

Alp Botany (2012) 122:109–117
DOI 10.1007/s00035-012-0105-0

ORIGINAL PAPER

Population structure and genetic diversity of relict populations of *Alyssum montanum* on limestone cliffs in the Northern Swiss Jura mountains

Hans-Peter Rusterholz · Denis Aydin ·
Bruno Baur

Received: 30 January 2012 / Accepted: 2 May 2012 / Published online: 27 May 2012
© Swiss Botanical Society 2012

Abstract Exposed cliffs in the Northern Swiss Jura mountains harbour a highly diverse flora with numerous rare and relict plant species. The genetic structure of cliff populations is of particular interest because in a variety of plant species the populations are small, isolated and separated from their main distribution area in the Alps and the Mediterranean. We examined possible relationships between population size and size structure, sexual reproduction and genetic diversity of *Alyssum montanum*, a relict plant species occurring on limestone cliffs in the Northern Swiss Jura mountains. The population size of *A. montanum* ranged from 8 to 248 plant individuals on the 12 cliffs examined. Most populations contained a high proportion of small-sized (=young) plants indicating a successful establishment of seedlings. Fitness-related traits of *A. montanum* (percentage of reproductive individuals, number of fruits, fruit weight) varied widely between cliffs and were neither related to the size of the populations nor to the percentage of vegetation cover on the cliffs. RAPD-PCR analysis revealed that *A. montanum* populations exhibit a remarkably high genetic diversity. However, genetic diversity decreased with decreasing population size. Moreover, the positive relationship found between genetic variability and fitness-related traits indicates that population size is a key factor for the persistence of *A. montanum* on limestone cliffs.

Keywords Population size · RAPD-PCR · Relict plant · Sexual reproduction

Introduction

The cliffs of the Jura mountains in Northwestern Switzerland harbour unique and diverse plant communities (Zoller 1989). A variety of plants growing on these cliffs are inter- or postglacial relicts nowadays with an arctic-alpine or mediterranean distribution (Walter and Straka 1970). In contrast to the large rocky area of the Alps, the cliffs of the Jura mountains are small and mostly surrounded by forests. Several relict plant species growing on these limestone cliffs occur only in small and isolated populations which are exposed to a high risk of extinction due to genetic, demographic or environmental stochasticity (Soulé 1986; Dannemann 2000). The type of population structure is another important factor for the persistence of rare and relict plant species (Oostermeijer et al. 1994). However, the relationship between population size and population structure is still in most plant species not known (e.g. Hegland et al. 2001). The large number of rare plants and the rarity of this habitat type give the limestone cliffs a high conservation value (Wassmer 1998; Baur 2003). The Fauna-Flora-Habitat guidelines of the European Union consider limestone cliffs as habitats of “European importance” [Council Directive 92/43/EEC (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.html)].

Genetic differentiation may occur in relict species as a result of their fragmented distribution (Le Corre et al. 1997; Pérez-Collazos and Catalan 2007). Partial extinctions, recurrent immigration, different migration routes, long-distance dispersal events, bottleneck situations, founder effects, in situ survival in refugia and genetic drift are crucial

Electronic supplementary material The online version of this article (doi:10.1007/s00035-012-0105-0) contains supplementary material, which is available to authorized users.

H.-P. Rusterholz (✉) · D. Aydin · B. Baur
Department of Environmental Sciences, Section of Conservation
Biology, University of Basel, St. Johannis-Vorstadt 10,
4056 Basel, Switzerland
e-mail: hans-peter.rusterholz@unibas.ch

processes for the genetic differentiation and evolution of populations (Ellstrand 1992; Luijten et al. 2000; Lutz et al. 2000). Furthermore, the genetic diversity in a plant population may be influenced by the breeding system, mode of dispersal, ability for clonal growth and life form of the species (Vogel et al. 1999; Hardy et al. 2006). Population size is one of the most important factors for the genetic diversity in numerous species (Leimu et al. 2006). However, the relative importance of population size and genetic diversity for plant fitness-related traits is still unclear. Some studies reported a clear positive relationship between genetic diversity and fitness-related traits (e.g. Dostálek et al. 2010), whereas other studies found no association between genetic diversity and plant fitness (e.g. Britten 1996).

In the present study, we examined the population sizes, size structure and reproductive traits of the relict plant *Alyssum montanum* on 12 isolated limestone cliffs in the Northern Swiss Jura mountains. Using RAPD-PCR, we estimated the genetic diversity of the 12 populations and assessed potential correlations between population size, fitness-related traits and genetic diversity in this threatened plant. In particular, we addressed the following questions:

1. Is the population size of *A. montanum* influenced by the size of the tree-free area and the cover of ground vegetation on the cliffs?
2. Are fitness-related traits such as the number of blooming shoots and fruits per plant, inflorescence length and fruit weight related to the local population size of *A. montanum*?
3. Is genetic diversity related to population size and fitness-related traits in the populations examined?
4. Do the 12 cliffs examined harbour genetically distinct populations of *A. montanum*?

