means clusters (codes as in Table 4). The size of the filled part of the circles is proportional to the number of boulder specialists on the erratic boulders (minimum: 0; maximum: 8). Boulder specialists with more than 5 occurrences are given in bold, and non-specialist species with more than 10 occurrences are given in normal font. Arrows indicate the direction and relative strength (length) of linear correlations with direct radiation (Radiation) and with mean indicator values for hemeroby (EM) and moisture (F).

Boulder specialists occurred across the whole range of direct radiation present in the dataset, but the individual species showed differences in their preferences and amplitudes for direct radiation (Fig. 6). Forest boulders generally had lower radiation values than non-forest boulders, two specialists were exclusively found on boulders in forests, and seven specialists were exclusively found on boulders outside forests.

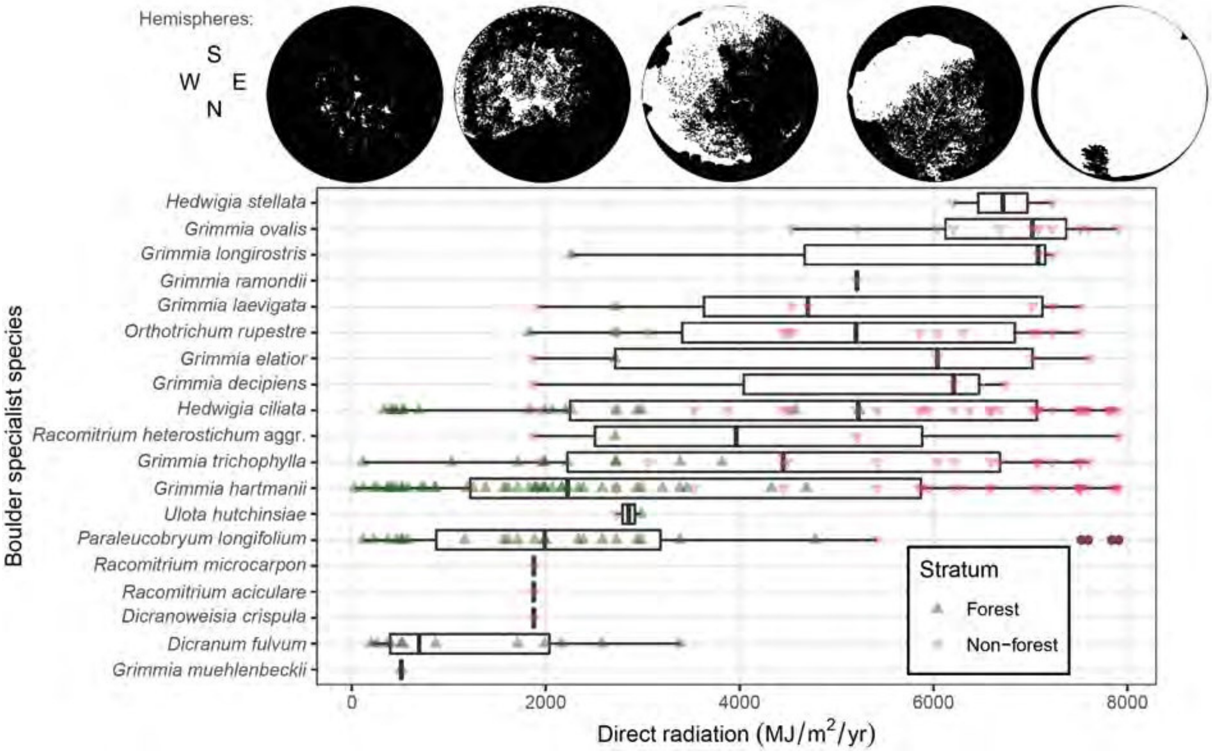


Fig. 6 Boxplots for the potential annual direct radiation for erratic boulders on which boulder specialist species were recorded. Triangles depict occurrences along the radiation gradient and their colours indicate whether the corresponding boulder was located in the forest or the non-forest stratum. The images above the plots are examples of the synthetic hemispherical images from which the radiation values were derived.

Discussion

Diversity of boulders and species

The current diversity and distribution of Swiss erratic boulders is mainly shaped by their massive historical depletion and was aptly summarised by Akçar et al. (2011), who stated that most of the extant erratic boulders are in forests, along property boundaries, or of poor stone quality. This pattern was also evident in our study areas, where 82% of the erratic boulders for which coordinates were available were in forests (Table S1). During fieldwork we observed cases of erratic boulders that were obviously removed from agricultural fields (pers. observation), and ongoing destruction of erratic boulders in open land even seemed to be an issue. This may explain why 24% of boulders in the non-forest stratum were not found at the original coordinates, compared with only 10% for the boulders within forests (Table S1).

By focusing on sites that are known to contain abundant and large erratic boulders and by using a stratified sampling approach, we deliberately enhanced the chances of detecting many boulder specialists. Therefore, our study rather documents the maximum boulder specialist diversity that can be expected in the Jura Mountains and the Swiss Plateau. Especially in the Swiss Plateau, large areas have been depleted of erratic boulders to such a degree that it is hardly possible to select a 50 km^2 area that contains 10 siliceous erratic boulders outside of forests (Swisstopo 2011).

We found 19 specialist bryophyte species of boulders, among which 4 are nationally threatened (vulnerable; Table 2; Schnyder et al. 2004). Meylan (1912) mentioned four further boulder specialist species that were recently confirmed outside our sample, namely *Andreaea rupestris* (LC; Hepenstrick 2018), *Grimmia alpestris* (LC; Hepenstrick 2021), *Syzygiella autumnalis* (EN; Swissbryophytes 2021) and *Orthotrichum urnigerum* (CR; Swissbryophytes 2021). For three further boulder specialists mentioned by Meylan (1912) – *Bazzania flaccida* (LC), *Frullania jackii* (NT) and *Pterogonium gracile* (VU) – no records are known to exist from after 1950 from the Swiss Plateau and Jura Mountains (Swissbryophytes 2021). Including additional potential boulder specialist species that have not yet been recorded (e.g. further species of the genus *Grimmia*; Hepenstrick 2021), the total number of boulder specialist bryophytes present in the Swiss Plateau and Jura Mountains may be around 30 species. Regarding the small total habitat area sampled here (5000 m^2; Fig. 3), the 19 specialist species can be considered a major contribution to biodiversity at the landscape level, which underlines the special biological conservation value of erratic boulders.

Boulder size and species richness

In agreement with Weibull and Rydin (2005) and Virtanen and Oksanen (2007), we confirmed positive species–area relationships on erratic boulders. This finding is not surprising, considering that the size of the sampled boulders spanned three orders of magnitude and the ubiquity of positive species–area relationships in ecology (Drakare et al. 2006). The comparably steep slope of our species–area curve for specialist bryophyte species of siliceous erratic boulders (Fig. 2), corresponding to a z-value of 0.40, matched well with the z-value of 0.39 for bryophyte and lichen species restricted to calcareous erratic boulders in Finland reported by Virtanen and Oksanen (2007). While the Finnish study ignored species also occurring in the matrix around the boulders, we compared boulder specialist species richness to total species richness, for which we found a distinctively lower z-value of 0.21. This pattern is in agreement with island biogeography theory (MacArthur and Wilson 1967), as it underlines the island-like properties of erratic boulders for specialist species: the colonisation and extinction of specialist species are more strongly influenced by the size of their special habitat island than by the colonisation and extinction of species that also occur in the matrix, which results in a steeper species–area curve for island specialists. This pattern of higher z-values for habitat specialists in habitat islands was also found by Dembicz et al. (2020) for vascular plant species in insular steppe fragments that exhibited $z = 0.32$ for total species richness and $z = 0.43$ for specialist species of steppes.

SLOSS analysis showed that several small boulders harbour more species than a single large boulder or few large boulders adding up to the same surface area (i.e. SL < SS; Fig. 3). This result is contrary to the original theory (SL > SS; Diamond 1975), but it is in agreement with most empirical studies conducted to explore this relationship (reviewed by Fahrig 2020). In our case, we explain the observed relationship of SL < SS with the pronounced ecological and floristic gradients among sampled boulders (Fig. 5) that likely exceed the maximum gradient possibly present on a single large boulder. In other words, a set of several small patches is environmentally more heterogeneous than a one single large patch (Fahrig 2020).

In summary, our analyses of boulder size and richness of boulder specialists suggest that large and small erratic boulders are both important for boulder species richness at the landscape scale. On the one hand, a strong positive species–area relationship showed that large boulders are more likely to harbour boulder specialist species (Fig. 2). The 20 largest boulders in our sample of 160 boulders harboured 14 of the 19 boulder specialist species found in total (Fig. 3). In fact, on the largest boulder in our sample we found the most boulder specialist species. On the other hand, the SLOSS analysis demonstrated that a set of small boulders harbour more and different boulder specialist species than a few large boulders. The importance of small boulders is exemplified with *Grimmia ramondii,* whose only record in our study was on a boulder of only 4.4 m^2 surface area (corresponding to a cube with a side length of 0.94 m), which also harboured three other boulder specialist bryophytes.

Ecological drivers

In congruence with our univariate analysis of boulder size (Fig. 2), boulder size was the dominant positive predictor in the GLMM analyses (Table 3). For the other predictors, however, total species richness, specialist species richness and single species responded differently and sometimes in contrasting directions. Such diverse responses were also reported by Kiebacher et al. (2017) for bryophytes on island-like trees in pastures.

Besides boulder size, total species richness was only affected by shrub cover. We presume that this positive effect on species richness is due to a microclimatic balancing effect of the shrub leaf canopy on the otherwise rather extreme rock microclimate (Larson et al. 2000). This balancing effect thus enables the growth of a wider variety of species on boulders, including epiphytic species (e.g. *Orthotrichum* spp.).

Boulder specialist species richness was affected negatively by nearby buildings and positively by elevation. The negative influence of nearby buildings may be due to frequent human disturbance. For instance, boulders may only recently have been excavated during the construction of the nearby buildings, and cleaning of boulders in gardens may impede the growth of bryophytes (Wächter 1996). The positive effect of elevation on specialist species may be caused by different aspects of this multifaceted predictor that correlates with e.g. temperature, deposition of atmospheric pollutants (Lovett and Kinsman 1990) and land-use intensity (Körner 2007).

In line with the negative response of boulder specialist richness to nearby buildings, the responses of single species to buildings were also negative. The response of boulder specialist species to elevation was also positive for three of the four species tested. Only *Hedwigia ciliata* showed a negative, but statistically not significant, response to elevation, which may reflect the thermophilic preferences of this species. For other predictors, boulder specialist species displayed rather diverse responses, reflecting their different environmental niches (Nebel and Philippi 2000). The typical forest species *Grimmia hartmanii* and *Paraleucobyrum longifolium* were negatively affected by direct radiation, while *H. ciliata* showed a positive response. The positive effect of tree cover found for *G. hartmanii* is in line with the findings of Weibull (2001), who stated that this species thrives on boulders influenced by rain throughfall and litter of deciduous trees. While Weibull and Rydin (2005) found reduced species richness on boulders under evergreen trees (*Picea abies*), in our analyses we only found a marginally significant negative effect of the cover of evergreen trees in *G. trichophylla*.

Regarding the pronounced positive influences of boulder size, the absence of significant effects of nearby erratic boulders was surprising and in contrast to the results of Virtanen and Oksanen (2007), who found a positive effect of connectivity among boulders on species richness in their study area, in which they sampled every single boulder. However, with our sampling design we did not specifically aim at addressing connectivity among boulders, and the fact that we sampled the largest boulder in cases where multiple boulders were present further hampered the chances of detecting a connectivity effect by investigating nearby boulders.

The two predictors that were thought to describe water availability, i.e. precipitation and distance to the nearest river, did not show significant effects on response variables, despite a humidity gradient in our dataset that was indicated by moisture indicator values (Fig. 5). Consequently, we conclude that the microclimatic influence on humidity, governed by local radiation and canopy cover, are more important for the bryophyte species composition on erratic boulders than the more general predictors precipitation and distance to the nearest river.

Characterisation of bryophyte communities

The major ecological and floristic bipartition into forest boulders with high bryophyte cover and open land boulders with high lichen cover (Fig. 4) is in agreement with the original description of the typical bryophyte communities of siliceous erratic boulders in the Jura Mountains by Meylan (1912). As a third group, Meylan (1912) mentioned a hygrophilous community, characterised by *Dicranoweisia crispula* and *Blindia acuta*, which was not part of our dataset because we excluded boulders touching water surfaces. Notably, one boulder in our sample (described in Hepenstrick 2020) matched this hygrophilous community, as it harboured *D. crispula* and the only hygrophilous boulder specialist found in our dataset, *Racomitrium aciculare* (Table 2; Nebel and Philippi 2000).

While Meylan (1912) did not further split forest and open land bryophyte communities on erratic boulders, we found an additional bipartition of both groups into communities that differed in the presence or absence of boulder specialists.

The four clusters found in our study can be assigned to floristically and ecologically described syntaxonomical units (Mucina et al. 2016) by their indicator species (Table 4) and the ecological variables (Fig. 4). Clusters c2 and c3, characterised by boulder specialists, match communities within the class *Racomitrietea heterostichi*, which comprises bryophyte communities on siliceous rock: the open land cluster c2 corresponds to the alliance *Grimmion commutatae*, which comprises associations on dry and sunny siliceous rock, and the forest cluster c2 corresponds to the alliance *Grimmio hartmanii–Hypnion cupressiformis*, which comprises associations on shaded siliceous rock. In contrast, clusters c1 and c4, characterised by the absence of boulder specialists, match communities that typically occur on calcareous rock or bark: the open land cluster c1 corresponds to the alliance *Schistidion apocarpi*, which comprises associations on exposed limestone rock and mortar walls, and the forest cluster c4 corresponds to the alliance *Neckerion complanatae*, which comprises bryophyte communities on shaded, base-rich rock or bark. Hence, c2 and c3 represent the actual insular specialist communities on siliceous erratic boulders in calcareous areas, while c1 and c4 represent generalist communities composed of species that source from the landscape matrix around the boulders.

This bipartition into specialist and generalist communities can be explained by the disturbances to boulders that prevent the establishment of specialist communities. For the open land generalist community c1, which is associated with buildings and high indicator values for nutrients and hemeroby (Fig. 4), our findings are in agreement with those of Wächter (1996), who found that anthropogenically disturbed siliceous erratic boulders in settlements contain species of the *Schistidion apocarpi* alliance but no boulder specialist bryophytes. For the forest generalist community c4, which is associated with a high shrub and a lower forest cover, indicating their occurrence at forest edges and in young forest, our results are in agreement with those of Meylan (1912), who observed that boulders in recently clearcut forests stocked with young trees lose their boulder specialists and only harbour generalist species. Additionally, both specialist communities (c2 and c3) occur on larger boulders than generalist communities (Fig. 4), which again underlines the island properties of erratic boulders whose specialist species are more likely to go extinct on small than on large boulders.

Conclusions and recommendation for conservations

We found that large boulders are more likely to harbour numerous boulder specialists than small boulders (Fig. 2), but also that, at the landscape level, many small boulders contribute more specialist species than a few large boulders (Fig. 3). For conservation practice, we translate these findings into the need to prioritise large boulders while not neglecting small ones. First, prioritising large boulders efficiently maximises the number of species covered with a minimal number of boulders considered. Second, large boulders are more prone to direct anthropogenic impacts that require mitigation, such as the removal of vegetation for sport climbing (Blum 2015) or for uncovering of archaeological and mystic aspects (Fässler 2020). Third, large boulders are often protected as geosites (Reynard et al. 2004; Gonggrijp 2000), which facilitates the protection of their vegetation as a genuine characteristic of the geosite itself. And fourth, large boulders well known by the public may act as flagships that raise awareness for rare bryophytes that are also present on small erratic boulders. Direct conservation of the boulder species diversity contributed by small erratic boulders is challenging because information on their precise location is often lacking (Swisstopo 2011). However, small boulders may be particularly prone to anthropogenic destruction. Hence, conserving the boulder specialist diversity contributed by small boulders may be best achieved by raising the general awareness of the conservation value of erratic boulders amongst stakeholders in forestry, agriculture and nature conservation.

We found that erratic boulders are about five times less abundant outside of forests than in forests (Table S1), that erratic boulders near settlements are less likely to harbour boulder specialist species (Table 3), and that the boulder specialist communities in open land harbour different and more specialist species than boulder specialist communities in forests (Table 4; Fig. 4). From these findings we conclude that special conservation attention should be placed on open land erratic boulders outside of settlements. First, these rare boulders are likely to harbour species that are rare at the landscape level (Fig. 6). Second, open land erratic boulders are still threatened by removal from agricultural areas. Finally, siliceous erratic boulders in full sun are hotspots not only for specialist bryophytes but also for many specialist lichen species (Meylan 1922; Epard et al. 2020).

Bryophytes are efficiently conserved through the conservation of their habitats (Hallingbäck and Hodgetts 2000). Hence, maintaining the integrity and position of erratic boulders is the foundation for the conservation of their special vegetation. Boulder vegetation should not be removed or disturbed, which happens when a boulder becomes covered by timber or agricultural deposits. In addition, major changes to a boulder's surroundings, such as largescale forest clearing or tree planting, should be avoided, as the specialist species present on an erratic boulder reflect its position on an ecological gradient from shady and humid to sunny and dry habitats (Fig. 6). Consequently, active conservation measures targeting the vegetation of a single boulder should be based on its species composition and on what is known on the ecological preferences of the species (Hallingbäck and Hodgetts 2000). For example, removing surrounding trees may be detrimental or beneficial, depending on the light requirements of the boulder specialists present.

Traditionally, erratic boulders have only received conservation attention as geosites witnessing the Ice Ages (Reynard 2004; Akçar et al. 2011). However, our study highlights the boulders' biological value, i.e. their function as habitat for specialised bryophytes, lichens (Krawiec 1938; Epard et al. 2020) and ferns (Mazenauer et al. 2014). Hence, erratic boulders unite geodiversity and biodiversity (Alahuhta et al. 2020) in a uniquely condensed way, and the conservation of both aspects can go hand in hand. Considering the vast areas which have experienced Pleistocene glaciations (Ehlers and Gibbard 2007), erratic boulders are a global phenomenon. Thus, the conclusions of our study may well apply to erratic boulders elsewhere and to the conservation of insular rock-dwelling cryptogam communities in general.

Acknowledgements

We thank Patrice Descombes and Dirk Karger for sharing processed climate data, and Thomas Kiebacher for identifying *Orthotrichum* species. We are grateful for financial support from several foundations, associations, and governmental and non-governmental organisations.

References

Akçar N, Ivy-Ochs S, Kubik PW, Schlüchter C (2011) Post-depositional impacts on 'Findlinge'(erratic boulders) and their implications for surface-exposure dating. Swiss Journal of Geosciences 104:445-453 https://doi.org/10.1007/s00015-011-0088-7

Alahuhta J, Toivanen M, Hjort J (2020) Geodiversity–biodiversity relationship needs more empirical evidence. Nature Ecology & Evolution 4:2-3 https://doi.org/10.1038/s41559-019-1051-7

Alvarez M (2020) R package lexiguel: miscellaneous functions. v.0.1.0., https://github.com/kamapu/lexiguel

Antz W, Trachsel P, Schmid R (2019) Bimano, smartphone app. v.2.2, bimano GmbH, Ittingen

BAFU (2006) Die biogeographischen Regionen der Schweiz (CH). Bundesamt für Umwelt, Bern

Bartoń K, (2015) Package 'MuMIn'. v.1.43.4., https://cran.r-project.org/web/packages/MuMIn

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48 https://doi.org/10.18637/jss.v067.i01

Bergamini A (2015) Moose im Kanton Schaffhausen. Naturforschende Gesellschaft Schaffhausen, Schaffhausen

Bini A, Buoncristiani JF, Couterrand S, Ellwanger D, Felber M, Florineth D, Graf HR, Keller O, Kelly M, Schlüchter, C (2009) Die Schweiz während des letzteiszeitlichen Maximums (LGM), Karte 1 : 500 000. Bundesamt für Landestopographie, Wabern

Blum C (2015) Steinhof - Steineberg Klettern, Familienklettern, Bouldern am grössten Findling im Mittelland und seinen Vasallen. Topo, Basel

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution 24:127-135 https://doi.org/10.1016/j.tree.2008.10.008

Borcard D, Gillet F, Legendre P (2018) Numerical ecology with R. Springer, Berlin

Brockmann-Jerosch H, Brockmann-Jerosch M (1926) Die Geschichte der schweizerischen Alpenflora. In: Schröter C (ed) Das Pflanzenleben der Alpen. Raustein, Zürich, pp 1149-1199

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Colpa JG, van Zanten BO (2006) Mossen op de Nederlandse hunebedden in 2004/2005. Buxbaumiella 75:34-50

Culmann P, Weber J (1901) Verzeichnis der Laubmoose des Kantons Zürich. Mitteilungen der Naturwissenschaftlichen Gesellschaft in Winterthur 3:3-79

Dembicz I, Moysiyenko II, Kozub Ł, Dengler J, Zakharova M, Sudnik-Wójcikowska B (2020) Steppe islands in a sea of fields: where island biogeography meets the reality of a severely transformed landscape. Journal of Vegetation Science 32:e12930 https://doi.org/10.1111/jvs.12930

Dengler J (2009) Which function describes the species–area relationship best? A review and empirical evaluation. Journal of Biogeography 36:728-744

Descombes P, Walthert L, Baltensweiler A, Meuli RG, Karger DN, Ginzler C, Zurell D, Zimmermann NE (2020) Spatial modelling of ecological indicator values improves predictions of plant distributions in complex landscapes. Ecography 43:1448-1463

Diamond JM (1975) The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. Biological Conservation 7:129-146

Drakare S, Lennon JJ, Hillebrand H (2006) The imprint of the geographical, evolutionary and ecological context on species–area relationships. Ecology Letters 9:215-227

Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366

Ehlers J, Gibbard PL (2007) The extent and chronology of Cenozoic Global Glaciation. Quaternary International 164:6-20 https://doi.org/10.1016/j.quaint.2006.10.008

Epard JL, Gex P, Vust M (2020) Les blocs erratiques propriété de la Société Vaudoise des Sciences Naturelles. Bulletin de la Société Vaudoise des Sciences naturelles 99:29-66

Fahrig L (2020) Why do several small patches hold more species than few large patches? Global Ecology and Biogeography 29:615-628

Fässler B (2020) Schalensteine und Megalithkultur im Kanton Solothurn. Corvo di Notte, Oensingen

Gonggrijp GP (2000) Planning and management for geoconservation. In: Barettino D, Wimbledon WAP, Gallego E (eds) Geological Heritage: its conservation and management. Instituto Tecnológico Geominero de España, Madrid, pp 29-45

Geheeb A (1864) Die Laubmoose des Cantons Aargau. Sauerländer, Aarau

Hallingbäck T, Hodgetts N (2000) Status survey and conservation action plan for bryophytes: mosses, liverworts and hornworts. IUCN, Cambridge

Hepenstrick D (2018) *Andreaea rupestris*. In: Bergamini A, Hepenstrick D, Hofmann H, Joss S, Kiebacher T, Meier M, Müller N, Roloff F, Schnyder N (2018) Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 13. Meylania 61:5-18 https://doi.org/10.21256/zhaw-2028

Hepenstrick D (2020) *Racomitrium microcarpon*. In: Bergamini A, Hepenstrick D, Hofmann H, Kiebacher T, Moser T, Müller N, Schnyder N, Stix S, Urmi E (2020) Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 15. Meylania 65:5-17 https://doi.org/10.21256/zhaw-21319

Hepenstrick D (2021) *Grimmia montana*. In: Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 16. Meylania 67: accepted

Hepenstrick D, Urmi E, Meier MK, Bergamini A (2016) Die Moosflora des silikatischen Findlings Alexanderstein in Küsnacht (ZH). Meylania 57:15-23 https://doi.org/10.21256/zhaw-2029

Hill MO, Preston CD, Bosanquet SDS, Roy DB (2007) BRYOATT Attributes of British and Irish mosses, liverworts and hornworts. Centre for Ecology and Hydrology, Cambridgeshire

Hjort J, Gordon JE, Gray M, Hunter Jr ML (2015) Why geodiversity matters in valuing nature's stage. Conservation Biology 29:630-639 https://doi.org/10.1111/cobi.12510

Imbrie J, Imbrie KP (1986) Ice Ages: solving the mystery. Harvard University Press, Cambridge

Ingerpuu N, Vellak K, Ehrlich L (2018) Revised red data list of Estonian bryophytes. Folia Cryptogamica Estonica 55:97-104 https://doi.org/10.12697/fce.2018.55.10

Jarvis A, Reuter HI, Nelson A, Guevara E (2008) Hole-filled seamless SRTM data V4. International Centre for Tropical Agriculture http://srtm.csi.cgiar.org.

