Tempo and drivers of plant diversification in the European mountain system

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There is still limited consensus on the evolutionary history of species-rich temperate alpine floras due to a lack of comparable and high-quality phylogenetic data covering multiple plant lineages. Here we reconstructed when and how European alpine plant lineages diversified, i.e., the tempo and drivers of speciation events. We performed full-plastome phylogenomics and used multi-clade comparative models applied to six representative angiosperm lineages that have diversified in European mountains (212 sampled species, 251 ingroup species total). Diversification rates remained surprisingly steady for most clades, even during the Pleistocene, with speciation events being mostly driven by geographic divergence and bedrock shifts. Interestingly, we inferred asymmetrical historical migration rates from siliceous to calcareous bedrocks, and from higher to lower elevations, likely due to repeated shrinkage and expansion of high elevation habitats during the Pleistocene. This may have buffered climate-related extinctions, but prevented speciation along elevation gradients as often documented for tropical alpine floras.

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Mountain regions across the world are important biodiversity hotspots, owing to their high species richness, endemism and faster pace of species diversification compared to lowlands1–4, with tropical mountain regions harboring by far the highest concentrations of plant diversity5–7. The diversity of temperate mountain floras is less prominent at the global scale, but still remarkably high compared to surrounding lowlands, specifically when considering the stressful climatic and edaphic conditions prevailing at high elevations and the dramatic climate and glacier oscillations of the Pleistocene period8–10. The exceptional plant diversity and endemism of the European mountains was early recognized, and its emergence has challenged the understanding of botanists and biogeographers since the pioneering work of von Haller 250 years ago11. Yet, how and when plant lineages have diversified in temperate mountains still remains insufficiently documented. While the drivers (how) and the tempo (when) of species diversification have already been explored for several European mountain plant lineages (e.g.11–13), no consensus has yet emerged on the evolutionary history of the species-rich European alpine flora. This is mostly due to previous clade-specific studies using both sparse and divergent genetic data, diverse analytical methods, and as a result, sometimes even yielding non-conclusive results11–15. To allow generalizations about the tempo and drivers of alpine plant diversification, we thus need to collect high-quality genomic data covering multiple plant lineages, estimate reliable and comparable phylogenies, and apply a multi-clade framework of modern phylogenetic comparative methods across study lineages.

The tempo of species diversification within mountain biotas is often described as a continuous, typically fast, process following the uplift of mountain ranges16–18, which slowed down after saturation of the whole available physical and ecological space (e.g.19), eventually being modulated by climatic changes. A dominant climatic process that affected current mountain biota worldwide1,11,17,18,20 was the gradual cooling of global temperatures since the middle Miocene (15 Ma BP), that culminated with the onset of Pleistocene (2.6 Ma BP), a geological epoch characterized with low average temperatures and cyclical fluctuation of global temperatures21. The effect of Pleistocene could have been positive in terms of net diversification rates with speciation being stimulated by climate-induced range dynamics (speciation pump mechanism,22,23), or negative with decreased speciation or increased extinction rates due to the impact of Pleistocene glacial periods. The latter perspective is motivated by the observation of greater plant endemism in areas that have experienced relative climatic stability or less glaciation7,24–26. Contrary to subtropical and tropical mountains such as the Northern Andes where massive plant diversification occurred during the Pleistocene (e.g.27,28), it has long been considered that temperate mountains such as the European Alps were so severely glaciated during Pleistocene cold periods that this posed limits to recent plant diversification29. However, the evidence for refugia at the peripheries of glaciated European mountain ranges3,7,30,31 and also on ice-free mountain tops protruding from glaciers (so-called nunataks32–35) suggests that unglaciated mountain habitats persisted during glacial periods and may even have triggered plant speciation. Whether Pleistocene climatic oscillations slowed down or spurred the diversification of European mountain plants, and what was the role of pre-Pleistocene gradual cooling, still remains unclear, with most recent reliable analyses concerning Primulaceae only11. Documenting the tempo of alpine plant diversification in European mountains therefore requires estimating accurate divergence times for a set of distinct plant clades. This would allow to carefully assess how diversification rates have varied through time and also between regions and environments that have been differently impacted by Pleistocene climate and glacial oscillations.

The unique flora of alpine environments was likely assembled through a complex interaction between spatial and ecological drivers that have influenced the divergence and migration of mountain plant lineages, but the relative influence of these drivers requires further investigation. It has long been considered that allopatric divergence was the main speciation driver in European mountains11,15,36,37. However, this view is challenged by the evidence that genetic structure and local adaptation across different elevation belts and bedrocks is pervasive in European mountain plants38,39, and that parapatric speciation along elevational gradient seems to be common in tropical and subtropical mountain floras15,40–44. In addition, the Pleistocene climatic oscillations may have unevenly impacted the rates of migration, speciation and extinction of plant lineages across the major ecological gradients (e.g. bedrocks, elevation) and between mountain ranges, depending on whether the particular areas were heavily glaciated or remained ice-free during most of the Pleistocene—e.g. high elevation, siliceous areas of central Alps vs. low and mid-elevation, calcareous areas of peripheral Alps, respectively45. It thus remains unclear whether species diversification in European mountains was dominantly driven by geographic divergence, or whether it was significantly complemented by speciation events occurring along ecological gradients as known from tropical mountain systems (e.g.5,41–44). Moreover, it is not clear how these evolutionary mechanisms interacted with glaciation dynamics, particularly how climatic oscillations influenced diversification and migration in the regions and parts of ecological gradients heavily impacted by glaciation. Disentangling these effects requires simultaneously estimating past rates of migration and cladogenesis within and between mountain ranges, elevation belts and bedrock types, which has never been performed so far on any temperate mountain flora.