Materials and methods

Study species

Alyssum montanum ssp. *montanum* (Brassicaceae) is a perennial chamaephyte (hereafter referred as *A. montanum*). It is a diploid species ($2n = 16$) occurring on limestone rocks and rocky slopes in South-western Germany, Northern Switzerland and Eastern France (Španiel et al. 2011, 2012). In Switzerland, it can mainly be found on south-exposed limestone cliffs situated in the Northern Jura mountains. Individual plants may have up to 70 shoots and each of them has the potential to develop a racemous inflorescence composed of up to 50 bright yellow coloured flowers. Flowering occurs from the beginning of March to the end of May in the Jura mountains. The flowers are

visited by a wide variety of pollinating insects including bees, hover flies, flies, wasps and beetles. Pollinated flowers produce one to two seeds in a silique (Hegi 1986). However, autogamy is assumed to occur frequently (Hegi 1986). The species is considered as vulnerable in the Red List of Switzerland (Moser et al. 2002).

Study sites

The study was carried out on 12 limestone cliffs situated in the Northern Swiss Jura mountains (Table 1; Fig. 1) in spring 2007. Due to the limited access of the cliff walls, the local distribution of *A. montanum* is still not well known in the Jura mountains. We selected our sampling sites based on regional inventories of cliff vegetation (Wassmer 1998; Knecht 1999). We could not find any individuals of *A. montanum* at another three cliffs. Further three cliffs were not considered because the plant has been introduced by humans (Knecht 1999, M. Zemp, personal communication).

The cliffs examined differ considerably in size (expressed as length of the rock wall; Table 1). At larger cliffs, *A. montanum* occurred in 2–6 sites, while at smaller cliffs the plants were restricted to a single site. At each site, we drew a detailed map showing the spatial distribution of *A. montanum*. To assess population characteristics the area occupied by *A. montanum* was measured and subdivided into 1 m² plots at each cliff. The number of plant individuals and the number of shoots and inflorescences of each plant individual were counted in each 1 m² plot. We measured the length of the longest shoot of each plant individual. In each plot, the cover of ground vegetation was estimated using the Domin scale (Müller-Dombois and Ellenberg 2002; Appendix 1 in the Supplementary Material). To estimate the extent of sexual reproduction, we measured the length of the inflorescence, counted the number of ripe fruits and determined fruit weight of one randomly selected individual in each plot (Appendix 2 in the Supplementary Material).

Sampling schedules and genetic analysis

We sampled leaf material from 10 randomly selected plant individuals at each site resulting in a total of 238 samples. To avoid repeated sampling from the same individual, the minimum distance between individuals was set to 2 m. Leaf samples were stored at -80°C until required for genetic analysis.

We applied the CTAB method of Doyle and Doyle (1990) to isolate high-molecular-weight DNA from leaf tissue. Template quality and quantity were measured using a spectrophotometer. We used the RAPD-PCR method (random amplified polymorphic DNA) to investigate the genetic population structure of *A. montanum*. To ensure the reproducibility of our RAPD-PCR analysis, 12 randomly chosen

Table 1 Characteristics of the *Alyssum montanum* populations surveyed on limestone cliffs in the Northern Jura mountains, Switzerland

Cliff (abbreviation)	Altitude ^a (m a. s. l.)	Exposition	Length of exposed cliff (m)	Cliff height (m)	Climbing activity ^b	Number of sampling sites	Area occupied by <i>A. montanum</i> (m ²)	Population size ^c	Plant density (number per m ²) ^d
Hofstetter Chöpfl (HO)	468	SW	1,100	35	70 (6)	6	288	147	3 (1–15)
Baflue (BA)	589	SSW	445	30	0	1	96	209	5 (1–21)
Sissacher Flue (SF)	700	SW	210	40	0	2	71	28	2 (1–4)
Tannenflue (TA)	664	SSW	850	25	60 (6)	4	254	177	3 (1–12)
Portiflue (PO)	876	SW	280	55	14 (1)	2	247	248	5 (1–19)
Schöntalflue (SC)	909	S	175	50	0	1	140	53	3 (1–13)
Ankenballen (AN)	1,032	S	270	50	0	1	23	33	3 (1–10)
Höchi Flue (HF)	952	SW	930	35	0	2	94	114	7 (1–13)
Rämel (RM)	832	S	720	40	41 (1)	2	190	217	6 (1–16)
Belchenflue (BE)	1,099	SW	205	100	0	1	385	10	1 (1–3)
Schmutzbergflue (SM)	982	SSW	70	50	0	1	70	8	2 (1–5)
Ämmenegg (AM)	1,053	S	210	30	0	1	77	15	4 (1–7)

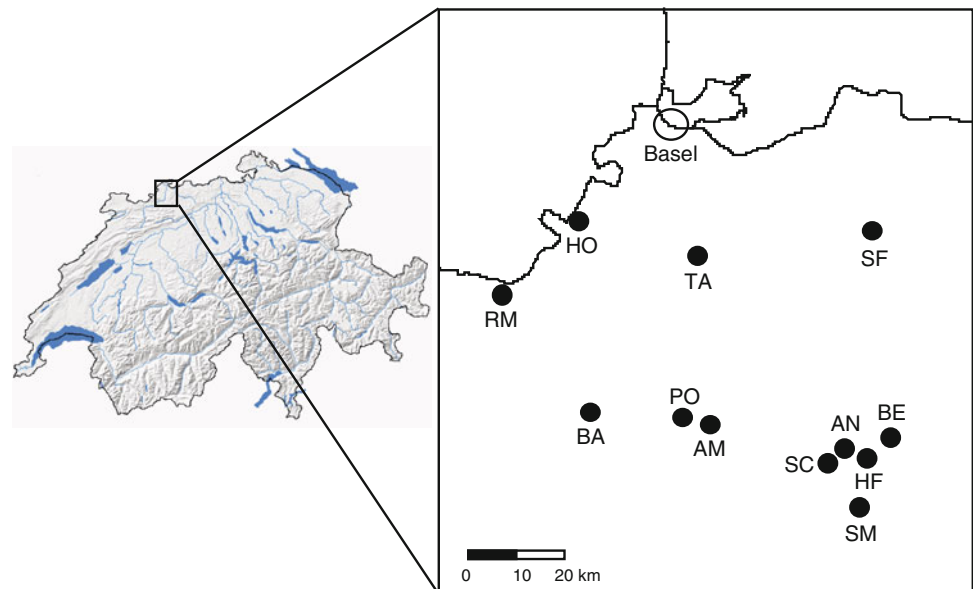
^a Top of the cliffs

^b Number of climbing routes and climbing intensity (in brackets) according to Knecht (1999): 1 = very low; 6 = extremely high

