Allopatric speciation with little niche divergence is common among alpine Primulaceae

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ABSTRACT

Aim Despite the accumulation of cases describing fast radiations of alpine plants, we still have limited understanding of the drivers of speciation in alpine floras and of the precise timing of their diversification. Here, we investigated spatial and temporal patterns of speciation in three groups of alpine Primulaceae.

Location Mountains of the European Alpine System.

Methods We built a new phylogeny of Primulaceae including all species in three focal groups: Androsace sect. Aretia, Primula sect. Auricula and Soldanella. Combining phylogenetic information with a detailed climatic data set, we investigated patterns of range and ecological overlap between sister-species using an approach that takes phylogenetic uncertainty into account. Finally, we investigated temporal trajectories of diversification in the three focal groups.

Results We found that a large majority of sister-species pairs in the three groups are strictly allopatric and show little differences in substrate and climatic preferences, a result that was robust to phylogenetic uncertainty. While rates of diversification have remained constant in Soldanella, both Androsace sect. Aretia and Primula sect. Auricula showed decreased diversification rates in the Pleistocene compared to previous geological epochs.

Main conclusions Allopatric speciation with little niche divergence appears to have been by far the most common mode of speciation across the three groups studied. A few examples, however, suggest that ecological and polyploid speciation might also have played a role in the diversification of these three groups. Finally, extensive diversification likely occurred in the late Miocene and Pliocene coinciding with the later phases of the Alpine uplift, while diversification slowed down during subsequent glacial cycles of the Pleistocene.

Keywords alpine plants, Androsace sect. Aretia, diversification rates, European Alpine System, phylogenetic uncertainty, Primula sect. Auricula, sister-species comparison, Soldanella, Speciation

INTRODUCTION

Understanding why certain groups of organisms and some regions contain more species than others is a fundamental question in biology and one of the major challenges for science in general (Pennisi, 2005). Mountains harbour more biodiversity than expected based on the area they occupy (Körner, 2004) and plants are no exception to this rule (Kier et al., 2005). Indeed, recent phylogenetic studies have revealed that in all mountain ranges on Earth groups of alpine plants underwent rapid radiations (reviewed in Hughes & Atchison, 2015), raising two important questions on the origin of alpine floras, namely: (1) which factors best explain the high rates of diversification in alpine plant clades, and (2) when did this diversity arise?

Allopatric speciation, either through vicariance or long-distance dispersal, has traditionally been considered an important driver of alpine plant diversification, for many
sister-taxa have allopatric distributions (Ozenda, 1995; Kadereit et al., 2004). This pattern of allopatry could be explained by the fact that alpine habitats constitute complex archipelagos of ‘sky islands’. If allopatric speciation were the dominant mode of speciation in the alpine flora, we would expect most sister-species pairs to be allopatrically distributed, yet they might show little ecological niche differentiation because this would not be needed for speciation to occur (Wiens & Graham, 2005). However, ecological niche divergence may also have contributed to speciation, regardless of the geographical mode of speciation. Indeed, the extreme climatic and edaphic heterogeneity of mountain regions leads to local adaptations in plant populations (Körner, 1999) and evidence suggests that this might be responsible for population divergence in the Cape Floristic Region ( Lexer et al., 2014) and for speciation in the New Zealand Alps (Voelckel et al., 2008).

Although it seems clear that the majority of alpine plant radiations are relatively young and fast, dating to the late Miocene, Pliocene or later (Hughes & Atchison, 2015), the relative importance of late Miocene/Pliocene mountain uplifts and later Pleistocene climatic fluctuations in driving alpine plant diversification remains unclear. Some studies suggested that mountain uplifts of the middle and late Cenozoic (Ollier, 2006) played a major role in stimulating the diversification of alpine floras (e.g. Hughes & Eastwood, 2006; Joly et al., 2009; Favre et al., 2010; Roquet et al., 2013; Bentley et al., 2014; Linder et al., 2014). Indeed, mountain uplifts created both highly fragmented environments and a considerable diversity of habitats along steep environmental gradients, factors that usually promote high speciation rates (Coyne & Orr, 2004). Other studies emphasized the importance of Pleistocene glacial cycles for speciation, as they have triggered drastic range contractions and expansions in alpine plants, driving population fragmentation (e.g. Kadereit et al., 2004; Joly et al., 2009).

