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Contemporary gene flow and mating system of *Arabis alpina* in a Central European alpine landscape

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• *Background and Aims* Gene flow is important in counteracting the divergence of populations but also in spreading genes among populations. However, contemporary gene flow is not well understood across alpine landscapes. The aim of this study was to estimate contemporary gene flow through pollen and to examine the realized mating system in the alpine perennial plant, *Arabis alpina* (Brassicaceae).
• *Methods* An entire sub-alpine to alpine landscape of 2 km^2 was exhaustively sampled in the Swiss Alps. Eighteen nuclear microsatellite loci were used to genotype 595 individuals and 499 offspring from 49 maternal plants. Contemporary gene flow by pollen was estimated from paternity analysis, matching the genotypes of maternal plants and offspring to the pool of likely father plants. Realized mating patterns and genetic structure were also estimated.
• *Key Results* Paternity analysis revealed several long-distance gene flow events (≤ 1 km). However, most outcrossing pollen was dispersed close to the mother plants, and 84 % of all offspring were selfed. Individuals that were spatially close were more related than by chance and were also more likely to be connected by pollen dispersal.
• *Conclusions* In the alpine landscape studied, genetic structure occurred on small spatial scales as expected for alpine plants. However, gene flow also covered large distances. This makes it plausible for alpine plants to spread beneficial alleles at least via pollen across landscapes at a short time scale. Thus, gene flow potentially facilitates rapid adaptation in *A. alpina* likely to be required under ongoing climate change.

Key words: Alpine, *Arabis alpina*, contemporary gene flow, genetic structure, mating system, paternity analysis, pollen dispersal, spatial autocorrelation.

INTRODUCTION

Gene flow, the transfer of genes among populations, is a key factor in determining genetic differentiation of populations (Mayr, 1963) and in spreading genes across a landscape (Wright, 1984). In heterogeneous environments, populations of sessile organisms like plants may be naturally isolated. Fragmented populations are more likely to be subject to genetic erosion and loss of genetic diversity owing to genetic drift (Ellstrand, 1992; Frankham *et al.*, 2002). Thus, gene flow is important to maintain a network of connected local populations, thereby increasing a species' chances for survival.

In alpine ecosystems, the heterogeneity of the landscape and the resulting spatio-temporal isolation of plant populations (Till-Bottraud and Gaudeul, 2002; Körner, 2003) have resulted in contrasting hypotheses on the relevance of gene flow in establishing or maintaining population connectivity beyond the local scale. On the one hand, gene flow is considered a rare event because of natural fragmentation of populations, which results in genetic divergence and substantial genetic structure of populations. At a large spatial scale, the genetic structure of populations of alpine species has mainly resulted from historical factors such as the location of glacial refugia and subsequent recolonization along post-glacial expansion

routes (Körner, 2003; Schönswetter *et al.*, 2005; Alvarez *et al.*, 2009; Thiel-Egenter *et al.*, 2011). These historical processes have left distinct imprints in the genetic structure of alpine species, which are still detectable today (Schönswetter *et al.*, 2005; Thiel-Egenter *et al.*, 2011). Even at a smaller spatial scale, the alpine landscape is characterized by topographic, edaphic and microclimatic heterogeneity, which potentially causes population divergence and local adaptation in alpine plants (Körner, 2003). These environmental gradients maintain population genetic structure (Till-Bottraud and Gaudeul, 2002; Körner, 2003) as a consequence of limited gene flow by pollen or seed and mating patterns favouring selfing and clonality. This assumption is supported by the low abundance and short flying distances of pollinators observed in the alpine landscape (Bingham and Orthner, 1998). In addition, clonality and selfing have been shown to increase at higher altitudes, impeding the dispersal of genetic variation but ensuring local population persistence (Schröter, 1926; Bliss, 1962; Bliss, 1971; García-Camacho and Totland, 2009).

In alpine plants, low gene flow and the resulting lack of functional connectivity have rarely been demonstrated by empirical data. Recent studies in alpine plants report restricted (Ohsawa and Ide, 2008; Gonzalo-Turpin and Hazard, 2009) to substantial long-distance gene flow events (Raffl *et al.*,

2008). Therefore, it is possible that gene flow facilitates the dispersal of genes more frequently and at larger distances in alpine landscapes than expected. This latter assumption is in agreement with evidence from non-alpine study organisms suggesting that gene flow occurs over large distances and at evolutionarily significant rates (Ellstrand, 1992, 2003). Gene flow via pollen in particular has been shown to disperse genes across large distances in non-alpine studies (e.g. Watrud *et al.*, 2004; Kamm *et al.*, 2009). In studies on alpine plant species, gene flow is indirectly studied from pollinator foraging distances or directly estimated from pollen movement measured by pollen dye (Ellstrand, 1992). Alternatively, gene flow has been estimated from population structure based on genetic differentiation such as Wright's F_{ST} (Wright, 1984). The parameter F_{ST} gives a historical estimate of gene flow based on pollen and seed dispersal, which is insensitive to small changes in allele frequencies in the contemporary landscape (Sork *et al.*, 1999). Therefore, this parameter is not suited to study contemporary patterns of gene flow (Whitlock and McCauley, 1999). In contrast, pollinator foraging distance and pollen movement give a contemporary estimate of gene flow by pollen, but severely underestimate the real amount of gene movement (Ellstrand, 1992).