^c Number of plant individuals

^d Median and range

Fig. 1 Location of the cliffs in the Northern Swiss Jura mountains. For population abbreviations see Table 1



leaf samples from four cliffs were screened for decamer primers (Operon Technologies Inc., USA). Out of the 50 primers examined, 6 primers were selected yielding polymorphic and reproducible bands (Appendix 3 in the Supplementary Material). DNA was amplified twice with the selected primers to assess reproducibility between runs. The reproducibility of the primers selected ranged from 93 to 100 % (Appendix 3). We used these six primers in the subsequent analyses of the 238 samples. One DNA sample served as an additional control to the blanks in every PCR run.

The RAPD-PCR mixture contained 25 ng DNA, 0.2 μ M primer, 200 μ M of each dNTPs (peqLab) and 1.0 unit Taq polymerase (peqLab). Amplification was achieved in a Genius cyclor (Techne, UK) under the following conditions: 5 min at 93 °C followed by 36 cycles of 1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. PCR was finished with an extension of 6 min at 72 °C and after the final cycle, samples were stored at 4 °C.

The amplified products were separated on 1.2 % agarose gels in 0.5 M TBE buffer (tris–borate–EDTA buffer)

containing ethidium bromid (concentration: 0.0001 %), using a molecular size standard (Roche). Each DNA sample was repeated once in separate amplification reactions. DNA from a few leaf samples showed poorly reproducible banding patterns. DNA from these leaves was extracted again and the RAPD-PCR procedure was repeated. Individuals that did not provide clear or reproducible signals were excluded from the analysis.

For data scoring, the banding patterns were recorded using the gel documentation system AlphaImager™ (Alpha Innotech, California, USA). The image profile and molecular weight of each band were determined with the fingerprinting software of Bio-Rad (Bio-Rad Laboratories, California, USA).

Statistical analyses

We used the R package (R Development Core Team 2011, version 2.12.2) for all analyses. The statistical analyses were conducted on the cliff level. Data on plant characteristics from different sites within a cliff were combined. Spearman rank correlation analysis was used to examine possible relationships between population size of *A. montanum*, size of the tree-free area and percentage of ground vegetation cover. The same analysis was used to examine the correlations between plant density (number of plant individuals/m²) and percentage of ground vegetation cover and between molecular variance, population size and fitness-related traits. One-way ANOVA was applied to examine whether plant density differed between populations. We used general linear models to examine potential effects of population, plant density and ground vegetation cover on fitness-related traits including the number of blooming shoots per plant, inflorescence length, number of fruits and mean fruit weight per plant. Fisher's exact tests (using Monte Carlo simulation with 10,000 replicates) were applied to examine whether both the proportion of plant individuals belonging to different plant size classes and the percentage of reproductive plant individuals differ between populations.

Preliminary analyses of molecular data from different sites within the cliffs revealed no differences in the genetic structure of sites within cliffs. We therefore combined the molecular data from different sites within a cliff but corrected then for differences in sampling size (Fischer and Matthies 1998). We calculated the percentage of polymorphic loci and Nei's genetic diversity of the populations using Popgene Version 1.32 (Yeh et al. 1997). Clonal diversity [i.e. the number of clones detected (G) divided by the sample size (N)] was determined using the program GenoType-GenoDive with a threshold value of 5 % (Meirns and Van Tienderen 2004). We used GENALEX (Version 6.0; Peakall and Smouse 2006) to calculate the genetic distance between the individuals using the procedure proposed by Huff et al.

(1993). The same software was used to perform a hierarchical analysis of variance (AMOVA) and a principal component analysis (PCA) based on the matrix of genetic distances (Huff et al. 1993).