Here, we investigated patterns of speciation in plants of the Alpine system sensu Ozenda (1995), i.e. the system formed by all West-European mountain ranges, from the Sierra Nevada to the mountains of the Balkans (Fig. 1). In the Alpine system, the young ages inferred for many groups suggest that the Pleistocene represented a period of active diversification for the Alpine flora (Stebbins, 1984; Vargas, 2003; Kadereit et al., 2004). For the reasons explained above, it is hypothesized that allopatric speciation played a major role in plants of the Alpine system (Ozenda, 1995; Vargas, 2003). Seven groups of plants are endemic to the Alpine system (Ozenda, 1995). In this article, we used three of these clades as model system and focussed on species from Androsace sect. Aretia, Primula sect. Auricula, and Soldanella, all of which belong to Primulaceae. Comprising more than 15 described species each, they represent the three largest plant clades in the Alpine system (Ozenda, 1995), hence the best possible examples of plant diversification in this region.

To determine the relative contribution of geographical and environmental factors to speciation, we first built a new dated phylogeny for these three clades. Using an approach to consider phylogenetic uncertainty in sister-species comparison, we compared patterns of geographical and ecological overlap over all possible sister-species pairs. We specifically asked the following questions: (1) Is there general support for a particular geographical mode of speciation in these three groups? (2) Do sister-species strongly differ in their climatic or soil preferences, as would be expected if environmental factors played an important role in speciation? Finally, (3) we investigated the timing of diversification for these three groups and asked whether Pleistocene glacial cycles were associated with increased diversification rates. By investigating the spatial and temporal patterns of diversification in the three largest plant clades of the Alpine system, this study sheds new light on the drivers of speciation in temperate alpine floras and helps us to understand why they are generally so diverse.

MATERIALS AND METHODS

Study groups

We chose Androsace sect. Aretia (hereafter /Aretia/), Primula sect. Auricula (hereafter /Auricula/) and Soldanella for two main reasons. First, these three groups are closely related within Primulaceae (DeVos et al., 2014), which allowed us to reconstruct a single phylogenetic tree including all of them, thereby leading to more consistent estimation of divergence times across clades (see below). Second, these groups represent the three most species-rich plant radiations in the Alpine system (Ozenda, 1995). All species in these three groups are perennials found in the mountains of the Alpine system, except Primula palinuri, which grows on coastal cliffs in southern Italy (Fig. 1). /Aretia/ has been shown to have originated in the late Miocene, hence it has been proposed that this group largely diversified during the last active phases of the Alpine uplift (Roquet et al., 2013). In contrast, both /Auricula/ and Soldanella are thought to have appeared more recently (i.e. during the Pliocene and Pleistocene respectively) and it has been suggested that their diversification largely occurred during Pleistocene glacial cycles (Zhang & Kadereit, 2002, 2004).

Phylogenetic inference

To identify species pairs and estimate their divergence times, we needed a dated phylogeny including our three focal groups. A large dated phylogeny including Androsace s.l., Primula s.l. and Soldanella has been recently published (DeVos et al., 2014), but it only includes c. 30% of species in our focal groups. Detailed phylogenetic trees are available for /Aretia/ (Boucher et al., 2012), /Auricula/ (Zhang & Kadereit, 2004; Crema et al., 2013), and Soldanella (Zhang & Kadereit, 2002), but they were obtained using different genetic markers and dating techniques, preventing the possibility of combining them into a single dated tree for
our target groups. We thus collected ITS2 sequences from Genbank for all described species in /Aretia, /Auricula, and Soldanella, as well as two outgroups (Ardisia crenata Sims. and Anagallis arvensis L.). For some species in /Aretia and /Auricula, multiple subspecies were included to verify species monophyly. We also included additional species in Androsace s.l. and Primula s.l. to break up long branches leading to our three groups in the phylogeny, a practice aimed at increasing phylogenetic accuracy (Poe, 2003). These species were chosen to sample all basal divergences identified in previous studies (Boucher et al., 2012; DeVos et al., 2014). To produce a well-resolved phylogenetic hypothesis, sequences of trnL–F and trnG–R were added for Androsace s.l. and sequences of trnL and rpl16 were added for Primula s.l. (all sequences obtained through Genbank, accession numbers available in Table S1 in Supporting Information). Sequences were aligned using MUSCLE (Edgar, 2004) and alignments were edited manually to remove ambiguous sites. The combined data set, comprising 3301 aligned nucleotide positions in 81 taxa (61% missing data), was analysed in a Bayesian framework using BEAST (Drummond et al., 2012), with two partitions: ITS2 versus all chloroplast markers. For each partition, we employed a GTR+Γ+I model of nucleotide substitution (Tavaré, 1986) and a lognormal relaxed molecular clock (Drummond et al., 2006).