In our study, we aim at providing a window into the evolutionary history of European mountain plants by inferring the tempo and drivers of speciation of six study plant lineages considered as representative cases of in situ diversification within European mountains (Fig. 1). These clades are: Androsace sect. Aretia (hereafter Androsace), Campanula sect. Heterophylla (hereafter Campanula), Gentiana sections Gentiana, Ciminalis and Calanthinae (hereafter Gentiana), Phyteuma, Primula sect. Auriculata (hereafter Primula) and Saxifraga sect. Saxifraga (hereafter Saxifraga). We reconstruct their phylogenies using a full-plastome phylogenomic dataset obtained by low coverage shotgun sequencing. Using a likelihood-based multi-clade comparative model approach developed specifically for this purpose, we estimate past rates and tempo of species diversification across all study lineages, and also lineage-specific deviations from general trends. We show that overall, there is a weak or no effect of past climate cooling on diversification rates in our study lineages. Further, we use state-dependent diversification models to show that the evolutionary assembly of these plant lineages across different bedrocks and elevational belts was strongly driven by migration. Finally, we examine the importance of different modes of speciation based on sister species comparisons, and demonstrate that the dominant mode of speciation was allopatry, complemented with bedrock-driven speciation. Our results suggest that past migrations across ecological gradients might have buffered climate-related extinctions on one hand, but prevented ecological speciations on the other hand.

Results

Tempo of species diversification. The six study lineages started to diversify in Europe at variable dates within the last 40 Ma (Fig. 2a). The credibility intervals of crown age estimate largely overlap between study lineages, but Saxifraga (95% highest
probabilistic density of crown age 12.5–38.1 Ma BP, *Phyteuma* (13.7–24.5 Ma BP), *Gentiana* (6.4–28.5 Ma BP) and *Campanula* (10.1–16.5 Ma BP) were probably older than both Primulaceae clades, namely *Androsace* (4.7–10.7 Ma BP) and *Primula* (3.2–7.9 Ma BP). All lineages accumulated considerable amounts of diversity both before and during the Pleistocene (2.6 Ma BP) so that none of them results from exclusively Tertiary nor exclusively Quaternary diversification. Our estimates integrate sources of uncertainty stemming from both molecular and fossil data in a conservative way and seem to be robust to alternative interpretations of fossil record—for details about dating analyses and handling of various sources of uncertainty, see Methods and Supplementary Methods 1.

To better understand the dynamics of clade diversification, we tested a range of hypotheses about diversification changes through time by contrasting five models depicting different diversification scenarios (Table 1). The model support was evaluated by difference in AIC between the focal model and a nested null model not containing the focal parameter(s) (referred to as AICdiff throughout the paper, see Methods for details). We first ran all the models in a multi-clade setup with parameters shared across the six lineages to seek for general patterns. We then separately re-ran the five models with lineage-specific parameters and compared their combined fit to the multi-clade model. Our multi-clade approach is based on calculating joint likelihood function as a product of individual likelihood functions of models fitted on each of the six lineages (see Methods for details). In order to validate the performance of multi-clade approach and to address general issues with identifiability in diversification models 46, we explored the models behavior with simulated data, showing that our approach can perform unbiased model selection and accurately estimate model parameters (Supplementary Methods 2).

The best performing multi-clade model assumed constant speciation and extinction rates (Table 1). Time- or temperature-dependent models (median AICdiff between −1.16 and −1.71 [Δdf=1] across 100 sets of Bayesian posterior trees) cannot in principle be rejected with confidence, but our sensitivity analysis showed that the used modeling approach and dataset have sufficient statistical power to detect temperature-dependent scenarios where Quaternary speciation rate dropped to 63% of Tertiary rate or less (Supplementary Methods 3). The universal temperature-dependence of speciation across all clades in our dataset considered together was thus either absent or weaker than such drop. Importantly, the combination of lineage-specific models slightly outperformed the models with shared parameters (median AICdiff = 9.68 [Δdf=19]), suggesting that the estimated diversification parameters in reality differed across lineages (Fig. 2b). In particular, the diversification dynamics of two lineages was better explained by models with non-constant rates (Table 1): *Primula* showed a slowdown of speciation either with time (median AICdiff = 2.04 [Δdf=1]) or during colder periods (median AICdiff = 2.09 [Δdf=1]); and *Androsace* showed support for a speciation slowdown in colder periods, although model performance was only slightly better than the constant rate model (median AICdiff = 0.40 [Δdf=1]) in this clade.