To study contemporary gene flow by pollen, segregating molecular markers among parental and offspring populations are suitable to be analysed. The genotypes of the mother plant and offspring are compared with a pool of all potential father plants within a population to detect the most likely pollen parent through paternity analysis (Sork *et al.*, 1999; Holderegger *et al.*, 2010). While population structure, relating to historical processes, has also been analysed widely in alpine plants, studies on contemporary gene flow in herbs across natural landscapes are rare. Moreover, studies in alpine plants that have been conducted with paternity analysis or by pollinator observation are limited to small-scale studies in field sites (e.g. Schmitt, 1980; Hirao *et al.*, 2006; Brunet and Holmquist, 2009; Stöcklin *et al.*, 2009).

Investigating contemporary gene flow is especially relevant in alpine ecosystems because alpine plants are particularly vulnerable to climate change (Theurillat and Guisan, 2001; Till-Bottraud and Gaudeul, 2002; Byars *et al.*, 2007). In alpine organisms, climate change has resulted in specific biological effects such as range contractions and local extinctions (Hughes, 2000; McCarthy, 2001; Parmesan, 2006). By measuring contemporary gene flow, the dynamics of gene movement in environmentally heterogeneous alpine landscapes can be estimated (Sork *et al.*, 1999). This will help in estimating whether alpine plants can adapt locally or migrate to new habitats at short time scales, i.e. a few generations, given the rapid changes caused by factors such as global warming (Till-Bottraud and Gaudeul, 2002; Thuiller, 2007).

This is the first study to investigate contemporary gene flow by pollen in an alpine plant on the basis of an exhaustive sampling at the landscape scale. We chose the alpine perennial plant *Arabis alpina* (Brassicaceae) to describe contemporary gene flow patterns with paternity analysis because of its recent development as an evolutionary and ecological model species for alpine plants (Ansell *et al.*, 2008; Poncet *et al.*, 2010; Tedder *et al.*, 2011). Only recently, *A. alpina* was found to be largely selfing in populations of the Alps (Ansell

et al., 2008; Buehler *et al.*, 2011), presumably owing to the loss of a functional self-incompatibility system in the course of post-glacial re-colonization (Tedder *et al.*, 2011). Accordingly, we expected to find limited gene flow through pollen because of high selfing and restricted outcrossing, which would probably result in strong spatial autocorrelation. In turn, information on effective seed dispersal is not available in *A. alpina*, which is potentially a main determinant of spatial genetic structure. The objectives of our study were (1) to investigate the extent to which individuals are functionally connected by contemporary pollen transfer at the landscape scale; (2) to estimate the realized mating pattern; and (3) to determine the fine-scale spatial genetic structure of this species in an alpine landscape of approx. 2 km^2. For this, we sampled all individuals of *A. alpina* in a sub-alpine to alpine landscape and genotyped adult individuals and their offspring/seeds at 18 nuclear microsatellites.

MATERIALS AND METHODS

Study species

The alpine perennial *Arabis alpina* (Brassicaceae) is a pioneer plant species with a wide distribution in the northern hemisphere, ranging from eastern Canada to the Ural Mountains and tropical East Africa (Koch *et al.*, 2006). In Central Europe, *A. alpina* occurs in the montane to alpine zones in calcareous open habitats (Schultze-Motel, 1986). It is found on scree fields, along small streams and wells, in moist ravines and on humus-rich rock floors. *Arabis alpina* is described as diploid ($2n = 16$) and reproduces both sexually through insect-pollinated seed and asexually by stoloniferous growth (Schultze-Motel, 1986). It has small white flowers frequently visited by insects from various groups, and its seeds are about 1 mm in size with wings extending around the edges, potentially facilitating wind dispersal (Schultze-Motel, 1986). Previous studies showed that *A. alpina* is self-compatible (Tedder *et al.*, 2011) and highly selfing in populations studied from the European Alps (Ansell *et al.*, 2008; Buehler *et al.*, 2011).