Karger DN et al. (2017) Climatologies at high resolution for the earth's land surface areas. Scientific Data 4:1-20

Kiebacher T, Keller C, Scheidegger C, Bergamini A (2017) Epiphytes in wooded pastures: isolation matters for lichen but not for bryophyte species richness. PloS ONE 12:e0182065 https://doi.org/10.1371/journal.pone.0182065

Kimmerer RW, Driscoll MJL (2000) Bryophyte species richness on insular boulder habitats: the effect of area, isolation, and microsite diversity. Bryologist 103:748-756 https://doi.org/10.1639/0007-2745(2000)103[0748:Bsroib]2.0.Co;2

Korner-Nievergelt F, Roth T, Von Felten S, Guélat J, Almasi B, Korner-Nievergelt P (2015) Bayesian data analysis in ecology using linear models with R, BUGS, and STAN. Elsevier, Amsterdam

Körner C (2007) The use of ‘altitude’ in ecological research. Trends in Ecology & Evolution 22:569-574

Krawiec F (1938) Flora epilityczna glazow narzutowych zachodniej polski. Jachowskiego, Poznan

Lawyer J, Haas J (2008) Adirondack rock, a rock climber’s guide. Adirondack Rock Press, Pompey NY

Landolt E, Bäumler B, Erhardt A, Hegg O, Klötzli F, Lämmler W, Nobis M, Rudmann-Maurer K, Schweingruber F, et al. (2010) Flora indicativa: ökologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen. Haupt, Bern

Larson DW, Matthes U, Kelly PE (2000) Cliff ecology: pattern and process in cliff ecosystems. Cambridge University Press, Cambridge

Lovett GM, Kinsman JD (1990) Atmospheric pollutant deposition to high-elevation ecosystems. Atmospheric Environment Part A 24:2767-2786 https://doi.org/10.1016/0960-1686(90)90164-I

MacArthur RH, Wilson E (1967) The theory of island biogeography. Princeton University Press, Princeton

Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K (2019) Cluster: cluster analysis basics and extensions, v.2.1.0. https://CRAN.R-project.org/package=cluster

Mazenauer D, Holderegger R, Krüsi B, Hepenstrick D (2014) Populationsentwicklung und Gefährdung von *Asplenium septentrionale* auf Findlingen im Schweizer Mittelland und Jura. Bauhinia 25:37-50

Meier MK, Urmi E, Schnyder N, Bergamini A, Hofmann H (2013) Checkliste der Schweizer Moose. Nationales Inventar der Schweizer Moosflora, Zürich

Meylan C (1912) La flore bryologique des blocs erratiques du Jura. Bulletin de la Société Vaudoise des Sciences Naturelles 48:49-70 https://dx.doi.org/10.5169/seals-269347

Meylan C (1922) Contribution à la connaissance des lichens du Jura. Bulletin de la Société Vaudoise des Sciences Naturelles 45:287-294 https://dx.doi.org/10.5169/seals-270919

Meylan C (1926) La flore bryologique et lichénologique du bloc erratique de La Grange-de-la-Côte. Bulletin de la Société Vaudoise des Sciences Naturelles 56:165-172 https://dx.doi.org/10.5169/seals-271595

Mucina L, Bültmann H, Dierßen K, Theurillat JP, Raus T, Čarni A, Šumberová K, Willner W, Dengler J, et al. (2016) Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. Applied Vegetation Science 19:3-264 https://dx.doi.org/10.1111/avsc.12257

Nebel M, Philippi G (eds) (2000) Die Moose Baden-Württembergs. Ulmer, Stuttgart

Oksanen J et al. (2019) Vegan: community ecology package. v.2.5-6. https://cran.r-project.org/package=vegan

Philippe M (2010) Bryophytes saxicoles calcarifuges sur substrats exotiques dans l’Ain. Les Nouvelles Archives de la Flore jurassienne et du nord-est de la France 8:213-218

Quinn JF, Harrison SP (1988) Effects of habitat fragmentation and isolation on species richness: evidence from biogeographic patterns. Oecologia 75:132-140

R Core Team (2017) R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Wien. https://www.R-project.org/

Reynard E (2004) Protecting stones: conservation of erratic blocks in Switzerland. In: Prikryl R (ed) Dimension stone 2004. New perspectives for a traditional building material. Balkema, Leiden, pp 3-7

Roberts D (2019) R package labdsv: ordination and multivariate analysis for ecology. v.2.0-1. http://ecology.msu.montana.edu/labdsv/R/

Schielzeth H (2010) Simple means to improve the interpretability of regression coefficients. Methods in Ecology and Evolution 1:103-113 https://dx.doi.org/10.1111/j.2041-210X.2010.00012.x

Schnyder N, Bergamini A, Hofmann H, Müller N, Schubiger-Bossard C, Urmi E (2004) Rote Liste der gefährdeten Moose der Schweiz. Bundesamt für Umwelt, Wald und Landschaft, Bern

Siebel HN, Bijlsma RJ, Sparrius LB (2013) Basisrapport voor de rode lijst mossen 2012. Bryologische en Lichenologische Werkgroep, Utrecht

Swissbryophytes (2021) Moosflora der Schweiz. www.swissbryophytes.ch.

Swisstopo (2011) GeoCover geologischer Atlas der Schweiz im Vektorformat. Bundesamt für Landestopografie, Wabern

Tukiainen H, Kiuttu M, Kalliola R, Alahuhta J, Hjort J (2019) Landforms contribute to plant biodiversity at alpha, beta and gamma levels. Journal of Biogeography 46:1699-1710 https://dx.doi.org/10.1111/jbi.13569

Ulvinen T, Syrjänen K, Anttila S (eds) (2002) The bryophytes of Finland: distribution, ecology, threats (in Finnish). Edita Publishing, Helsinki.

Virtanen R, Oksanen J (2007) The effects of habitat connectivity on cryptogam richness in boulder metacommunity. Biological Conservation 135:415-422 https://doi.org/10.1016/j.biocon.2006.10.013

Wächter HJ (1996) Zur Moosvegetation von Findlingen zwischen Ems und Weser. Osnabrücker Naturwissenschaftliche Mitteilungen 22:89-110

Webster C, Mazzotti G, Essery R, Jonas T (2020) Enhancing airborne LiDAR data for improved forest structure representation in shortwave transmission models. Remote Sensing of Environment 249:e112017 https://doi.org/10.1016/j.rse.2020.112017

Weibull H (2001) Influence of tree species on the epilithic bryophyte flora in deciduous forests of Sweden. Journal of Bryology 23:55-66 http://dx.doi.org/10.1179/jbr.2001.23.1.55

Weibull H, Rydin H (2005) Bryophyte species richness on boulders: relationship to area, habitat diversity and canopy tree species. Biological Conservation 122:71-79 https://doi.org/10.1016/j.biocon.2004.07.001

Wildi O (2017) Data analysis in vegetation ecology. CABI, Wallingford

Supplementary Material

Table S1 Overview of the study areas and the number of erratic boulders. The ID (as in Fig. 1), name (abbreviation of Swiss canton in brackets), central coordinates (latitude/longitude WGS84) and area of each study area is given, along with the number of 50×50 m grid cells with erratic boulders from which the sample for the study was drawn; the number of grid cells that were replaced in the field due to replacement criteria (see Note S1), with the number for the replacement criterion “no boulder found” given in brackets; and the number of erratic boulders sampled per stratum (i.e. forest, non-forest; number of boulders with at least one specialist species given in brackets).

Study area				**Number of grid cells with erratic boulders**			**Number of grid cells with erratic boulders replaced**			**Number of sampled erratic boulders**		
ID	**Name**	**Coordinates**	**Area (km^2)**	**Total**	**Forest**	**Non-forest**	**Total**	**Forest**	**Non-forest**	**Total**	**Forest**	**Non-forest**
A	Aubonne (VD)	46.52/6.41	62	130	79	51	33 (9)	10 (1)	23 (8)	20 (7)	10 (6)	10 (1)
C	Corcelles (NE)	47.00/6.88	51	297	251	46	4 (1)	1 (0)	3 (1)	20 (16)	10 (9)	10 (7)
E	Evilard (BE)	47.17/7.27	40	117	96	21	4 (2)	0 (0)	4 (2)	20 (12)	10 (8)	10 (4)
H	Herrliberg (ZH)	47.30/8.64	44	275	227	48	29 (13)	14 (5)	15 (8)	20 (13)	10 (8)	10 (5)
L	Langendorf (SO)	47.23/7.51	44	806	731	75	40 (9)	4 (0)	36 (9)	20 (13)	10 (8)	10 (5)
N	Niederwil (AG)	47.37/8.28	49	157	119	38	20 (5)	5 (2)	15 (3)	20 (6)	10 (4)	10 (2)
R	Riedtwil (BE)	47.12/7.69	58	96	68	28	26 (19)	8 (4)	18 (15)	20 (15)	10 (9)	10 (6)
V	Vuiteboeuf (VD)	46.81/6.55	59	113	73	40	5 (2)	0 (0)	5 (2)	20 (15)	10 (6)	10 (9)
		Sum:	**407**	**1991**	**1644**	**347**	**161 (60)**	**42 (12)**	**119 (48)**	**160 (97)**	**80 (58)**	**80 (39)**

Note S1 Detailed information on boulder selection and sampling

The stratified sampling of erratic boulders was set up in a geographic information system (GIS). Stratification of forest and non-forest boulders was conducted based on the forest polygons in the digital landscape model Vector25 (Swisstopo 2007). Each study area was overlayed with a 50 × 50 m grid. Each grid cell was classified as containing forest boulders, non-forest boulders or no boulders, with cells containing both forest and non-forest boulders classified as non-forest. Finally, within each study area and stratum, grid cells containing boulders were randomly assigned continuous ID-numbers and the ten lowest ID-numbers per stratum and site were chosen as the initial sample. Where this procedure selected adjacent grid cells, the cell with the lower ID-number was kept in the sample and the other one was replaced with the cell within the same stratum with the lowest ID-number of all the cells that had not already been selected.

Field work took place from November 2016 to April 2017 and from January 2018 to April 2018. Selected grid cells were visited in the field and the boulders' coordinates were found using a differential GNSS device (Geo 7X, Trimble, Sunnyvale, CA, USA), which was also used to record the precise coordinates of the boulders sampled. For a selected boulder to be sampled, a series of criteria had to be fulfilled. First, a minimum of one erratic boulder had to be present in a radius of 50 m around the selected boulders' coordinates. The aboveground dimensions had to be at least 0.5 m in height and 0.5 m in length. The boulders had to be non-calcareous (i.e. no reaction with 10% HCl). We excluded boulders in contact with water surfaces, as well as boulders protruding from slopes, which are influenced by runoff water. Boulders that were clearly outside their assigned stratum (either forest or non-forest) were excluded, but boulders in habitat types that were ecologically between the two strata, such as forest edges or hedges, were kept in the sample and the assigned stratum. If multiple boulders within a grid cell fulfilled these criteria, the largest boulder was chosen for sampling. If there was no boulder that fulfilled the above criteria within the 50 m radius around the coordinates, the selected grid cell was excluded and replaced with the nearest grid cell of the same stratum that had not already been sampled, selected or excluded. The criteria were applied again and, if necessary, the replacement procedure was repeated until the nearest replacement boulder for the originally selected boulder was found.

Table S2 Datasets from which coordinates of erratic boulders were retrieved.

Dataset
Canton de Vaude (2009) Inventaire des blocs erratiques. Direction générale de l'environnement URL: http://www.asitvd.ch/md/72 (date last accessed: 8.2.2021)
Kanton Aargau (1997). Geomorphologisches Inventar. Abteilung Landschaft und Gewässer. https://www.ag.ch/geoportal/rest/services/alg_geomorphologie/MapServer (date last accessed: 8.2.2021)
Kanton Bern (2010). Geschützte geologische Objekte des Kantons Bern. Amt für Landwirtschaft und Natur des Kantons Bern Abteilung Naturförderung. URL: https://www.geo.apps.be.ch/de/geodienste/geodienstangebot.html?view=sheet&preview=search_list&catalog=geocatalog&type=complete&guid=77ffa066-b993-466d-bc1f-33737bcb97bd (date last accessed: 8.2.2021)
Kanton Solothurn (2002) Findlinge in der Region Solothurn. Amt für Umwelt. URL: https://geoweb.so.ch/geodaten/datenbeschreibung.php?usertyp=intern&seite=descr_auswahl&id=200215 (date last accessed: 8.2.2021)
Kanton Zürich (1997) Inventar der Natur- und Landschaftsschutzgebiete von überkommunaler Bedeutung. Amt für Landschaft und Natur. URL: http://www.geolion.zh.ch/geodatensatz/show?gdsid=127 (date last accessed: 8.2.2021)
Mazenauer D. (2013) Vorkommen und Gefährdung von *Asplenium septentrionale* auf den Findlingen im Schweizer Mittelland und Jura. Wädenswil: Semesterarbeit, Zürcher Hochschule für angewandte Wissenschaften ZHAW.
Swisstopo (2011) GeoCover geologischer Atlas der Schweiz im Vektorformat. Bundesamt für Landestopografie, Wabern

Table S3 Definition of the species aggregates used.

Aggregate	**Reference / Definition**
Brachythecium salebrosum aggr.	Meier et al. (2013)
Bryum capillare aggr.	Meier et al. (2013) excluding *B. moravicum* Podp.
Bryum subapiculatum aggr.	Meier et al. (2013)
Hypnum cupressiforme aggr.	Meier et al. (2013)
Plagiochila asplenioides aggr.	Meier et al. (2013)
Plagiomnium affine aggr.	Meier et al. (2013)
Racomitrium heterostichum aggr.	Meier et al. (2013)
Radula complanata aggr.	Unpublished aggregate including *R. complanata* (L.) Dumort. and *R. lindenbergiana* C.Hartm.
Schistidium apocarpum aggr.	Meier et al. (2013) additionally including *Schistidium lancifolium* (Kindb.) H.H.Blom and *S. papillosum* Culm.
Syntrichia ruralis aggr.	Meier et al. (2013)
Ulota crispa aggr.	Meier et al. (2013)

Table S4 Bryophytes recorded on 160 siliceous erratic boulders in the Swiss Jura Mountains and on the Swiss Plateau. Frequencies are given in brackets.

Abietinella abietina (Hedw.) M.Fleisch. (1)
Amblystegium serpens (Hedw.) Schimp. (17)
Anomodon attenuatus (Hedw.) Huebener (13)
Anomodon longifolius (Schleich. ex Brid.) Hartm. (1)
Anomodon viticulosus (Hedw.) Hook. & Taylor (9)
Antitrichia curtipendula (Hedw.) Brid. (6)
Atrichum undulatum (Hedw.) P.Beauv. (7)
Barbula unguiculata Hedw. (1)
Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen *(10)*
Brachythecium albicans (Hedw.) Schimp. (2)
Brachythecium rutabulum (Hedw.) Schimp. (82)
Brachythecium salebrosum aggr. (6)
Brachythecium tommasinii (Sendtn. ex Boulay) Ignatov & Huttunen (2)
Bryum argenteum Hedw. (11)
Bryum barnesii J.B.Wood ex Schimp. (1)
Bryum capillare aggr. (35)
Bryum moravicum Podp. (28)
Bryum subapiculatum aggr. (4)
Calliergonella cuspidata (Hedw.) Loeske (1)
Ceratodon purpureus (Hedw.) Brid. (19)
Cinclidotus fontinaloides (Hedw.) P.Beauv. (1)
Cirriphyllum crassinervium (Taylor) Loeske & M.Fleisch. (3)
Climacium dendroides (Hedw.) F.Weber & D.Mohr (1)
Ctenidium molluscum (Hedw.) Mitt. (7)
Dicranoweisia cirrata (Hedw.) Lindb. (2)
Dicranoweisia crispula (Hedw.) Milde (1)
Dicranum fulvum Hook. (13)
Dicranum montanum Hedw. (4)
Dicranum scoparium Hedw. (27)
Dicranum viride (Sull. & Lesq.) Lindb. (6)
Didymodon rigidulus Hedw. (1)
Entodon concinnus (De Not.) Paris (3)
Eurhynchium angustirete (Broth.) T.J.Kop. (4)
Eurhynchium striatum (Hedw.) Schimp. (12)
Fissidens dubius P.Beauv. (12)
Frullania dilatata (L.) Dumort. (32)
Frullania tamarisci (L.) Dumort. (1)
Grimmia decipiens (Schultz) Lindb. (3)
Grimmia elatior Bruch ex Bals.-Criv. & De Not. (5)
Grimmia hartmanii Schimp. (68)
Grimmia laevigata (Brid.) Brid. (7)
Grimmia longirostris Hook. (3)
Grimmia muehlenbeckii Schimp. (1)
Grimmia ovalis (Hedw.) Lindb. (11)
Grimmia pulvinata (Hedw.) Sm. (38)
Grimmia ramondii (Lam. & DC.) Margad. (1)
Grimmia trichophylla Grev. (25)
Hedwigia ciliata (Hedw.) Ehrh. ex P.Beauv. (48)
Hedwigia stellata Hedenäs (2)
Homalia trichomanoides (Hedw.) Brid. (9)
Homalothecium lutescens (Hedw.) H.Rob. (6)
Homalothecium sericeum (Hedw.) Schimp. (31)
Homomallium incurvatum (Schrad. ex Brid.) Loeske (2)
Hygroamblystegium varium (Hedw.) Mönk. (2)
Hylocomium splendens (Hedw.) Schimp. (1)
Hypnum cupressiforme aggr. (134)
Isothecium alopecuroides (Lam. ex Dubois) Isov. (74)
Kindbergia praelonga (Hedw.) Ochyra (1)
Lejeunea cavifolia (Ehrh.) Lindb. (9)
Leucodon sciuroides (Hedw.) Schwägr. (17)
Loeskeobryum brevirostre (Brid.) M.Fleisch. (3)
Lophocolea bidentata (L.) Dumort. (2)
Lophocolea heterophylla (Schrad.) Dumort. (2)
Metzgeria conjugata Lindb. (1)
Metzgeria furcata (L.) Dumort. (63)
Metzgeria temperata Kuwah. (3)
Mnium hornum Hedw. (2)
Mnium stellare Hedw. (2)
Neckera complanata (Hedw.) Huebener (21)
Neckera crispa Hedw. (3)

Table S4 (continued).

Neckera pumila Hedw. (2)
Orthotrichum affine Schrad. ex Brid. (42)
Orthotrichum anomalum Hedw. (28)
Orthotrichum diaphanum Schrad. ex Brid. (15)
Orthotrichum lyellii Hook. & Taylor (14)
Orthotrichum obtusifolium Brid. (4)
Orthotrichum pallens Bruch ex Brid. (11)
Orthotrichum patens Bruch ex Brid. (10)
Orthotrichum pumilum Sw. ex anon. (1)
Orthotrichum rupestre Schleich. ex Schwägr. (14)
Orthotrichum schimperi Hammar (3)
Orthotrichum speciosum Nees (6)
Orthotrichum stramineum Hornsch. ex Brid. (8)
Orthotrichum striatum Hedw. (11)
Orthotrichum tenellum Bruch ex Brid. (1)
Oxystegus tenuirostris (Hook. & Taylor) A.J.E.Sm. (1)
Paraleucobryum longifolium (Hedw.) Loeske (27)
Phascum cuspidatum Schreb. ex Hedw. (2)
Plagiochila asplenioides aggr. (17)
Plagiomnium affine aggr. (10)
Plagiomnium cuspidatum (Hedw.) T.J.Kop. (1)
Plagiomnium rostratum (Schrad.) T.J.Kop. (1)
Plagiomnium undulatum (Hedw.) T.J.Kop. (13)
Plagiothecium denticulatum (Hedw.) Schimp. (3)
Plagiothecium laetum Schimp. (12)
Plagiothecium nemorale (Mitt.) A.Jaeger (23)
Plasteurhynchium striatulum (Spruce) M.Fleisch. (1)
Platygyrium repens (Brid.) Schimp. (1)
Pleurozium schreberi (Willd. ex Brid.) Mitt. (3)
Polytrichum formosum Hedw. (24)
Polytrichum piliferum Schreb. ex Hedw. (2)
Porella platyphylla (L.) Pfeiff. (32)
Pseudoleskeella catenulata (Brid. ex Schrad.) Kindb. (1)
Pseudoleskeella nervosa (Brid.) Nyholm (5)
Pseudoscleropodium purum (Hedw.) M.Fleisch. (8)
Pseudotaxiphyllum elegans (Brid.) Z.Iwats. (1)
Pterigynandrum filiforme Hedw. (39)
Pylaisia polyantha (Hedw.) Schimp. (8)
Racomitrium aciculare (Hedw.) Brid. (1)
Racomitrium canescens (Hedw.) Brid. (1)
Racomitrium heterostichum aggr. (4)
Racomitrium microcarpon (Hedw.) Brid. (1)
Radula complanata aggr. (34)
Rhizomnium punctatum (Hedw.) T.J.Kop. (11)
Rhodobryum ontariense (Kindb.) Kindb. (1)
Rhynchostegium confertum (Dicks.) Schimp. (5)
Rhynchostegium murale (Hedw.) Schimp. (1)
Rhytidiadelphus squarrosus (Hedw.) Warnst. (5)
Scapania nemorea (L.) Grolle (1)
Schistidium apocarpum aggr. (73)
Sciuro-Hypnum flotowianum (Sendtn.) Ignatov & Huttunen (1)
Sciuro-Hypnum populeum (Hedw.) Ignatov & Huttunen (58)
Sciuro-Hypnum reflexum (Starke) Ignatov & Huttunen (1)
Syntrichia papillosa (Wilson) Jur. (9)
Syntrichia ruralis aggr. (19)
Syntrichia virescens (De Not.) Ochyra (3)
Taxiphyllum wissgrillii (Garov.) Wijk & Margad. (3)
Thuidium assimile (Mitt.) A.Jaeger (2)
Thuidium delicatulum (Hedw.) Schimp. (2)
Thuidium tamariscinum (Hedw.) Schimp. (19)
Tortella bambergeri (Schimp.) Broth. (1)
Tortella tortuosa (Hedw.) Limpr. (10)
Tortula caucasica Lindb. ex Broth. (2)
Tortula muralis Hedw. (12)
Ulota crispa aggr. (11)
Ulota hutchinsiae (Sm.) Hammar (2)
Weissia longifolia Mitt. (1)
Zygodon rupestris Schimp. ex Lorentz (1)

Table S5 Results of generalised linear mixed-effects models (GLMMs) fitted to species numbers with Poisson regression and to the occurrence of four boulder specialist species with logistic regression. M: number of best candidate models (Δ AIC < 2); K: number of predictors in the best candidate model set. Relative variable importance (RVI) is the sum of Akaike weights over all possible candidate models containing the predictor. Estimates are standardised coefficient estimates after model averaging of the best candidate models (in bold: $p < 0.1$). Adjusted standard error (SE_adj), z-value, *p* and lower and upper limits of 95% confidence intervals are given for the estimates.