Results

Population size and size structure

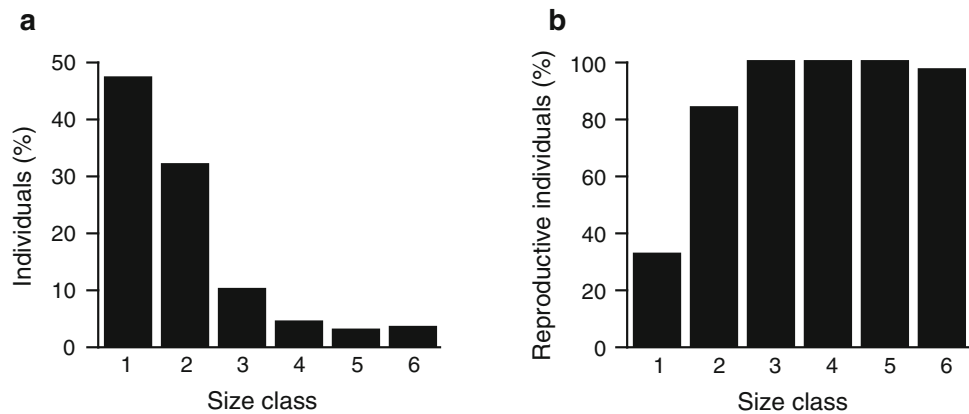
The population size of *A. montanum* ranged from 8 to 248 plant individuals on the cliffs examined (Table 1). Population size was neither related to the size of the tree-free area nor to the percentage of ground vegetation cover on the cliffs (Spearman rank correlation; area: $r_s = 0.41$, $n = 12$, $P = 0.18$; vegetation cover: $r_s = -0.23$, $n = 12$, $P = 0.48$). In contrast, the population size decreased with increasing altitude of the cliffs ($r_s = -0.66$, $n = 12$, $P = 0.022$). The density of *A. montanum* individuals decreased with increasing ground vegetation cover ($r_s = -0.67$, $n = 12$, $P = 0.016$) and differed significantly between the populations ($F_{11,23} = 6.80$, $P < 0.0001$). Furthermore, a highly skewed distribution of the size classes was found (Fig. 2a). Out of the total of 1,253 individuals recorded, 593 individuals (47.3 %) belonged to the size class 1 (small plants) and only 77 (6.2 %) could be assigned to the classes 5 and 6 (large plants; Fig. 2a). The populations differed in the proportions of individuals belonging to different size classes (Fisher's exact test with simulated P values based on 10,000 replicates, $P < 0.0001$). For example, in the populations Ankenballen and Baflue, 72 % of the individuals belonged to the smallest size class, whereas the corresponding figures for the populations Belchenflue and Sissacherflue were 20 and 25 %.

The proportion of reproductive individuals ranged from 33.3 to 80.7 % and differed significantly between the populations (Fisher's exact test with simulated P value based on 10,000 replicates, $P < 0.0001$). Considering single plant size classes, only 32 % of the plants belonging to size class 1 were able to develop flowers. The corresponding figures for size class 2 were 84 and 97.5–100 % for the size classes 3–6 (Fig. 2b). However, none of the assessed reproductive characteristics of *A. montanum* including number of blooming shoots per plant, inflorescence length, number of fruits and fruit weight per plant individual differed between populations (generalized linear model, all cases $P > 0.43$). Furthermore, neither of the recorded reproductive characteristics was related to the plant density or to the size of the populations (generalized linear model, all cases $P > 0.31$).

Genetic population structure

In total, 98 scorable bands were obtained from the six primers used in the RAPD-PCR analysis of the 228 samples

Fig. 2 Percentage of plant individuals (a) and percentage of reproductive individuals (b) belonging to the different size classes. Pooled data from all populations are shown. Size class 1: 1–5 shoots/plant individual; 2: 6–10; 3: 11–15; 4: 16–20; 5: 21–25 and 6: >26



(Appendix 3 in the Supplementary Material). Seventy-nine of these bands (80.6 %) were polymorphic and used for further analyses. The number of scorable bands per primer ranged from 11 (X03) to 19 (X04). Considering single populations, the percentage of polymorphic loci ranged from 1.4 to 14.6 %, Nei's genetic diversity from 0.018 to 0.215 and molecular variance from 3.2 to 65.8 (Table 2). A total of different 225 multilocus RAPD phenotypes were recorded. Identical RAPD profiles were only detected in the two populations Ämmenegg and Schmutzbergflue causing a reduced clonal diversity (Table 2).

There was a positive correlation between population size and molecular variance (Spearman rank correlation, corrected for unequal sampling size; $r_s = 0.66$, $n = 12$, $P = 0.022$; Fig. 3). In contrast, plant density did not affect molecular variance ($r_s = 0.16$, $n = 12$, $P = 0.62$). Further correlation analyses revealed that the proportion of reproductive individuals tended to increase with increasing molecular variance ($r_s = 0.52$, $n = 12$, $P = 0.08$; Fig. 4a) and that the number of fruits per plant increased significantly with increasing molecular variance ($r_s = 0.59$, $n = 12$, $P = 0.05$, Fig. 4b). In contrast, fruit weight was not affected by molecular variance ($r_s = 0.29$, $n = 12$, $P = 0.35$).

Analysis of molecular variance (AMOVA) revealed that the within-population variation accounted for 53 % and the among-population variation for 47 % of the total variation. All components of variation were found to be highly significant (AMOVA, $P < 0.0001$). Genetic distances (Nei 1978) among populations were not correlated with the geographical distances (Mantel test: $r_M = -0.06$, $n = 12$, $P = 0.37$). This finding was supported by the principal coordinate analysis (PCO) of Nei's genetic distance showing three distinct clusters which were not related to the geographic origin of the plant samples (Fig. 5). The first three axes of the multivariate analysis explained 44.3 % of the total variation (axis 1: 19.9 %; axis 2: 17.4 %; axis 3: 7.0 %). Cluster A consists exclusively of samples derived from the cliff Hoffstetter Chöpfl, whereas clusters B and C are composed of a mixture of samples derived from different cliffs (Fig. 5).