Study area and sampling

Our study area was located on a sub-alpine to alpine landscape of approx. 2 km^2 in Urnerboden, Switzerland (Fig. 1). This landscape encompasses a riverbank, a small ravine and a scree field. Along the riverbank at around 1350 m a.s.l., *A. alpina* occurs frequently over a stretch of about 700 m. The small ravine is located along a mountainside extending from 1350 to 1700 m a.s.l., over a length of about 1 km. In the rock/scree field at 1750 m a.s.l., *A. alpina* occurs on dry scree as well as on nutrient-rich alpine pastures surrounding the scree field. In this landscape, *A. alpina* grows as scattered individuals or in small groups. We exhaustively mapped and sampled all *A. alpina* plants in summer 2008, including all distinctly separated rosettes. We repeatedly searched the entire area to be confident that full sampling was achieved. Fresh leaf material was collected from 595 plants considered to have potentially flowered and was immediately stored in silica gel. Open-pollinated seeds were collected from all
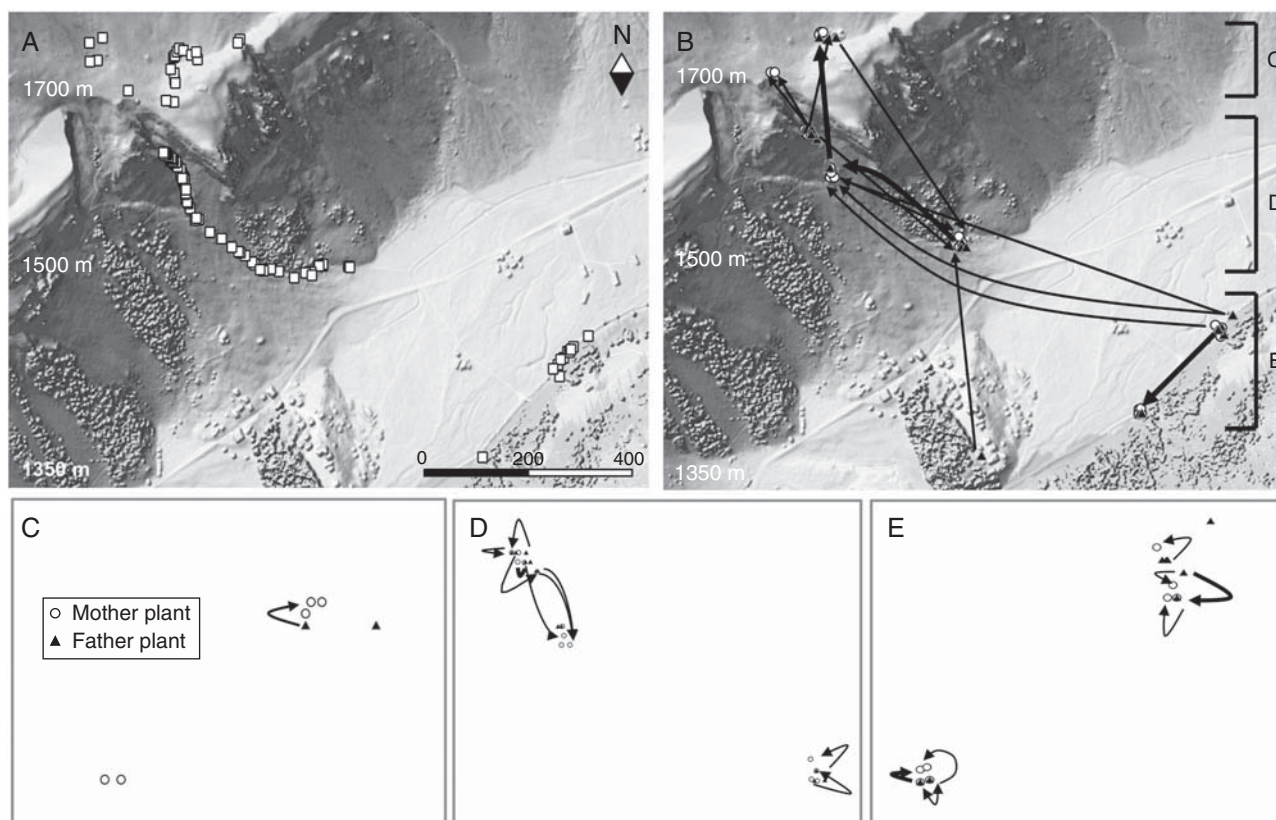
FIG. 1. Distribution of *Arabis alpina* in Urnerboden Switzerland. (A) Spatial clusters of individual *A. alpina* plants are represented by squares. (B–E) Patterns of contemporary gene flow by pollen of *A. alpina* are indicated by lines connecting mating partners (mother plants shown as circles and father plants shown as triangles) as detected in genetic paternity analysis. Thin lines represent one mating event and thick lines represent two mating events. (B) Long-distance gene flow events (>100 m) across the landscape, and close-ups of the scree field (C), ravine (D) and river (E) are shown for short-distance gene flow events (<100 m). The positions of the close-ups along the North–South axis are marked in B.

fruiting adult plants. The collected seeds were stored at room temperature until DNA extraction. We mapped the location of individuals and the relative distance to neighbouring plants by hand and recorded co-ordinates every 10 m as well as separately for every mother plant from which we sampled seeds using a hand-held GPS receiver. For paternity analysis, we randomly selected 49 mother plants, and we genotyped all adult plants and at least ten seeds per mother plant ($n = 499$ seeds).

DNA extraction and microsatellite genotyping

To genotype adult plants, DNA was isolated from 10 mg of lyophilized material ground using a disruptor mill (Retsch, Haan, Germany). Total genomic DNA was extracted following the DNeasy 96 plant kit protocol (Qiagen, Hombrechtikon, Switzerland). For paternity analysis, embryos and cotyledons were excised from seeds after placing them in H_2O at room temperature for 12 h. Disruption and DNA extraction of the seeds were done as described for adult plants.

We used 18 of the 19 polymorphic nuclear simple sequence repeat (nSSR) markers (excluding locus 3X6R) described in Buehler *et al.* (2011). The nSSR loci were amplified in four multiplex PCRs (Table 1). Multiplex PCRs with fluorescent dye-labelled primers were performed in 10 µL volumes containing 1–2 ng of DNA, 4·6 µL of 2× Master Mix

(Multiplex PCR Kit, Qiagen) and 0·1 µL of each primer (5 µM). PCRs were performed on Veriti thermocyclers (Applied Biosystems, Foster City, CA, USA). Initial denaturation was set at 94 °C for 15 min, and was followed by 27–29 cycles (multiplex 1 and 3, 28 cycles; multiplex 2, 29 cycles; multiplex 4, 27 cycles) at 94 °C for 30 s, 57 °C for 90 s, 72 °C for 30 s, ending with a final extension at 72 °C for 30 min. PCR products were run with ROX 400 HD as internal size standard (Applied Biosystems) on a 3130xl Genetic Analyzer (Applied Biosystems), and electropherograms were analysed using GENEMAPPER 3·7 (Applied Biosystems).