Response variable	**M**	**K**	**Predictor**	RVI	**Estimate**	**SE_adj**	**z-value**	***p***	**2.5% CI**	**97.5% CI**
All species	20	10								
			Boulder size	1.00	0.635	0.055	11.600	0.000	0.527	0.742
			Buildings	0.57	-0.087	0.054	1.620	0.105	-0.192	0.0183
			Distance to river	0.45	-0.087	0.062	1.410	0.159	-0.208	0.0339
			Shrubs above	1.00	0.184	0.050	3.670	0.000	0.086	0.283
			Direct radiation	0.38	-0.063	0.054	1.170	0.243		
			Elevation	0.28	-0.056	0.069	0.809	0.419	-0.191	0.0794
			Precipitation	0.30	-0.063	0.072	0.877	0.381	-0.203	0.0774
			Evergreens above	0.36	-0.057	0.056	1.010	0.314	-0.167	0.0536
			Boulders	0.31	-0.038	0.051	0.749	0.454	-0.137	0.0614
			Trees above	0.30	0.029	0.053	0.548	0.583	-0.0753	0.134
Boulder specialists	16	10								
			Elevation	0.91	0.365	0.128	2.860	0.004	0.115	0.615
			Boulder size	1.00	1.290	0.119	10.900	0.000	1.06	1.53
			Evergreens above	0.50	-0.253	0.172	1.480	0.140	-0.59	0.083
			Buildings	1.00	-1.030	0.360	2.850	0.004	-1.73	-0.322
			Trees above	0.52	-0.220	0.153	1.440	0.150	-0.521	0.0801
			Distance to river	0.36	-0.194	0.170	1.140	0.253	-0.528	0.139
			Direct radiation	0.34	-0.176	0.173	1.020	0.309	-0.516	0.163
			Precipitation	0.35	0.146	0.163	0.896	0.370	-0.173	0.466
			Boulders	0.29	-0.076	0.123	0.619	0.536	-0.317	0.165
			Shrubs above	0.28	-0.074	0.172	0.427	0.669	-0.412	0.264
Grimmia hartmanii	8	8								
			Elevation	1.00	1.990	0.592	3.370	0.001	0.833	3.15
			Boulder size	1.00	3.050	0.573	5.320	0.000	1.92	4.17
			Buildings	0.68	-1.160	0.763	1.520	0.129	-2.65	0.336
			Direct radiation	0.73	-1.050	0.543	1.940	0.053	-2.12	0.0124
			Trees above	0.91	1.430	0.604	2.370	0.018	0.25	2.62
			Distance to river	0.41	0.613	0.490	1.250	0.211	-0.348	1.57
			Evergreens above	0.35	0.382	0.419	0.912	0.362	-0.438	1.2
			Precipitation	0.27	-0.263	0.451	0.582	0.561	-1.15	0.622
Grimmia trichophylla	9	9								
			Boulder size	1.00	2.150	0.530	4.060	0.000	1.11	3.19
			Evergreens above	0.96	-3.940	2.270	1.740	0.082	-8.38	0.503
			Distance to river	0.53	0.958	0.622	1.540	0.124	-0.261	2.18
			Trees above	0.36	-0.430	0.485	0.885	0.376	-1.38	0.522
			Buildings	0.36	-0.637	0.824	0.773	0.439	-2.25	0.978
			Boulders	0.28	0.329	0.483	0.682	0.496	-0.617	1.28
			Elevation	0.29	0.407	0.438	0.930	0.353	-0.451	1.27
			Precipitation	0.28	-0.231	0.549	0.421	0.673	-1.31	0.844
Hedwigia ciliata	13	9								
			Boulder size	1.00	2.840	0.654	4.340	0.000	1.56	4.12
			Buildings	0.99	-2.570	1.190	2.160	0.031	-4.9	-0.233
			Direct radiation	0.94	1.570	0.531	2.950	0.003	0.526	2.61
			Distance to river	0.43	-0.740	0.539	1.370	0.170	-1.8	0.316
			Evergreens above	0.43	-0.776	0.641	1.210	0.226	-2.03	0.48
			Elevation	0.32	-0.454	0.521	0.871	0.384	-1.48	0.568
			Trees above	0.32	-0.374	0.533	0.702	0.483	-1.42	0.671
			Boulders	0.27	-0.249	0.443	0.561	0.575	-1.12	0.62
			Shrubs above	0.28	-0.282	0.558	0.506	0.613	-1.38	0.811

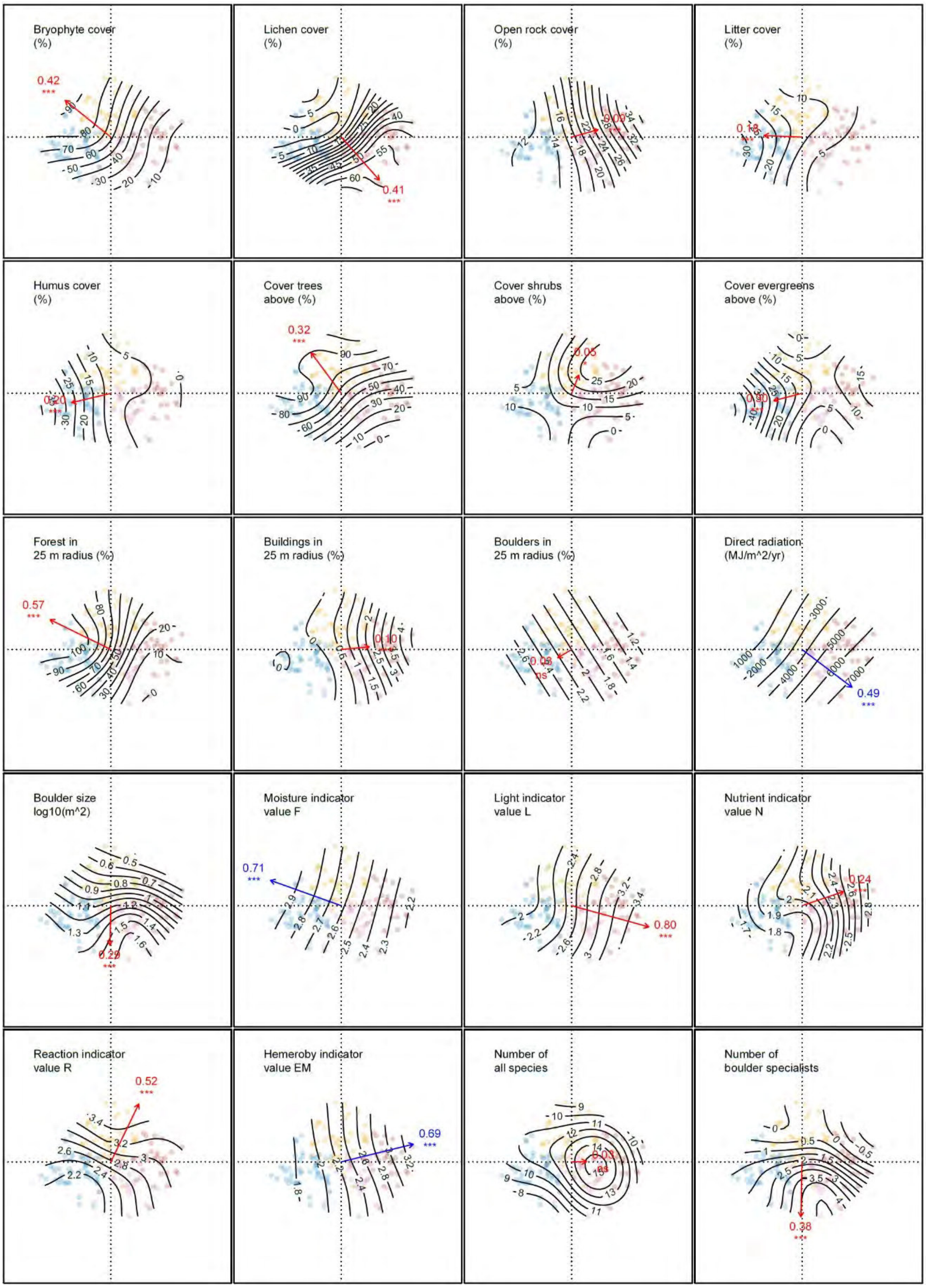


Fig. S1 Projections of variables on a detrended correspondence analysis (DCA) of bryophyte species composition of 151 siliceous erratic boulders, generated with the R package *vegan* (Oksanen et al. 2019). Trend surfaces were fitted with the 'ordisurf' function and arrows with the 'envfit' function. For the arrows, R^2 and significance levels (*** $p < 0.001$,** $p < 0.01$, * $p < 0.05$, ns $p > 0.05$) for the indicated linear correlations are given. The blue arrows were chosen for visualisation in Fig. 5.

References

Meier MK, Urmi E, Schnyder N, Bergamini A, Hofmann H (2013) Checkliste der Schweizer Moose. Nationales Inventar der Schweizer Moosflora, Zürich.

Oksanen J et al. (2019) Vegan: community ecology package. v.2.5-6. https://cran.r-project.org/package=vegan

Swisstopo (2007) Vector25, das digitale Landschaftsmodell der Schweiz. Bundesamt für Landestopografie, Wabern

Chapter II

Photo: Daniela Mazenauer

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

Daniel Hepenstrick, Niklaus Zemp, Alex Widmer, Rolf Holderegger

Manuscript published in Conservation Genetics (2021)
https://doi.org/10.1007/s10592-021-01414-6

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 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 for *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 efficient 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 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 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 species richness and boulder size. In addition, Virtanen and Oksanen (2007) reported a positive effect of connectivity on 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 Swiss lowlands, 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). Over the last centuries, these erratic boulders and their cryptogam communities have declined as a result of multiple factors. Historical exploitation of countless erratic boulders for construction material substantially reduced their numbers (Reynard 2004; Lugon et al. 2006). Losses due to farmland clearance are reflected in geological maps that indicate around five times fewer erratic boulders in open land than in forests (Akçar et al. 2011; Swisstopo 2013). More recently, 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 only 5 out of 17 historically documented populations of *A. septentrionale* on erratic boulders in the Swiss lowlands 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).

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 are 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 and 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 the mainlands.

Materials and Methods

Study species

Asplenium septentrionale 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). 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 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 gives rise to short-lived sporophytes, which meiotically produce spores that give rise to 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 with 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 continue 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 tetraploidy. 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 a hybridisation event (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. 1; Labhart 1992). 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. 1).

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. 1). We sampled the more abundant *Hedwigia ciliata* at the same sites and from 13 additional sites. For *A. septentrionale* we sampled 10 individuals (sporophytes) per population. *Hedwigia ciliata* often only forms small populations on erratic boulders, and we therefore sampled just five individuals (gametophytes) per population. Where populations were smaller than the target sample size, we sampled all available individuals. For each population, we sampled 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 sampled from three erratic boulders (Hepenstrick and Kiebacher 2019) with three samples per population (Table 1).

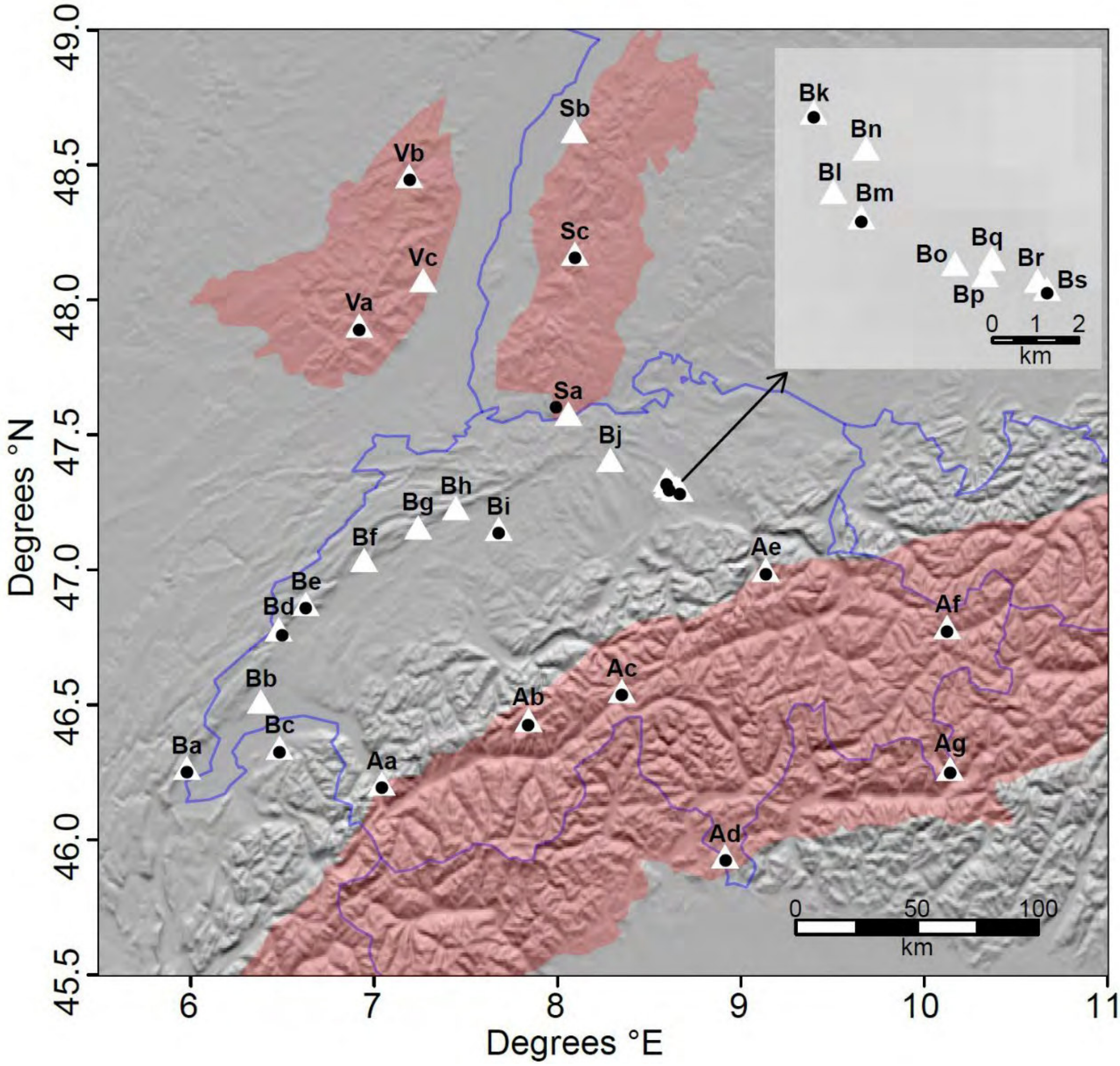


Fig. 1 Study area and sampling locations. Areas with predominantly calcareous bedrock are given in grey, areas with predominantly siliceous bedrock (mainland) in red (Asch 2005), and political borders in in blue (Becker et al. 2018). Sampled populations are indicated by white triangles (*Hedwigia ciliata*) 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), siliceous Black Forest (S) and siliceous Vosges (V). (Background map: Jarvis et al. 2008)

Table 1 Samples of *Asplenium septentrionale*, *Hedwigia ciliata* and *H. stellata* analysed in the present study. 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. 1), 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. 2), the number of multilocus genotypes (MLG), and the Simpson index for populations with four or more samples per lineage.

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	46.858851 / 6.627042	*H. ciliata*	5 (1)	0/0/0/5/0	0/0/0/2/0	0.48
				A. septentrionale	10 (1)		1	0
			46.759825/6.498386	*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
				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

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*, each two times. 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 570-bp size selection (Westergaard et al. 2019). First, to improve the reference catalogue and estimate the number of expected fragments, 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 on an Illumina MiSeq at the Genetic Diversity Centre ETH Zurich (Switzerland) using a 300-bp paired-end reads V3 protocol. Finally, for sequencing all 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 take into account the different catalogue sizes. We have 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. We obtained optimal results 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 mapped HiSeq paired-reads of all individuals against these references using BWA 0.7.12, 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 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. We addressed the high mean allelic error rate (9.3%) among technical replicates in *A. septentrionale* by applying an additional Hardy-Weinberg-equilibrium filter in order to remove further putative erroneous variant calls due to potential paralogs (Puritz et al. 2014). 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 possible occurrence of multiple lineages in *H. ciliata* prohibited genetic analyses based on diploid Mendelian inheritance and large population sample sizes. Consequently, we analysed the SNP datasets with more general methods that had 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. 2) 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 separates 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 two diploid lineages (d1 and d2) that were represented by larger sample sizes and more populations (Table 1).

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; Kamvar et al. 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 should 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, which was probably due to the high incidence of gene duplication in fern genomes (Nakazato et al. 2006). Rigid filtering because of otherwise high error rates resulted in a relatively small number of 404 SNPs. The slightly higher observed than expected heterozygosity (Table 2) was in congruence with the tetraploidy 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. 2). The first cluster contained the samples of the outgroup *H. stellata* and two samples that were collected as *H. ciliata* but were clearly actually *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 were diploid, based on their high observed heterozygosity of > 0.5 (Table 2, Fig. 2). 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. 2). 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 found.

Table 2 Overview of the analysed taxa and SNP datasets for *Asplenium septentrionale* and *Hedwigia*. Numbers after slashes refer to separate SNP datasets for the different *Hedwigia* lineages. For each taxon (column) the following information is given: the number of samples analysed (N; number of technical replicates in brackets), the number of loci in the SNP dataset (N loci), the observed heterozygosity (Hobs), the expected heterozygosity (Hexp), the fraction of loci heterozygous in all samples (Het fixed; fixed heterozygosity), the fraction of loci homozygous in all samples (Hom fixed; fixed homozygosity), the number of populations that contained the taxon (N pops), the number of different multilocus genotypes detected (N MLG), and the 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.

	A. septentrionale	***Hedwigia* all samples**	***H. ciliata* lineage d1**	***H. ciliata* lineage d2**	***H. ciliata* lineage h1**	***H. ciliata* lineage h2**	***Hedwigia 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

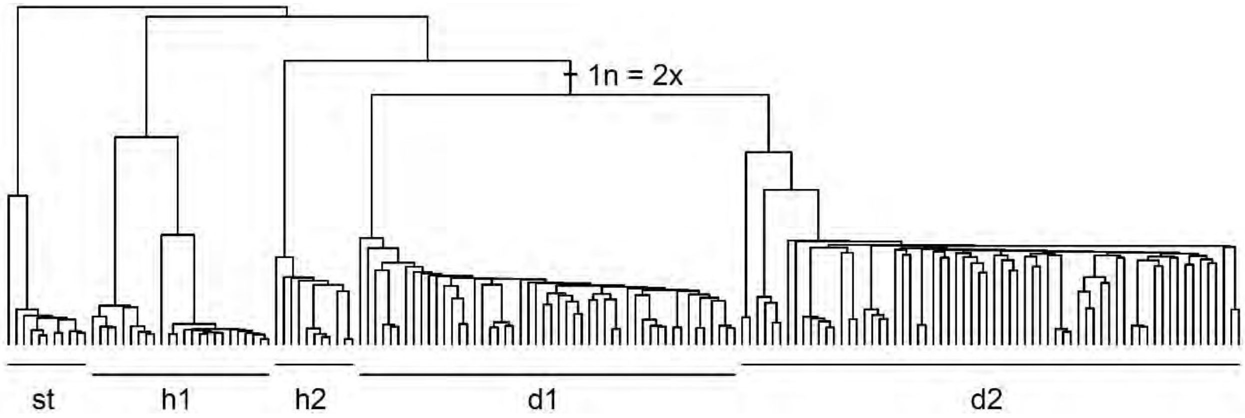


Fig. 2 UPGMA tree based on 4926 SNPs of all 162 *Hedwigia* samples. The cluster containing the polyploid lineages is indicated in the diagram (1n = 2x). Distinguished lineages are indicated below the diagram: the outgroup consists of *H. stellata* (st), *H. ciliata* clusters into two haploid (h1 and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

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. 3).

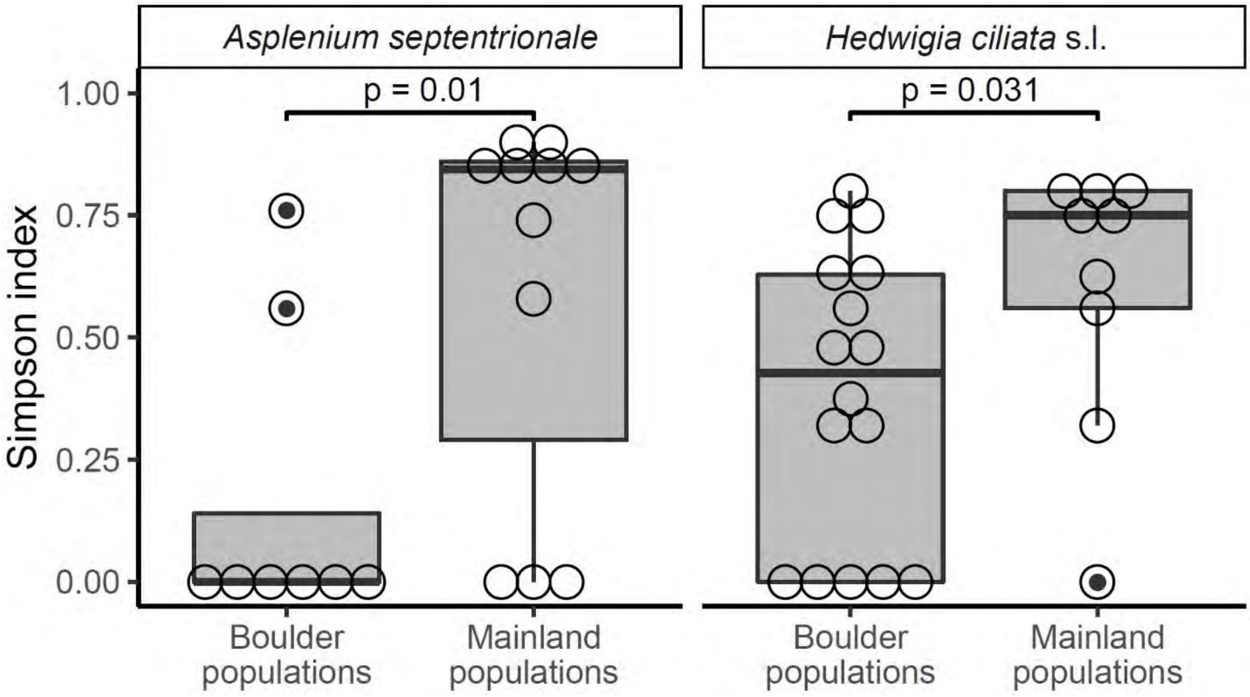


Fig. 3 Multilocus genotype diversity of *Asplenium septentrionale* and *Hedwigia ciliata* in boulder and mainland populations. Above the boxplots of Simpson diversity indices, p-values from one-sided Wilcoxon rank sum tests are given. Data points are given as circles. Populations with a Simpson index of 0 consist of only one multilocus genotype each.

DAPC

DAPC of *A. septentrionale* revealed a distinct spatial population structure, and BIC-values suggested between two and six genetic clusters (K; Fig. 4). 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 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 the population structure (Fig. 4).

DAPC of *H. ciliata* lineages d1 and d2 did not reveal any spatial genetic structure across populations, neither in the 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).

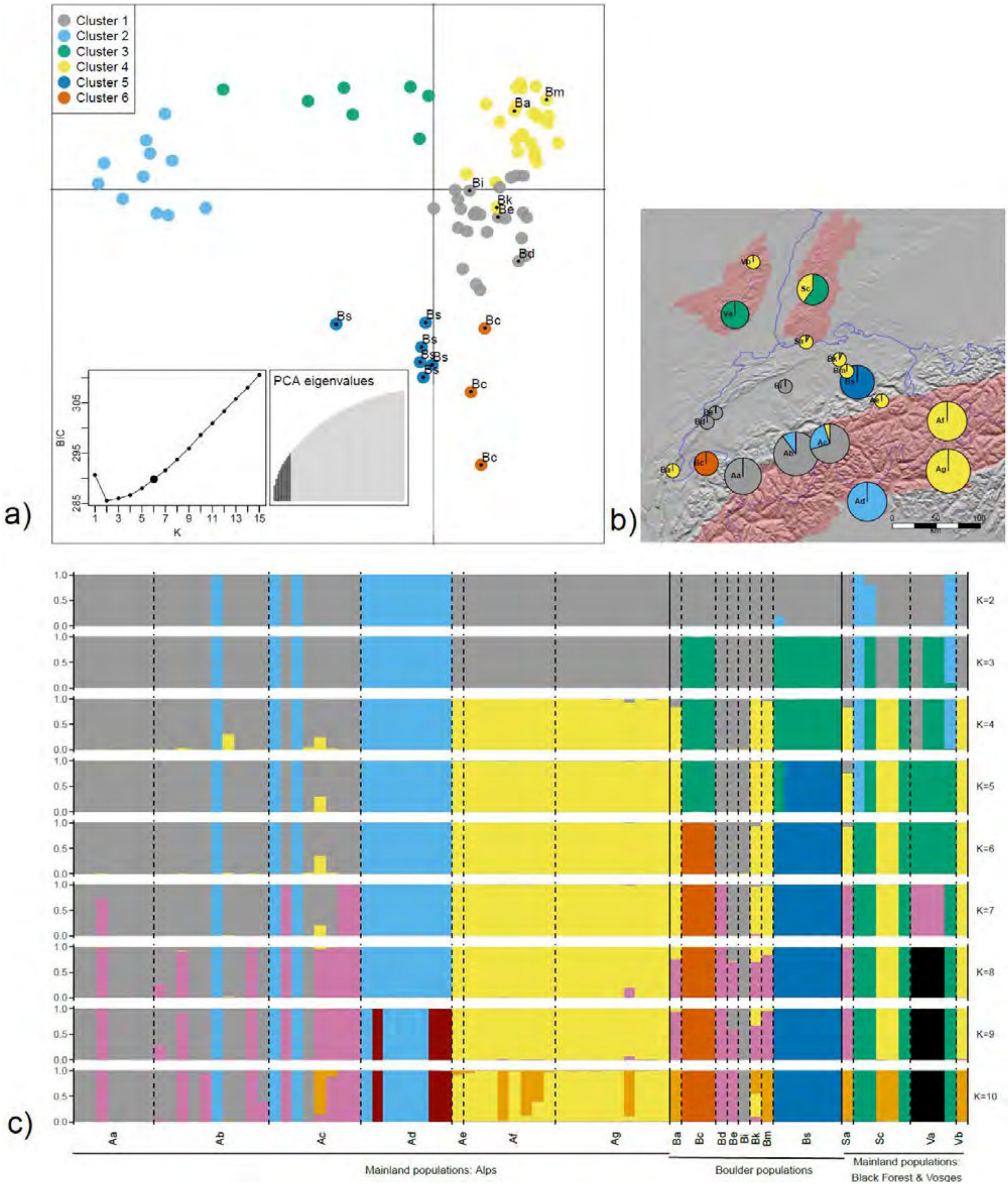


Fig. 4 DAPC results for 79 *Asplenium septentrionale* multilocus genotypes. (a) DAPC scatterplot showing the group assignment to six genetic clusters (different colours), with samples from boulder populations labelled. Insets: (left) Bayesian Information Criterion (BIC) as a function of the number of 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 Figure 1 and Table 1.