Discussion

The present study shows that populations of the relict plant *Alyssum montanum* growing on cliffs in the Northern Swiss

Table 2 Percentage of polymorphic loci, Nei's genetic diversity, molecular variance and clonal diversity (G/N) of *A. montanum* populations at limestone cliffs in the Northern Swiss Jura mountains

Cliffs	N	Polymorphic loci (%) ^a	Nei's genetic diversity ^a	Molecular variance ^a	Clonal diversity (G/N) ^a
Hofstetter Chöpfl (HO)	48	6.8	0.163	59.8	1.000
Baflue (BA)	10	14.6	0.169	24.4	1.000
Sissacher Flue (SF)	19	12.8	0.203	42.2	1.000
Tannenflue (TA)	40	10.0	0.215	59.5	1.000
Portiflue (PO)	17	8.4	0.128	65.8	1.000
Schöntalflue (SC)	10	12.6	0.157	17.7	1.000
Ankenballen (AN)	10	8.8	0.101	12.5	1.000
Höchi Flue (HF)	20	8.9	0.140	27.6	1.000
Rämel (RM)	20	10.3	0.183	35.9	1.000
Belchenflue (BE)	10	7.1	0.079	13.3	1.000
Schmutzbergflue (SM)	8	2.3	0.030	3.6	0.964
Ämmenegg (AM)	10	1.4	0.018	3.2	0.867

N the number of individuals sampled

^a Corrected for sampling size

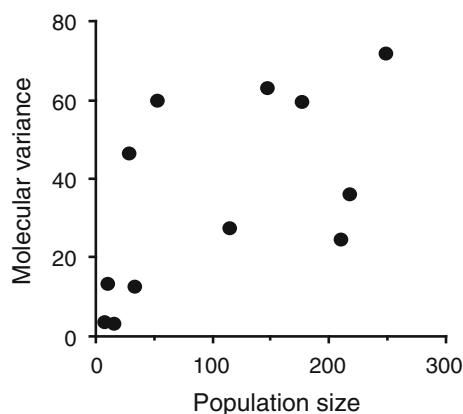


Fig. 3 Correlations between molecular variance (corrected for sample size) and population size (number of individuals) of *Alyssum montanum* on 12 cliffs ($r_s = 0.66$, $P = 0.022$)

Jura mountains are small and isolated. Our field survey indicates that this plant species occurred on more cliffs distributed in the Northern Swiss Jura mountains some decades ago and that the plant is threatened by overgrowing forest. The size structure of the populations and the reproductive traits examined were not related to the size of the populations. Furthermore, the genetic variability of *A. montanum* was positively correlated with local population size and with several reproductive characteristics of the plant.

Population size in relict plant species is in most cases highly variable. For example, the population size ranged from 4 to 370 individuals in *Draba aizoides*, from 1 to 150 in *Saxifraga paniculata* (Wezel 2007), from 26 to 257 in relict *Biscutella laevigata* populations (Dannemann 2000), from 60 to 5,000 in the alpine relict *Saxifraga cernua* (Bauert et al. 2007) and from 85 to 28,000 in the relict *Ligularia sibirica* (Smidová et al. 2011). The population sizes recorded for *A. montanum* (8–248 individuals) in our study are in the range of these estimates.

The population size of *A. montanum* decreased with increasing altitude of the cliff. However, the population size was not related to any of the others recorded habitat characteristics examined. This could be explained by the

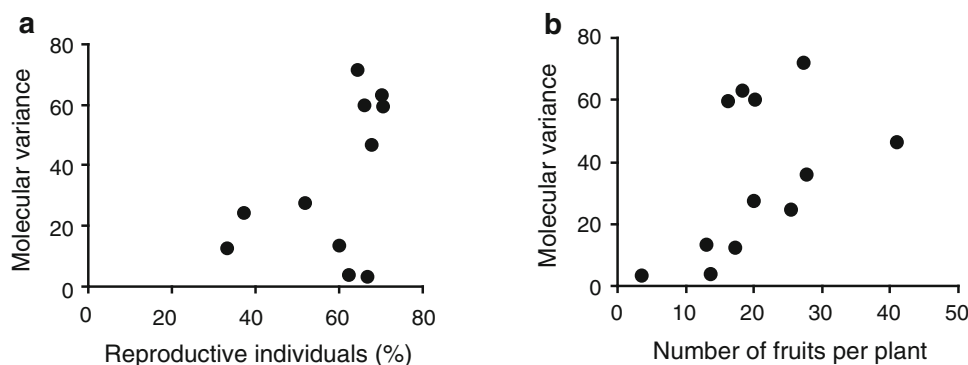
pronounced spatial aggregations of plant individuals on the cliffs. The majority of the populations investigated (9 out of 12) exhibits a so-called “invasive population structure” which is characterized by a high proportion of seedlings and small plants in relation to large plants (Oostermeijer et al. 1994). This particular type of population size structure most frequently occurs in early successional stages of different habitats (Oostermeijer et al. 1994; Brys et al. 2004; Eckstein et al. 2009). The cliff vegetation is disturbed by storms and/or rock climbing and resting hikers on the top of the cliffs. Storms and recreational activities may create gaps in the vegetation that allow the successful establishment of juvenile plants of *A. montanum*. Disturbance may not affect all species in a similar way. For example, the population size structure of *Draba aizoides* in the cliff vegetation of the Bavarian Alps was not affected by climbing activities (Vogler and Reisch 2011).

In our study, the fitness-related parameters examined were neither related to population size nor to plant density or vegetation cover. This result contrasts the findings of Leimu et al. (2006) who showed a positive relationship between population size and fitness-related traits in numerous plant species. Our unexpected finding could be a result of the combined effects of the ability of *A. montanum* to produce a substantial amount of seeds by self-fertilization and the dispersal of the seeds by gravity resulting in a highly clumped distribution of individuals on the cliffs. It seems, therefore, that spatial population attributes are more important for reproduction and fitness of *A. montanum* than population size (Luzuriaga et al. 2006).