**Evolutionary assembly: the relative influence of bedrock, elevation and geography.** We used clado genetic state-dependent diversification models (ClaSSE, 47,48) to analyze the evolutionary assembly of our study lineages across bedrock types (calcareous vs. siliceous), elevation belts (high elevation vs. mid-elevation habitats), and geographic regions (five major European mountain regions, see Fig. 1). Using AIC comparisons, we quantified the importance of speciation associated with splits between bedrock types, elevation belts, or regions (which we term state-change speciation); speciation within the same bedrock type, elevation belt or region (constant-state speciation); and the importance of change of bedrock type, elevation belt or region occurring along branches of the phylogeny, that is without been linked to speciation events (which we term migration). The ClaSSE models for bedrocks and elevation belts were equivalent to a GeoSSE model 47, while the model for geographic regions represents a generalization of GeoSSE for more than two regions (see Methods and Supplementary Methods 4 for details). The best models inferred from model selection were then re-run in a Bayesian framework to obtain credibility intervals around parameter estimates. As in the previous analyses, all models were evaluated in the multi-clade framework with a model sharing parameters between clades, and subsequently as lineage-specific models with distinct parameter set for each clade. To validate that parameters of multi-clade and lineage-specific models are identifiable, we tested these approaches with simulated phylogenies reflecting the size and structure of our dataset (Supplementary Methods 4).

The state-change speciation between siliceous and calcareous bedrocks appeared to be an important driver of plant diversification (AICdiff = 8.96 [Δdf=1]). Model parameters suggest that a bedrock generalist lineage split into descendant lineages specializing on calcareous or siliceous habitats on average cca 0.5 times
per Ma and such speciation events constituted 19% of all speciation events (Table 2). However, we found no evidence for allopatric speciation between elevation belts (AICdiff = $-2 \left[ df = 1 \right]$) or between the five major mountain regions of Europe (AICdiff = $-1.62 \left[ df = 1 \right]$). The latter result suggesting no allopatric speciation between mountain regions is however likely biased due to the coarse scale of the considered mountain regions caused by computational limitations (see Methods for details). For this reason we addressed the prevalence of allopatric speciation and its spatial scaling with complementary analysis of sister species (see below).

Constant-state speciation rates differed between bedrock types (AICdiff = $3.51 \left[ df = 1 \right]$) and regions (AICdiff = $17.87 \left[ df = 4 \right]$), but not between elevation belts (AICdiff = $1.02 \left[ df = 1 \right]$). In particular, we inferred higher speciation rates on siliceous than on calcareous bedrock (mean estimate and credibility interval in Fig. 3a) and also higher speciation rates in the Alps and the Iberian mountains than in any other European mountain region (Supplementary Fig. 1), but no notable difference in speciation rates between high- and mid-elevation habitats (AICdiff = $19.79 \left[ df = 1 \right]$). This result captures events of secondary migration of high altitude ancestors toward lower elevations, leading to relatively high present-day and equilibrium proportions of species inhabiting either mid-elevation habitats or occurring in both elevation belts (Fig. 3f, Supplementary Fig. 2).

Models of evolutionary assembly slightly differed among the six study lineages (AICdiff = $7.39 \left[ df = 30 \right]$ for bedrock, AICdiff = $21.07 \left[ df = 20 \right]$ for elevation). The most notable outliers concerning the assembly across bedrock types were *Androsace* and *Phyteuma*, which exhibit higher proportions of species occurring on siliceous than on calcareous bedrock (Fig. 3e). In *Androsace*, this was linked to marginally faster diversification rates on siliceous than on calcareous bedrock and symmetrical migration between the two bedrock types. *Phyteuma* showed marginally faster diversification on calcareous bedrock, but strong directional migration towards siliceous bedrock, thus explaining the higher species diversity of this genus on siliceous bedrock.
among all six study lineages, the degrees of overlap slightly showed no overlap in the occupied elevation belts. Only 9% of sister species pairs with no overlap. Similar patterns were found at the coarse geographic scale, with over 22% sister species differing less across bedrock types. In ClaSSE models, sister lineages were particularly important for these two lineages. The distribution of overlapping sister species varied in terms of elevation belt, bedrock type, and geographic range, with the latter being particularly important for fine geographic assembly.

**Sister-species overlap in geographical and ecological space.** Due to computational limitations, the ClaSSE models used above could only be run with a maximum of five geographic regions. Hence, we performed a complementary analysis to infer how sister species vary in the dimension of overlap in terms of elevation belt, bedrock type, and geographic range, the latter being estimated both with coarse-scale and fine-scale geographic regions (five and 87 mountain regions, see Fig. 1 and Supplementary Methods 5). Most sister pairs diverged over the fine geographic scale (Fig. 4), with 37% sister species pairs showing no geographic overlap at this scale. Similarly, as in ClaSSE models, sister species differed less across bedrock types (27% of sister pairs with no overlap) and over the coarse geographic scale (22% of sister pairs with no overlap). Only 9% of sister species pairs showed no overlap in the occupied elevation belts.

Although the order of speciation modes remained the same among all six study lineages, the degrees of overlap slightly differed between lineages (Fig. 4). Notably, the lineages with lowest geographic overlap at the fine scale were Primula (63% of pairs with no overlap) and Androsace (43% of pairs with no overlap), suggesting that allopatric speciation at finer geographic scale was particularly important for these two lineages. The lineage Campanula exhibited high variance in sister species overlap distribution at fine geographic scale, on the one hand having a high proportion of sister pairs with no overlap (43%), but on the other hand having the highest proportion of sister species pairs showing complete geographic overlap (36%).