Genotypic diversity and clonal structure

The average number of alleles per locus, expected (H_E) and observed (H_O) heterozygosity, and the global inbreeding coefficient (F_{IS}) were calculated separately for adult plants and offspring using GENEPOP 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) and FSTAT 2.9.3 (Goudet, 1995). To characterize clonal structure, we searched for identical multilocus genotypes (ignoring missing data). We calculated the cumulative probability of finding two matching multilocus genotypes by chance and the estimated expected number of individuals considering the sample size. These calculations, considering both unrelated and sibling individuals,

TABLE 1. *Characteristics of 18 nuclear microsatellite loci for Arabis alpina in a sub-alpine to alpine landscape*

Locus	Adults				Offspring				Labels	Multiplex
	N_A	H_E	H_O	F_{IS}	N_A	H_E	H_O	F_{IS}		
3JUY	6	0.74	0.22	0.70	6	0.68	0.23	0.67	NED	1
4MDH	5	0.48	0.13	0.73	4	0.41	0.07	0.83	FAM	1
5GTC	10	0.70	0.26	0.63	6	0.54	0.15	0.72	HEX	1
9VSH	12	0.45	0.18	0.61	8	0.44	0.13	0.71	FAM	1
A1T8T	4	0.64	0.24	0.62	3	0.65	0.15	0.77	NED	1
A4JW7	5	0.50	0.15	0.71	5	0.58	0.13	0.77	HEX	1
DJSE	4	0.55	0.20	0.64	4	0.57	0.09	0.84	FAM	2
3Q19	6	0.40	0.16	0.61	5	0.47	0.14	0.69	NED	2
6U3A	5	0.41	0.14	0.66	5	0.53	0.06	0.88	NED	2
A25GM	6	0.71	0.22	0.69	6	0.67	0.23	0.65	HEX	2
DEET	7	0.45	0.18	0.59	5	0.59	0.08	0.86	FAM	3
46HI	5	0.49	0.19	0.61	4	0.38	0.09	0.76	HEX	3
7PIQ	7	0.64	0.22	0.66	7	0.63	0.12	0.81	HEX	3
BWFI	7	0.45	0.12	0.74	9	0.34	0.10	0.72	FAM	3
79PO	10	0.73	0.25	0.66	5	0.76	0.17	0.77	HEX	4
A8E90	4	0.67	0.17	0.75	2	0.69	0.13	0.81	FAM	4
A93Q	3	0.18	0.06	0.65	6	0.30	0.06	0.79	FAM	4
EB54	5	0.61	0.20	0.66	5	0.54	0.17	0.69	HEX	4
Mean	6.17 (2.38)	0.54 (0.15)	0.18 (0.05)	0.66 (0.05)	5.28 (1.67)	0.54 (0.13)	0.13 (0.05)	0.76 (0.07)		

Shown are the number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (F_{IS}) for each locus in the adult plants and offspring, with standard errors in parentheses. Furthermore, for each locus, the fluorescent labelling (Labels) and the multiplex PCR in which the locus was amplified (Multiplex) are given.

were performed with GENALEX 6.41 (Peakall and Smouse, 2006). The probability of identity, i.e. finding the same multi-locus genotype by chance and not as ramets of the same genet, was 1.5×10^{-11}. In our sample, it was thus expected to find 9.4×10^{-9} individuals showing the same multilocus genotype. Respective values considering siblings were 1.4×10^{-5} and 8.7×10^{-3}. Given these estimates, we henceforth assume that any individuals showing the same allele combinations across all 18 loci resulted from clonal propagation.

Paternity analysis

Paternity analysis was carried out with 49 mother plants and ten open-pollinated offspring per mother plant using CERVUS 3.0.3 (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007). CERVUS uses a likelihood-based approach and assigns paternity according to the highest logarithm of the likelihood (LOD score). LOD scores are calculated by determining the likelihood of assignment of a parent relative to the likelihood of arbitrary parents. We applied the following simulation parameters to find the confidence level of paternity analysis assignment: 10 000 simulated mating events; 1000 candidate father plants; eight as the minimum number of loci; 0·8 as the proportion of candidate parents sampled; genotyping error rate of zero (Slate *et al.*, 2000; Slavov *et al.*, 2005); 0·66 as the rate of inbreeding (F_{IS} value given by the GENEPOP analysis above); all adult plants treated as candidate father plants. While setting the genotyping error rate to 0·01 (as calculated from replicates) did not change the outcome (D. Buehler, unpubl. res.), we used the population-wide inbreeding coefficient to avoid generating a bias when paternity analysis relied on the maternal plants sampled. However, mating system analysis (see below) gave a very similar inbreeding estimate (0·67), which also did not affect the outcome of our study. As in Rathmacher *et al.* (2010), allele frequencies used in the simulation step were based on the *A. alpina* adult plants. We subsequently added alleles private to the offspring as $P = 0.0001$ to the frequency data file. In the paternity analysis, we used 95 % as strict and 80 % as relaxed confidence levels as recommended by Marshall *et al.* (1998). Effective pollen dispersal distances were calculated as the straight line distance from the mother plant to the most likely pollen parent, reflecting minimum flight distances of pollen-carrying insects. Curve fitting estimation for the distribution of pollen dispersal distances was done using TABLECURVE 2D (Systat, Erkrath, Germany).