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.

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

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. 5). 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. 5). In *H. ciliata* lineage d2, a group of conspicuously high distance values in Fig. 5 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. 2).

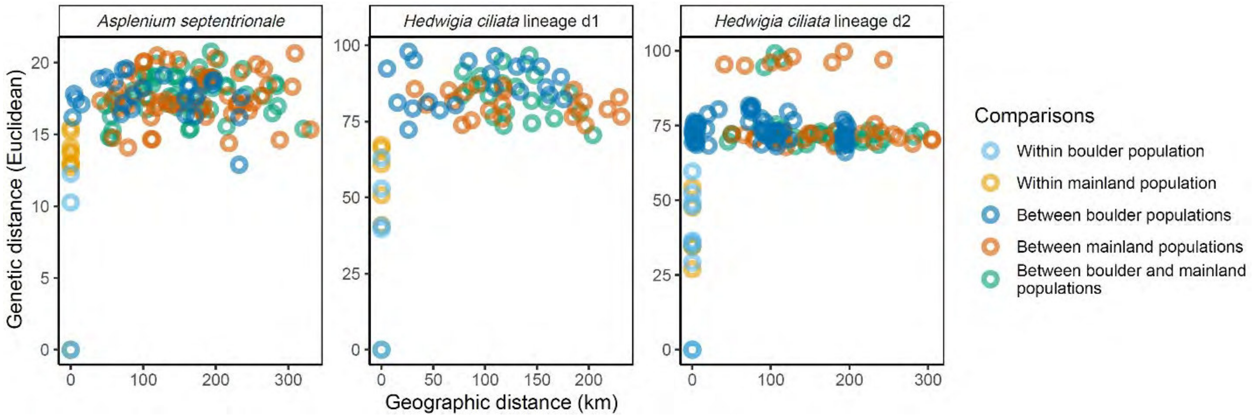


Fig. 5 Geographic distances vs. genetic distances (mean pairwise individual Euclidean distances) for *Asplenium septentrionale* and for *Hedwigia ciliata* lineages d1 and d2.

Discussion

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 had 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. 4) 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. 4) 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 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. 4). 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. 4). In accordance, AMOVA did not reveal a significant differentiation of boulder populations from mainland populations (Table 3). Concerning the origin of the 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. 5) 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 along 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 greater 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. Because eastern boulders tended to cluster with eastern Alpine populations and western boulders with western Alpine populations in the DAPC analyses, we hypothesise that the erratic boulders have been colonised by Alpine mainland populations, probably facilitated by intensive, Alpine down-slope winds (Foehn; Brinkmann 1971).

Spatial genomic patterns in Hedwigia ciliata

Across all samples, the *Hedwigia* dataset was structured by a phylogenetic signal (Fig. 2). We found haploid and diploid samples, a result that is 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. 1). We thoroughly checked our protocols and found no indication that these genotypes were erroneous. In 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. 5). 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 the context of our genomic study, this area–diversity relationship is mirrored in a lower multilocus genotype diversity in boulder populations than in mainland populations (Fig. 3). 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 polyploidy of *A. septentrionale* that promotes its highly selfing breeding system: Masuyama and Watano (1990) found that polyploid 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 ($r_{\text{boulders}} < r_{\text{mainland}}$), 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 ($a_{\text{boulders}} << a_{\text{mainland}}$). Furthermore, the dispersal kernels of *A. septentrionale* and *H. ciliata* must nevertheless be sufficiently "fat tailed" (small b) such that relatively frequent 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, then – 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 polyploid *A. septentrionale*. In *H. ciliata* we found four presumably independent lineages, which underline the biodiversity contributed by the 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; Meylan 1926a; Meylan 1926b; Epard et al. 2020). We do not recommend reintroducing *A. septentrionale* to isolated erratic boulders where it has become extinct, as only the original populations witness the impressive long-distance dispersal capabilities of this edaphically specialised cryptogam species (Brockmann-Jerosch and Brockmann-Jerosch 1926). Finally, our study showcases 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.

Acknowledgements

We thank the many people and institutions that enabled the collection of samples: Info Flora and Swissbryophytes in Switzerland, Marc Philippe and Jean-François Thomas in France, and the Bundesamt für Naturschutz and Thomas Breunig in Germany. We thank Sabine Brodbeck and René Graf for help with DNA extraction and Claudia Michel for preparing ddRAD libraries. We are grateful for financial support from several foundations, associations, and governmental and non-governmental organisations (listed on www.zhaw.ch/findlingsflora).

References

Akçar N, Ivy-Ochs S, Kubik PW, Schlüchter C (2011) Post-depositional impacts on 'Findlinge' (erratic boulders) and their implications for surface-exposure dating. Swiss J Geosci 104:445-453. https://doi.org/10.1007/s00015-011-0088-7

Asch K (2005) 1:5 million international geological map of Europa and adjacent areas. Bundesanstalt Geowissenschaften und Rohstoffe, Hannover

Bainard JD, Fazekas AJ, Newmaster SG (2010) Methodology significantly affects genome size estimates: quantitative evidence using bryophytes. Cytometry A 77:725-732. https://doi.org/10.1002/cyto.a.20902

Baker HG (1955) Self-compatibility and establishment after 'long-distance' dispersal. Evolution 9:347-349. https://doi.org/10.2307/2405656

Baughman JT, Payton AC, Paasch AE, Fisher KM, McDaniel SF (2017) Multiple factors influence population sex ratios in the Mojave Desert moss *Syntrichia caninervis*. Am J Bot 104:733-742. https://doi.org/10.3732/ajb.1700045

Becker RA, Wilks AR, Brownrigg R, Minka TP, Deckmyn A (2018) R package maps: draw geographical maps, 3.3.0. https://cran.r-project.org/web/packages/maps/

Blum C (2015) Steinhof - Steineberg Klettern, Familienklettern, Bouldern am grössten Findling im Mittelland und seinen Vasallen. Topo, Basel

Bornand C, Eggenberg S, Gygax A et al. (2019) Regionale Rote Liste der Gefässpflanzen der Schweiz. Info Flora, Bern

Brinkmann, WAR (1971). What is a foehn? Weather 26: 230-240.

Brockmann-Jerosch H, Brockmann-Jerosch M (1926) Die Geschichte der schweizerischen Alpenflora. In: Schröter C (ed) Das Pflanzenleben der Alpen. Raustein, Zürich, pp 1149-1199

Buchbender V, Hespanhol H, Krug M et al. (2014) Phylogenetic reconstructions of the Hedwigiaceae reveal cryptic speciation and hybridisation in *Hedwigia*. Bryophyt Divers Evol 36:1-21. http://dx.doi.org/10.11646/bde.36.1.1

Catchen J, Hohenlohe PA, Bassham S et al. (2013) STACKS: an analysis tool set for population genomics. Mol Ecol 22:3124-3140. https://doi.org/10.1111/mec.12354

Clarc LV, Jasieniuk M (2011) polysat: an R package for polyploid microsatellite analysis. Mol Ecol Res 11:562-566. https://doi.org/10.1111/j.1755-0998.2011.02985.x

Clark J, Hidalgo O, Pellicer J et al. (2016) Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny. New Phytol 210:1072-1082. https://doi.org/10.1111/nph.13833

Clarke LJ, Ayre DJ, Robinson SA (2009) Genetic structure of East Antarctic populations of the moss *Ceratodon purpureus*. Antarct Sci 21:51-58. https://doi.org/10.1017/S0954102008001466

Colpa JG, van Zanten BO (2006) Mossen op de Nederlandse hunebedden in 2004/2005. Buxbaumiella 75:34-50.

Colgan PM (2009) Glacial erratics. In: Gornitz V (ed) Encyclopedia of paleoclimatology and ancient environments. Springer, Dordrecht. pp 354. https://doi.org/10.1007/978-1-4020-4411-3_90

Danecek P et al. (2011) The variant call format and VCFtools. Bioinformatics 27:2156-2158. https://doi.org/10.1093/bioinformatics/btr330

Dos Santos ACR, Goldman GH, Riaño-Pachón DM (2017) ploidyNGS: visually exploring ploidy with next generation sequencing data. Bioinformatics 33:2575-2576. https://doi.org/10.1093/bioinformatics/btx204

Ehlers J, Gibbard PL (2004) Quaternary glaciations – extent and chronology part I: Europe. Elsevier, Amsterdam

Epard J-L, Gex P, Vust M (2020) Les blocs erratiques propriété de la Société Vaudoise des Sciences Naturelles. Bull Soc VaudSci Nat 99:29-66.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491. https://pubmed.ncbi.nlm.nih.gov/1644282/

Fritsch R (1991) Index to bryophyte chromosome counts. Bryophytorum Bibliotheca, Cramer, Berlin

Garrison E (2012) Vcflib: A C++ library for parsing and manipulating VCF files. https://github.com/ekg/vcflib

Garrison E, Marth G (2012) Haplotype-based variant detection from short-read sequencing. arXiv:1207.3907[q-bio.GN]

Glime JM (2017) Bryophyte ecology. Michigan Technological University, Michigan. http://www.bryoecol.mtu.edu/.

Grundmann M, Ansell SW, Russell SJ et al. (2007) Genetic structure of the widespread and common Mediterranean bryophyte *Pleurochaete squarrosa* (Brid.) Lindb. (Pottiaceae) – evidence from nuclear and plastidic DNA sequence variation and allozymes. Mol Ecol 16:709-722. https://doi.org/10.1111/j.1365-294X.2007.03181.x

Hedenäs L (1994) The *Hedwigia ciliata* complex in Sweden, with notes on the occurrence of the taxa in Fennoscandia. J Bryol 18:139-157. https://doi.org/10.1179/jbr.1994.18.1.139

Heer O (1865) Die Urwelt der Schweiz. Schulthess, Zürich

Hepenstrick D, Kiebacher T (2019) *Hedwigia stellata*. In: Bergamini A et al. (2019) Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 14. Meylania 63:6-8. https://doi.org/10.21256/zhaw-19787

Hepenstrick D, Urmi E, Meier MK, Bergamini A (2016) Die Moosflora des silikatischen Findlings Alexanderstein in Küsnacht (ZH). Meylania 57:15-23. https://doi.org/10.21256/zhaw-2029

Holderegger R, Schneller JJ (1994) Are small isolated populations of *Asplenium septentrionale* variable? Biol J Linn Soc 51:377-385. https://doi.org/10.1111/j.1095-8312.1994.tb00969.x

Hunt HV, Ansell SW, Russell SJ et al. (2009) Genetic diversity and phylogeography in two diploid ferns, *Asplenium fontanum* subsp. *fontanum* and *A. petrarchae* subsp. *bivalens*, in the Western Mediterranean. Mol Ecol 18:4940-4954. https://doi.org/10.1111/j.1365-294X.2009.04402.x

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898-1914. https://doi.org/10.1111/j.1558-5646.1999.tb04571.x

Ignatova EA, Kuznetsova OI, Fedosov VE, Ignatov MS (2016) On the genus *Hedwigia* (Hedwigiaceae, Bryophyta) in Russia. Arctoa 25:241–277. https://doi.org/10.15298/arctoa.25.20

Jarvis A, Reuter HI, Nelson A, Guevara E (2008) Hole-filled seamless SRTM data V4. International Centre for Tropical Agriculture. http://srtm.csi.cgiar.org.

Joanne M, Mehltreter K, Walker LR (2010) Ecological importance of ferns. In: Mehltreter K, Walker L, Sharpe J (eds) Fern Ecology. Cambridge University Press, Cambridge, pp 1-20 https://doi.org/10.1017/CBO9780511844898

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart T, Collins C (2015) A tutorial for discriminant analysis of principal components (DAPC) using adegenet 2.0.0. MRC Centre for Outbreak Analysis and Modelling Imperial College, London. https://adegenet.r-forge.r-project.org.

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94. https://doi.org/10.1186/1471-2156-11-94

Kamvar ZN, Brooks JC, Grünwald NJ (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Front Genet 6:208. https://doi.org/10.3389/fgene.2015.00208

Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281. https://doi.org/10.7717/peerj.281

Kang M, Huang H, Jiang M, Lowe AJ (2008) Understanding population structure and historical demography in a conservation context: population genetics of an endangered fern. Divers Distr 14:799-807. https://doi.org/10.1111/j.1472-4642.2008.00484.x

Karlin E, Andrus R, Boles S, Shaw A (2011) One haploid parent contributes 100% of the gene pool for a widespread species in northwest North America. Mol Ecol 20:753-767. https://doi.org/10.1111/j.1365-294X.2010.04982.x

Kimmerer RW, Driscoll MJL (2000) Bryophyte species richness on insular boulder habitats: the effect of area, isolation, and microsite diversity. Bryologist 103:748-756. https://doi.org/10.1639/0007-2745(2000)103[0748:Bsroib]2.0.Co;2

Klekowski EJ, Lloyd RM (1968) Reproductive biology of the Pteridophyta 1. General considerations and a study of *Onoclea sensibilis* L. Bot J Linn Soc 60:315-324. https://doi.org/10.1111/j.1095-8339.1968.tb00093.x

Knaus BJ, Grünwald NJ (2017) vcfr: a package to manipulate and visualize variant call format data in R. Mol Ecol Res 17:44-53. https://doi.org/10.1111/1755-0998.12549

Krawiec F (1938) Flora epilityczna glazow narzutowych zachodniej polski (Die epilithische Flora der erratischen Blöcke Westpolens). Jachowskiego, Poznań

Labhart TP (1992) Geologie der Schweiz. Ott, Bern

Lugon R, Pralong J-P, Reynard E (2006) Patrimoine culturel et géomorphologie: le cas valaisan de quelques blocs erratiques, d'une marmite glaciaire et d'une moraine. Bull Murith 124:73-87. http://data.rero.ch/01-R004569656/html

Luo D, Xu B, Rana SK et al. (2018) Phylogeography of rare fern *Polystichum glaciale* endemic to the subnival zone of the Sino-Himalaya. Plant Syst Evol 304:485-499. https://doi.org/10.1007/s00606-018-1495-2

MacArthur RH, Wilson EO (1963) An equilibrium theory of insular zoogeography. Evolution 17:373-387. https://doi.org/10.2307/2407089

Maechler M, Rousseeuw P, Struyf A et al. (2019) cluster: Cluster analysis basics and extensions, R package version 2.1.0. https://svn.r-project.org/R-packages/trunk/cluster/

Masuyama S, Watano Y (1990) Trends for inbreeding in polyploid pteridophytes. Plant Spec Biol 5:13-17. https://doi.org/10.1111/j.1442-1984.1990.tb00188.x

Mazenauer D, Holderegger R, Krüsi B, Hepenstrick D (2014) Populationsentwicklung und Gefährdung von *Asplenium septentrionale* auf Findlingen im Schweizer Mittelland und Jura. Bauhinia 25:37-50

Meylan C (1912) La flore bryologique des blocs erratiques du Jura. Bull Soc Vaud Sci Nat 48:49-70. https://dx.doi.org/10.5169/seals-269347

Meylan C (1926a) La flore bryologique et lichénologique du bloc erratique de La Grange-de-la-Côte. Bull Soc Vaud Sci Nat 56:165-172. https://dx.doi.org/10.5169/seals-271595

Meylan C (1926b) Nouvelle contribution à la connaissance des lichens du Jura, avec quelques indications de localités des Alpes. Bull Soc Vaud Sci Nat 56:173-178. https://dx.doi.org/10.5169/seals-271596

Milde J (1870) Die erratischen Moose. Bot Z 28:129-134. http://www.biodiversitylibrary.org/item/105619

Miller NG, Robinson SC (2015) Bryophytes of Martha's Vineyard and Nomans Land, Massachusetts, USA. Rhodora 117:431-453. http://dx.doi.org/10.3119/15-09

Monsen KJ, Blouin MS (2004) Extreme isolation by distance in a montane frog *Rana cascadae*. Conserv Genet 5:827-835. https://doi.org/10.1007/s10592-004-1981-z

Nakazato T, Jung M-K, Housworth EA et al. (2006) Genetic map-based analysis of genome structure in the homosporous fern *Ceratopteris richardii*. Genetics 173:1585-1597. https://doi.org/10.1534/genetics.106.055624

Nathan R (2006) Long-distance dispersal of plants. Science 313:786-788. https://doi.org/10.1126/science.1124975

Nebel M, Philippi G (eds) (2000) Die Moose Baden-Württembergs. Ulmer, Stuttgart

Norros V, Rannik Ü, Hussein T, Petäjä T, Vesala T, Ovaskainen O (2014) Do small spores disperse further than large spores? Ecology 95:1612-1621. https://doi.org/10.1890/13-0877.1

O'Leary SJ, Puritz JB, Willis SC et al. (2018) These aren't the loci you're looking for: principles of effective SNP filtering for molecular ecologists. Mol Ecol 27:3193-3206. https://doi.org/10.1111/mec.14792

Oksanen J et al. (2019) vegan: community ecology package. R package version 2.5-6. https://cran.r-project.org/package = vegan

Parisod C (2008) Postglacial recolonisation of plants in the western Alps of Switzerland. Bot Helv 118:1-12. https://doi.org/10.1007/s00035-008-0825-3

Patiño J, Vanderpoorten A (2018) Bryophyte biogeography. Crit Rev Plant Sci 37:175-209. https://doi.org/10.1080/07352689.2018.1482444

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PloS ONE 7:e37135. https://doi.org/10.1371/journal.pone.0037135

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959. https://doi.org/10.1093/genetics/155.2.945

Puritz JB, Hollenbeck CM, Gold JR (2014) dDocent: a RADseq, variant-calling pipeline designed for population genomics of non-model organisms. PeerJ 2:e431. https://doi.org/10.7717/peerj.431

R Core Team (2017) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Ramsay HP (2011) Australian mosses – new chromosome numbers and a compilation of chromosome data. Telopea 13:577-619

Reichstein T (1984) *Asplenium*. In: Kramer KU (ed) Gustav Hegi, Illustrierte Flora von Mitteleuropa I. Parey, Berlin, pp 211-266

Reynard E (2004) Protecting stones: conservation of erratic blocks in Switzerland. In: Prikryl R (ed) Dimension stone 2004. New perspectives for a traditional building material. Balkema, Leiden, pp 3-7

Rünk K, Pihkva K, Liira J, Zobel K (2016) Selection of source material for introduction of the locally rare and threatened fern species *Asplenium septentrionale*. Plant Ecol Div 9:167-173. https://doi.org/10.1080/17550874.2016.1182225

Såstad S (2005) Patterns and mechanisms of polyploid speciation in bryophytes. In: Bakker FT, Chatrou LW, Gravendeel B, Pelser PB (eds) Plant species-level systematics: new perspectives on pattern and process. Gantner, Ruggell, pp 317-334

Schneller JJ, Holderegger R (1996) Genetic variation in small, isolated fern populations. J Veg Sci 7:113-120. https://doi.org/10.2307/3236423

Schönswetter P, Stehlik I, Holderegger R, Tribsch A (2005) Molecular evidence for glacial refugia of mountain plants in the European Alps. Mol Ecol 14:3547-3555. https://doi.org/10.1111/j.1365-294X.2005.02683.x

Sharbel TF, Haubold B, Mitchell-Olds T (2000) Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. Mol Ecol 9:2109-2118. https://doi.org/10.1046/j.1365-294X.2000.01122.x

Soltis PS, Soltis DE (2000) The role of genetic and genomic attributes in the success of polyploids. PNAS 97:7051-7057. https://doi.org/10.1073/pnas.97.13.7051

Sorsa P (1964) Studies on the spore morphology of Fennoscandian fern species. Ann Bot Fenn 1:179-201. https://www.jstor.org/stable/23724600

Sundberg S (2005) Larger capsules enhance short-range spore dispersal in *Sphagnum*, but what happens further away? Oikos 108:115-124. https://doi.org/10.1111/j.0030-1299.2005.12916.x

Suter M, Schneller J, Vogel J (2000) Investigations into the genetic variation, population structure, and breeding systems of the fern *Asplenium trichomanes* subsp. *quadrivalens*. Int J Plant Sci 161:233-244. https://www.jstor.org/stable/10.1086/314258

Swisstopo (2013) GeoCover, vectorised geological atlas of Switzerland. swisstopo, Wabern

Szövényi P, Sundberg S, Shaw AJ (2012) Long-distance dispersal and genetic structure of natural populations: an assessment of the inverse isolation hypothesis in peat mosses. Mol Ecol 21:5461-5472. https://doi.org/10.1111/mec.12055

Trewick SA et al. (2002) Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. Mol Ecol 11:2003-2012. https://doi.org/10.1046/j.1365-294x.2002.01583.x

Tribsch A, Schönswetter P (2003) Patterns of endemism and comparative phylogeography confirm palaeoenvironmental evidence for Pleistocene refugia in the Eastern Alps. Taxon 52:477-497. https://doi.org/10.2307/3647447

Vanderpoorten A, Devos N, Goffinet B et al. (2008) The barriers to oceanic island radiation in bryophytes: insights from the phylogeography of the moss *Grimmia montana*. J Biogeogr 35:654-663. https://doi.org/10.1111/j.1365-2699.2007.01802.x

Vanderpoorten A et al. (2019) To what extent are bryophytes efficient dispersers? J Ecol 107:2149-2154. https://doi.org/10.1111/1365-2745.13161

Virtanen R, Oksanen J (2007) The effects of habitat connectivity on cryptogam richness in boulder metacommunity. Biol Conserv 135:415-422. https://doi.org/10.1016/j.biocon.2006.10.013

Vogel JC et al. (1999) Genetic structure, reproductive biology and ecology of isolated populations of *Asplenium csikii* (Aspleniaceae, Pteridophyta). Heredity 83:604-612. https://doi.org/10.1038/sj.hdy.6886120

Wächter HJ (1996) Zur Moosvegetation von Findlingen zwischen Ems und Weser. Osnabrücker Naturwiss Mitt 22:89-110

Weber J (1912) Neue Standorte von *Asplenium septentrionale*. Mitt Naturwiss Ges Winterthur 9:140-145

Weibull H, Rydin H (2005) Bryophyte species richness on boulders: relationship to area, habitat diversity and canopy tree species. Biol Conserv 122:71-79. https://doi.org/10.1016/j.biocon.2004.07.001

Westergaard KB, Zemp N, Bruederle LP et al. (2019) Population genomic evidence for plant glacial survival in Scandinavia. Mol Ecol 28:818-832. https://doi.org/10.1111/mec.14994

Wickham H et al. (2019) Welcome to the tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Willis SC, Hollenbeck CM, Puritz JB, Gold JR, Portnoy DS (2017) Haplotyping RAD loci: an efficient method to filter paralogs and account for physical linkage. Mol Ecol Res 17:955-965. https://doi.org/10.1111/1755-0998.12647

Wyatt R (1994) Population genetics of bryophytes in relation to their reproductive biology. J Hattori Bot Lab 76:147-157. https://doi.org/10.18968/jhbl.76.0_147

Supplementary Material

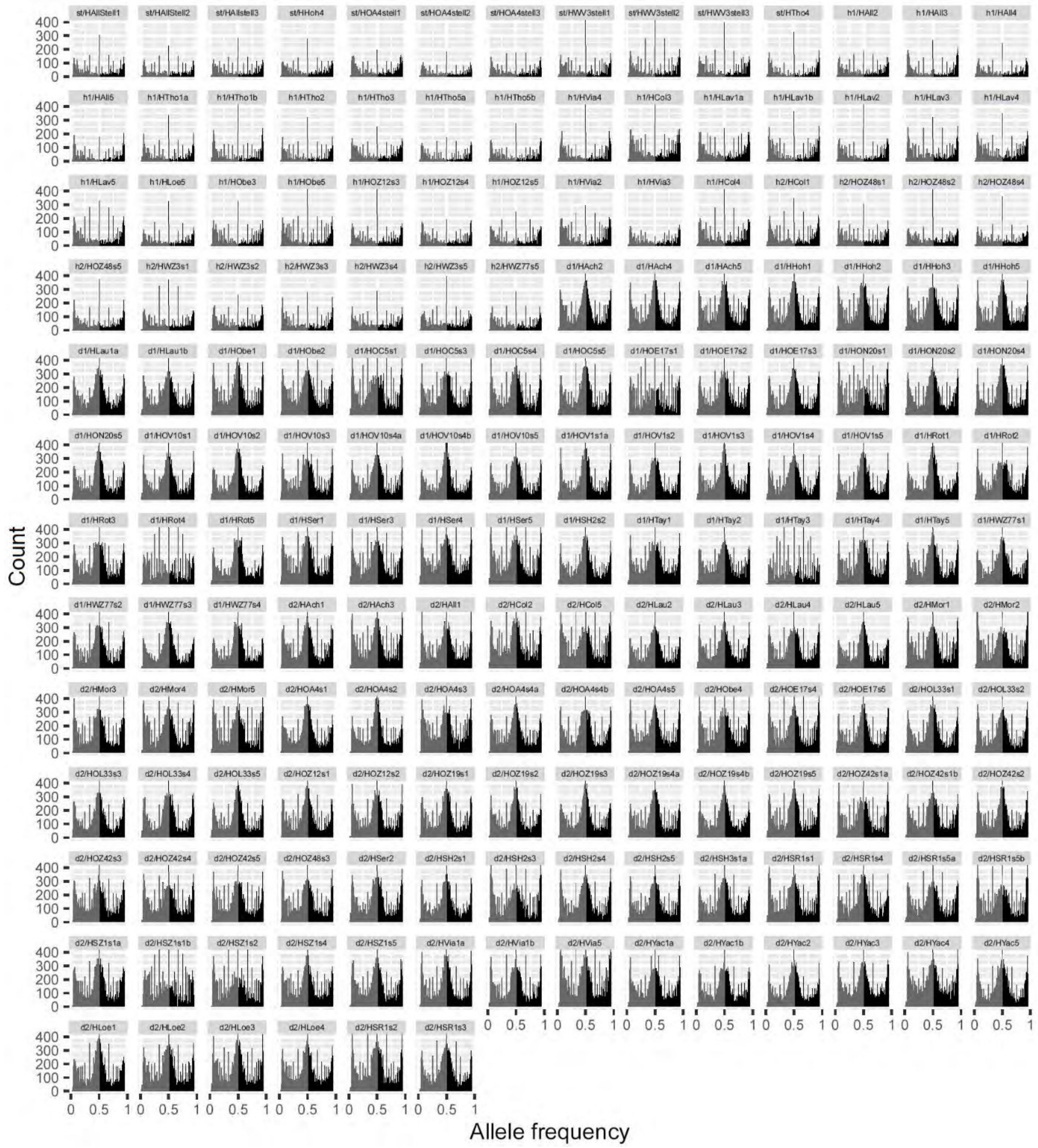


Fig. S1 PLOYDYNGS results for all analysed *Hedwigia* samples. The histograms show frequencies of the most frequent (black) and the second most frequent (grey) putative allele of the reads mapped to 20,000 fragments of the *Hedwigia* catalogue (which had a total of 85,938 fragments). Diploid samples are characterised by a peak at 0.5 that reflects the putative alleles of heterozygote loci, which do not occur in haploid samples. Samples are sorted according to their lineage assignment (st = *H. stellata*; h1 = *H. ciliata* haploid lineage h1; h2 = *H. ciliata* haploid lineage h2; d1 = *H. ciliata* diploid lineage d1; d2 = *H. ciliata* haploid lineage h2).

