Biochemical Systematics and Ecology 126 (2026) 105230

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Metabolomic discrimination of the *Primula auricula* genetic complex

Stéphanie Morelon [a,b,1,*], Gaëtan Glauser [c], Emmanuel Defossez [a], Jérémy Gauthier [d,e], Jason Grant [b], Nadir Alvarez [d,e], Sergio Rasmann [a,2]

[a] Laboratory of Functional Ecology (LEF), University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, CH-2000, Switzerland
[b] Laboratory of Evolutionary Genetics (LEG), University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, CH-2000, Switzerland
[c] Neuchâtel Platform of Analytical Chemistry, Rue Emile-Argand 11, Neuchâtel, 2000, Switzerland
[d] Naturéum – State Museum of Natural Sciences, Palais de Rumine, Place de la Riponne 6, 1005, Lausanne, Switzerland
[e] Department of Ecology and Evolution, University of Lausanne, 1015, Lausanne, Switzerland

ARTICLE INFO

Keywords:
Chemotaxonomy
Flavonoids
Glacier refugia
Phylogeography
Phytochemical diversity

ABSTRACT

Understanding how evolutionary history and ecological pressures shape plant chemical diversity is central to ecology and evolution, yet it remains unclear whether metabolomic data can reliably detect fine-scale, intraspecific divergence, particularly in morphologically cryptic taxa. While metabolomics has revealed broad patterns of chemical evolution across lineages, its power to resolve genetically structured variation within species is still underexplored. Here, we investigated the alpine *Primula auricula* complex, a morphologically uniform but genetically subdivided taxon distributed across the Alps. Using ultra-high-performance liquid chromatography coupled with mass spectrometry, we profiled the metabolomes of individuals sampled from 37 populations spanning three main genetic clades and an outlier group, previously identified through ddRADseq phylogeography. We found that metabolomic diversity carries a strong phylogenetic signal: each clade exhibited distinct chemical profiles, with exclusive or enriched metabolite superclasses such as carotenoids in one clade and phenylpropanoids in another. Outlier populations displayed reduced metabolomic richness, consistent with potential genetic drift or bottlenecks. While phylogenetic structure was the dominant driver of chemical variation, climatic variables, particularly temperature and precipitation, modulated certain stress-related metabolite groups, such as octadecanoids. Our results demonstrate that metabolomic profiling can capture both historical divergence and ecological adaptation in cryptic alpine taxa. By linking chemical, genetic, and environmental variation, this study highlights metabolomics as a cost-effective and high-resolution approach to uncover cryptic diversity, refine taxonomy, and inform conservation strategies in biodiversity hotspots increasingly threatened by climate change.

1. Introduction

Species adapt to environmental variation along ecological gradients, including shifts in climate (Carcaillet and Brun, 2000; Theodoridis et al., 2019; Baudière and Serve, 1971), soil properties (Cunningham et al., 1999; Anacker et Strauss, 2014), and biotic pressures such as herbivory and competition (García-Ramos et Huang, 2013). These adaptations often involve phenotypic changes (Lande et Shannon, 1996; Agrawal, 2001; Ghalambor et al., 2007; Frei et al., 2012). For instance, climatic variation may drive physiological adaptations to temperature or precipitation extremes (Reyer et al., 2013), while soil gradients may favour different nutrient uptake strategies (Jobbagy et Jackson, 2001). Biotic interactions, including herbivory or pathogens, further shape plant survival through the evolution of enhanced plant defence mechanisms (e.g., Gatehouse, 2002). Collectively, these selective pressures can generate distinct genetic lineages (Benton, 2009; Morlon et al., 2024), with varying degrees of local adaptation (Nosil, 2008; Galtier, 2019; Barton, 2020; Hernández-Hernández et al., 2021). Understanding how