Although the fossil record for Primulaceae is sparse, we could calibrate three interior nodes in the phylogeny: Androsace s.l. was given a minimum age of 5.3 Ma based on fossil seeds from the Miocene (Dorofeev, 1963a), Primula s.l. was given a minimum age of 15.97 Ma based on fossil seeds from Primula rosiae from the Middle Miocene (Czaja, 2003), and the group formed by Androsace + Primula + Soldanella was given a minimum age of 28 Ma based on fossil seeds of Lysimachia angulata (Primulaceae) from the Early Oligocene (Dorofeev, 1963b). For these three groups, we fixed an absolute maximum age to 72 Ma, based on a fossil flower from the Late Cretaceous, which could be attributed to primuloids (i.e. Maesaceae, Theophrastaceae, Primulaceae, Myrsinaceae) but not to Primulaceae sensu stricto (Fris et al., 2010). In each case, stem nodes were calibrated and we applied uniform priors between the minimum and maximum bounds because we consider that the paucity of fossil Primulaceae does not allow for more informative priors.
To facilitate analysis, the monophyly of *Aretia, Auricula,* *Soldanella* and *Primula s.l. + Soldanella* was enforced based on previous phylogenetic evidence (DeVos et al., 2014). All other parameters in BEAST were set to their default values and two independent MCMC chains were run for 20 million generations each. One hundred trees were randomly sampled from the posterior distribution generated by the BEAST analysis, and were trimmed to species level prior to further analyses.

**Occurrence and environmental data**

Distribution data for species in *Aretia, Auricula* and *Soldanella* was obtained from various sources (see Appendix S2). All points with >1 km positional uncertainty were discarded. If several points were located in the same 100 m pixel we only kept one presence point for further analyses. The final data set included 17,300 occurrence points for all species in the three groups, except for *Soldanella angusta,* *S. marroissensis,* *S. oreodoxa* and *S. rugosa* for which no occurrence data could be retrieved (63 spp. in total, Fig. 1).

To accurately quantify differences in the climatic niche between species, we used a climatic data set for Europe with a 100 m spatial resolution, including monthly values for potential evapotranspiration, moisture index (precipitation – potential evapotranspiration), solar radiation, precipitation, relative air humidity and temperature. These layers were generated by first downscaling long-term monthly mean, minimum, and maximum temperature and monthly precipitation from WorldClim (Hijmans et al., 2005) across Europe. Downscaling was performed by local regressions in a moving window (see Zimmermann et al., 2007 for details). Solar radiation, potential evapotranspiration and moisture index were calculated following the procedures described in Zimmermann et al. (2007). To reduce the number of climatic variables used (96 initially), we measured all variables over 10,000 randomly chosen points in Western Europe, and investigated their correlations (see Appendix S2). From that we selected six variables to represent the climate of our chosen study area: solar radiation in June, minimum temperature in January, maximum temperature and monthly precipitation in June, relative air humidity in June, and precipitation both in January and in June. Minimum and maximum temperatures are indeed more important than mean temperatures for the physiology of alpine plants (Körner, 1999) and values of climatic variables from January and June were selected to characterize climate in the coldest month of the year (January) and during the flowering period of most species from our three focal groups (June). These six variables were then transformed using principal component analysis (PCA) in each pairwise niche comparison (see below).

Finally, information on species’ substrate preferences was obtained from the literature (Smith & Lowe, 1997; Zhang & Kadereit, 2002, 2004; Richards, 2003) and personal field observations. Each species was classified as being either calcifuge, calcicole or indifferent to substrate type.

**Geographical overlap**

If allopatry were the dominant mode of speciation we would expect most species pairs in Alpine Primulaceae to have allopatric distributions. Following Anacker & Strauss (2014), we estimated the geographical range of each species by placing a 10 km round buffer around each occurrence points and merging all overlapping circles obtained. Range overlap was then measured as the area of overlap between the ranges of two species divided by the range size of the species with the smaller range.

**Environmental overlap**

If selection for different environmental preferences were an important driver of speciation in alpine Primulaceae, we would expect sister-species to exhibit little environmental overlap. Overlap in substrate preferences was measured as 1 if two sister-species had overlapping substrate preferences, and as 0 if the species had divergent substrate preferences.

To quantify niche overlap between sister-species in multidimensional environmental space we used the method of Broennimann et al. (2012). This method compares the density of species occurrences against the available environment in a study area along multiple environmental axes in a multivariate analysis, and measures niche overlap along the axes of this multivariate analysis. Here, for each sister-pair we considered the first two axes of a PCA calibrated on the entire environmental space sampled over the ranges of each sister-species pair. Climatic niche overlap between the two taxa was measured using Schoener’s $D$ (Schoener, 1970) in this reduced environmental space. However, two taxa might exhibit differences in their climatic niches only because they live in regions where different climates occur (i.e. different realized niches), not because they have different physiologies (i.e. different fundamental niches; see Soberón, 2007; Boucher et al., 2014). To control for the potentially confounding effect of background climate, we used a niche similarity test (Warren et al., 2008), which compares the observed climatic niche overlap between two species to the overlap measured between one of the species’ niche and the randomized niche of its sister. This randomized niche is obtained by randomly sampling occurrence points in the region where the species occurs. For each sister-pair comparison, we used 99 randomizations to test whether the niches of the two taxa are more similar than expected by chance, given their geographical distributions.