**Discussion**

Here we explored the tempo and drivers of species diversification across a representative sample of six diverse plant lineages whose evolutionary history is tied to the European mountain system. As we purposely narrowed our study down to clades that previously received a systematic revision, it is clear that many of our studied species had already been sequenced, although always for a few genes only and using variable methodologies. Instead, we employed a uniform next-generation sequencing approach to all study clades and produced over two hundred whole plastid genomes. Chloroplast genomes provide a common ground molecular information that can be applied across various groups and phylogenetic scales. While the resulting topologies may be prone to biases introduced by incomplete lineage sorting or hybridization events in some cases (see Methods for more information and a brief discussion), our chloroplast-based approach avoids some of the pitfalls of nuclear phylogenomics (e.g. paralogy). Our study therefore constitutes a significant step forward toward documenting the evolutionary origins of alpine plant diversity in European mountains, because it is the first to harness next-generation sequencing on multiple plant clades in parallel and to apply a multi-clade comparative framework to explicitly model the tempo and drivers of mountain plant diversification. To make sure that our data collection strategy and analytical approaches can inform us about patterns of mountain plant evolution, we also extensively validated the identifiability and sensitivity of our comparative models using simulated datasets. Our results may thus allow certain generalizations across our study lineages, confirming previous knowledge or botanists intuitions regarding plant diversification in European mountains, documenting some quite surprising, and perhaps counter-intuitive patterns, and finally identifying some interesting variations between the evolutionary histories of different study clades.

The onset of cold and variable Pleistocene climate has caused massive losses of plant diversity in European lowlands and was long thought to have induced a diversification slowdown in European mountains, which was also demonstrated with particular plant lineages. Surprisingly, we detected neither strong nor homogeneous effects of past temperature on plant diversification rates across our six study lineages. Diversification dynamics in all study clades together were best explained by a

| Table 2 Comparison of ClaSSE models of evolutionary assembly containing parameters of state-change speciation between bedrocks (siliceous vs. calcareous), elevation belts (high elevation vs. mid-elevation) or five coarse geographic regions, with the nested models lacking state-change speciation terms. |

| Bedrock types | Rate of state-change speciation (Ma⁻¹) | Proportion of state-change to all speciation events | AICdiff |
|---------------|---------------------------------------|-----------------------------------------------|--------|
| Elevation belts | 0.453 | 0.19 | 8.96 |
| Geographical regions (coarse scale) | <0.001 | <0.001 | -2.00 |

Values with AICdiff > 2 are depicted in bold.
constant rates model, and showed no indication for overall slowdown of species diversification with decreasing global temperature. When analyzed at the level of individual lineages, only the two Primulaceae clades showed certain support for diversification slowdown, as was also demonstrated in earlier studies\textsuperscript{11,15}. Yet more interestingly, the other four lineages showed no individual sign of slowdown with decrease of temperature. The past temperature thus did not universally influence the diversification rates of our study lineages, which was not only true for the gradual, and perhaps hardly detectable, the effect of cooling since middle Miocene, but also for the onset of dramatically colder Pleistocene climate. This result was further corroborated by a sensitivity analysis, suggesting that if any temperature-driven Pleistocene reduction of diversification rates took place across all study lineages, it was most likely weaker than a drop to 63% of pre-Pleistocene rates, given the observed data.

The weak and ambiguous effect of Pleistocene climate on diversification rates was also detectable in analyses across elevational gradient and geographical space: the high elevation habitats and also the Alps \textit{sensu stricto}, which were most severely impacted by Pleistocene glaciers, did not show lower rates of
speciation or higher rates of extinction. Instead, high elevation lineages exhibited high rates of directional migration to lower elevations. This may suggest that the habitats suitable for high mountain plants were not severely reduced during glacial periods but rather shifted downwards. Such shifts instead of loss in habitats may thus have resulted in relatively weaker and more lineage-specific declines in diversification rates. Strictly speaking, our findings are based on lineages that significantly diversified in the European mountain system, and thus do not contradict the paleontological evidence for massive Pleistocene extinctions in the European lowland flora. The high rates of endemism and genetic diversity in low elevation refugia at mountain peripheries and the presence of mountain plants in lowland glacial palynological record (Androsace sp., Gentiana sp., Saxifraga sp., but also Dryas octopetala, Polygonum viviparum or Saussurea sp.) however suggest that the above described migration, diversification and survival processes may represent a general model for other extant mountain plant lineages not included in our study, and perhaps also for other components of European mountain biotas. In fact, it is interesting to note the similarity between the scenario of lineage migrations suggested by our results and the one reported by on Mediterranean mountain birds.

Why did diversification of some lineage slowdown while other remain steady? Given that we have six cases for comparison and we only observed a slowdown in the Primulaceae family (Primula and Androsace), we can only hypothesize the answer. A scenario of ecological opportunity driving species diversification following mountain uplift could provoke a diversification slowdown after saturation of the available ecological space. But such an effect should be observed in the oldest and most diverse lineages of our study clades, rather than in the youngest and relatively least diverse ones such as Primula and Androsace. The observed diversification slowdown could alternatively be explained by a decreasing rate of allopatric speciation, as the two lineages that slowed down towards the present (Primula, Androsace) also show the highest rates of sister species allopatry. The prevalence of allopatric speciation can theoretically lead to an intrinsic slowdown of speciation rates, particularly in lineages with low dispersion capacity, once the spatial setting of species’ geographic ranges no longer enhances further genetic isolation. Another explanation could be that Pleistocene climatic oscillations promoted migrations between populations in areas that would otherwise remain isolated, thus reconnecting populations and inhibiting allopatric diversification. Simulations of diversification across dynamically fragmented landscapes indeed showed that more connected landscapes or a faster pace of connection-disconnection events can impede species diversification under certain conditions.