Mating system

To characterize the effective mating system in our study population of *A. alpina*, the mother plants and the open-pollinated offspring were analysed with MLTR v3.3 (Ritland, 2002). On the basis of maximum likelihood procedures, the program estimates the multi- and single-locus outcrossing rates (t_m and t_s, respectively), biparental inbreeding ($t_m - t_s$), maternal inbreeding (F), correlated selfing rate (r_s) and the multilocus paternity correlation (r_p). The correlated selfing rate indicates among-family variation in mating system; $r_s = 1$ signifies that siblings are all either selfed or outcrossed and $r_s = 0$ indicates that selfing rates do not vary

among families. The multilocus paternity correlation is the proportion of full-sibs among outcrossed sibs and indicates whether offspring are the result of single or multiple paternities. The reciprocal of r_p ($N_{ep} = 1/r_p$) gives the effective number of pollen donors (Smouse *et al.*, 2001; Fernández-Manjarres and Sork, 2005). All seeds belonging to a mother plant were grouped as a family, and default settings for the estimated parameters were used in MLTR for the analysis of families (i.e. $t = 0.9$, $r_t = 0.1$, $r_p = 0.1$, $F = 0.1$). We used 1000 bootstraps to derive confidence intervals with resampling done over families.

Spatial genetic structure

To estimate the genetic structure of the adult plants in our study landscape, we used spatial autocorrelation analysis (Smouse and Peakall, 1999) as implemented in GENALEX 6.41 (Peakall and Smouse, 2006). Pairwise spatial autocorrelation coefficients (r) were computed as a multilocus average for diploid individuals and co-dominant markers. Correlograms were generated with eight size classes of even sample size: 0–30, 30–60, 60–120, 120–240, 240–480, 480–960 and 960–1920 m for a data set with all individuals and a data set excluding clonal individuals. A second analysis was performed, also with and without clonal individuals, to test for spatial genetic structure for the data set with all individuals at distances <30 m, with size classes of 5 m. We used 1000 bootstraps to estimate 95 % confidence intervals for the significance of r against no spatial structuring, i.e. random distribution.

RESULTS

Genotypic diversity and clonal structure

Over the entire landscape, adult plants and offspring of *A. alpina* showed high inbreeding coefficients ($F_{IS} = 0.66$ and $F_{IS} = 0.76$, respectively), while mean genetic diversity was identical for both categories ($H_E = 0.54$; Table 1). The number of alleles per locus ranged from three to 12, with a total of 111 and 95 alleles (genotyped for adults and offspring, respectively) over the 18 loci (Table 1). Five alleles were private to the offspring. Five cases of triplicate samples taken from rosettes assumed to be ramets of the same plant gave identical multilocus genotypes, providing high confidence in the reliability of our markers. Only 28 clonal groups of adult plants were determined. There were 16 clonal groups with individuals located within 5 m, and 12 clonal groups with individuals located at distances of 40–330 m between them. Nineteen clonal groups consisted of only two plants, while nine clonal groups consisted of 3–7 plants.

Paternity analysis and pollen flow distance

Ten offspring were typed at fewer than eight loci and were excluded from the analysis. CERVUS analysis resulted in an exclusion probability of 0.969 and a clear assignment of 249 (51 %) of the 499 offspring analysed to one most likely father plant. The remaining 240 offspring (49 %) were unassigned to a potential father plant. Of these, 122 (51 %) offspring had several potential father plants, whereas 118 (49 %) offspring had no potential father plants assigned in the study area. Of the assigned offspring, 86 (35 %) were assigned with a confidence level of 95 %, and 163 (65 %) were assigned at an 80 % confidence level. Overall, 210 (84 %) offspring resulting from selfing were detected. Outcrossing events were rare, as only 39 of the assigned offspring (16 %) were outcrossed (23 at a confidence level of 95 % and 16 more at a confidence level of 80 %). Eighteen of the 49 mother plants produced only selfed offspring and mother plants had 1–3 outcrossed offspring.

The distance of effective pollen dispersal, i.e if not selfed, ranged from ≤5 m to about 1000 m (Figs 1 and 2). Altogether, 36 % (14 counts) of the 39 effective outcrossed pollen travelled ≤5 m and about 51 % (20) were dispersed to ≤20 m. Two pollen (6 % of outcrossing gene flow) were dispersed over a distance of 1000 m. Pollen dispersal occurred over an altitudinal difference of up to 350 m (Fig. 1). The curve best fitting the empirical data on pollen flow was a general non-linear power equation; pollen dispersal distance = $1.548 + 1672.60x^{-3.045}$ ($R^2 = 0.804$; $P = 0.001$, Fig. 2).