**Integrating phylogenetic uncertainty in sister-pair comparisons**

To understand patterns of speciation in alpine Primulaceae, we first measured the proportion of strictly allopatric sister-species pairs, the proportion of sister-pairs that overlap in substrate preferences, and the proportion of sister-pairs that
have significantly similar climatic niches. In addition, we also investigated whether these metrics are correlated with time since divergence of the sister-pairs. We expect that, if speciation is allopatric, range overlap should be zero for recently diverged species pairs and may increase with time since divergence if the two sister-species shift their ranges (Barraclough et al., 1998). Similarly, if divergence in ecological preferences is an important driver of speciation, we expect recently diverged species pairs to show little ecological overlap, but ecological overlap may increase with time.

We first evaluated all of these metrics over the species pairs that received > 85% posterior probability in BEAST’s maximum clade credibility tree (Table 1, Fig. 2). This procedure considers phylogenetic uncertainty, as sister-species are inferred from the posterior distribution of trees. Yet, if groups are poorly resolved, very few sister-pairs may be recovered with high posterior probability, reducing sample size for comparative analyses. In addition, sister-pairs present in trees sampled from the posterior distribution may show clear patterns of geographical or environmental overlap. For example, all species in a clade may be allopatric, strongly supporting allopatric speciation irrespective of the precise sister-species relationships. To account for phylogenetic uncertainty in our analyses, we calculated metrics of range and ecological overlap for all species pairs retrieved in each of the 100 trees randomly sampled from the posterior distribution. This provides a complete assessment of the robustness of these metrics to uncertainty in sister-pair estimation.

**Temporal patterns of diversification**

To test whether diversification rates of alpine Primulaceae increased during the Quaternary glacial cycles, we compared two different diversification models: (1) a constant birth-death model during the entire history of each group and (2) a birth-death model where rates of speciation and extinction can differ before and after the Pliocene/Pleistocene boundary (i.e. 2.4 Ma ago). As extinction is difficult to estimate from molecular phylogenies (Rabosky, 2010) we also compared models without extinction, i.e. pure-birth models. All models were fitted on the three groups separately. We note here that *Aretia* also includes the North American *Douglasia* Lindley, which was not included in our phylogeny. However, *Douglasia* is monophyletic (Boucher et al., 2012), hence our analysis may only miss one speciation event in the Alpine system (the one leading to the stem lineage of *Douglasia*), while it includes the remaining 19 speciation events. Models were fitted on 100 different trees from BEAST trimmed to the species level and their fit was compared using the Akaike Information Criterion (AIC).

Unless otherwise specified, all analyses were run in R (R Core Team, 2014) using packages ade4 (Dray & Dufour, 2007), raster (Hijmans & van Etten, 2012) and TreePar (Stadler, 2011).

**Table 1** Range and niche differences in the 11 sister-species pairs from the MCC tree with more than 85% posterior support. For each sister-species pair, columns indicate: the posterior probability that the two species are sister, the median divergence time inferred from the posterior of BEAST, range overlap, overlap in climatic niches, the P of the niche similarity test and overlap in substrate preferences respectively.