Genetic population structure

Our study shows that the relict *A. montanum* exhibits a considerable high genetic diversity on the cliffs investigated. The range in the proportion of polymorphic loci (4–62 %) is comparable with the variation in polymorphic loci found in other European glacial relict species: 12–54 % in *Saxifraga paniculata* (Reisch et al. 2003), 28–60 % in *Draba aizoides* (Müller 2006) and 72–92 % in *Biscutella*

Fig. 4 Correlations between molecular variance (corrected for sampling size) and **a** percentage of reproductive individuals ($r_s = 0.52$, $P = 0.08$) and **b** mean number of fruits per plant ($r_s = 0.59$, $P = 0.05$) in *Alyssum montanum* on 12 cliffs



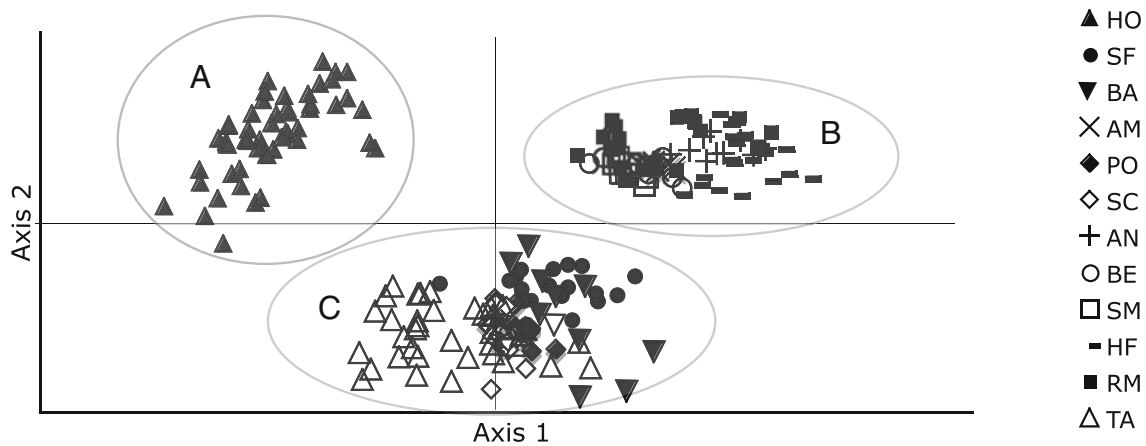


Fig. 5 Principal component analysis (PCA) based on the matrix of genetic distances between 12 populations of *Alyssum montanum* in the Northern Swiss Jura mountains. For population abbreviations see [Table 1](#)

laevigata (Dannemann 2000). Furthermore, similar high genetic diversity as found in the present study has been reported in other relict species (Pérez-Collazos and Catalan 2007; Garzia-González et al. 2008; Smidová et al. 2011). In our study, genetic diversity was significantly lower in smaller populations than in larger ones (Fig. 3). This finding supports the general positive relationship between population size and genetic diversity reported in numerous plant species (Fischer and Matthies 1998; Hensen et al. 2005; Smidová et al. 2011). The low genetic diversity recorded in small *A. montanum* populations could be a result of genetic drift, inbreeding depression, lack of pollinators and/or poor habitat quality (Avice and Hamrick 1996; Young and Clarke 2001; Hardy et al. 2006).

The within-population molecular variability detected in *A. montanum* populations (53 %) was relatively low compared with those found within populations of several other relict plant species: 71.9 % in *Ramonda myconi* (Dubreuil et al. 2008) and >80 % in *Stipa capillata*, *Physaria bellii* and *Ligularia sibirica* (Hensen et al. 2005; Kothera and Richards 2007; Smidová et al. 2011). However, such low within-population genetic variability is typical for self-compatible species and species with the ability for clonal growth (Peterson et al. 2008). The calculated Φ_{st} value of 0.46 indicates a high population subdivision in *A. montanum*. Similar Φ_{st} values have been reported in plant species with a restricted geographical range and in species characteristic for early to middle successional stages of a particular habitat type (0.40–0.45; Nybom and Bartish 2000). In contrast, much lower Φ_{st} values have been found in plant species with life-history characteristics similar to *A. montanum* (0.19–0.23; Nybom and Bartish 2000).

The analysis of the spatial genetic structure revealed three distinct clusters of plant individuals whose affiliation could not be explained by their geographic origin (Fig. 5). We found no positive correlation between geographic and

genetic distance between *A. montanum* populations. The period of isolation after the last glaciation was probably not sufficiently long to cause genetic divergence in relation to geographic distances, as found in long-lived perennial species (Gabrielsen et al. 1997; Schmidt and Jensen 2000). Knecht (1999) assumed that some populations of *A. montanum* were founded by botanists with seeds obtained from the Hofstetter Chöpfli. However, our analysis does not support this assumption, because individuals from the Hofstetter Chöpfli were all assigned to one distinct cluster (Fig. 5).