Allopatric speciation has long been regarded as a prevalent mode of speciation in mountain environments, and the high importance of allopatric speciation demonstrated by our results is generally consistent with this view. An additional interesting finding of our study is the relatively small spatial scale of allopatric speciation, which did not take place between major mountain ranges such as the Alps or Carpathians, but rather within these ranges. It would be interesting to further study the same lineages with nuclear markers, as spatial structure may sometimes emerge due to the usage of chloroplast-based maternal lineage phylogenies. Although the small-scale allopatric speciation appeared to be the most important mode of speciation (37% of speciation events) in sister species of our study lineages, our results further suggest that bedrock-driven divergence has been another important driver (27%) of sister-species speciations. This contrasts with a previous study finding an almost exclusive mode of allopatric speciation and little bedrock divergence in three European alpine Primulaceae genera. These previous results may however reflect a peculiar situation in Primulaceae - a family containing Androsace and Primula - the two lineages in our dataset for which we found allopatry to be by far the most important mode of speciation.

We estimated that bedrock shifts account for about one fourth (27% in sister species analyses) or one fifth (19% in CladSSE analyses) of all speciation events across our study clades. Bedrock or edaphic species differentiation is well known from Mediterranean floras, while in European mountain species it has mostly been studied at the intraspecific level, or in studies using non-quantitative comparisons to other speciation drivers. To our knowledge, our result thus provides the first quantitative evaluation of the importance of bedrock-driven speciation in European mountain plants. Still, it remains unclear to what extent these speciation events were truly parapatric, that is with reduced gene flow between adjacent populations due to bedrock adaptation, or whether bedrock specialization originated as a consequence of allopatry. Experimental evidence suggests that new
species can indeed arise due to strong divergent selection of calcareous and siliceous ecotypes, even in the presence of gene flow\(^67\). Our findings have far reaching implications: it shows that contrary to long-standing expectations, many speciation events may not or not only have been due to selectively neutral geographic divergence, but also due to divergent evolution across a complex geological landscape requiring specific physiological adaptations to the divergent proportions of important nutritional elements\(^68\).

Another important finding of our study is the inference of frequent migration events between bedrock types. Intriguingly, we found that these migrations were asymmetrical, with silicate habitats being more often the source of limestone-dwelling lineages than the other way around. This finding seemingly contrasts with previous literature suggesting that plant adaptation to calcareous soils is more restrictive than adaptation to silicate bedrocks\(^67,69-71\). The higher pleiotropic fitness costs of adaptation to calcareous bedrock may however prevent lineages from switchback to siliceous habitats, and calcareous habitats might thus function as the evolutionary trap\(^72,73\). Moreover, the stronger migration from silicates to limestones we detected here may also be linked to geographic contingencies: the peripheral lower mountain systems in Europe may be both calcareous and siliceous, while central high elevation parts of European mountains (such as Central Alps, Central Pyrenees or High Tatra) consist almost exclusively of silicate bedrocks\(^45\). Due to this triangular relationship, the elevation belts may constitute a confounding hidden factor \(\text{sensu}^{74}\) in the models of evolutionary assembly across bedrock types, where the inferred migrations from high to low elevations and from silicaceous to calcareous bedrocks may partly reflect the same process of historical migration out of the central ranges of the mountain systems, possibly due to Pleistocene glaciations. The interaction between bedrock, elevational niche and possibly habitat type may in fact be rather complex, as demonstrated by Androsace and Phyteuma, both which deviate from the general trend and yielded greater migration rates towards the silicate bedrocks. In Androsace this pattern is likely linked to lineages surviving in high elevation silicate rocky habitats, probably even during Pleistocene (discussed below). In Phyteuma, silicate specialists are often species inhabiting sub-alpine grasslands in lower silicate mountains with mild slopes, such as Massif Central, Sudetes or eastern Carpathians, where these lineages may also have survived Pleistocene. More work based on an extended population sampling is certainly needed to further disentangle different historical scenarios of evolutionary assembly of alpine floras.

The evolutionary assembly of our study clades across elevation belts was apparently marked by massive directional migration from high to mid-elevations, causing the mid-elevation habitats to host more species despite speciation rates being roughly equivalent in both elevation belts. This directional migration was likely fostered by Pleistocene glaciations causing downward shifts of communities during glacial periods, which then may have left relictual populations at lower elevations during interglacials. Such climate-induced range shifts may have had two-fold consequences on the dynamics of species diversification. On the one hand, it may have prevented species extinctions due to habitat loss during glacial periods, but on the other hand, intense altitudinal migration may have hampered speciation across elevation belts, as we did not find evidence for speciation along elevational gradients. This finding makes an important distinction with known mechanisms of species diversification in the Andes or other tropical mountains, where speciation across elevation gradients seems to have been a relatively important driver of plant speciation\(^75,41-44\). More specifically, high and mid-elevation populations in the European mountain system may have differentiated at intraspecific levels\(^38,75\), but the strong migration from high to lower elevations in response to the severe Pleistocene climate oscillations has likely prevented the emergence of reproductive barriers necessary for speciation events. Important historical migrations along elevation gradients may thus provide an explanation why species diversification has not been as explosive in the European mountain system as in tropical ones\(^15,29\).