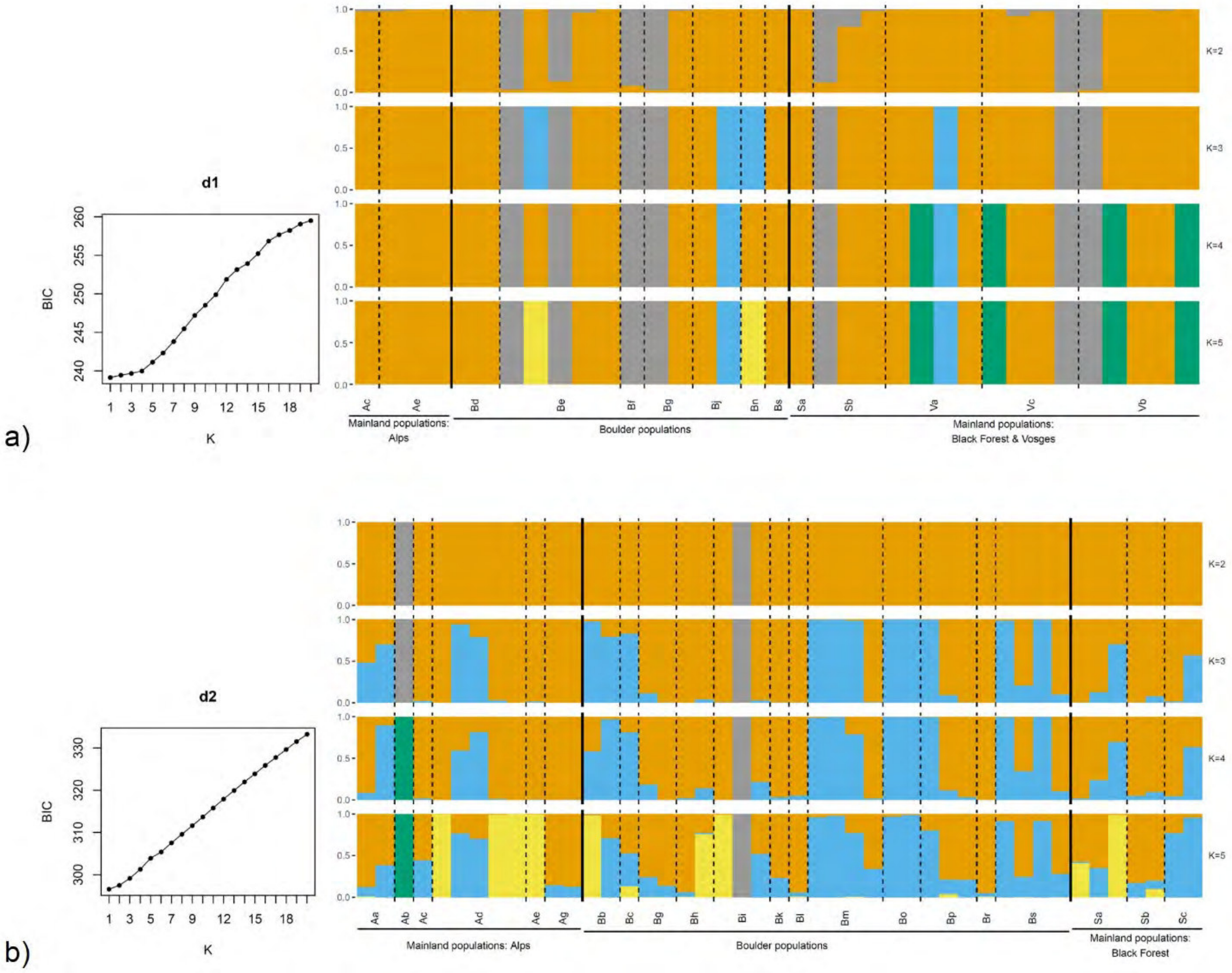


Fig. S2 DAPC results for *Hedwigia ciliata* lineages d1 (a) and d2 (b). Bayesian Information Criterion (BIC) as a function of the number of clusters K (left) and bar plots of posterior assignment probabilities for an increasing number of clusters (K = 2 to K = 5; right). Each vertical bar represents 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 Figure 1 and Table 1.

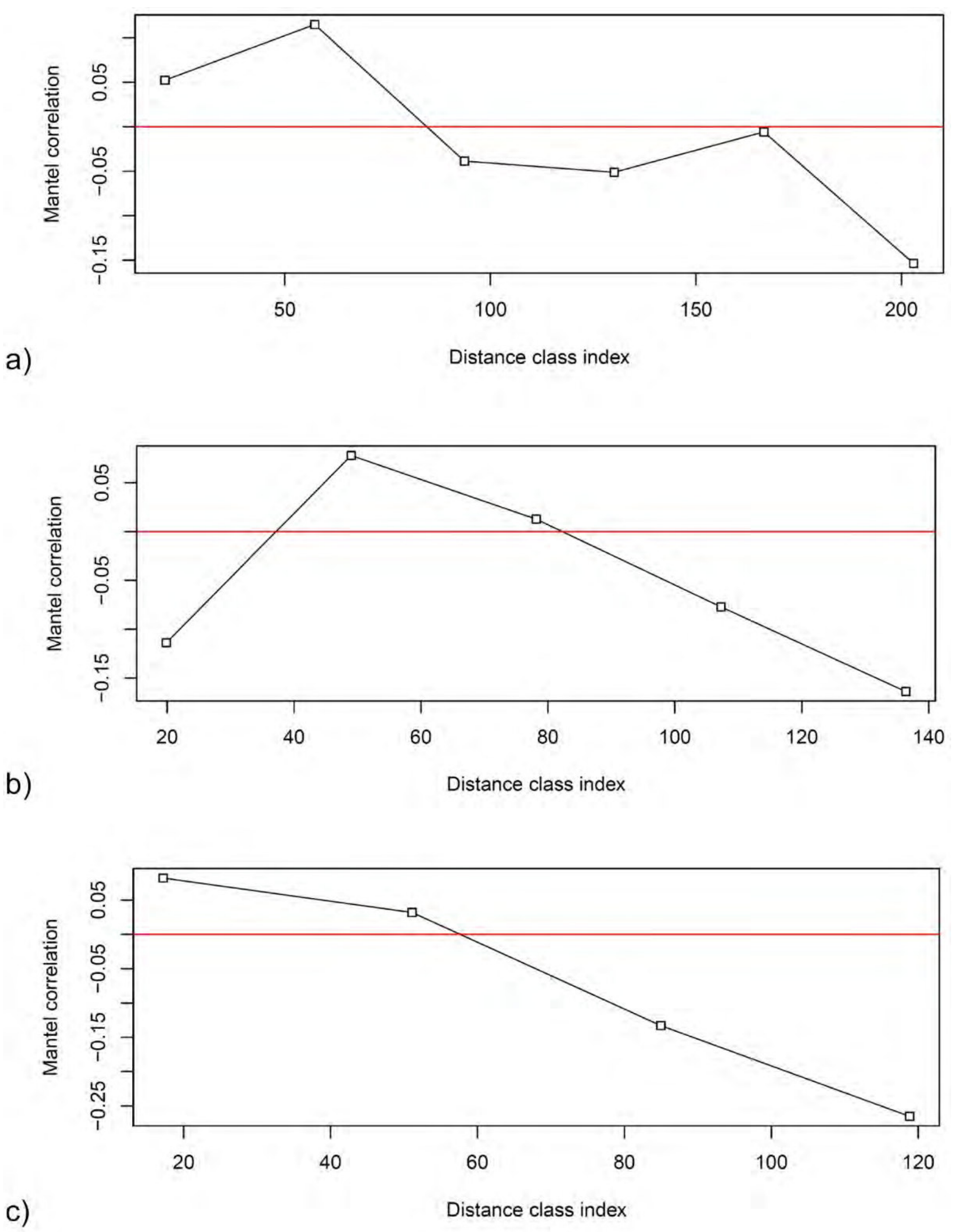


Fig. S3 Mantel correlograms for *Asplenium septentrionale* (a), and *Hedwigia ciliata* lineages d1 (b) and d2 (c). None of the tested distance classes were significant.

Chapter III

The distribution of climbing chalk on climbed boulders and its impact on rock-dwelling fern and moss species

Daniel Hepenstrick, Ariel Bergamini, Rolf Holderegger

Manuscript published in Ecology and Evolution (2020)
https://doi.org/10.1002/ece3.6773

Abstract

Rock climbing is a popular sport and the number of climbers is rising worldwide. Numerous studies on the impact of climbing on rock-dwelling plants have indicated negative effects, which were mainly attributed to mechanical disturbances such as trampling and removal of soil and vegetation. However, climbers also use climbing chalk (magnesium carbonate hydroxide), whose potential chemical effects on rock-dwelling species have not been assessed so far. Climbing chalk is expected to alter the pH and nutrient conditions on rocks, which may affect rock-dwelling organisms. We investigated two fundamental aspects of climbing chalk: (i) Its distribution along non-overhanging climbing routes was measured on regularly spaced raster points on gneiss boulders used for bouldering (ropeless climbing at low height). These measurements revealed elevated climbing chalk levels, even on 65% of sampling points without any visual traces of climbing chalk. (ii) The impact of climbing chalk on rock-dwelling plants was assessed for four fern and four moss species in an experimental set-up in a climate chamber. The experiment showed significant negative, though varied effects of elevated climbing chalk concentrations on the germination and survival of both ferns and mosses. Our findings thus suggest that elevated climbing chalk concentrations along climbing routes can occur even where no chalk traces are visible and that climbing chalk can have negative impacts on rock-dwelling organisms.

Keywords: bouldering, bryophytes, cliff ecosystem, human disturbance, magnesia, magnesium carbonate, plant conservation

Introduction

The number of climbers is rising worldwide, since the popularity of rock climbing, as a sport activity, is continuously increasing. Its Olympic debut in Tokyo 2021 will probably further amplify this trend (Attarian and Keith 2008; IOC 2016). Along with this rise in rock climbing, previously uninfluenced rock habitats are increasingly frequented by climbers and the adverse impacts of this activity require mitigation (Hanemann 1999; Holzschuh 2016). Several authors have studied the impact of climbing on multiple organismic groups (reviewed in Holzschuh 2016) and predominantly reported negative effects. However, Holzschuh (2016) pointed out that most of the studies on the impact of climbing are based on the direct comparison between climbed and unclimbed rocks (e.g. Nuzzo 1996; Müller et al. 2004; Rusterholz et al. 2004) and thereby might overestimate the impact of climbing because of confounded abiotic differences between climbed and unclimbed rocks such as terrain roughness. Nevertheless, recent studies accounting for this methodological drawback still demonstrated negative effects of climbing activities on rock vegetation (Tessler and Clark 2016; March-Salas et al. 2018) and showed that increasing climbing intensity corresponds to increasing alterations of species communities on rocks (Lorite et al. 2017; Schmera et al. 2018). While for rock-nesting bird species, simple human presence leads to disturbance (Camp and Knight, 1998; Covy et al. 2019), negative effects of rock climbing on sessile rock-dwelling organisms is mainly attributed to mechanical disturbances such as trampling and removal of soil and vegetation (Holzschuh 2016). However, climbing chalk – a component unique to climbing among all outdoor activities – has so far been considered merely a visual indicator of climbing activities on rocks (Camp and Knight 1998; Thiel and Spribille 2007; Adams and Zaniewski 2012; Clark and Hessl 2015), while its potential chemical impact on rock-dwelling species has rarely been mentioned (Holzschuh 2016; Tessler and Clark 2016). In fact, the only published study on the potential chemical impact of climbing chalk was conducted by Fickert (2014), who investigated the potential increase in soil pH at the base of climbed boulders due to climbing chalk and found no difference between soil pH at the base of climbed and unclimbed boulders. Given the scarcity of information, the chemical impact of climbing chalk on rock-dwelling vegetation remains largely unknown.

Climbing chalk is a fluffy white powder that consists of magnesium carbonate hydroxide ($1\text{-}4MgCO_3 \cdot Mg(OH)_2 \cdot 3\text{-}5H_2O$), which is also known as magnesia alba or basic magnesium carbonate (Shand 2006; Ropp 2013). It dries hand sweat and thereby enhances grip friction. Traditionally used in gymnastics, in the 1950s it was introduced to bouldering, which is a low-height (generally < 4 m, often on boulders), ropeless subdiscipline of climbing (Gill 1969; Niegl 2009; Tessler and Clark 2016). Nowadays, climbing chalk is perceived as an inherent component of all kinds of rock climbing, but its use is probably most extensive in bouldering (Attarian and Keith 2008; Niegl 2009). Magnesium carbonate hydroxide is barely soluble in pure water, in aqueous suspension it has a pH of around 10.5, its solubility is better if water contains CO_2, and it readily dissolves in diluted acid (Budavari et al. 1996; Ropp 2013; Shand 2006). With regard to the pH dependency of plant nutrient uptake and the vital role of magnesium as a macronutrient, climbing chalk can be expected to impact plant growth (Barker and Pilbeam 2015). This potential impact should be interdependent with rock chemistry, as acidic conditions on siliceous rock (e.g. granite or gneiss) strongly contrast the alkaline properties of climbing chalk, while alkaline conditions on carbonate rock (e.g. limestone or dolomite) are more in line with the chemical properties of climbing chalk (Kinzel 1983). Hence, calcifuge species might be more susceptible to climbing chalk than calcicoles. Among the diverse life forms of rock-dwelling plants, ferns and mosses – common life forms in most cliff ecosystems (Larson et al. 2000) – should be particularly sensitive to climbing chalk, as their early gametophytic stages (prothallia and protonema) lack regulatory mechanisms and directly absorb water with their single-cell-layer plant bodies (Jahns 1983).

In the present study, we addressed two fundamental aspects contributing to the understanding of the potential impact of climbing chalk on rock-dwelling ferns and mosses. First, in order to gain information on the extent of climbing chalk present along climbing routes, we measured its presence, concentration and distribution on gneiss boulders used for bouldering. Second, in order to explore a potential chemical effect of climbing chalk on rock-dwelling ferns and mosses, we experimentally tested if different climbing chalk concentrations affected the germination and survival of rock-dwelling ferns and mosses in a climate chamber experiment.

Materials and Methods

Study site and measurements of climbing chalk on climbed boulders

In order to obtain information on the presence, distribution and concentration of climbing chalk on climbed rock, we used the following sampling scheme. We sampled bouldering routes, due to their greater accessibility compared with roped climbing routes, on siliceous boulders, which enabled swab-sampling with diluted acid, as the acid dissolves climbing chalk traces potentially present on the rock but does not react chemically with the rock itself (as it would with limestone). We selected non-overhanging rock surfaces with flat topographies of similar slope and equal size in order to increase comparability (Fig. 1, Supplementary Fig. S1).

Field sampling took place in southern Switzerland (Ticino) in two well-known bouldering areas, Cresciano and Chironico, which each comprise > 1000 bouldering routes (Ambrosio et al. 2006a; Ambrosio et al. 2006b). Both bouldering areas are holocene landslide deposits of gneiss boulders of the Penninic Leventina nappe whose granitic orthogneisses generally have a low magnesium content (Claude et al. 2014; Rütti et al. 2008). In each bouldering area, we sampled the first three rock faces we came across that were not markedly overhanging (i.e. slope < 95°) and had a rather flat topography on a surface 2.5 m in height and 1.5 m in width including a substantial part of a bouldering route (Table 1). Within the 2.5 m × 1.5 m area (Fig. 1) on each boulder, two sets of sampling points were collected: (i) The general distribution of climbing chalk along the bouldering route was sampled on a 0.5 m raster grid. (ii) The climbing holds were targeted by sampling one point at the centre of each climbing hold, as well as vertically 10 cm above, 10 cm below and 20 cm below the climbing hold. As a control, we additionally sampled a total of 20 sampling points on unclimbed boulders (10 points per bouldering area). For each sampling point, we inspected an area of 2 cm × 1 cm, noted whether climbing chalk deposits were visible or not, and carefully tabbed off the 2 cm × 1 cm area with a medical swab (FLOQSwabs 502CS01, Copan, Brescia, Italy) that was slightly wetted in 2% nitric acid in order to dissolve and take up a maximum amount of climbing chalk potentially present on the sampling point. The swabs were put into individual 15 ml centrifuge tubes containing 10 ml pure water. In the laboratory, the magnesium content was determined with ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry; Optima 7300 DV, Perkin Elmer, Waltham, USA). As a conservative threshold level for elevated climbing chalk concentrations, we defined the empirical 99.7th percentile (mean plus three standard deviations) of the magnesium concentrations measured on the 20 control sampling points on unclimbed boulders (i.e. 0.00126 mg/cm^2 magnesium).

Table 1 Sampled bouldering routes in the two bouldering areas Cresciano and Chironico. Climbing grade (Fontainebleau-Scale), aspect and inclination of the sampled 2.5 m × 1.5 m areas, coordinates (latitude/longitude WGS84) and sampling date.

ID	Climbing grade*	Aspect	Inclination	Coordinates	Sampling date
Cresciano					
A	6a	N	Vertical (~90°)	46.28573/9.00702	22.10.2016
B	6b+	SW	Inclined (<90°)	46.28695/9.00664	22.10.2016
C	6b	S	Vertical (~90°)	46.28584/9.00689	13.10.2017
Chironico					
D	6a+	SW	Vertical (~90°)	46.430220/8.846502	13.10.2017
E	NA	SO	Inclined (<90°)	46.427499 /8.849307	13.10.2017
F	7c+	W	Vertical (~90°)	46.430650/8.848645	14.10.2017

* Ambrosio et al. (2006a); Ambrosio et al. (2006b)

Germination and survival experiment

In order to explore the potential impact of climbing chalk on rock-dwelling fern and moss species under controlled conditions, we set up a factorial experiment in which we sowed spores on agar plates with different climbing chalk concentrations and assessed their germination and survival. Spores of four rock-dwelling fern species (*Asplenium septentrionale*, *A. trichomanes* ssp. *quadrivalens*, *Cystopteris fragilis* and *Polypodium vulgare*) and four rock-dwelling moss species (*Grimmia pulvinata*, *Hedwigia ciliata*, *Hypnum cupressiforme* and *Orthotrichum anomalum*) covering a broad spectrum of rock habitat types were collected in Switzerland (Table 2). At each site, several fertile fern fronds with freshly opened sporangia or moss sporophytes from about five different individuals were pooled, dried and stored at room temperature. Fern spores were purified using different sieves (1 cm to 50 µm), and moss spores were purified by separating sporophyte debris from spores using forceps.

Table 2 Rock-dwelling ferns and mosses used in the germination and survival experiment. Typical habitat and pH preferences (calcicole or calcifuge), site (locality, latitude/longitude WGS84), date of accession, and year in which the experiment was conducted (run).

Species	Habitat preferences*	Accession	Run
Ferns:			
Asplenium septentrionale (L.) Hoffm.	Exposed siliceous rocks; calcifuge	Ausserberg (VS), 46.31463/7.84271 1.7.2014	2017
Asplenium trichomanes subsp. *quadrivalens* D. E. Mey.	Calcareous rocks and mortared walls; calcicole	Wädenswil (ZH) 47.22331/8.67640 12.10.2012	2018
Cystopteris fragilis (L.) Bernh.	Shady basic rocks and walls; calcicole	Wädenswil (ZH) 47.22288/8.67689 12.7.2018	2018
Polypodium vulgare L.	Acidic rocks, also epiphytic; calcifuge	Arth-Goldau (SZ) 47.04790/8.55601 16.9.2017	2018
Mosses:			
Grimmia pulvinata (Hedw.) Sm.	Calcareous rocks, walls and concrete; calcicole	Wädenswil (ZH) 47.22131/8.67664 4.5.2017	2018
Hedwigia ciliata (Hedw.) P.Beauv.	Exposed siliceous rocks; calcifuge	Bellinzona (TI) 23.10.2016 46.18851/9.03073	2017
Hypnum cupressiforme Hedw.	Siliceous rocks, rotting wood, trees and soil; calcifuge	Wädenswil (ZH) 47.21805/8.67905 2.3.2017	2017
Orthotrichum anomalum Hedw.	Calcareous rocks, walls and concrete; calcicole	Wädenswil (ZH) 47.22151/8.67691 4.5.2017	2017

* Ellenberg et al. (1992); Jahns (1983); Lauber et al. (2018)

The experiment was conducted in 35 mm diameter Petri dishes each containing 4 ml of 0.45% agar medium (A 7002 Sigma-Aldrich, St. Louis, USA). We prepared four different types of agar media that varied in their climbing chalk concentrations: The 0% climbing chalk medium (control) was based on pure water, the 100% climbing chalk medium was based on pure water saturated with climbing chalk (26 mg/l magnesium; Loose White Gold Chalk, Black Diamond, Innsbruck, Austria), and the 50% and 25% media were based on 1:1 and 1:3 dilutions of the saturated climbing chalk solution, respectively. Because of the nutrient poverty of rock habitats (Larson et al. 2000) and because agar contains some nutrients (Bridson and Brecker 1970), no additional nutrients were added.