<table>
<thead>
<tr>
<th>Species pair</th>
<th>Posterior probability</th>
<th>Divergence time (Ma)</th>
<th>Geographical overlap</th>
<th>Climatic overlap</th>
<th>P value similarity</th>
<th>Substrate overlap</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Androsace wulfeniana</em></td>
<td>0.87</td>
<td>2.98</td>
<td>0%</td>
<td>27%</td>
<td>0.02</td>
<td>Same substrate</td>
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<tr>
<td><em>A. brevis</em></td>
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<tr>
<td><em>A. hausmannii</em></td>
<td>0.99</td>
<td>4.07</td>
<td>0%</td>
<td>0.1%</td>
<td>0.52</td>
<td>Same substrate</td>
</tr>
<tr>
<td><em>A. komovensis</em></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td><em>A. cylindrica</em></td>
<td>0.98</td>
<td>5.31</td>
<td>2%</td>
<td>10%</td>
<td>0.1</td>
<td>Different substrates</td>
</tr>
<tr>
<td><em>A. vandelli</em></td>
<td></td>
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<tr>
<td><em>A. pubescens</em></td>
<td>0.91</td>
<td>1.56</td>
<td>44%</td>
<td>79%</td>
<td>0.01</td>
<td>Same substrate</td>
</tr>
<tr>
<td><em>A. helvetica</em></td>
<td></td>
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<tr>
<td><em>A. laggeri</em></td>
<td>1</td>
<td>0.88</td>
<td>7%</td>
<td>35%</td>
<td>0.01</td>
<td>Same substrate</td>
</tr>
<tr>
<td><em>A. halleri</em></td>
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<tr>
<td><em>A. lactea</em></td>
<td>0.86</td>
<td>4.72</td>
<td>0%</td>
<td>10%</td>
<td>0.01</td>
<td>Different substrates</td>
</tr>
<tr>
<td><em>A. mathildae</em></td>
<td></td>
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<tr>
<td><em>A. adfinis</em></td>
<td>1</td>
<td>3.29</td>
<td>0%</td>
<td>4%</td>
<td>0.01</td>
<td>Same substrate</td>
</tr>
<tr>
<td><em>A. cantabrica</em></td>
<td></td>
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<tr>
<td><em>Primula latifolia</em></td>
<td>0.99</td>
<td>2.04</td>
<td>20%</td>
<td>49%</td>
<td>0.01</td>
<td>Same substrate</td>
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<tr>
<td><em>P. marginata</em></td>
<td></td>
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<tr>
<td><em>P. daonensis</em></td>
<td>1</td>
<td>0.52</td>
<td>4%</td>
<td>41%</td>
<td>0.01</td>
<td>Same substrate</td>
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<tr>
<td><em>P. hirsuta</em></td>
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<tr>
<td><em>P. glutinosa</em></td>
<td>0.97</td>
<td>4.05</td>
<td>3%</td>
<td>48%</td>
<td>0.01</td>
<td>Different substrates</td>
</tr>
<tr>
<td><em>P. spectabilis</em></td>
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</tr>
<tr>
<td><em>P. glaucescens</em></td>
<td>0.99</td>
<td>3.08</td>
<td>0%</td>
<td>0.3%</td>
<td>0.02</td>
<td>Different substrates</td>
</tr>
<tr>
<td><em>P. deorum</em></td>
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</table>
RESULTS

Phylogenetic inference

Convergence of the two BEAST runs was verified and the first 10 millions generations of each run were discarded as burn-in. The two runs were combined to produce a maximum clade credibility tree (hereafter MCC). Most nodes in the phylogeny outside of the three focal groups received 100% posterior support (Fig. 2). In /Aretia, 17 out of 25 nodes received >85% posterior support, while this was the case for 16 out of 29 nodes in /Auricula. In Soldanella, however, only four of the 15 nodes received > 85% support (see Fig. 2). Previous relationships in /Aretia were mostly confirmed, and the reciprocal monophyly of the two subsections of /Auricula (Zhang & Kadereit, 2004) was recovered with more than 97% posterior support. In Soldanella, the western-central group (Zhang & Kadereit, 2002) was confirmed to be monophyletic with 94% support, while the eastern group was poorly resolved and inferred to be paraphyletic (Fig. 2). In the MCC, 11 sister-species pairs were inferred with more than 85% posterior probability and could be used for sister-species comparisons (hereafter ‘main sister-pairs’, see Table 1, Fig. S1). The choice of this threshold represents a compromise between the level of support required and the number of sister-pairs retained in...
the MCC. All species for which multiple subspecies were included (i.e. Androsace adnata, A. cylindrica, Primula auricula and P. lutea) were found to be monophyletic with more than 88% posterior support. Dating yielded a median crown age of 8.2 Ma (95% HPD: 4.4–14.5 Ma) for /Aretia, 6.6 Ma (3.4–12.0) for /Auricula and 4.3 Ma (1.4–9.5) for Soldanella (see Fig. 2 and Fig. S2 for uncertainty in divergence times).

**Geographical overlap**

Most sister-pairs from the three groups were allopatric: among the 11 main sister-pairs, five were strictly allopatric and four had less than 10% range overlap (Table 1). Only two sister-pairs showed substantial range overlap: Androsace helvetica and A. pubescens (44%), and Primula latifolia and P. marginata (20%). The same pattern was found when considering phylogenetic uncertainty: on average over the 100 trees, 57% of sister-pairs were strictly allopatric (range: 47–68% of sister-pairs), and up to 85% of sister-pairs had less than 10% geographical overlap (range: 75–91% of sister-pairs). Range overlap was not correlated with time since divergence of the two sister-species.

**Environmental overlap**

In contrast, environmental overlap was high between sister-species. Indeed, over the 11 main sister-pairs, only four species pairs did not overlap in their substrate preferences (Table 1). These were: Androsace cylindrica (calcicole) and A. vandelli (calcifuge), A. lactea (calciluge) and A. mathildae (calcifuge), Primula glutinososa (calcifuge) and P. spectabilis (calcicole), and P. glaucescens (calcicole) and P. deorum (calcifuge). Results were the same over the 100 trees: on average 71% of sister-species shared the same substrate preferences (range: 53–83% of sister-pairs). We found that sister-pairs differing in their substrate preferences diverged earlier than sister-pairs sharing the same substrate: this was true for the 11 main sister-pairs (mean difference in divergence times = 1.8 Ma, $P = 0.017$, $R^2 = 43.2$%), as well as over each of the 100 trees (range: 0.3–5.0 Ma, significant in 59 trees).