Mating system

Outcrossing rates were considerably low ($t_m = 0.315$; $t_s = 0.118$) indicating a high degree of selfing in *A. alpina* (Table 2). Biparental inbreeding was substantial ($t_m - t_s = 0.197$) and suggests frequent mating among relatives. The estimate for inbreeding in the mother plants was also high ($F = 0.674$). The correlated selfing rate r_s was 0.305, and the multilocus paternity correlation r_p was 0.712. Hence, a large number of offspring were the result of single paternity. The number of effective pollen donors ($N_{ep} = 1/r_p$) was 1.4 individuals.

Spatial genetic structure

In a correlogram showing autocorrelation coefficients as a function of distance classes and a corresponding 95 % confidence interval, the distance class at which the estimate of r is no longer significant provides an approximation of the extent of detectable positive spatial genetic structure (Peakall *et al.*, 2003). In the data set considering all samples, the intercept occurred at 261 m, indicating that in distance classes 0–240 m, individuals were genetically more related than expected from random distribution at a significance level of $P = 0.05$ (Fig. 3A). The correlogram for the spatial distance class for <30 m showed that individuals located at 5 m distance were substantially genetically related (Fig. 3B). The correlograms excluding clonal individuals gave similar results, supporting that clones did not affect spatial structuring (results not shown).

DISCUSSION

A considerable number of studies have been published describing historical gene flow in alpine plants as inferred from population genetic structure at large spatial scales or by direct observation of pollinators at small spatial scales (e.g. Hirao *et al.*, 2006; Ohsawa and Ide, 2008; Paun *et al.*, 2008;
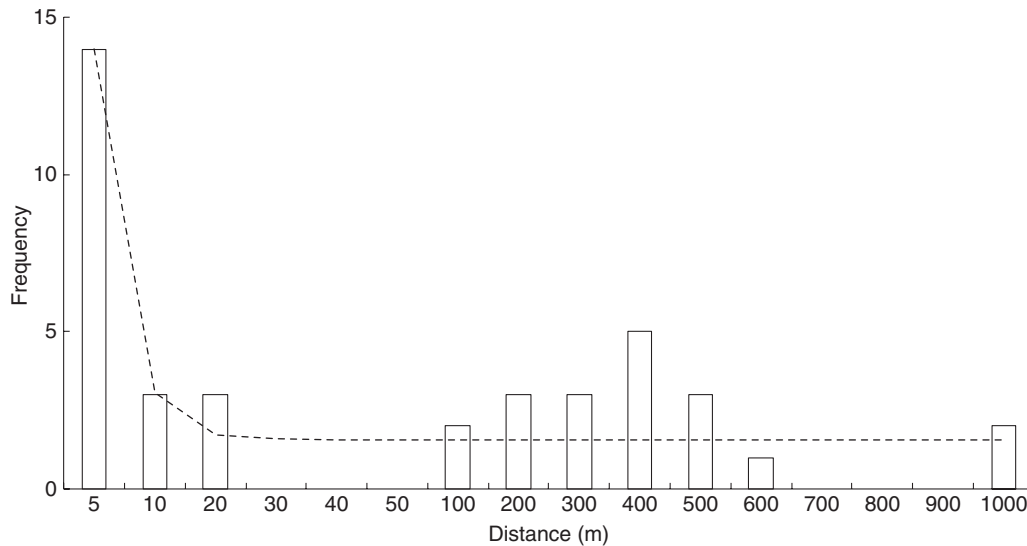
FIG. 2. Frequency distribution of distances of contemporary gene flow by pollen in *Arabis alpina* as detected in genetic paternity analysis. The dashed line represents the best-fitting curve ($y = 1.548 + 1672.60x^{-3.045}$; $R^2 = 0.804$, $P = 0.001$).

TABLE 2. *Realized mating pattern analysis of* Arabis alpina *on a sub-alpine to alpine landscape*

Parental inbreeding, F	0·674 (0·050)
Multilocus outcrossing rate, t_m	0·315 (0·035)
Single-locus outcrossing rate, t_s	0·118 (0·019)
Biparental inbreeding, $t_m - t_s$	0·197 (0·025)
Correlated selfing rate, r_s	0·305 (0·062)
Multilocus paternity correlation, r_p	0·712 (0·059)
Number of effective pollen donors, N_{ep}	1·400

Errors of estimates based on 1000 bootstraps are shown in parentheses.

Gonzalo-Turpin and Hazard, 2009; Stöcklin *et al.*, 2009; Meirmans *et al.*, 2011). However, to our knowledge, studies describing contemporary gene flow at the landscape scale are lacking. The aim of this study was to estimate contemporary gene flow by pollen using paternity analysis and to examine the mating system in the alpine plant *A. alpina*. We detected maximum gene flow distances up to 1 km. However, *A. alpina* had a high selfing rate ($F_{IS} = 0.66$) and a low number of outcrossing events (16 %) within the study population. Spatial genetic structure, indicative of gene flow within a given area, showed that individuals growing in close proximity were genetically more related than expected from random distribution. Below we will discuss these findings in the context of alpine plants.