We did not sterilise spores in order to avoid unwanted influences due to the sterilisation process (Camloh 1999). Therefore, spore batches were first checked for contamination with e.g. fungi by sowing 10 Petri dishes per spore batch. Batches that showed contamination after one week on more than three dishes were discarded. For sowing, a pinch of spores was suspended in 500 µl pure water containing 0.05% of the non-ionic detergent Tween 80 (Sigma-Aldrich, Louis, USA), which prevented spores from clumping. After soaking for two hours, spore suspensions were diluted to a concentration of 10–15 spores/µl. One droplet of 2 µl spore suspension was placed into the Petri dish with a piston pipette, resulting in ca. 20–30 spores dispersed in a circle of 4 mm diameter. Petri dishes were sealed with parafilm.

In the experiment, every combination of plant species and climbing chalk concentration was replicated in 12 petri-dishes (i.e. 384 dishes in total). Germinated and living plants were counted under a stereo microscope weekly for six weeks. The number of sown spores was determined during the first counting. *Hypnum cupressiforme* (Table 2) was counted twice a week for only 2.5 weeks, because later on it was impossible to distinguish between individuals as a result of its filamentous and fast growing protonema (Supplementary Fig. S2).

The experiment was carried out in two runs (due to limited availability of the climate chamber; 02.05.2017–11.07.2017 and 18.10.2018–04.12.2018; Table 2) in the same four-shelf climate chamber (RUMED 1301, Rubarth, Laatzen, Germany) at a constant temperature of 22°C, a relative humidity of 80% and a 16/8 h day/night cycle (eight fluorescent tubes; Philips, Amsterdam, Niederlande, 58W, TLD480 REFLEX, each about 5240 lumens). The 48 Petri dishes per species were equally distributed among four trays of acrylic glass, each tray containing three randomly assigned replicates of each of the four climbing chalk concentrations. Each of the four trays was randomly assigned to one of the four shelves (one tray per species per shelf). A potential temperature gradient among shelves was accounted for by weekly moving the bottom shelf to the top position and the other shelves one position downwards. Tray positions on shelves were randomised weekly and dish position per tray was randomised during counting.

From the six counting events per Petri dish, we derived two response variables: (i) germination rate, i.e. the maximum number of plants observed at any counting event divided by the number of sown spores; and (ii) survival rate, i.e. the number of surviving plants at end of the experiment divided by the maximum number of plants observed at any counting event.

The experiment corresponded to a split plot design (Altman and Krzywinski 2015), and we thus analysed the germination and survival rates, which were based on count data of a binomial nature, with generalised linear mixed models using the binomial family with the logit link function (package lme4; Bates et al. 2015) in R (R Core Team 2017). As fixed effects, we considered the block effect ‘shelf’ (eight levels: four climate chamber shelves times two runs), the treatment effect ‘species’ (eight levels, one per species), the treatment effect ‘climbing chalk concentration’ (four levels: 0%, 25%, 50%, 100%) and the interaction between species and climbing chalk concentration. As random effects, we included the following further factors given by the experimental design: the two runs (i.e. the two time periods in which the experiment was conducted; see above), the 32 plexiglass trays on which the Petri dishes were placed (four trays per species, each tray corresponding to a plot), and, in order to account for overdispersion, the individual Petri dishes (corresponding to split plots). The fixed effects were tested in sequential likelihood ratio tests (deviance tables; Nelder and Wedderburn 1972) and the differences between adjacent factor levels of climbing chalk concentrations were tested with forward difference contrasts (Venables and Ripley 2002).

Results

Measurements of climbing chalk on climbed boulders

The measurements on climbed boulders revealed distinctively elevated climbing chalk (magnesium) concentrations, with the highest values on climbing holds and raster points with visible climbing chalk traces (Fig. 1, Supplementary Fig. S1). On and around all 41 climbing holds, climbing chalk visibility and measured concentrations were highest at the middle of the holds, followed by the sampling points 10 cm below, then 20 cm below and finally 10 cm above the climbing holds (Fig. 2, Table 3). The sampling of 141 raster points revealed that 85 (65%) of the 130 sampling points without any visible climbing chalk traces showed elevated climbing chalk concentrations above the defined threshold level of 0.00126 mg/cm^2 magnesium (Fig. 2, Table 3). One of the six surveyed boulders exceeded the climbing chalk concentration threshold at every sampling point (Fig. 1c).

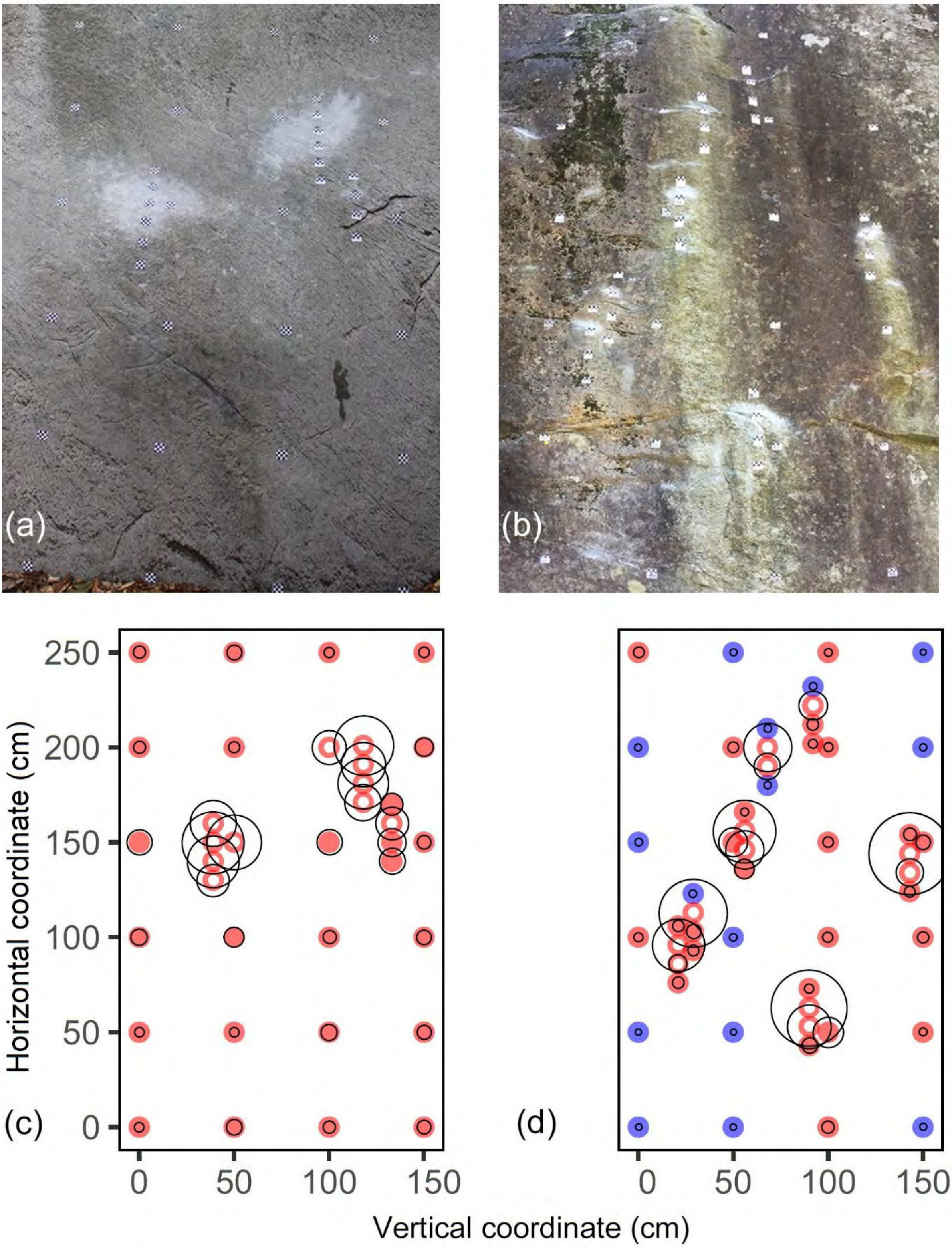


Fig. 1 Example of the climbing chalk (magnesium) distribution on two climbed boulders (boulders B and E, Table 1). In the photos (a, b) of the assessed 2.5 m × 1.5 m rectangles, stickers mark sampling points, and white climbing chalk traces are visible predominantly around the climbing holds. The corresponding graphics (c, d) visualise the amount of climbing chalk (magnesium) measured at the sampling points. Red: value above threshold level; blue: value below threshold level; white dots: visible climbing chalk traces at sampling points; the area within each black circle is proportional to the amount of climbing chalk measured at the corresponding sampling point.

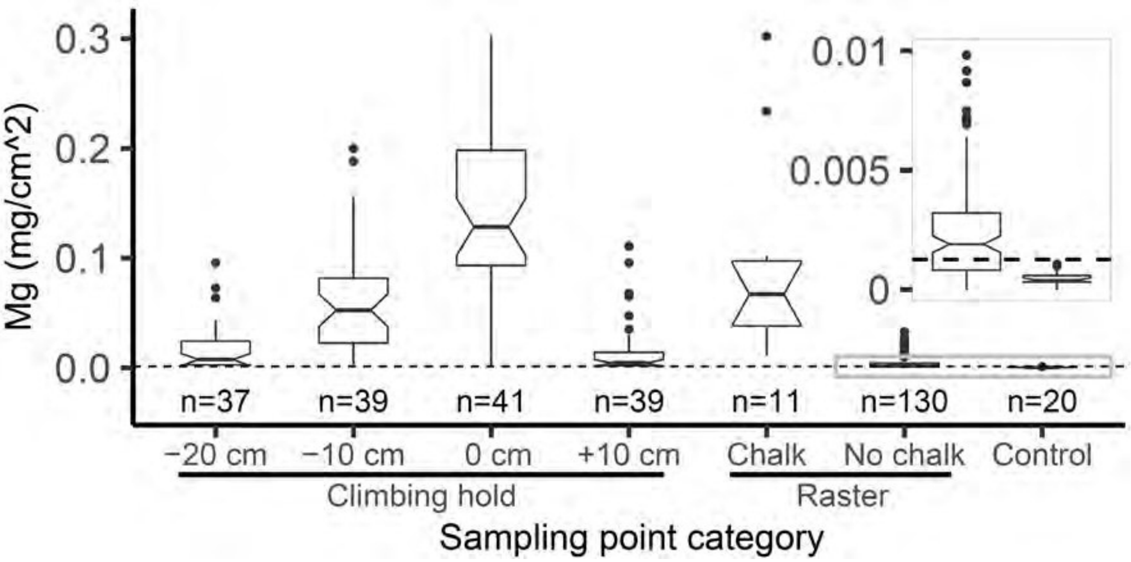


Fig. 2 Amount of climbing chalk (magnesium) measured on climbed and unclimbed (control) boulders. Sampling points on and around climbing holds are grouped by their vertical distance to the climbing hold (-20 cm, -10 cm, 0 cm, +10 cm), and raster sampling points are grouped by the visibility of climbing chalk traces (chalk, no chalk). The dashed line indicates the threshold above which climbing chalk measurements were considered elevated. The inset shows the content of the grey box at an enlarged scale.

Table 3 Summary of the 317 sampling points assessed on gneiss boulders. Percentages of sampling points with visible climbing chalk traces and elevated climbing chalk concentrations (magnesium) for each of the different types of sampling points: on (0 cm), above (+10 cm) and below climbing holds -10 cm, -20 cm), on raster points with and without visible traces of climbing chalk, and on unclimbed boulders (control).

		N	**Climbing chalk visible [%]**	**Magnesium elevated [%]**
Climbing hold		156	57	95
	+10 cm	39	13	82
	0 cm	41	100	100
	-10 cm	39	79	100
	-20 cm	37	32	97
Raster		141	8	67
	Chalk	11	100	100
	No chalk	130	0	65
Control		20	0	0

Germination and survival experiment

Responses to climbing chalk differed among the fern species (Fig. 3). The germination rate and survival rate of *Asplenium septentrionale* declined with increasing climbing chalk concentration. *Asplenium trichomanes* showed generally low germination and almost no survival across all media. *Cystopteris fragilis* germinated equally well under all climbing chalk concentrations, while survival was considerably reduced on media containing climbing chalk. Finally, the response of *Polypodium vulgare* in terms of both germination and survival was rather uniform across all climbing chalk concentrations. The moss species showed no clear systematic difference from the fern species but also rather diverse response patterns (Fig. 3). *Grimmia pulvinata* and *Hedwigia ciliata* germinated equally well on all media, while survival was reduced on media containing climbing chalk. The germination rate and survival rate of *Hypnum cupressiforme* showed a slight decline with increasing climbing chalk concentration, and *Orthotrichum anomalum* showed lower germination and survival on media containing climbing chalk. Notably, the specimens of all species appeared less vigorous on climbing chalk media (examples in Supplementary Fig. S2).

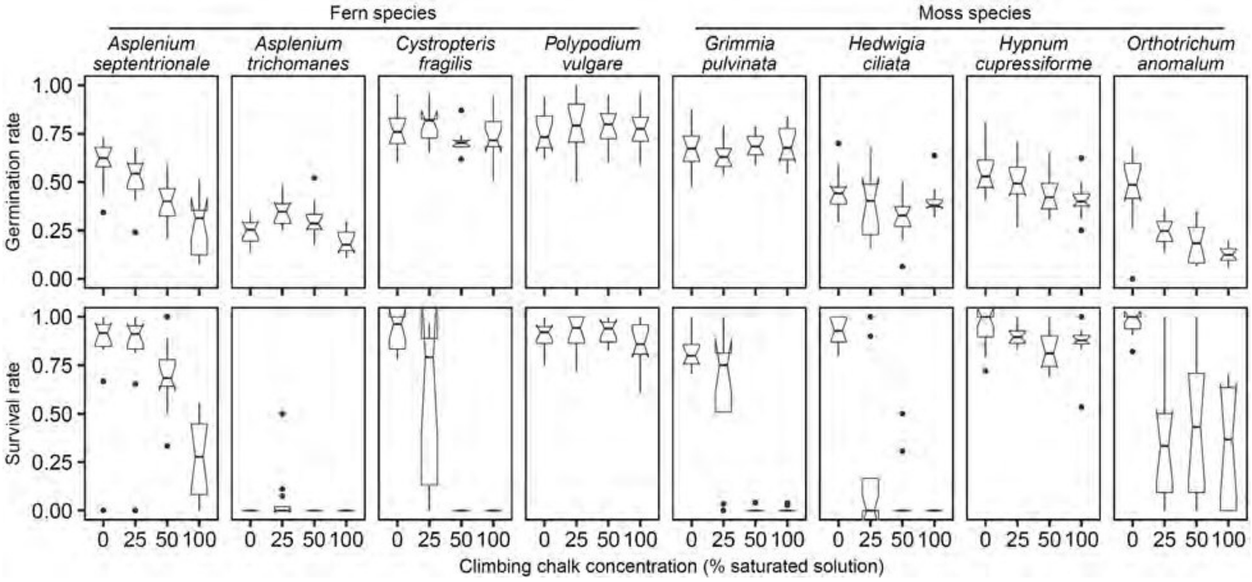


Fig. 3 Germination and survival rates of four fern and four moss species on agar with four climbing chalk concentrations.

The effects of species, climbing chalk concentration and their interaction were significant for both germination and survival rates, while the block effect of climate chamber shelf had no significant influence (Table 4). The effect of climbing chalk concentration was negative: between the 25% and 50% media and between the 50% and 100% media germination and survival rates decreased significantly, while the difference between 0% and 25% was not significant (Table 5).

Table 4 Analysis of deviance of fixed effects, sequentially added to the random effect model, on the germination and survival rates of fern and moss species.

	Fixed effect	**df model**	**Deviance model**	**$\chi 2$**	**df**	**p**
Germination rate						
	Intercept	4	2091	-	-	-
	Climate chamber shelf	11	2085	6.2	7	0.52
	Species	17	1981	103.9	6	<0.0001
	Mg concentration	20	1922	58.9	3	<0.0001
	Species : Mg concentration	41	1797	125.2	21	<0.0001
Survival rate						
	Intercept	4	1513	-	-	-
	Climate chamber shelf	11	1508	4.6	7	0.71
	Species	17	1408	99.8	6	<0.0001
	Mg concentration	20	1244	164.4	3	<0.0001
	Species : Mg concentration	41	1024	220.2	21	<0.0001

Table 5 Coefficients obtained by forward difference contrasts of the four levels of climbing chalk concentration in the full generalised linear mixed models for germination and survival rates of all four fern and four moss species analysed together.

	Contrast	**Estimate**	**Std. Error**	**Z-value**	**p**
Germination rate					
	25% vs. 0%	-0.300	0.176	-1.703	0.088
	50% vs. 25%	-0.570	0.177	-3.224	0.001
	100% vs. 50%	-0.516	0.188	-2.750	0.006
Survival rate					
	25% vs. 0%	-0.079	0.598	-0.132	0.895
	50% vs. 25%	-1.542	0.570	-2.707	0.007
	100% vs. 50%	-2.044	0.564	-3.624	0.0002

Discussion

Our findings demonstrate that elevated climbing chalk concentrations can occur along climbing routes even where no chalk traces are visible and that climbing chalk can have negative impacts on the germination and early survival of rock-dwelling ferns and mosses.

Measurements of climbing chalk concentrations on climbed boulders

To our knowledge, this is the first study presenting measurements of climbing chalk (magnesium) on climbed rock. We deduced climbing chalk input by climbers by measuring the amount of magnesium on climbed boulders, which we compared with the amount of naturally occurring magnesium detected on unclimbed boulders of the same rock type. In the samples taken in the middle of climbing holds, vertically 10 cm above, 10 cm below and 20 cm below, the highest concentrations were detected in the middle of the climbing holds, where climbing chalk traces were always visible (Fig. 2, Table 3). Above climbing holds, both visible climbing chalk traces and concentrations diminished rapidly. In contrast, below climbing holds, visible climbing chalk traces and climbing chalk concentrations diminished gradually. This trend of primarily downward dispersal of climbing chalk on rock faces was also noticeable in raster sampling points without visible climbing chalk traces but often with elevated climbing chalk concentrations (Fig. 1d, Supplementary Fig. S1). We hypothesise that three interdependent main factors influence the distribution of climbing chalk on climbed rocks: (i) Climbing intensity (Schmera et al. 2018) is positively correlated with the input of climbing chalk. (ii) The climbing routes' microtopography (including slope; Kuntz and Larson 2006) will affect the amount and frequency with which a climber applies climbing chalk and also the way that climbing chalk is dispersed. (iii) The dispersion pathway – either leaching with runoff rainwater or as dust during climbing (Weinbruch et al. 2008) or brushing/cleaning of climbing holds (Niegl 2009) – influences the extent to which climbing chalk spreads on a rock away from climbing routes and holds. The examples given in Figure 1 illustrate these aspects. The popular route called "the never ending story" (Fig. 1a; Ambrosio et al. 2006b) exceeded the climbing chalk concentration threshold at every sampling point (Fig. 1c). It is climbed intensively, its microtopography necessitates two extensive holds, and runoff rainwater and dust from climbing and brushing of holds further spread climbing chalk on the < 90° inclined rock. In contrast, on a less popular, unnamed route (Fig. 1b; Ambrosio et al. 2006a), elevated climbing chalk concentrations were more confined to climbing holds and adjacent areas below them (Fig. 1d). The route is only rarely climbed, its climbing holds are small, and there were no signs of brushing activity. Here, runoff rainwater on the < 90° inclined rock might not only distribute climbing chalk but also cause magnesium to leach and levels to decrease over time.

Germination and survival experiment

We are not aware of other studies on the effect of climbing chalk on mosses and ferns or on any other rock-dwelling organisms. Methodologically, we approached this question with a germination experiment with different climbing chalk concentrations on agar in a climate chamber. On the one hand, conditions on agar plates in a climate chamber are far from the environmental conditions in rock habitats, but on the other hand, the chosen approach made it possible to test varying climbing chalk concentrations in a controlled way and to quantitatively measure germination and survival. While in standard ecotoxicological germination tests germination rate is assessed as the fraction of germinated seeds after a defined time span (OECD 2006; Wang and Keturi 1990), we defined germination rate as the ratio between the maximum number of germinated, living plants and the number of spores sown per Petri dish. This was necessary because of the different germination behaviour of the assessed species. For instance, *Hypnum cupressiforme* had already started germinating three days after sowing, and after only one week 83% of the Petri dishes with spores from this species reached their maximum number of living plants. In contrast, spores of *Orthotrichum anomalum* took two weeks to start germination (Supplementary Fig. S3). Across all species, except *H. cupressiforme*, 87% of the Petri dishes had reached their maximum number of living plants after four weeks. Hence, the germination process in this study can be considered rather complete. However, survival rate – the fraction of plants surviving until the end of the experiment – reflected an ongoing process. This becomes obvious when considering the development of the studied species over time (Supplementary Fig. S3). While nearly all *Asplenium trichomanes* individuals had died after six weeks, the number of living plants per Petri dish of most other species was steadily decreasing, particularly under higher climbing chalk concentrations.

Germination and survival rates differed among species (Fig. 3, Table 4), but there was a lack of correspondence between a species' ecology (Table 2) and its response to elevated climbing chalk concentrations in the experiment (Fig. 3). One would expect calcicoles to be more tolerant of higher pH values and magnesium concentrations, because they are adapted to high pH levels and high ion concentrations of calcium, which is chemically similar to magnesium (Barker and Pilbeam 2015; Lee 1999). Nevertheless, the calcicoles *Cystopteris fragilis* and *Grimmia pulvinata* showed a response similar to that of the calcifuge *Hedwigia ciliata*, with almost no survival on 50% and 100% climbing chalk media, whereas the calcifuge *A. septentrionale* responded with a gradual decline in germination and survival, and the calcicole *O. anomalum* exhibited an intermediate pattern. The calcicole subspecies of *A. trichomanes* had generally low germination and survival at all climbing chalk concentrations, a result that could also have been caused by the rather old spores of this species used in the experiment (Table 2; Camloh 1999). Only *Polypodium vulgare* and *H. cupressiforme* showed congruence of their response patterns (only slight differences in germination and survival among different climbing chalk concentrations) with their ecology (weakly calcifugeand also growing epiphytic). In summary, a simple calcicole-calcifuge classification (Lee 1999) did not predict the species' responses to elevated climbing chalk concentrations in the present study. The observed differences rather reflect species-specific reactions to pH and ion concentrations, as documented for diverse fern and moss species (Suo et al. 2015; Wiklund and Rydin 2004).

Synthesis and outlook

On six climbing routes on gneiss used for bouldering, on regularly spaced raster points, elevated climbing chalk levels were measured even at 65% of the sampling points without any visual traces of climbing chalk (Table 3). In the laboratory experiment with four fern and four moss species, increasing climbing chalk concentrations impaired germination and survival (Table 5). These two results may be set into relation through the amount of climbing chalk involved. For instance, the amount of magnesium present at the threshold level of elevated climbing chalk concentrations (0.00126 mg/cm^2) is equivalent to a ca. 1 mm thick layer of 50% saturated climbing chalk medium, which significantly impaired germination and survival in the experiment (13 mg/l). One could thus conclude that even on 65% of the raster sampling points without any visual traces of climbing chalk, rock-dwelling plant species (Table 2) may well be negatively affected. However, unlike thawing salt, which simply increases the concentration of sodium (Na^+) and chloride (Cl^-) ions (Blomqvist 1998), the situation with climbing chalk is more complex. The solubility of climbing chalk increases with increasing water acidity (Budavari et al. 1996), which is influenced by the acidity of rain and the runoff through substrates and on rock (Larson et al. 2000). When dissolved, the ions involved in magnesium carbonate hydroxide are magnesium cations (Mg^{2+}), which increase the ion concentration, and the anions carbonate (CO_3^{2-}) and hydroxide (OH^-), which additionally increase pH. In solution, the anionic components of climbing chalk are in acid–base equilibria: hydroxide with water (H_2O) and carbonate with bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3), which is in solution equilibrium with CO_2 in the air. Hence, depending on the solution and acid–base equilibria, climbing chalk can evolve into other compounds than the initial magnesium carbonate hydroxide. This interplay of water acidity and solubility of climbing chalk should be strongly influenced by rock type. Acidic conditions on siliceous rock, like the gneiss rock on which we conducted our measurements, enhance the solubility of climbing chalk and may therefore also enhance the dislocation of magnesium with runoff water. In contrast, the alkaline conditions on limestone lower the solubility of climbing chalk, which may thereby enhance its persistence on rock. Dislocation with runoff water on limestone may thus be predominantly in the form of climbing chalk particles, rather than its dissolved ions. Further, on porous sandstone the persistence of climbing chalk has been reported to be particularly enhanced, as it irreversibly stains and alters sandstone surfaces (Attarian and Keith 2008;Niegl 2009; Huddart and Stott 2019) In addition, rock type is a main determinant of plant community composition on rocks (Spitale and Nascimbene 2012). Calcicole and calcifuge communities might react differently to climbing chalk, due to the different chemical behaviour of climbing chalk on different rock types rather than to calcicole-calcifuge adaptations of plant species. We found no evidence of the latter explanation in our experiment.