Overlap in climatic niches was on average 28% over the 11 main sister-pairs, and climatic niches were significantly similar in all sister-pairs (niche similarity test: $P < 0.05$, see Table 1) except Androsace cylindrica and A. vandelli ($P = 0.1$) and A. hausmanni and A. komovensis ($P = 0.52$). This tendency was confirmed when taking phylogenetic uncertainty into account: on average 77% of sister-species had similar climatic niches (range: 58–90% of sister-pairs). None of the 61 possible sister-pairs found across the 100 trees had significantly divergent climatic niches. Overlap in climatic niches was not correlated with time since divergence of the two sister-species, nor with overlap in substrate.

**Temporal patterns of diversification**

In the three groups, pure-birth models provided a better fit than birth-death models. In Soldanella, a time-constant model and a model with a shift in diversification rates at the Pleistocene had very similar AIC scores (median $\Delta$AIC = 0.74 over the 100 trees). Under the constant pure-birth model, the net diversification rate in Soldanella was 0.48 species/Myr on average over the 100 trees (range: 0.23–0.93, see Table 2). In /Aretia and /Auricula, however, the model with the lowest AIC was a pure-birth model with different diversification rates in different epochs (median $\Delta$AIC> 3.71 for /Aretia, median $\Delta$AIC> 3.38 for /Auricula).

In both clades a decrease in diversification rates was observed in the Pleistocene (Table 2): in /Aretia the net diversification rate before the Pleistocene was 0.34 sp./Myr (range: 0.11–0.71), and dropped to 0.11 sp./Myr (range: 0.001–0.29) during the Pleistocene; in /Auricula, this rate dropped from 0.51 sp./Myr (range: 0.17–1.18) to 0.19 sp./Myr (range: 0.05–0.37).

**DISCUSSION**

We investigated the three largest plant radiations in the European Alpine system to gain insights into the relative contributions of geographical barriers versus ecological preferences to their diversification. Our analyses largely relied on comparing current ranges and ecological preferences in sister-species pairs. This methodology has known limitations, since it ignores the possibility that species’ ranges and niches have evolved since speciation. We attempted to control for possible post-speciation range movement and niche evolution by measuring the correlation between time since divergence and range or niche overlap (Barraclough et al., 1998). However, range overlap was always very low between sister-species pairs while their niches were most often similar, regardless of time since divergence. Although we remain aware of the potential caveats listed above, this finding increases our confidence in our results. In addition, we have

**Table 2** Diversification rates in the three studied groups. For each group, net diversification rates (estimated using a pure-birth model, see Material and Methods) are presented separately for the periods before and during the Pleistocene. The average net diversification rate estimated over the 100 trees from the BEAST posterior is presented along with its standard deviation. Values are identical in both periods for Soldanella as a model with no rate shift in the Pleistocene had an AIC score very close to a model with a shift (see Results). All rates are expressed in species/Myr.

<table>
<thead>
<tr>
<th>Clade</th>
<th>Net diversification rate before the Pleistocene</th>
<th>Net diversification rate in the Pleistocene</th>
</tr>
</thead>
<tbody>
<tr>
<td>/Aretia</td>
<td>$0.34 \pm 0.14$</td>
<td>$0.11 \pm 0.06$</td>
</tr>
<tr>
<td>/Auricula</td>
<td>$0.51 \pm 0.21$</td>
<td>$0.19 \pm 0.09$</td>
</tr>
<tr>
<td>Soldanella</td>
<td>$0.48 \pm 0.14$</td>
<td>$0.48 \pm 0.14$</td>
</tr>
</tbody>
</table>
used an approach to sister-pair comparisons that fully accounts for phylogenetic uncertainty. This showed that our results are robust to uncertainty in the estimation of sister-species, which further increases our confidence in the main patterns of speciation found in the study groups. Although these all belong to Primulaceae, patterns of diversification, range and niche overlap are so clear that they enable us to make strong inferences and provide insights into the drivers of speciation in temperate alpine floras in general.