Long-distance gene flow

Approximately 5 % of outcrossing pollen found in this study was dispersed at much larger maximum distances (≤ 1 km) than detected in other studies on alpine plants (e.g. Schmitt, 1980; Hirao *et al.*, 2006; Brunet and Holmquist, 2009; Stöcklin *et al.*, 2009). For instance, in the alpine plants *Epilobium fleischeri*, *Geum reptans* and *Campanula thyrsoides*, pollen dispersal was inferred from pollinator movement at a maximum distance of only 40 m (Stöcklin *et al.*, 2009). In another study, paternity analysis was used to measure contemporary gene flow in the alpine plant *Aquilegia coerulea* across population patches located up to 150 m apart (Brunet and Holmquist, 2009). The results of this study showed that pollinators frequently dispersed pollen among patches. However, gene flow detection was limited to the maximum distance between population patches. Therefore, studies using pollen flow measurements on small spatial scales or pollen flow inferred from pollinator observation bias information about the potential of gene flow in alpine plants, ultimately rendering gene flow a rare event (Ellstrand, 1992). The long-distance dispersal detected in our study is, thus, reasonable, even though our results also in part reflect both the size of our study landscape (2 km²) and the method used to quantify pollen flow. It remains to be studied the ways in which attractive flowers enhance – by attracting pollinators over large distances – or limit long-distance gene flow by pollen – owing to the local supply of sufficient (but see Wirth *et al.*, 2011). Moreover, it is unknown how much of the selfing results from autonomous selfing or from insect-induced pollen deposition (within or among flowers of the same genet). Only field experiments including insect exclusion will be informative in this regard.

Outcrossing pollen in *A. alpina* was mostly dispersed within a 5-m radius of the mother plant. This is reflected in the general findings of other gene flow studies, which describe pollen flow as a numerically frequent event close to the source plant and decreasing with distance (Ellstrand, 1992; Kamm *et al.*, 2009). In alpine plants, gene flow inferred from pollinator movement has shown that pollinators frequently visit flowers which are located close together (Schmitt *et al.*, 1980; Stöcklin *et al.*, 2009). Pollinator abundance, pollinator flight distances and differences in plant phenology directly limit gene flow in alpine areas (Hirao and Kudo, 2004; García-Camacho and Totland, 2009). In alpine environments, low pollinator abundance has been detected and
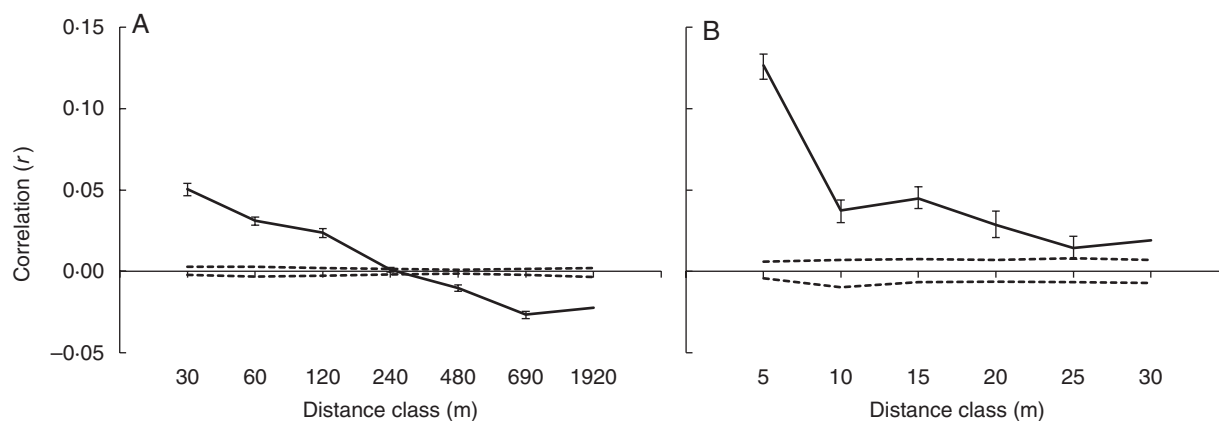
Fig. 3. Analysis of spatial genetic structure of *Arabis alpina* in a sub-alpine to alpine landscape using spatial autocorrelation analysis. The correlograms show average correlation coefficients between genetic distance among pairs of individuals plotted against distance classes: (A) for eight distance classes across the entire range of the study area, and (B) for a section of the correlogram with 5 m distance classes up to 30 m. Dashed lines delimit 95 % confidence intervals under the hypothesis of randomly distributed genotypes.

pollinator activity depends on the frequency of adverse weather conditions and the seasonal phenology of alpine plants (Hirao *et al.*, 2006). However, long-distance pollen flow was not restricted by differences in elevation in our study (Fig. 1). Therefore, asynchronous flowering patterns may have been only partially responsible for restricting gene flow in *A. alpina*. Instead, a combination of the above-described factors may explain the low number of outcrossing events and high frequency of pollen dispersal over short distances, while the dominance of selfing remains important in describing gene flow in *A. alpina*.

We detected a high percentage of offspring (49 %) with unresolved paternity. These offspring resulted either from gene flow of unsampled individuals within the landscape (cryptic gene flow) or from pollen immigration via gene flow from outside the study landscape (pollen inflow). This same result was also detected when a 1 % genotyping error was assumed in paternity analysis. In our landscape, cryptic gene flow is plausible as a result of working with a small herb over a large area. Some individuals might have been left unsampled within the landscape despite an exhaustive search. However, pollen inflow may have occurred originating from scree fields at higher elevations or from ravines located at approx. 2 km distance. Again, genotyping errors could account for undetected paternity to a certain degree, but this effect cannot be adequately quantified.