* Corresponding author. Laboratory of Functional Ecology (LEF), University of Neuchâtel, Rue Emile-Argand 11, Neuchâtel, CH-2000, Switzerland.
E-mail addresses: stephanie.morelon@gmail.com, stephanie.morelon@unine.ch (S. Morelon), gaetan.glauser@unine.ch (G. Glauser), emmanuel.defossez@unine.ch (E. Defossez), mr.gauthier.jeremy@gmail.com (J. Gauthier), jason.grant@unine.ch (J. Grant), nadir.alvarez@vd.ch (N. Alvarez), sergio.rasmann@unine.ch (S. Rasmann).
[1] This author should be considered as first author.
[2] This author should be considered as senior author.

https://doi.org/10.1016/j.bse.2026.105230
Received 24 October 2025; Received in revised form 16 January 2026; Accepted 17 January 2026
Available online 21 January 2026

such genetic diversity is maintained across heterogeneous landscapes remains critical, particularly in the context of complex ecological gradients and historical perturbations.

In addition to spatial variation, temporal factors also shape genetic lineages within species. Historical climatic fluctuations during the Quaternary, for example, profoundly influenced genetic diversity and population structure in many plant species (Médail and Diadema, 2009). Glaciations created isolated refugia, providing safe havens where populations persisted and genetically diversified (Hewitt, 2000; Schönswetter et al., 2005; Boucher et al., 2021; Schneeweiss et al., 2017; Smyčka et al., 2017). As a result, several montane species surviving cycles of glacial and interglacial periods have given rise to geographically distinct lineages (Schönswetter et al., 2004; Schönswetter et al., 2005; Dépraz et al., 2008; Boucher et al., 2021; Záveská et al., 2021; Baumel et al., 2023; Luqman et al., 2023; Voisin et al., 2023), often exhibiting complex patterns of genetic structure and cryptic diversity (Gugerli et al., 2008; Rogivue et al., 2018). Peripheral refugial populations may have adapted to extreme abiotic conditions, while post-glacial recolonization facilitated secondary contact and occasional hybridization (Schneeweiss et al., 2017). Yet, despite such genetic divergence, morphological differentiation is often incomplete or subtle, obscured by secondary contact and shared ecological pressures (De Queiroz, 2007; Boucher et al., 2021; Kadereit and Abbott, 2021). This underscores the limitations of relying solely on phenotypic traits to detect taxonomic divergence (He et al., 2022; Liu et al., 2023; Nevado et al., 2024). Therefore, it is essential to explore alternative tools and methodologies to uncover cryptic phenotypic diversity in recently differentiated species, offering deeper insights into the processes driving speciation and biodiversity across evolutionary scales (Fišer et al., 2018; Boucher et al., 2021).

To differentiate species or subspecies, taxonomists typically rely on heritable morphological traits such as leaf shape, flower colour, trichome density (Zhang et Kadereit, 2004). In rarer cases, the chemical profiles are also employed (Fico et al., 2007; Yuan et al., 2017; Ušjak et al., 2020). Phytochemical profiles, indeed, have been offering valuable insights into how plants adapt to varying ecological abiotic and biotic conditions and pressures (Walker et al., 2022). Specialized metabolites of plants, in particular, play key roles in mediating interactions with the biotic and abiotic environment (Baldwin and Schultz, 1983; Karban et al., 2014). These chemical traits are not only influenced by environmental pressures but also encode the evolutionary history of plant lineages, providing a novel framework for studying adaptation and divergence (Walker et al., 2023). Accordingly, recent advances in chemical ecology have revealed patterns of phytochemical variation along ecological gradients at both inter and intraspecific levels (Moreira et al., 2018; Aartsma et al., 2019; Defossez et al., 2021). Distinct chemical profiles have been linked to habitat-specific pressures, such as drought or herbivory, while also reflecting evolutionary divergence within and between species (Kergunteuil et al., 2019, 2020). As a result, phytochemistry has emerged as a promising tool for resolving taxonomic challenges, particularly in morphologically cryptic groups where molecular methods alone may not suffice (Martucci et al., 2014; Yuan et al., 2017; Chervin et al., 2019; Evergetis and Haroutounian, 2020; Ušjak et al., 2020). Integrating chemical traits into taxonomy use offers a promising framework for linking ecological adaptation with evolutionary divergence (Orians, 2000; Berenbaum, 2002).