Species with a fragmented distribution are particularly vulnerable to extinction when gene flow between populations is reduced or even lacking. The observed low within- and among-population genetic variation is characteristic for plant species which produce a high proportion of self-fertilized seeds and have a moderate capacity for clonal growth. Our study revealed that population size is the key factor responsible for the recorded high genetic variability and for the persistence of the populations. Our results also showed a close relationship between genetic variability and fitness-related traits. The presumed local extinction of *A. montanum* at three cliffs in the Northern Swiss Jura mountains might be the result of overgrowing forest. We therefore suggest that adequate management actions should be developed and implemented to promote *A. montanum* on cliffs. The action plans should preserve the specialized plants from intensive mechanic disturbances by sport climbing and hiking (McMillan and Larson 2002; Müller et al. 2004; Rusterholz et al. 2004, 2011). Furthermore, an adequate forest management should prevent the rocky cliffs from being shadowed by overgrowing forest (Müller et al. 2006).

Acknowledgments We thank A. Baur and three anonymous reviewers for their valuable comments on the manuscript.

References

- Avise J, Hamrick JL (1996) Conservation genetics: case histories from nature. Chapman & Hall, New York
- Bauert MR, Kälin M, Edwards PJ, Baltisberger M (2007) Genetic structure and phylogeography of alpine relict populations of *Ranunculus pygmaeus* and *Saxifraga cernua*. Bot Helv 117:181–196
- Baur B (ed) (2003) Freizeitaktivitäten im Baselbieter Wald. Ökologische Auswirkungen und ökonomische Folgen. Verlag des Kantons Basel-Landschaft, Liestal
- Britten HB (1996) Meta-analyses of the association between multilocus heterozygosity and fitness. Evolution 50:2158–2164
- Brys R, Jaquemyn H, Edels P (2004) Reduced reproductive success in small populations of the self-incompatible *Primula vulgaris*. J Ecol 92:5–14
- Council Directive 92/43/EEC (http://ec.europa.eu/environment/nature/legislation/habitatsdirective/index_en.html)
- Dannemann A (2000) Der Einfluss von Fragmentierung und Populationsgrösse auf die genetische Variation und Fitness von seltenen Pflanzenarten am Beispiel von *Biscutella laevigata* (Brassicaceae). Dissertationes Botanicae, Band 330
- Dostálek T, Münzbergová Z, Placková I (2010) Genetic diversity and its effect on fitness in an endangered plant species, *Dracocephalum austriacum* L. Conserv Genet 11:773–783
- Doyle JJ, Doyle JL (1990) A rapid total DNA preparation procedure for fresh plant tissues. Focus 12:13–15
- Dubreuil M, Riba M, Mayol M (2008) Genetic structure and diversity in *Ramonda myconi* (Gesneriaceae): effects of historical climate change on a preglacial relict species. Am J Bot 95:577–587
- Eckstein RL, Danihelka J, Otte A (2009) Variation in life-cycle between three rare and endangered floodplain violets in two regions: implication for population viability and conservation. Biologica 64:69–80
- Ellstrand NC (1992) Gene flow by pollen: implications for plant conservation genetics. Oikos 63:77–86
- Fischer M, Matthies D (1998) RAPD variation in relation to population size and plant fitness in the rare plant *Gentianella germanica* (Gentianaceae). Am J Bot 84:1685–1692
- Gabrielsen TM, Bachmann K, Jakobsen KS, Brochmann C (1997) Glacial survival does not matter: RAPD phylogeography of Nordic *Saxifraga oppositifolia*. Mol Ecol 6:831–842
- Garzia-González R, Carrasco B, Penailillo P, Letelier L, Herrera R, Lavandero B, Moya M, Caligaria PDS (2008) Genetic variability and structure of *Gomortega keule* (Molina) Baillon (Gomortegaceae) relict populations: geographical and genetic fragmentation and its implications for conservation. Botany 86:1299–1310
- Hardy OJ, Maggia L, Bandou E, Bryne H, Caron H, Chevallier MH, Doligez A, Dutech C, Kremer A, Latouch-Hallé C, Troispoux V, Veron V, Degen B (2006) Fine-scale genetic structure and gene dispersal interference in 10 Neotropical tree species. Mol Ecol 15:559–571
- Hegi G (1986) Illustrierte Flora von Mitteleuropa. Lehmann's Verlag, Munich
- Hegland SJ, Van Leeuwen M, Oostermeijer JGB (2001) Population structure of *Salvia pratensis* in relation to vegetation and management of Dutch dry floodplain grasslands. J Appl Ecol 38:1277–1289
- Hensen I, Oberprieler C, Wesche K (2005) Genetic structure, population size and seed production of *Pulsatilla vulgaris* Mill. (Ranunculaceae) in Central Germany. Flora 200:3–14
- Huff DR, Peakall R, Smouse P (1993) RAPD variation within and among natural populations of outcrossing Buffalograss *Buchloea dactyloides* (Nutt.) Englm. Theor Appl Genet 86:927–934
- Knecht D (1999) Felsinventar Basler Jura und Schutzkonzept. Unpublished report
- Kothera L, Richards CM (2007) Genetic diversity and structure in the rare Colorado endemic plant *Physaria bellii* Mulligan (Brassicaceae). Conserv Genet 8:1043–1050
- Le Corre V, Dumoulin-Lapegue S, Kremer A (1997) Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. Mol Ecol 6:519–529
- Leimu R, Mutikanen P, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? J Ecol 94:942–952
- Luijten SH, Dierick A, Gerard ADJ, Oostermeijer JGB, Raijman LEL, Den Nijs HCM (2000) Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in the Netherlands. Conserv Biol 14:1776–1787
- Lutz E, Schneller JJ, Holderegger R (2000) Understanding population history for conservation purposes: population genetics of *Saxifraga aizoides* (Saxifragaceae) in the lowlands and lower mountains north of the Alps. Am J Bot 87:583–590
- Luzuriaga AL, Escudero A, Albert MJ, Giménez-Beanivides L (2006) Population structure effect on reproduction of a rare plant: beyond population size effect. Can J Bot 84:1371–1379
- McMillan MA, Larson DW (2002) Effects of rock climbing on the vegetation of the Niagara escarpment in southern Ontario, Canada. Conserv Biol 16:389–398
- Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for analysis of genetic diversity of asexual organisms. Mol Ecol Notes 4:792–794
- Moser DM, Gygas A, Bäumler B, Wyler N, Palese R (2002) Rote Liste der gefährdeten Arten der Schweiz. Farn und Blütenpflanzen, Bundesamt für Umwelt, Wald und Landschaft, Bern
- Müller SW (2006) Human impact on the vegetation of limestone cliffs in the northern Swiss Jura mountains. University of Basel, Basel
- Müller SW, Rusterholz HP, Baur B (2004) Rock climbing alters the vegetation of limestone cliffs in the northern Swiss Jura Mountains. Can J Bot 82:862–870
- Müller SW, Rusterholz HP, Baur B (2006) Effects of forestry practices on relict plant species on limestone cliffs in the northern Swiss Jura mountains. Forest Ecol Manage 237:227–236
- Müller-Dombois D, Ellenberg H (2002) Aims and methods of vegetation ecology. Wiley, New York
- Neis M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590
- Nyblom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect Plant Ecol Evol Syst 3:93–114
- Oostermeijer JGB, Veer RV, den Nijs JCM (1994) Population structure of the rare, long-lived perennial *Gentiana pneumonanthe* in relation to vegetation and management in the Netherlands. J Appl Ecol 31:428–438
- Peakall R, Smouse PE (2006) GENEALX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295
- Pérez-Collazos E, Catalan P (2007) Genetic diversity analysis and conservation for the Iberian threatened populations of the irano-turanian relict *Krascheninnikovia ceratoides* (Chenopodiaceae). Bot J Linn Soc 92:419–429
- Peterson A, Bartish IV, Peterson J (2008) Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L. Plant Ecol 198:101–110