The lineage Androsace sect. Aretia constitutes a notable exception regarding the general scenario of evolutionary assembly highlighted above, probably because this lineage shows strong affinity to high elevation habitats. In comparison to the others, this lineage shows a greater species richness in high elevations, likely due to directional migration from mid- to high elevation habitats. Moreover, according to the ancestral state reconstruction it also contains two sub-lineages that likely evolved exclusively in high elevation habitats. In fact, several species of Androsace stretch to the highest limits of vascular plant life in the European mountain system (above 4000 m a.s.l.\(^76\)), and in Himalaya (6350 m a.s.l.\(^77\)). Unlike other study lineages, Androsace has likely undergone specific adaptations facilitating the repeated entrance into the harsh adaptive zone of high elevation habitats and continued population persistence at high elevations throughout the Pleistocene. A clear manifestation of such adaptation is that Androsace repeatedly developed a dense cushion life form, an architecture seemingly well adapted to plant life at high elevations not only in this genus\(^78\) but also in many other angiosperm lineages\(^79\). Given the inferred long-term affinity of Androsace to high alpine environments, it can be reasonably hypothesized that high-elevation sub-lineages of Androsace have survived glacial periods in situ in so-called nunatak refugia—i.e. rocky outcrops at high elevations protruding the glaciers\(^80\)—rather than in peripheral refugia, which may have been the prevalent scenario for the majority of the other study lineages. The survival in the limited area of nunataks could also explain Pleistocene diversification slowdown observed in Androsace, although the consequences of nunatak survival for species diversification remains unclear. The high elevation sub-lineages of Androsace sect. Aretia identified in this study thus constitute a perfect system for further tests of the nunatak survival hypothesis at smaller spatial and phylogenetic scales.

In conclusion, our study provides an unprecedented window to the history of diversification of the temperate mountain flora in Europe. It shows that plant diversification within European mountains was a complex evolutionary process, with a strong interplay between altitudinal migration, allopatric speciation and bedrock adaptation of different lineages. Importantly, the onset of Pleistocene climate did not cause a strong diversification slowdown, as was previously expected, but rather stimulated strong migrations of mountain biota across elevation gradients, particularly towards lower altitudes. We hypothesize that these massive altitudinal migration events on the one hand buffered extinctions due to habitat loss during glacial periods, but on the other hand impeded Pleistocene adaptive radiations across the elevation gradient, as is classically known from tropical mountains. We also found speciation events to be mostly driven by geographic divergence but almost as frequently by bedrock shifts. Overall, the absence of obvious adaptive diversification across the elevation gradient and the prevalence of allopatric speciation likely contributed to the lower richness and slower diversification dynamics generally observed in temperate mountains compared to tropical ones.

**Methods**

**Clade selection and sampling.** The study lineages were selected as representative cases of plant species diversification in the European mountains by the following...
DNA extraction and sequencing. DNA was extracted from collections of leaf tissues gathered during our sampling campaigns, using DNA extraction kits from Macherey-Nagel (Duren, Germany) and Qiagen (Hilden, Germany). The shotgun libraries were prepared and sequenced with methodology depending on the sequencing facility (see Supplementary Data 1 for sequencing facility used for each sample):

For the samples sequenced in Genoscope (Paris, France), the library preparation protocol applied before sequencing was chosen on the basis of the DNA extraction yield. When available, 250 ng of genomic DNA were sonicated using the E210 Covaris instrument (Covaris, Inc., Woburn, MA, USA) and the NEBNext DNA Library Prep Kit for Illumina (New England Biolabs). After two consecutive 1x AMPure® Ultra II Q5 Master Mix clean ups, the ligated products were PCR-amplified by 12 cycles PCR using Kapa HiFi Hotstart NGS library Amplification kit (Kapa Biosystems, Wilmington, MA, USA), followed by a 0.6x AMPure® XP purification. When the extraction yielded low DNA quantities, 10–50 ng of genomic DNA were sonicated. Fragments were end-repaired, 3’-adenylated and NextFlex DNA barcoded adapters were added by using NEBNext Ultra II DNA Library Prep kit for Illumina (New England Biolabs). After two consecutive 1x AMPure® XP (Beckmann Coulter Genomics, Danvers, Massachusetts, USA) clean ups, the ligated products were PCR-amplified by 12 cycles PCR with Kapa Hotstart NGS library Amplification kit (Kapa Biosystems, Wilmington, MA, USA), followed by a 0.6x AMPure® XP purification. For the samples sequenced in Fasteris (Geneva, Switzerland) and BGI (Shenzhen, China), custom commercial protocol for preparation was applied. The prepared libraries were sequenced on Illumina HiSeq2000 sequencer (Illumina, San Diego, CA, USA), using 101 base-length read chemistry in a paired-end flow cell.