In the diverse studies documenting impacts of climbing on rock vegetation (Nuzzo 1996; Rusterholz et al. 2004; Müller et al. 2004; Tessler and Clark 2016; Lorite et al. 2017; March-Salas et al. 2018; Schmera et al. 2018), the potential chemical impact due to climbing chalk and the mechanical impacts of climbing, such as trampling and removal of soil and vegetation, are usually confounded on climbed rocks (Holzschuh 2016). In contrast, in our study we assessed the distribution of climbing chalk separately from its impact on species in an experiment; however, the real impact of climbing chalk on rock-dwelling plants under natural conditions remains difficult to deduce. To this end, studies in climbing areas that only allow climbing chalk on a subset of their routes (Werdermann 1993; Heinicke 2001) might enable comparisons of the impact of climbing with and without climbing chalk. Furthermore, it would be worthwhile to continue with an experimental approach by applying climbing chalk on unclimbed rock and studying its in-situ impact on rock-dwelling species (bryophytes and ferns, but also lichens and flowering plants) on different rock types. Finally, recent advances in drone and imaging technology may facilitate the mapping and analysis of visible climbing chalk traces and vegetation health along climbing routes with hyperspectral imaging (Peng et al. 2020, Strumia et al. 2020, Zhang et al. 2020).

Recommendations for conservation practice

Our study indicates that climbing chalk might negatively affect mosses and ferns growing along climbing routes. Although there is a clear necessity for further research on this issue, we suggest taking into account the potential impact of climbing chalk when developing conservation measures for rare rock-dwelling species in the close vicinity of climbing routes (e.g. when erratic boulders that harbour rare species are used for bouldering; Hepenstrick et al. 2016; Mazenauer et al. 2014). In order to judge the potential threat imposed by climbing chalk, it helps to consider climbing intensity, microtopography and dispersion pathways (leaching with runoff rainwater and dust from climbing or brushing). In our experiment with different climbing chalk concentrations, negative impacts of climbing chalk were apparent for both calcicole and calcifuge species. Hence, potential impacts of climbing chalk are not necessarily restricted to calcifuge plant species. Furthermore, alternatives to climbing chalk, such as adhesive colophony resin and absorptive balls which remove excess moisture from hands (Niegl 2009), could be assessed as a potential solution in cases where the use of climbing chalk is problematic.

Acknowledgements

We thank Christian Lusti and Jonathan Pachlatko for assistance in the field, Nicolas Phan-huy and Dominik Penkov for help with the climate chamber experiment, and Lilian Gasser for statistical help. We are grateful for financial support from several foundations, associations, and governmental and non-governmental organisations (listed below), and we thank two anonymous referees for valuable comments on an earlier version of the manuscript.

References

Altman N, Krzywinski M (2015) Split plot design. Nat Methods 12:165-166. http://dx.doi.org/10.1038/nmeth.3293

Ambrosio A, Cameroni C, Grizzi R, Lodi R, Vonarburg N (2006a) Chironico boulder. Ticino Boulder, Bellinzona

Ambrosio A, Cameroni C, Grizzi R, Lodi R, Vonarburg N (2006b) Cresciano boulder. Ticino Boulder, Bellinzona

Attarian A, Keith J (2008) Climbing management, a guide to climbing issues and the development of a climbing management plan. The Access Fund, Boulder

Ballantyne M, Pickering CM (2015) The impacts of trail infrastructure on vegetation and soils: current literature and future directions. J Environ Manage 164:53-64. http://dx.doi.org/10.1016/j.jenvman.2015.08.032

Barker AV, Pilbeam DJ (2015) Handbook of plant nutrition. CRC Press, Boca Raton

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:1-48. https://doi.org/10.18637/jss.v067.i01

Blomqvist G (1998) Impact of de-icing salt on roadside vegetation: A literature review. Swedish National Road and Transport Research Institute, Linköping

Bridson EY, Brecker A (1970) Design and formulation of microbial culture media. In: Norris JR, Ribbons DW (eds) Methods in microbiology 3A. Cambridge University Press, London, pp 229-295

Budavari S, O'Neil MJ, Smith A, Heckelman PE, Kinneary JF (eds) (1996) The Merck index, an encyclopedia of chemicals, drugs and biologicals. Merck, Whitehouse Station

Camloh M. (1999) Spore age and sterilization affects germination and early gametophyte development of *Platycerium bifurcatum*. Am Fern J 89:124-132. https://doi.org/10.2307/1547346

Camp RJ, Knight RL (1998) Rock climbing and cliff bird communities at Joshua Tree National Park, California. Wildl Soc Bull 26:892-898 https://jstor.org/stable/3783567

Claude A, Ivy-Ochs S, Kober F, Antognini M, Salcher B, Kubik PW (2014) The Chironico landslide (Valle Leventina, southern Swiss Alps): age and evolution. Swiss J Geosci 107:273-291. https://doi.org/10.1007/s00015-014-0170-z

Covy N, Benedict L, Keeley WH (2019) Rock climbing activity and physical habitat attributes impact avian community diversity in cliff environments. PLoS ONE 14: e0209557. https://doi.org/10.1371/journal.pone.0209557

Clark P, Hessl A (2015). The effects of rock climbing on cliff-face vegetation. Appl Veg Sci 18:705-715 https://doi.org/10.1111/avsc.12172

Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulissen D (1992) Zeigerwerte von Pflanzen in Mitteleuropa. Goltze, Göttingen

Fickert T (2014) Zum Einfluss des Klettersports auf silikatische Felsökosysteme. Mitt Fränkischen Geogr Ges 59:47-58 http://fgg-erlangen.de/fgg/ojs/index.php/mfgg/article/view/262

Gill J (1969) The art of bouldering. Am Alp J 16:355-357

Hanemann B (1999) The sustainable management of climbing areas in Europe. IUCN, Gland

Heinicke D, Friedrich J, Glaser C, Heinicke F, Scharnweber M, Seifert F. (2001) Kletterführer Sächsische Schweiz, Gebiet der Steine. Berg- und Naturverlag, Dresden

Hepenstrick D, Urmi E, Meier MK, Bergamini A (2016) Die Moosflora des silikatischen Findlings Alexanderstein in Küsnacht (ZH). Meylania 57:15-23. https://doi.org/10.21256/zhaw-2029

Holzschuh A (2016) Does rock climbing threaten cliff biodiversity? A critical review. Biol Conserv 204:153-162. https://doi.org/10.1016/j.biocon.2016.10.010

IOC (2016) IOC approves five new sports for Olympic Games Tokyo 2020. https://www.olympic.org/news/ioc-approves-five-new-sports-for-olympic-games-tokyo-2020. Accessed 22 November 2019

Jahns HM (1983). Collins guide to the ferns, mosses, and lichens of Britain and North and Central Europe. Collins, London:

Kinzel H (1983) Influence of limestone, silicates and soil pH on vegetation. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds). Encyclopedia of plant physiology, physiological plant ecology III (12). Springer, Berlin, pp 201-244

Kuntz KL, Larson DW (2006) Influences of microhabitat constraints and rock-climbing disturbance on cliff-face vegetation communities. Conserv Biol 20:821-832. https://doi.org/10.1111/j.1523-1739.2006.00367.x

Larson DW, Matthes U, Kelly PE (2000) Cliff ecology: Pattern and process in cliff ecosystems. University Press, Cambridge

Lauber K, Wagner G, Gygax A (2018) Flora Helvetica, illustrierte Flora der Schweiz. Haupt, Bern

Lee JA (1999) The calcicole-calcifuge problem revisited. In: Callow JA (ed) Advances in botanical research, 29. Academic Press, San Diego, pp 1-30

Lorite J, Serrano F, Lorenzo A, Canadas EM, Ballesteros M., Peñas J (2017). Rock climbing alters plant species composition, cover, and richness in Mediterranean limestone cliffs. PLoS ONE 12:e0182414. https://doi.org/10.1371/journal.pone.0182414

March-Salas M, Moreno-Moya M, Palomar G, Tejero-Ibarra P, Haeuser E, Pertierra LR. (2018) An innovative vegetation survey design in Mediterranean cliffs shows evidence of higher tolerance of specialized rock plants to rock climbing activity. Appl Veg Sci 21:289-297. https://doi.org/10.1111/avsc.12355

Mazenauer D, Holderegger R, Krüsi B, Hepenstrick D (2014) Populationsentwicklung und Gefährdung von *Asplenium septentrionale* auf Findlingen im Schweizer Mittelland und Jura. Bauhinia 25:37-50

Müller S, Rusterholz,H-P, Baur B (2004) Rock climbing alters the vegetation of limestone cliffs in the northern Swiss Jura Mountains. Can J Bot 82:862-870. https://doi.org/10.1139/b04-058

Nelder JA, Wedderburn RW (1972) Generalized linear models. J R Stat Soc Ser A-G 135:370-384

Niegl G (2009) Bouldering: one of the last sports defying technology? Interview with Kilian Fischhuber. Sports Tech 2:63-65. https://doi.org/10.1002/jst.116

Nuzzo VA (1996) Structure of cliff vegetation on exposed cliffs and the effect of rock climbing. Can J Bot 74:607-617. https://doi.org/10.1139/b96-077

OECD (2006) OECD Guidelines for the testing of chemicals. Organisation for Economic Cooperation and Development, Paris

Peng Y, Zhang M, Xu ZY, Yang TT, Su YL, Zhou T, Wang H, Wang Y, Lin Y (2020) Estimation of leaf nutrition status in degraded vegetation based on field survey and hyperspectral data. Sci Rep 10:1-12. https://doi.org/10.1038/s41598-020-61294-7

R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ropp RC (2013) Encyclopedia of the alkaline earth compounds. Elsevier, Oxford

Rusterholz HP., Müller SW, Baur B (2004). Effects of rock climbing on plant communities on exposed limestone cliffs in the Swiss Jura Mountains. App Veg Sci 7:35–40. https://doi.org/10.1111/j.1654-109X.2004.tb00593.x

Rütti R, Marquer D, Thompson AB (2008) Tertiary tectono-metamorphic evolution of the European margin during Alpine collison: example of the Leventina Nappe (Central Alps, Switzerland). Swiss J Geosci 101:157-171. https://doi.org/10.1007/s00015-008-1278-9

Schmera D, Rusterholz HP, Baur A, Baur B (2018) Intensity-dependent impact of sport climbing on vascular plants and land snails on limestone cliffs. Biol Conserv 224:63-70. https://doi.org/10.1016/j.biocon.2018.05.012

Shand MA (2006) The chemistry and technology of magnesia. Wiley, Hoboken

Suo J, Chen S, Zhao Q, Shi L, Dai S (2015) Fern spore germination in response to environmental factors. Front Biology 10:358-376. https://doi.org/10.1007/s11515-015-1342-6

Spitale D, Nascimbene J (2012) Spatial structure, rock type, and local environmental conditions drive moss and lichen distribution on calcareous boulders. Ecol Res 27:633-638. https://doi.org/10.1007/s11284-012-0935-7

Strumia S, Buonanno M, Aronne G, Santo A, Santangelo A (2020) Monitoring of plant species and communities on coastal cliffs: is the use of unmanned aerial vehicles suitable? Diversity. 12:149. https://doi.org/10.3390/d12040149

Thiel H, Spribille T (2007) Lichens and bryophytes on shaded sandstone outcrops used for rock climbing in the vicinity of Göttingen (southern Lower Saxony, Germany). Herzogia 20:159-77.

Tessler M, Clark TA (2016) The impact of bouldering on rock-associated vegetation. Biol Conserv 204:426-433. https://doi.org/10.1016/j.biocon.2016.10.004

Venables WN, Ripley BD (2002) Modern applied statistics with S. Springer, Berlin

Wang W, Keturi PH (1990) Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample. Water Air Soil Pollution 52:369-376

Weinbruch S, Dirsch T, Ebert M, Hofmann H, Kandler K (2008) Dust exposure in indoor climbing halls. J Environ Monit 10:648-654. https://doi.org/10.1039/b719344k

Werdermann P (1993). Böhmischer Sandstein: die schönsten Klettereien in der Tschechei: Panico-Alpinverlag, Köngen

Wiklund K, Rydin H (2004) Ecophysiological constraints on spore establishment in bryophytes. Funct Ecol 18:907-913

Zhang H, Zhang B, Wei ZQ, Wang CZ, Huang Q (2020) Lightweight integrated solution for a UAV-borne hyperspectral imaging system. Remote Sens 12:1-17 http://doi.org/10.3390/rs12040657

Supplementary Material

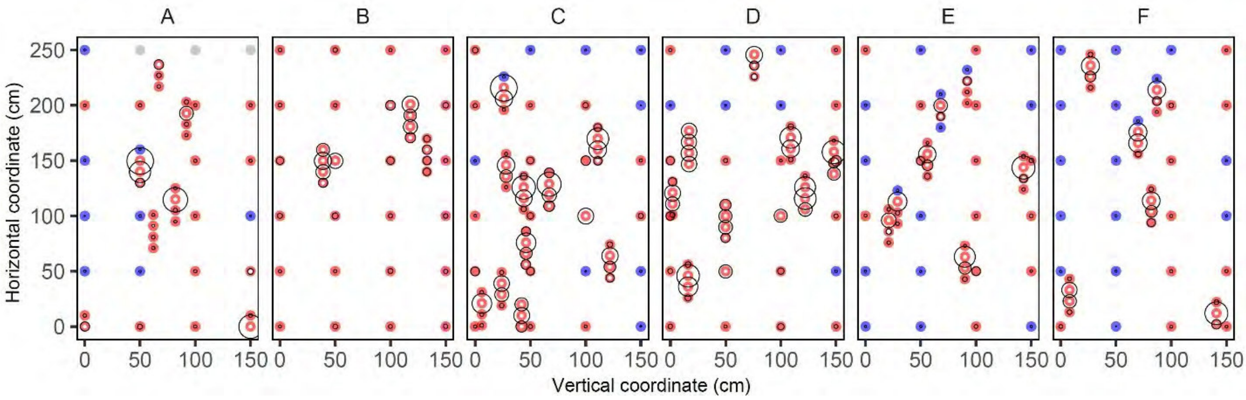


Fig. S1 Climbing chalk (magnesium) distribution on six climbed boulders. Red: value above threshold level; blue: value below threshold level; grey: no data; white dot: visible climbing chalk traces at sampling point. The area within each black circle is proportional to the amount of climbing chalk measured at the corresponding sampling point.

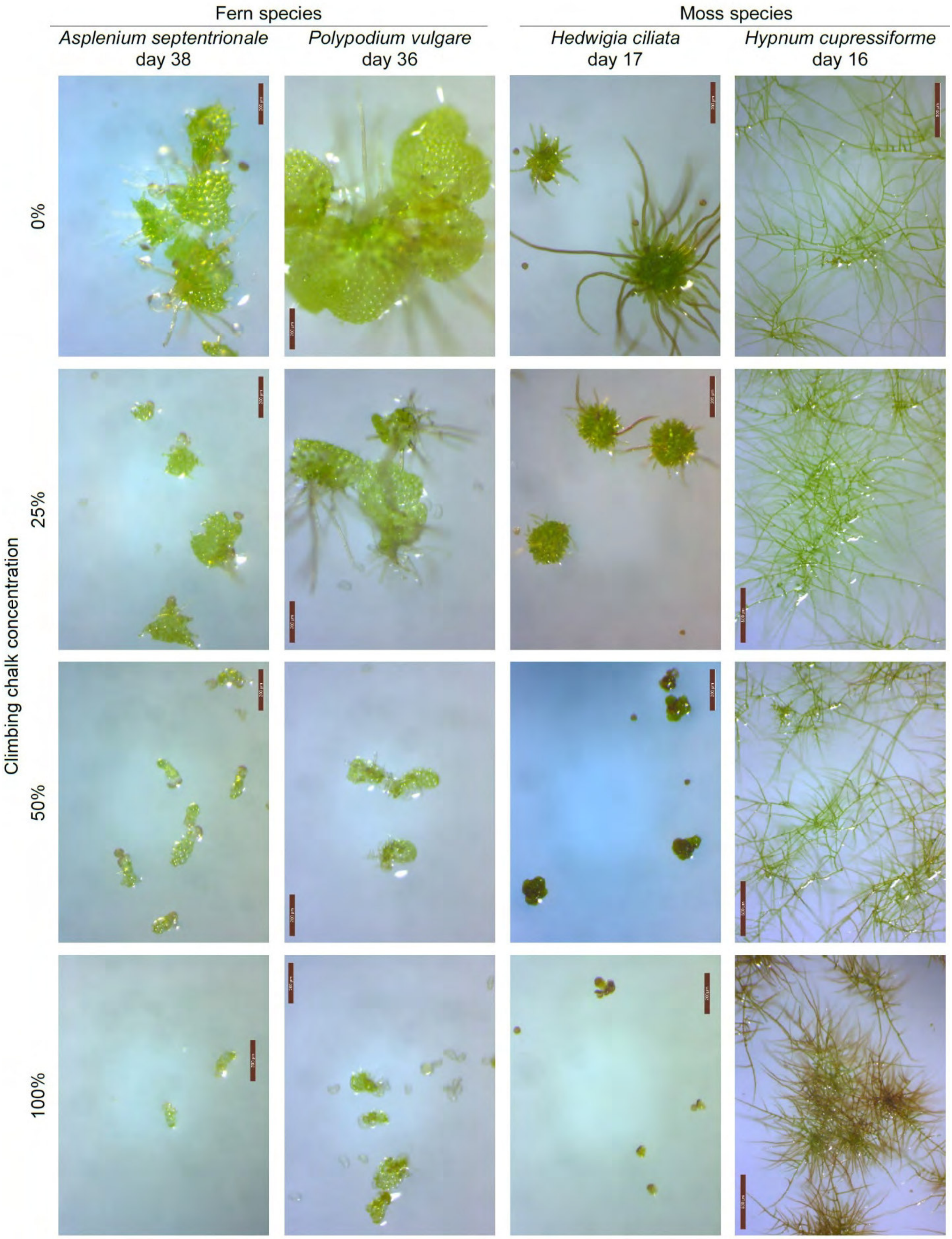


Fig. S2 Examples of fern and moss gametophytes growing on agar under different climbing chalk concentrations. The length of scale bars for fern species and *Hedwigia ciliata* is 200 µm, for *Hypnum cupressiforme* 500 µm.

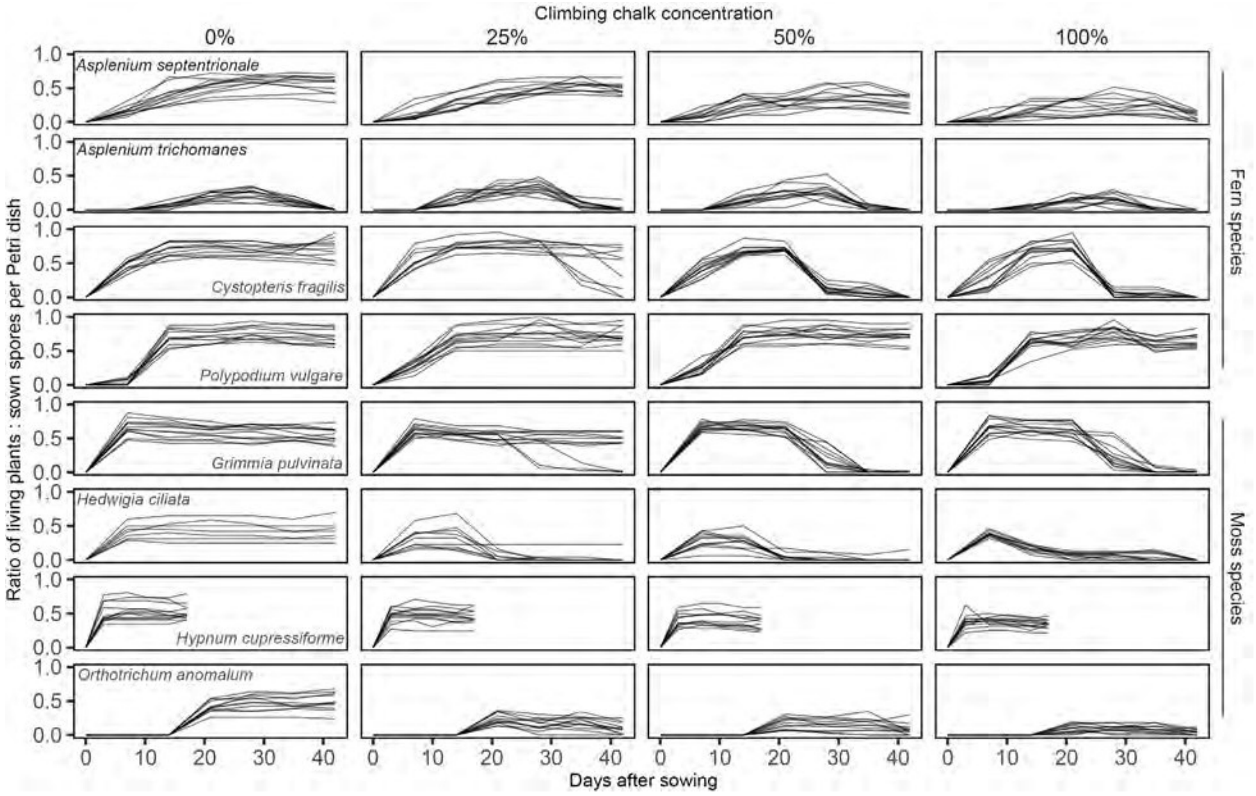
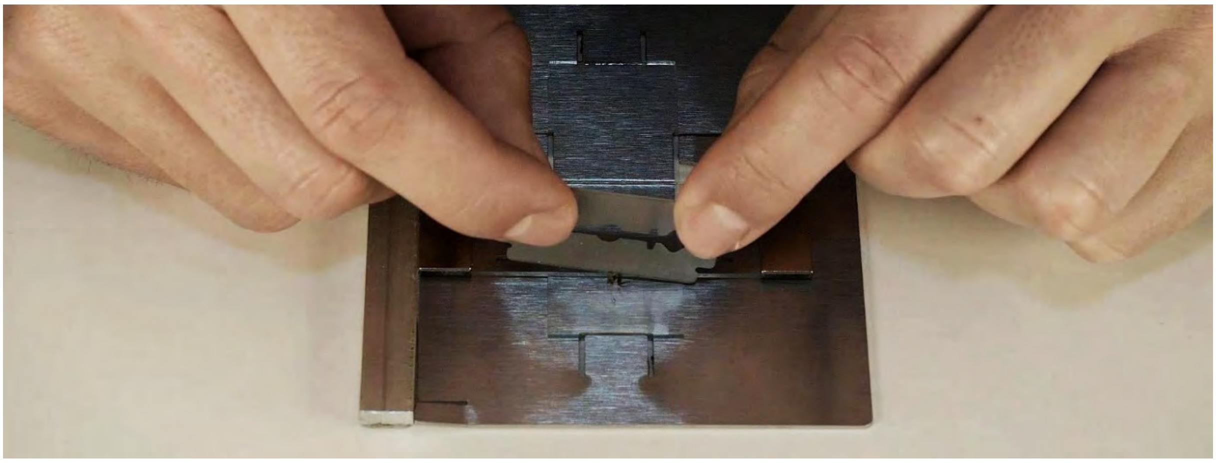


Fig. S3 Development of the eight assessed fern and moss species in four different climbing chalk concentrations on agar in a climate chamber experiment. Each line represents one Petri dish.

Appendix I



A simple, magnet-assisted bryophyte cross sectioning tool for beginners and for teaching in bryology

Daniel Hepenstrick

Manuscript published in Lindbergia (2018)
https://doi.org/10.25227/linbg.01099

Abstract

I describe the production and the application of a low-tech, easy-to-handle sectioning aid, which might be helpful for anyone who struggles with cross sectioning bryophytes. Technical plans and an instructional video are provided as supplementary material.

Keywords: bryophyte identification, cutting tool, free-hand sectioning, methods, microscopy

Introduction

High-quality cross sections of bryophyte leaves, stems or thalli are important for the identification of species and the study of bryophyte morphology (Figure 1). While experienced bryologists section bryophytes easily, fast and routinely, those new to the field often struggle with this task, which involves simultaneously fixing a tiny object under the dissection microscope with one hand while precisely cutting it with the other (Nishimura 1997; Pilkington 2013). Moreover, newcomers to bryology are frequently also beginners in microscopy. Thus, failure in appropriate sectioning (e.g. too thick sections) of bryophytes is common and often leads to frustration. Worst of all, it could dampen students' enthusiasm of working with bryophytes, leading them to give up the discipline. In order to enhance beginners' sectioning success, I developed a low-tech, easy-to-handle sectioning aid, whose production and application is described in the present article.