Evidence for allopatric speciation with little divergence in ecological niches

Considered together, our results allow us to make robust inferences about the main mode of speciation in alpine Primulaceae. First, a majority (i.e. 57% on average) of sister-species pairs had strictly allopatric distributions, regardless of the time since their divergence. Another 28% of species pairs had range overlap between 0 and 10%, which indicates either peripatric, parapatric or again allopatric distributions, three situations that we cannot distinguish since we placed buffers of 10 km around occurrence points to calculate range overlap. These results support the conclusion that geographical separation played a key role in the diversification of these three groups. The clearest examples are the divergence between Androsace adfinis, distributed in the Alps, and A. cantabrica, endemic of the Cantabrian mountains, or the split between Primula glaucescens, restricted to the Dolomites, and P. deorum, endemic of the Rila mountains. Second, our measurements of ecological niche overlap show that sister-species most often grow on the same substrate (71% of species pairs on average) and have similar climatic niches (77% on average). This similarity in climatic niches is especially noticeable since we used many more climatic variables and a better spatial resolution than commonly done. These two results suggest that allopatric speciation with little ecological niche divergence has been the main mode of speciation in /Aretia, /Auricula, and Soldanella, making these groups classic examples of non-adaptive radiations (Gittenberger, 1991). A similar scenario has been suggested for many other groups of alpine plants (Ozenda, 1995; Vargas, 2003; Kadereit et al., 2004; Gehrke & Linder, 2011; Verboom et al., 2015). One possible reason for this widespread pattern is that allopatric speciation, which is facilitated by the fragmentation of alpine habitats, does not require that the climatic niches of sister-species differ (Wiens & Graham, 2005). Furthermore, failure of climatic niches to evolve over short time-scales might indeed explain why some populations of a species become isolated from each other when the environment changes quickly and some environments become unsuitable in the middle of a species range, thus initiating allopatric speciation (Wiens & Graham, 2005). Interestingly, opposite results involving frequent niche divergence between sister-pairs has been found in floras from non-alpine regions, like the California Floristic Province (Anacker & Strauss, 2014), the Arctic (Theodoridis et al., 2013), or the lowlands of the Cape Floristic Region (Van der Niet & Johnson, 2009), suggesting that alpine floras might differ from lowland floras in their main speciation mechanisms.

Possible processes driving speciation in alpine Primulaceae

Although biogeographical and phylogenetic data alone do not provide enough information to fully elucidate the processes driving speciation in alpine Primulaceae, they allow us to make hypotheses regarding possible drivers of speciation. Allopatric speciation does not require divergent selection to act on the ecological characteristics of incipient species, and genetic drift alone can drive speciation. Drift is especially likely to be an important driver of speciation in alpine plants since they often have relatively small population sizes and/or probably underwent strong bottlenecks during glacial cycles, as suggested for several groups of high-elevation plants from the Cape Floristic Region (Verboom et al., 2015). However, the onset of reproductive isolation between incipient species via genetic drift might be rather slow, while selection might speed up the process (Coyne & Orr, 2004). In /Aretia and /Auricula, the occurrence of many naturally occurring hybrids suggests that reproductive isolation between closely related species is often incomplete, providing circumstantial evidence that genetic drift might have played an important role in speciation. Indeed, eight such hybrids have been described in /Aretia (Smith & Lowe, 1997) and as many as 32 in /Auricula (Kadereit et al., 2011).

However, genetic drift need not be the sole driver of speciation in allopatry and divergent selection on ecological attributes might also be involved in the building of reproductive isolation between incipient species, as recently suggested in a study of cryptic speciation the high-elevation cape sedge Tettraria triangularis (Britton et al., 2014). In plants of the European Alpine system, substrate type has been shown to be a major driver of genetic structure (Alvarez et al., 2009). Since we found that on average 29% of sister-species pairs in alpine Primulaceae grow on different substrates, substrate specialization could have contributed to speciation in the groups studied here. Indeed, different substrates require different physiological adaptations to cope with pH or chemical elements (e.g. excess of calcium and deficiency in phosphorus and iron in alkaline soils; Zohlen & Tyler, 2004). Hybrids between plants adapted to two different substrates might thus show poor performance on either substrate, as shown for hybrids between Primula hirsuta (silicicole) and P. lutea (calicicole, Kadereit et al., 2011). In addition, since different substrates are often spatially separated in mountains, divergence in substrate preferences would increase spatial isolation between diverging populations. However, since we found that sister-species pairs growing on different substrates tend to be significantly older than pairs growing on the same substrate, divergence in substrate preferences might not be initiating speciation in alpine Primulaceae, but rather occur after divergence. Finally, since our study only considered a few
dimensions of species’ ecological niches, it is still possible that divergent selection on other niche dimensions, like interactions with pollinators, micro-habitat, or finer characteristics of the substrate used by each species, might have been involved in speciation.