Chloroplast genome reconstruction, gene region selection and sequence processing. The resulting paired-end reads were used to reconstruct the chloroplast genomes using Org.Asm 1.0.3, a de novo organelle assembler based on De Bruijn graph implemented in Python 3. We retrieved complete circular plastomes for each species and extracted based on their positioning relative to the neighboring coding regions. Coding and non-coding regions of ingroup and outgroup samples were filtered by Gblocks 0.91 (specifying that the data type corresponded to codon alignments), and concatenated together using FastConCat. The non-coding regions were aligned by Mafft 79, quality filtered by Gblocks (specifying that the data corresponded to DNA alignments) and concatenated together. This sequence processing pipeline resulted in high-quality sequence matrices for each family ranging between 35471–47102 bp for coding and 2435–5112 bp for non-coding regions, with less than 3% missing data overall.

Phylogenetic analyses and molecular dating calibrations. The resulting coding and non-coding alignments for each of the four families were used for inferring plastome phylogenies in BEAST 2 88. Non-coding regions were removed from coding alignments were modeled with separate averaging-site models as implemented in bModeltest 1.2.0 89. We used Yule tree prior and lognormal clock model with uniform fossil dating priors on at least two nodes of each family. Note that we applied uniform priors between minimum and maximum bounds because the sparse fossil record of our study clades does not allow applying more informative priors—this therefore constitutes a very conservative approach. In some cases, the fossil record used for dating calibration allowed multiple interpretations of age and positioning in the phylogenetic tree. For consistency with literature, we specifically used the interpretations mirroring previous influential studies on the respective families. Only the interpretation with the best statistical support for the downstream analyses. To test the robustness of this choice, we however reran the phylogenetic analyses with alternative dating calibration where necessary and explored the differences. For a detailed description of used fossil calibrations and their comparison with alternative interpretations, see Supplementary Methods 1 and Supplementary Section 2.

The BEAST inference for each family was performed in four independent runs, each having 100 M generations and a burn-in of 30%. We evaluated convergence both visually and by checking whether the effective sample size (ESS) was >100 for all the parameters. We used TreeAnnotator to generate maximum credibility trees. The maximum credibility trees and reduced posterior trees were pruned to only include ingroup species and one individual per species, for details on species tree inference see Supplementary Methods 6. We obtained well resolved species-level phylogenies with 87% of nodes receiving >0.95 posterior probability (see Supplementary Software 1 for maximum credibility trees with node supports). Chloroplast genomes provide highly informative and accurate dating due to the large number of orthologous genomic regions with variable mutation rates, and the universality of the bioinformatic pipeline allowing to obtain homogeneous phylogenetic information across virtually any angiosperm group 89. The limitation of our approach is that chloroplast-based phylogenies are only tracking the evolution of maternal lineages, which might be problematic in clades with frequent hybridization, introgression, or incomplete lineage sorting. The shortcomings of plastome-based phylogeny might have impacted, even slightly, the relative branching times and topologies of reconstructed trees of our lineages (see e.g. 5 for Saxifraga), and hence some downstream analyses of species differentiation. It is important to point out that different downstream analyses that we present in our paper are differently prone to biases related to the usage of chloroplast markers: Time-dependent analyses only depend on distribution of branching events through time 86, and are thus least likely to be systematically influenced by the usage of maternal tree topologies. Species-dependent analyses are phylogeny-explicit, but their overall results across multiple clades are also relatively unlikely to be biased by the usage of chloroplast markers in a systematic manner. Moreover, the main result of high mobility of lineages across elevational gradient and bedrocks would likely be underestimated rather than overestimated by usage of presumably less mobile maternal lineages. Perhaps the most bias-prone analyses are species comparisons, where they directly depend on terminal tree topologies. The absence of polyphyletic species and the low number of identified paraphyletic species (see Supplementary Methods 6 for details) roughly suggest that neither hybridization and introgression nor incomplete lineage sorting are very common in the terminal parts of our phylogenies. More exact prevalence of these processes complete lineage sorting and introgression would need to be investigated in future phylogenomic studies using nuclear markers and denser population sampling, especially if results of interest are specific.
Ecological and geographic characteristics of species. The information about bedrock, elevational niche and geographical occurrences for each ingroup species was obtained from the following regional floristic literature: Flora Alpina97, Flora Ibérica98, Flora Slovenska99, Wildpflanzen Siebenbürgens100, and Flora Srbijski101. We used published ecological and biogeographical studies focused on certain lineages to obtain additional information about genera Androsace102, Phyteuma103, and Saxifraga104. The information about Campanula was in part compiled also based on information available in herbarium specimens, local taxonomic literature and from field observations. In several cases, we contacted local taxonomists and made categorization based on provided information. Where possible, the information from floristic literature was compared and supplemented with previously published11 or publicly accessible106 sources of point occurrence data.

The calcareous bedrock niche was defined by the regular presence of species on calcareous, dolomitic or ultrabasic rocks, the siliceous bedrock niche was defined by regular presence of species on any bedrocks that do not fall in the calcareous category, species in the remaining rock types were covered by Flora Alpina97. A species is considered present to a calcareous niche if regular presence is indicated from limestones (ca) or serpentinites (ser), and to siliceous niche if regular presence is indicated from silicates (si), intermediate substrates (ca/si) or volcanic rocks (bas). For details on the attribution of bedrock niches to individual species, see Supplementary Data 4.

The mid-elevation niche was defined by regular presence of species in habitats below timberline, i.e. up to subpulvinal elevation zone sensu Flora Alpina97. High-elevation niche was defined by regular presence above timberline, i.e. in alpine and nival zone sensu Flora Alpina97. In the cases in which floristic information did not refer to elevational belts, we defined Flora Alpina97 marked species presence in mid-elevation niche if the species was inhabiting habitats lower that 200 m below regional timberline and in high elevation niche if the species was inhabiting habitats 200 m above regional timberline. No such treated species was restricted to the range ±200 m around timberline. For details on the attribution of elevational niches to individual species, see Supplementary Data 4.