Figure 1. A leaf cross section showing the leaf anatomy of Polytrichaceae with lamellae and the forked terminal cells, which are diagnostic for *Polytrichum commune*. This section is the best result out of three trials with the magnetic sectioning aid by someone who had never sectioned a bryophyte before. In all three trials, sections with clearly recognisable diagnostic features were present.

Description and application of the tool

Among the large variety of sectioning methods applied by bryologists, technical sectioning aids such as microtomes, pith and wax embedding (O'Brien and McCully 1981) are rarely used, due to their time consuming application (Glime and Wagner 2013). Hence, the present method is kept simple and fast: it is basically an extension of the widely applied "double slide sectioning technique" (Glime and Wagner 2013) with a magnetic support plate. This allows a specimen to be fixed on a slide aided by a second slide which is placed at 90 degrees to the first slide and which is pressed down by means of magnets attached to it (Figure 2). This set-up fixes the specimen into position without the involvement of the hands and therefore allows the cutting of sections with a razor blade that is guided by both hands. The latter substantially facilitates sectioning.

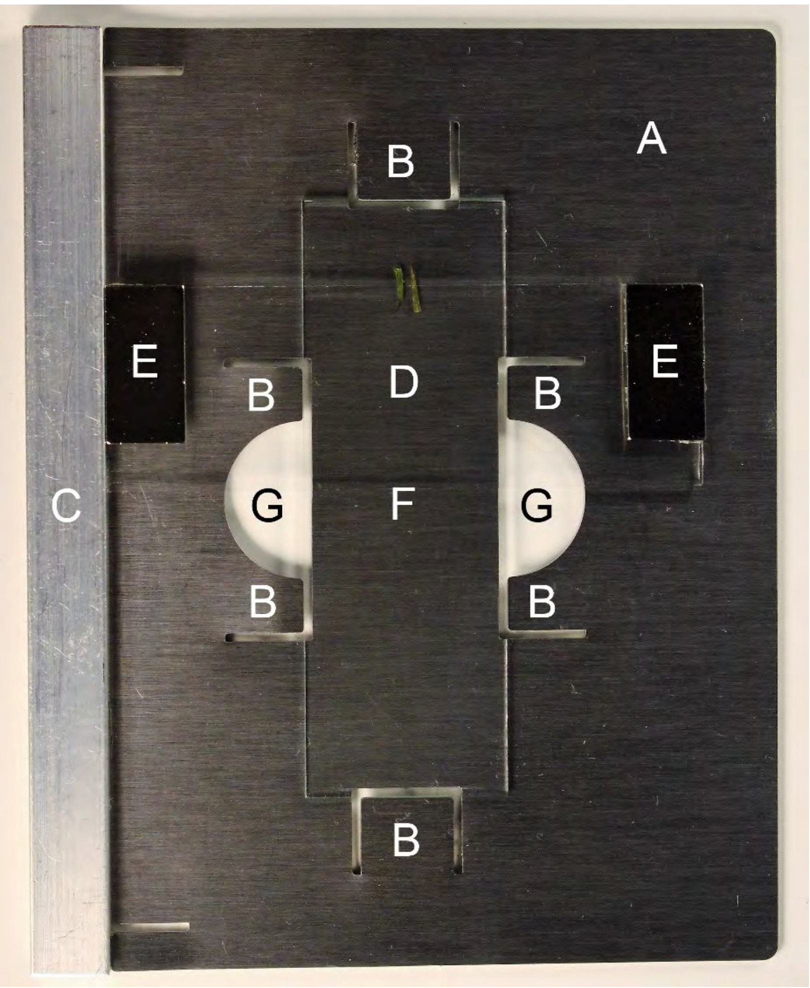


Figure 2. The magnetic sectioning aid with fixed *Polytrichum* leaves ready for sectioning. The support plate (A) consists of a magnetic, rust-free steel sheet with laser cut tongues (B) which are slightly bent upwards, and a non-magnetic strip (C) which helps to align the magnetic slide (D) with its attached neodymium magnets (E) centrally on the lower slide (F) in order to fix the specimen for sectioning. Sections are cut under a dissecting microscope with a razor blade that is guided with both hands. The semicircle slots (G) help to place and grasp the lower slide.

Production of the two involved components, a metallic support plate and a magnetic slide, is straightforward. The support plate is made of a 120 × 96 × 1 mm ferromagnetic rust-proof steel sheet type EN 1.4016 (AISI 430) tailored by an industrial laser cutter. This type of steel is widely used for domestic appliances, and many companies that sell steel sheet have this in stock and are able to cut it using a laser. By sending the technical plans from the supplementary material (PDF plan and corresponding CAD file) to the respective company, a set of 20 support plates can be produced for less than €10 per piece (mainly processing costs). The template is set up for standard 76 × 26 mm microscope slides (DIN ISO 8037-1), whereas for slides with other dimensions it has to be modified as specified in the PDF plan. After production, the eight laser-cut tongues are manually bent upwards (e.g. with the help of a slotted screwdriver), so that their underneath edges just reach the surface of the plate. The two lateral tongues are used to tightly align a non-magnetic (e.g. aluminium, wood or plastic) rectangular strip of ca. 120 × 10 × 3 mm, which is glued into its position, ideally with a two-part adhesive glue. The magnetic slide is a standard microscope slide with cut, not bevelled, edges. On each narrow side, a neodymium magnet (ferritic magnets are generally too weak) is aligned to the edges and glued to the slide. Commercially available self-adhesive 20 × 10 × 1 mm neodymium magnets (<€0.50 per piece; supermagnete.com) have proven suitable for this application, but other neodymium magnets can also be used (e.g. fixed with double-sided tape).

Application of the method is demonstrated in an instructional video in the supplementary material, which may help to harness the magnetic aid for teaching and personal use. Sectioning starts with the positioning of a standard microscope slide on the support plate in the area between the six central tongues. This prevents the slide from gliding away during sectioning and correctly aligns it with the lateral non-magnetic strip. On this lower slide, a specimen is placed and then fixed with the magnetic slide. Fixation starts by directly aligning one narrow side of the magnetic slide against the inner edge of the non-magnetic strip (magnets orientated upwards). Then, the magnetic slide is gently laid down on the specimen so that it is partly covered. Slight pressure on the middle of the magnetic slide will tilt it to a horizontal position. Now, the magnetic slide fixes the specimen by means of the lateral magnets, which symmetrically apply pressure on the orthogonally and centrally orientated lower slide. The support plate with the fixed specimen can now be placed under the dissecting microscope (in case the above steps have not already been conducted there). The part of the specimen that protrudes from the magnetic slide is set on focus and cut into sections, as thinly as possible, with a razor blade that is controlled with both hands. After cutting, the magnetic slide is removed and sections that remain stuck on the blade and/or the lower slide are pushed into a drop of water placed on the lower slide. Then, a cover slip is laid on the sections and the slide is grasped through semicircle slots for examination under the light microscope.

This method can be varied depending on the type of specimen involved and on personal preferences. Some of the possible variations are also shown in the instructional video. For extra stability or in case of thick specimens, the magnetic slide can be placed with downwards orientated magnets, which will prevent it from tilting. For this option, the magnets have to be equal in height to the slides being used. In order to increase the force by which the specimen is pressed down, additional magnets can be placed on the magnets attached to the magnetic slide. This also enhances the stability of the system and might be useful for fine specimens that need to be pressed down more powerfully for proper fixation. Notably, if the applied magnets are too strong, the magnetic slide will bend and therefore loose pressure. Further, reorientation of the specimen might be achieved by actively tilting and shifting the magnetic slide or by pushing it slightly backwards while sectioning, which will gradually expose the specimen. Also, the orientation of the support plate, the guidance and type of razor blade, and the amount of specimen material and of water can be varied according to individual preferences. I encourage users to find their own best setting, which will most probably vary for different types of specimen.

The method has been informally assessed in the Bachelor's course "Biology of Bryophytes and Ferns" 2016, which is taught at the Swiss Federal Institute of Technology in Zürich (ETH Zürich). After several practical days, in which students sectioned bryophytes in a traditional way (i.e. fixing specimen with forceps), they were introduced to the present method. The students readily applied the magnetic aid and in the subsequent oral evaluation they (n=19) concordantly agreed that it substantially enhanced their success in preparing cross-sections of bryophyte leaves and stems. Hence, in future courses the magnetic aid will be incorporated from the beginning.

Anyone who struggles with cross sectioning bryophytes, especially people new to bryology and microscopy, might profit from the presented method. Low priced production makes the aid an affordable method for use during teaching in class, and it might well be applied for sectioning materials other than bryophytes.

Caution note

The method involves sharp, fragile and magnetic objects. Their handling might cause injury from which the author declines any liability.

Acknowledgements

The technical plans were provided by courtesy of Diethelm AG (Hermetschwil, Switzerland). I would like to thank Ariel Bergamini for evaluating the method in his course, Nicolas Phan-huy for Figure 1, Aron Marty for help with the instructional video, and Rolf Holderegger and Caroline Hyde-Simon for valuable feedback on the manuscript.

References

Glime, J.M. and Wagner, D.H. 2013. Laboratory techniques: slide preparation and stains. In: J.M. Glime, Bryophyte Ecology. Volume 3, Methods, Chapter 2.2. (11 September 2013) [accessed 12 December 2016] pp 3-27. Available at www.bryoecol.mtu.edu

Pilkington, S. 2013. Hand lenses and microscopes part 2: choosing a microscope. Field Bryology 107: 32-35.

Nishimura, N. 1997. Easy ways to make transverse sections under the dissecting microscope. Bryological Research 7: 30-31.

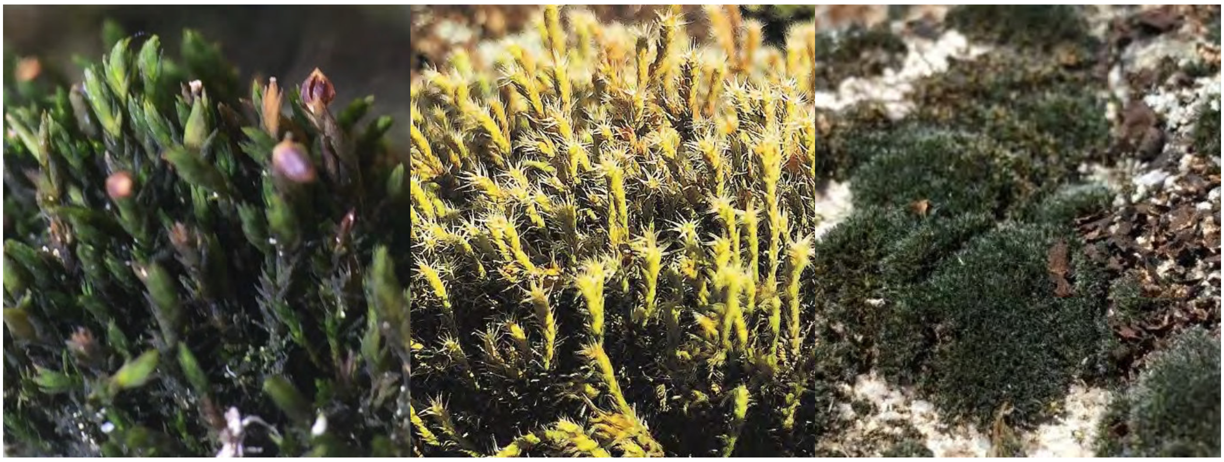
O'Brien, T.P. and McCully M.E. 1981. The study of plant structure principles and selected methods. Backwell, Oxford.

Supplementary Material

Instructional video: https://figshare.com/s/fbdcec502de355693de2

PDF and CAD plans of the support plate: https://figshare.com/s/bf9169dfb98eefa726f6

Appendix II



Contributions to the bryofloristic exploration of erratic boulders in Switzerland

The journal of the Swiss Association of Bryology and Lichenology publishes the yearly series 'Contributions to the bryofloristic exploration of Switzerland' with descriptions of new sites of rare, threatened or otherwise remarkable bryophyte species. In four short articles, I shared some of the special findings made during this thesis on erratic boulders with the bryological community. The published articles can be accessed via the DOI-links.

Hepenstrick D. (2021) *Grimmia montana*. In: Bergamini A., Boch S., Hepenstrick D., Kiebacher T., Lüth M., Moser T., Müller N., Schnyder N. Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 16. Meylania 67: 8-21. https://doi.org/10.21256/zhaw-23419

Hepenstrick D. (2020) *Racomitrium microcarpon*. In: Bergamini A., Hepenstrick D., Hofmann H., Kiebacher T., Moser T., Müller N., Schnyder N., Stix S., Urmi E. Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 15. Meylania 65: 12-3. https://doi.org/10.21256/zhaw-21319

Hepenstrick D., Kiebacher T. (2019) *Hedwigia stellata*. In: Bergamini A., Büschlen A., Hepenstrick D., Kiebacher T., Meier M., Schnyder N., Urmi E. Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 14. Meylania 63: 5-14. https://doi.org/10.21256/zhaw-19787

Hepenstrick D. (2018) *Andreaea rupestris*. In: Bergamini A., Hepenstrick D., Hofmann H., Joss S., Kiebacher T., Meier M., Müller N., Roloff F., Schnyder N. Beiträge zur bryofloristischen Erforschung der Schweiz – Folge 13. Meylania 61: 5-18. https://doi.org/10.21256/zhaw-2028

Appendix III

Outreach articles and public excursions

With outreach articles and on public excursions – 12.9.2015 in Meilen and 18.6.2021 in Solothurn – I informed the public generally interested in nature about the special flora of erratic boulders. The published articles can be accessed via the DOI-links.

Hepenstrick, D. (in press) Flore des blocs erratiques. In: Cercle Vaudois de Botanique (ed.). Atlas de la flore vaudoise. Cercle Vaudois de Botanique, Lausanne.

Hepenstrick, D., Schmit, F. (2020) Findlinge sind wertvolle Lebensräume. Umwelt Aargau 84: 49-52. (reprint) https://doi.org/10.21256/zhaw-21320

Hepenstrick, D., Schmit, F. (2020) Findlinge sind wertvolle Lebensräume. Milan 2020(3): 26-29. https://doi.org/10.21256/zhaw-21318

Hepenstrick, D., Walthard, P. (2017). Granitinsel im Kalkmeer / Ilots de granit dans une mer de calcaire / Isole granitiche nel mare calcareo. Die Alpen / Les Alpes / Le Alpi 2017(5): 60-61. https://doi.org/10.21256/zhaw-1368

Appendix IV

Information boards

Populations of *Asplenium septentrionale* on the two erratic boulders “Pflugstein” and “Alexanderstein” were acutely threatened by destruction by sport climbers. In collaboration with Naturnetz Pfannenstil, Pro Natura, the canton of Zürich and the local municipalities I created two information boards that provide information about the rare species on erratic boulders and may reduce the threat of the populations of *A. septentrionale*. Regarding a third population of *A. septentrionale* on a remote erratic boulder that is occasionally used by climbers, Pro Natura and I decided against an information board. Instead I mapped all individuals of *A. septentrionale* on the boulder, which will make it possible to monitor the development of local population size. The pdf-files of the information boards can be accessed via the DOI-links.

Hepenstrick D. et al. (2018) Naturschutzobjekt Alexanderstein. Information board in Küsnacht, Switzerland. https://doi.org/10.21256/zhaw-21955

Hepenstrick D. et al. (2017) Naturschutzobjekt Pflugstein. Information board in Herrliberg, Switzerland. https://doi.org/10.21256/zhaw-21954

Appendix V

Schutz für die grünen Inseln

Biologe Daniel Hepenstrick erforscht die Flora auf Findlingen. Diese ist im Mittelland einzigartig und gefährdet.

Alice Guldimann

Daniel Hepenstrick hält sein Auge ganz nahe an den moosbewachsenen Stein und blickt durch eine kleine Lupe, die er an einem Band um den Hals trägt. «Himbeer-Kissenmoos», erkennt er sofort. Seit vier Jahren beschäftigt sich Biologe Hepenstrick im Rahmen seiner Doktorarbeit mit der Flora auf Findlingen. Im Wald hinter

Media coverage

The subjects I treated in my thesis were picked up by mass media, which resulted in several reports about the special flora of erratic boulders and the impact of climbing chalk. The reports can be accessed at https://www.zhaw.ch/findlingsflora.

Mathys B. (2021) Seltenes Alpen-Moos wächst auf Findlingen im Mittelland. SRF News, 5.7.2021

Snow J. (2021) Rock climbing is getting more popular – and that concerns conservationists. National Geographic, 23.6.2021

Buehler J. (2020) Climbing chalk harmful to cliffside plants. Frontiers in Ecology and the Environment 18: 546.

Giaimo C. (2020) Don’t chalk the moss! Anthropocene, 9.12.2020.

Mathys B. (2020) Findlinge als Lebensräume. Radio SRF 1 Regionaljournal Aargau Solothurn 11.12.2020.

Neukom H.-P. (2020) Der Alexanderstein – ein Biotop für seltene Pflanzen. Küsnachter 13.8.2020: 7.

Guldimann A. (2019) Schutz für die grünen Inseln. Solothurner Zeitung 24.9.2019: 17.

Koechlin S. (2016) Botanische Besonderheiten auf steinernen Zeugen der Eiszeit. Tierwelt 21.4.2016: 28-29.

Acknowledgements

This thesis would not have been possible without the help of many people and organisations. I feel privileged for the great support I received. Thank you all!

First, I want to express great gratitude to Rolf Holderegger for being an excellent and inspiring “Doktorvater”, for accompanying me in my journey from the first draft of the project and the entire funding process, through the actual doctorate, to the final polishing of the thesis. Further, I thank the co-examiners, Ariel Bergamini and Alex Widmer, for congenial collaboration during the thesis and Gregor Kozlowski for being the external co-examiner.

This thesis was financed by the following foundations, associations, and governmental and non-governmental organisations (in alphabetical order): Amt für Landschaft und Natur Kanton Zürich; Amt für Landwirtschaft und Natur Kanton Bern; Bundesamt für Umwelt BAFU; Département du territoire et de l’environnement Canton de Vaud; Gemeinde Herrliberg; Gemeinde Meilen; Gemeinde Seeberg; Lotteriefonds Kanton Solothurn; NAKUSO-Stiftung; Pro Natura Schweiz; Schweizer Alpen Club SAC; Schweizerische Botanische Gesellschaft; Stiftung Binelli & Ehrsam; Stiftung Temperatio; Swisslos-Fonds Kanton Aargau; Wolfermann-Nägeli-Stiftung; and Zürcherische Botanische Gesellschaft. I am grateful for this funding and thank all the people involved who share enthusiasm for the flora of erratic boulders.

I thank Thomas Kiebacher for the identification of *Orthotrichum* and *Schistidium* samples and for help with climbing a large erratic boulder. I thank Christian Ginzler and Clare Webster for contributing geodata. For providing species distribution data I thank Info Flora and Swissbryophytes in Switzerland, Marc Philippe and Jean-François Thomas in France, and the Bundesamt für Naturschutz and Thomas Breunig in Germany. For genetic wet lab work I thank Sabine Brodbeck, René Graf and Claudia Michel, and for bioinformatic support I thank Niklaus Zemp from the Genetic Diversity Centre of ETH Zürich. I thank Christian Lusti and Jonathan Pachlatko for help with the climbing chalk measurements in the field, Nicolas Phan-huy and Dominik Penkov for help with the climate chamber experiment, and Lilian Gasser for help with statistics. I also thank Pro Natura and René Amstutz for collaboration and support in the “Pilotprojekt Auflichtung” and Pro Natura Seeland, the Puijenbroek family and Bürgergemeinde Solothurn for providing the sites for the experiment. I thank Candida Haritz, Daniela Mazenauer and Nicola Stäheli for their preliminary research before the thesis started.

For coaching I thank Silvia Callegari and for English editing I thank Melissa Dawes.

I thank ETH Zürich, WSL and ZHAW for the great research environment they provided for my thesis work. I especially want to thank the current and former members of the ZHAW research group Vegetation Ecology, the WSL research unit Biodiversity and Conservation Biology, and the ETH Zürich research group Plant Ecological Genetics for being such marvellous colleagues and friends, and for providing me with technical, intellectual and emotional support whenever I needed it.

I would like to thank the Swiss Association of Bryology and Lichenology (BRYOLICH) and its active members for boosting my bryological competency from 2013 onwards during fieldtrips and species determination events. A special thank you goes to Edi Urmi for introducing me to bryophyte identification at numerous determination evenings and to Christine Keller for organising these splendid evenings.

I also want to thank my family. My mother for raising me and for her unconditional support throughout my life. My father for the good times we spent together and his enthusiasm for every academic step I took. Christian, my brother, for being there, for sharing inspiring views on life and alpha errors, and for hand modelling.

I thank all my friends, for countless nice weekends, evenings and phone calls that enrich my life with adventure, inspirational discourses and the feeling of being understood and looked after. Special thanks to Melina and Vero.

Finally, I thank, Damian, my partner: thank you for bearing with me throughout the thesis and infinite thanks for all the vital non-botanical and non-scientific joy you bring to my life.

Curriculum vitae

Daniel Hepenstrick Swiss citizen, born 17. November 1983 in Zürich, Switzerland

Education

1/2016 – 5/2021	Doctoral thesis at the Department of Environmental Systems Science, ETH Zürich, Switzerland
4/2008 – 12/2009	MSc in Biology, ETH Zürich, Switzerland
9/2006 – 5/2007	Exchange semester, Imperial College London, United Kingdom
8/2004 – 3/2008	BSc in Biology, ETH Zürich, Switzerland
8/1999 – 9/2003	Matura, Mathematisch-Naturwissenschaftliches Gymnasium Rämibühl Zürich, Switzerland

Professional experience

1/2014 – present	Researcher, Vegetation Ecology, ZHAW Wädenswil, Switzerland
9/2011 – 12/2013	Research assistant, Vegetation Ecology, ZHAW Wädenswil, Switzerland
1/2010 – 12/2010	Technical staff assistant, Evolutionary Ecology, WSL Birmensdorf, Switzerland
10/2008 – 6/2011	Teacher for waste and sustainable consumption, Stiftung Praktischer Umweltschutz Schweiz Zürich, Switzerland
1/2009 – 9/2011	Independent field biologist for botany and Odonata, Zürich, Switzerland
4/2006 – 9/2010	Teaching assistant for systematic botany, ETH Zürich, Switzerland

Peer-reviewed publications

Hepenstrick D., Bergamini A., Webster C., Ginzler C., Holderegger R. (in press) Factors determining bryophyte species richness and community composition on insular siliceous erratic boulders in calcareous landscapes. Journal of Vegetation Science

Hepenstrick, D., Zemp N., Widmer A., Holderegger R. (2021) Neither connectivity nor genetic diversity matter in the conservation of a rare fern and a moss on insular erratic boulders. Conservation Genetics. https://doi.org/10.1007/s10592-021-01414-6

Hepenstrick, D., Bergamini, A., Holderegger, R. (2020) The distribution of climbing chalk on climbed boulders and its impact on rock-dwelling fern and moss species. Ecology and Evolution 10: 11362-11371.

Biurrun, I., Burrascano, S., Dembicz, I., Guarino, R., Kapfer, J., Pielech, R., Babbi, M., Hepenstrick, D., Widmer, S., Dengler, J., et al. (2019) GrassPlot v. 2.00: first update on the database of multi-scale plant diversity in Palaearctic grasslands. Palaearctic Grasslands 44: 26-47.

Dengler, J., Widmer, S., Staubli, E., Babbi, M., Gehler, J., Hepenstrick, D., Bergamini, A., Billeter, R., Boch, S., Rohrer, S., Dembicz, I. (2019) Dry grasslands of the central valleys of the Alps from a European perspective: the example of Ausserberg (Valais, Switzerland). Hacquetia 18: 155-177.

Hepenstrick, D. (2018) A simple, magnet-assisted bryophyte cross sectioning tool for beginners and for teaching in bryology. Lindbergia 41: 01099.

Mazenauer, D., Holderegger, R., Krüsi, B., Hepenstrick, D. (2014) Populationsentwicklung und Gefährdung von *Asplenium septentrionale* auf Findlingen im Schweizer Mittelland und Jura. Bauhinia 25: 37-50.

Hepenstrick, D., Thiel, D., Holderegger, R., Gugerli, F. (2012) Genetic discontinuities in roe deer (*Capreolus capreolus*) coincide with fenced transportation infrastructure. Basic and Applied Ecology 13: 631-638.

Hepenstrick, D., Holderegger, R., Keller, D. (2012) Monitoring von Populationen der Helm-Azurjungfer *Coenagrion mercuriale* (Odonata: Coenagrionidae): was taugen zwei Begehungen pro Saison? Entomo Helvetica 5: 139-145.

09.11.2021