Selfing, realized mating patterns and spatial genetic structure

In this study, a substantial number of offspring (84 %) analysed in the paternity analysis resulted from selfing. This corresponds to the generally held view that alpine plants often self, especially with increasing altitude, even though much evidence for such a presumed trend comes from observational or correlative inference (Bliss, 1971; but see Gugerli *et al.*, 1998; Bingham and Ranker, 2000). As a result of pollen limitation, selfing, but also clonal proliferation, may be seen as a reproductive assurance mechanism (García-Camacho and Totland, 2009).

The investigation of the realized mating system in *A. alpina* also confirmed that sexual reproduction occurred mostly by selfing. Mother plants had low outcrossing rates, which often resulted in offspring from single paternity. Due to few effective pollen donors, inferred pollen availability was low. In a recent study, Tedder *et al.* (2011) showed that the self-incompatibility system, as is found in other members of the Brassicaceae (Bateman, 1954), was largely non-functional in populations of *A. alpina* from the Central and Western Alps, while it still functions in populations from the Appenines in Italy. Accordingly, *A. alpina* populations in the Central and Western Alps, which comprised our study landscape, are characterized by high levels of inbreeding, suggesting a regional change in breeding system possibly during the course of postglacial re-colonization (Ansell *et al.*, 2008). This corresponds to the distinct phylogeographic patterns found in the Central Alps, indicating independent origins and/or local glacial refugia (Ehrich *et al.*, 2007; Ansell *et al.*, 2008; Alvarez *et al.*, 2009). Tedder *et al.* (2011) concluded that populations of *A. alpina* in the Central Alps lack an inbreeding avoidance mechanism. This suggests that in our landscape, self-compatible plants colonized the area, or, less likely, a loss of self-incompatibility has occurred locally.

In our data set, we detected that individuals were genetically more similar than expected under random distribution up to a distance of about 260 m (Fig 3). Spatial genetic structure is expected to be prominent in alpine plant populations as a result of habitat fragmentation and spatial isolation (Till-Bottraud and Gaudeul, 2002). However, there are several life history traits, such as mating system and means of dispersal, which determine the degree of spatial autocorrelation. For example, the outcrossing Brassicaceae species *Biscutella laevigata* shows spatial autocorrelation over much shorter distances than *A. alpina* (Parisod and Bonvin 2008), which may be explained by mating system. However, even within this species, Parisod and Joost (2010) found significant differences between two populations classified as leading vs. trailing edge. Clonally propagated individuals were rare in our landscape and did not visibly affect the correlogram, but clonal reproduction may become more important at even

higher elevations where *A. alpina* approaches its upper limit of occurrence. However, a specific, hypothesis-driven sampling with appropriate replication would be required for rigorous testing of increased clonality at higher elevations. A closer look at the genetic structure found for *A. alpina* in the study population showed that individuals located within 5 m were highly related, indicating that a large fraction of gene dispersal frequently occurs over such short distances. This assumption was congruent with the result of the paternity analysis, in which the highest frequency of pollen dispersal occurred at <5 m distance from the source (Fig. 2), and the mating system, which indicated high selfing. Therefore, the genetic structure in the landscape mirrors the contemporary pollen dispersal and mating patterns in *A. alpina*.

Conclusions

Alpine landscapes are especially prone to be affected by climate change (IPCC, 2007), and alpine organisms are showing the first signs of range contractions or distribution changes (Hughes, 2000; McCarthy, 2001; Parmesan, 2006; Frei *et al.*, 2010). Various studies have shown that alpine plants usually possess ample variation at neutral genetic loci (Stöcklin *et al.*, 2009), and genetic variation at putatively adaptive loci has also been described (e.g. Manel *et al.*, 2010; Poncet *et al.*, 2010). Therefore, alpine plants potentially harbour genetic variation suitable for adaptation to changing environmental and climatic conditions. However, the survival of alpine species under climate change does not only depend on suitable standing genetic variation, but also on an organism's ability to spread beneficial genes to those areas where they match future environmental conditions (Byars *et al.*, 2009). The question of whether alpine organisms are able to adapt at short time scales remains open (Till-Bottraud and Gaudeul, 2002; Thuiller, 2007) since it is strongly influenced by outcrossing gene flow and, thus, the mating system.

The present work challenges the common finding that gene flow only occurs across short distances in alpine areas (Pluess and Stöcklin 2004). To make general conclusions about gene flow in *A. alpina* and in alpine plants, additional landscapes or alpine species, representing a variety of mating and dispersal strategies, should be analysed. Unfortunately, there is a tremendous gap in empirical data on contemporary gene flow in alpine plants to date, particularly so at a large spatial scale. In predominantly outcrossing plants, gene flow is expected to occur more frequently than shown here for the highly selfing plant *A. alpina*. However, in this study, our landscape-scale analysis, using molecular markers, showed that contemporary gene flow can reach large distances in *A. alpina*. Even a few long-distance gene flow events can spread genes, such as potentially advantageous alleles, among populations (Morjan and Rieseberg, 2004). The findings of this study suggest that gene flow has the potential to spread (beneficial) alleles in *A. alpina* at a short time scale to larger distances than previously expected, which might help this species to cope rapidly with climate change.

ACKNOWLEDGEMENTS

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