**Timing of diversification of alpine Primulaceae**

The three studied groups of alpine Primulaceae probably originated before the Pleistocene (Fig. 2, Appendix S1). Contrary to our expectations, the Pleistocene was probably not a period of accelerated diversification, for we found that diversification actually slowed down at this time in both /Aretia and /Auricula (Table 2). This conforms with results from earlier phylogenetic analyses in /Aretia (Roquet et al., 2013) and /Auricula (Kadereit et al., 2004), which documented diversification slowdowns using phylogenetic methods that differ from those used in our study. In light of these findings, it appears that the general hypothesis that plants of the Alpine system diversified mostly during the glacial cycles of the Pleistocene may not represent the only possible explanation to the origin of species diversity in these environments. Rather, extensive diversification may have taken place in the late Miocene and Pliocene during the last phases of the Alpine uplift (Ozenda, 1995), while the Pleistocene might have been mostly marked by differentiation within species (Vargas, 2003). These results are consistent with evidence from the fossil record suggesting that few speciation events took place in response to Pleistocene glacial cycles (Bennet, 2004).

Slowdowns in diversification are often interpreted as evidence for diversity-dependence via intra-clade competition leading to the filling of geographical and/or ecological space (Moen & Morlon, 2014). However, many species in /Aretia and /Auricula grow in rock crevices that generally contain soil with reasonable levels of nutrients (Körner, 1999), but host a very low density of plant individuals. Hence, it is unlikely that plants in these groups experience strong competition for light or nutrients, although they might compete for pollinators. On the contrary, facilitative interactions often dominate in alpine plant communities (Callaway et al., 2002). We thus believe that diversity-dependence is not responsible for the diversification slowdowns observed in these two groups. Rather, the deceleration of diversification might be due to increased extinction during the Pleistocene following drastic range contractions imposed by glacial cycles, or to a progressive reduction in species range size as events of allopatric speciation occurred, reducing opportunities for further allopatric speciation events (Moen & Morlon, 2014). Our finding that pure-birth models had the best fit in the three groups should not be interpreted as evidence that extinction did not occur, as it is difficult to estimate extinction rates from molecular phylogenies (Rabosky, 2010). Therefore’, distinguishing between the two alternative explanations for diversification slowdowns presented above is not possible.

Our results, however, allow us to reject the alternative model in which speciation would have increased during the Pleistocene following secondary contact after glacial retreats (Stebbins, 1984), at least for our three study groups. This is further supported by the fact that only three recently derived polyploid taxa have been reported in these groups: Androsace adfinis subsp. brigantiaca, Primula elusiana and some populations of P. marginata. Polyploidization events are relatively few, compared to all speciation events in /Aretia, /Auricula and Soldanella, suggesting that polyploid speciation following secondary contact took place (see Casazza et al., 2012), but did not play a major role in the history of alpine Primulaceae.

We acknowledge that our interpretation is influenced by the details of molecular dating analyses, which yielded older crown ages for both /Auricula and Soldanella than earlier studies (see detailed discussion in Appendix S1). Indeed, a Pleistocene origin for these groups would yield higher support to the secondary contact model of speciation, as suggested previously for /Auricula (Kadereit et al., 2004, Casazza et al., 2012). Although our phylogenetic study of Primulaceae includes more fossil calibration points than previous ones and should thus produce the most accurate age estimates possible, uncertainty is still high (see Appendix S1). Given the scarcity of fossils in Primulaceae, it is, however, unlikely that higher accuracy can soon be reached. Furthermore, we would like to emphasize that the diversification slowdowns detected in both /Aretia and /Auricula do not depend on absolute nodal age estimates per se.

**CONCLUSION**

In this study we investigated patterns of diversification of the three largest plant clades of the European Alpine system: Androsace sect. Aretia, Primula sect. Auricula and Soldanella. Two clear patterns emerged across the three groups. First, we found strong evidence for allopatric speciation with little ecological divergence as the main mode of speciation, suggesting that these groups represent non-adaptive radiations. Second, the origins of these three clades likely trace back to the late Miocene or Pliocene and they had already started to diversify significantly before the Pleistocene. Although specific to the three studied groups, our results bear important implications for our understanding of diversification in plants of the Alpine system, as well as in temperate alpine floras in general. Finally, we note that two out of the three groups that we investigated (/Aretia and /Auricula) are of polyploid origins (Kress, 1963), as is the case for several other fast radiations of plants and vertebrates (Seehausen, 2004). Whether or not these groups are of hybrid origins and whether or not this influenced diversification rates, as suggested by Seehausen (2004), remains to be explored.

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REFERENCES


SUPPORTING INFORMATION
Additional Supporting Information may be found in the online version of this article:
Appendix S1 Phylogenetic inference and dating.
Appendix S2 Occurrence data and climatic quantification.

DATA ACCESSIBILITY
All data used in this article are available upon request via e-mail from the corresponding author.

BIOSKETCH
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Author contributions: F.B., N.Z. and E.C. conceived the ideas; F.B. built the phylogeny and analysed the data; F.B. led the writing with substantial contributions from N.Z. and E.C.

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