In order to compile information about species geographical distribution, we defined the smallest operational geographical units across Europe for which we could get credible presence/absence information of every species in our dataset. Specifically, we used operational geographical units in Flora Alpina97 for the Alps, and more recently defined the evolutionary extant and other European mountain regions (coarse scale vegetation) sensu Körner et al.107 for other European mountain regions (coarse scale vegetation) with subsequent modifications that better reflect structuring of biogeographic information in local floristic literature (see Supplementary Methods 5). We used this approach rather than grid-based processing of point occurrence data (as e.g. in ref. 11), because of imbalanced point data quality depending on the country and broader European region. For the purpose of ClaSSE models, operational geographical units were merged into five major mountain regions of Europe and surrounding areas (Fig. 1). For details on operational geographical units and how they were merged into five major regions, please refer to Supplementary Methods 5 and Supplementary Data 4.

Tempo of species diversification. Based on the inferred phylogenies of each lineage, we fitted five models depicting different temporal dynamics of species diversification (see Table 1). For the models with temperature-dependent speciation or extinction, we used exponential dependence on \( T_o \) isotope ratio time series from Greenland ice cores21. To accommodate for phylogenetic uncertainty, we fitted every model on 100 trees randomly selected from the Bayesian posterior distribution of phylogenetic trees, and report either the whole distribution (parameter values at various median values of AIC comparisons).

To explore the overall diversification dynamics across all study lineages, we developed a multi-clade framework to fit the above-described diversification models across multiple evolutionary lineages simultaneously. We assumed that each of our lineages is an independent realization of shared diversification dynamics, which we account for by constructing the joint likelihood functions of temporal diversification models as a product of the likelihood functions of each of the six lineages, and harnessing the lineage-specific parameters into shared values. A similar approach with joint likelihood was previously used for state-dependent diversification models47,108. We optimized model parameters using the joint likelihood function with a simplex routine, equivalent to the default correction strategies were proposed to address this issue47,116. However, this is an issue relevant mostly when the evolutionary states are stable3,11, which is clearly not the case of our study plant lineages with the important role of migration and state-change speciation across bedrocks, elevational belts and regions.

Moreover, all constant-state speciation and extinction rate differences presented in this paper turned unsupported or only weakly supported even without these corrections, and are interpreted as such. Instead, we used the SSE framework for testing all migration and state-change events and collapsed a burn-in of 5000 iterations. The proportions of state-change to all speciation events were obtained by multiplying the present-day numbers of species in different model states (e.g. calcareous specialist, siliceous specialist, bedrock generalist) by their respective speciation rates. The analyses were performed using the R package divcor 0.9-1111. The SSE models are known to suffer from elevated Type I errors when testing constant-state diversification rates differences between states, and various correction strategies were proposed to address this issue47,12,116. However, this issue is relevant mostly when the evolutionary states are stable3,11, which is clearly not the case of our study plant lineages with the important role of migration and state-change speciation across bedrocks, elevational belts and regions.

Sister-species overlap in geographical and ecological space. We performed an overlap analysis between sister species in order to address the importance and frequency of allopatric speciation at two different spatial scales, along with speciation between bedrock types and elevation belts. To do this, we identified all sister species pairs in maximum clade credibility trees, and calculated the Schoener’s D niche overlap index119 for 87 operational geographic units (fine scale geography), five European mountain regions (coarse scale geographic areas)
geography), elevational belts (high elevation vs. mid-elevation) and bedrocks (calcaceous vs. siliceous). The overlap estimates were calculated using the R package spaa 0.2.120.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Raw genomic data generated in this study are available in European Nucleotide Archive under project accession codes https://www.ebi.ac.uk/ena/browser/view/PRJEB43865, https://www.ebi.ac.uk/ena/browser/view/PRJEB46693, https://www.ebi.ac.uk/ena/browser/view/PRJEB50489. Accession codes for individual samples are provided in Supplementary Data 1. Species-level phylogenies, both maximum credibility and posterior samples, are available in Supplementary Software 1. Geographic and ecological characteristics of different species are available in Supplementary Data 4. Source data are provided with the paper.

**Code availability**

Custom R functions used for fitting multi-clade comparative models are available as a GitHub repository containing a R package (https://github.com/smyckaj/multidiv/tree/https://github.com/smyckaj/multidiv/tree/). Examples with simulated data are available in Supplementary Methods 2 and 4.

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**Author contributions**

J.S., C.R., and S.L. designed the study, J.S. performed the analyses, and J.S., C.R., and S.L. wrote the first draft of the manuscript. J.S., C.R., M.B., R.D., K.S., N.E.Z. W.T., L.G.A., S.L. and other members of PhyloAlps consortium contributed to the sample collection and its coordination. C.P., M.R., R.D., and J.G.V. coordinated and mounted the reference herbarium collection. M.B., A.A., F.D., P.W., F.B., and E.C. designed and performed the wet lab procedures, sequencing and bioinformatic pipelines. The project as a whole was coordinated by S.L. All authors contributed to the final version of the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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