- R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Reisch C, Poschold P, Wingender R (2003) Genetic variation of *Saxifraga paniculata* Mill. (Saxifragaceae): molecular evidence for glacial relict endemism in Central Europe. *Biol J Linn Soc* 80:11–21
- Rusterholz HP, Müller SW, Baur B (2004) Effects of rock climbing on plant communities on exposed limestone cliffs in the Swiss Jura mountains. *Appl Veg Sci* 7:35–40
- Rusterholz HP, Verhoustraeten C, Baur B (2011) Effects of long-term trampling on the above-ground forest vegetation and soil seed bank at the base of limestone cliffs. *Environ Manage* 48:1024–1032
- Schmidt K, Jensen K (2000) Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components. *Am J Bot* 87:678–689
- Smidová A, Münzbergová Z, Placková I (2011) Genetic diversity of a relict plant species, *Ligularia sibirica* (L.) Cass. (Asteraceae). *Flora* 206:151–157
- Soulé ME (1986) Viable populations for conservation. Cambridge University Press, Cambridge
- Španiel S, Marhold K, Filová B, Zozomova-Lihová J (2011) Genetic and morphological variation in the diploid–polyploid *Alyssum montanum* in Central Europe: taxonomic and evolutionary considerations. *Plant Syst Evol* 294:1–25
- Španiel S, Marhold K, Filová B, Zozomova-Lihová J (2012) A new circumscription of *Alyssum montanum* ssp. *montanum* and *A. montanum* ssp. *gmelinii* (Brassicaceae) in Central Europe: molecular and morphological evidence. *Bot J Linn Soc* 169:378–402. doi:10.1111/j.1095-8339.2012.01225.x
- Vogel JC, Rumsey FJ, Russel SJ (1999) Genetic structure, reproductive biology and ecology of isolated populations of *Asplenium csikii* (Aspleniaceae, Pteridophyta). *Heredity* 83:604–612
- Vogler F, Reisch C (2011) Genetic variation on the rocks—the impact of climbing on the population ecology of a typical cliff plant. *J Appl Ecol* 48:899–905
- Walter H, Straka H (1970) Arealkunde. Floristisch-historische Geobotanik. Eugen Ulmer, Stuttgart
- Wassmer A (1998) Zur Felsenflora des östlichen Kettenjuras. Grundlagen und Berichte zum Naturschutz. Band 17. Baudepartement, Sektion Natur und Landschaft, Aargau
- Wezel A (2007) Changes between 1927 and 2004 and effect of rock climbing on occurrence of *Saxifraga paniculata* and *Draba aizoides*, two glacial relicts on the limestone cliffs of the Swabian Jura, southern Germany. *J Nat Conserv* 15:84–93
- Yeh F, Yang R, Boyle T (1997) Popgene Version 1.31. Microsoft Window-based freeware for population genetic analysis, Alberta
- Young EW, Clarke GM (2001) Genetics, demography and viability of fragmented populations. Cambridge University Press, Cambridge
- Zoller H (1989) Die Verarmung der Pflanzenwelt. In: Imbeck-Löffler P (ed) Natur aktuell, Lagebericht zur Situation der Natur im Kanton Basel-Landschaft im Jahr 1988. Verlag des Kantons Basel-Landschaft, Liestal, pp 217–246