Because chemical profiles can reveal both adaptive responses to specific environmental conditions and signatures of genetic divergence (Martucci et al., 2014; Martinidou et al., 2023; Peters et al., 2023), it could be postulated that (phylo)genetic differentiation can be mirrored by metabolomic profiles differentiation, as evolutionary divergence often alters metabolic pathways and specialized metabolite production (Chae et al., 2014; Defossez et al., 2021; Holzbach et al., 2021). Such alignment between chemical and phylogenetic differentiation provides a valuable framework for studying diversification in phenotypically cryptic groups. Besides, while advances in genetic methods, such as next-generation sequencing, have greatly enhanced our understanding of phylogeographic patterns in plant lineages, these methods are often resource-intensive and may not fully capture functional differentiation (Peterson et al., 2012; Troisi et al., 2020). By contrast, phytochemical approaches offer a cost-effective (compared to genetic analyses) and complementary phenotypic differentiations, capable of identifying fine-scale population structures and functional traits (Moreira et al., 2018; Szymański et al., 2020; Peters et al., 2023). Despite this potential, the relationship between chemical and phylogeographic differentiation remains largely underexplored, particularly at the intraspecific level, underscoring the need for eco-metabolomic studies to bridge this gap (Colombo et al., 2017; Evergetis and Haroutounian, 2020; Szymański et al., 2020).

The *Primula auricula* (Primulaceae) species complex provides an ideal system for exploring the relationship between phytochemistry and phylogenetic divergence. Widely distributed across the Alps, this complex comprises several genetically distinct clades separated by major geographic and historical barriers, reflecting a history of Pleistocene glacial isolation and subsequent range dynamics (Zhang et al., 2004; Boucher et al., 2016a,b; Morelon et al., 2026). Phytochemical studies of Primula species, including *P. auricula*, have revealed a rich secondary metabolite composition dominated by flavonoids, particularly Primula-type flavones and flavonol 3-O-glycosides, as well as phenylpropanoids, carotenoids, triterpenoid saponins, and related compounds (Fico et al., 2007; Colombo et al., 2014, 2017; Holzbach et al., 2021). Within the *P. auricula* complex, kaempferol- and isorhamnetin-derived flavonoids are especially prominent and exhibit compartment-specific diversification in leaf tissues (Colombo et al., 2014; Holzbach et al., 2021). Previous phylogeographic work showed that the major genetic divergence within this complex dates back to the mid-Pleistocene and was likely driven by allopatric differentiation associated with glacial refugia (Morelon et al., 2026). Despite this deep genetic structure and documented chemical richness, lineages within the complex remain largely indistinguishable morphologically in the field (Zhang and Kadereit, 2004; Somlyay and Bauer, 2010). This combination of cryptic morphology, strong phylogeographic structure, and known phytochemical diversity makes *P. auricula* a powerful model to test whether metabolomic profiles can capture fine-scale evolutionary divergence beyond morphology alone.

Accordingly, we investigated whether chemical differentiation aligns with phylogenetic divergence within the *P. auricula* complex (Morelon et al., 2026). Using metabolomic analyses, we characterized chemical diversity across 37 Alpine localities and addressed three key questions: a) Does chemical differentiation mirror phylogenetic divergence? b) Are certain groups of metabolites consistently over- or under-expressed within particular clades? c) Are chemical differences better explained by phylogenetic divergence or by environmental factors such as climatic variation? We hypothesized that as lineages diverge phylogeographically, their metabolomes also evolve, leading to differential expression of metabolite groups. If metabolomic patterns strongly track phylogenetic signal, then divergence among clades should outweigh the influence of local ecological conditions, such as local climatic conditions. By bridging phylogeography and metabolomics, this study aims to clarify how evolutionary and ecological factors jointly shape chemical diversity across landscapes.

2. Material and methods

2.1. Material collection and phylogeographic delimitation

Leaf material of *P. auricula* was collected in the Jura mountains and the Alps between summer 2021 and summer 2023 across a total of 37 localities (Fig. 1, Table S1). Depending on population size, topography, and accessibility, two to five individuals were sampled per site. A minimum distance of 5 m between two samples was set when possible, and one to two fully expanded and healthy leaves per plant were collected to

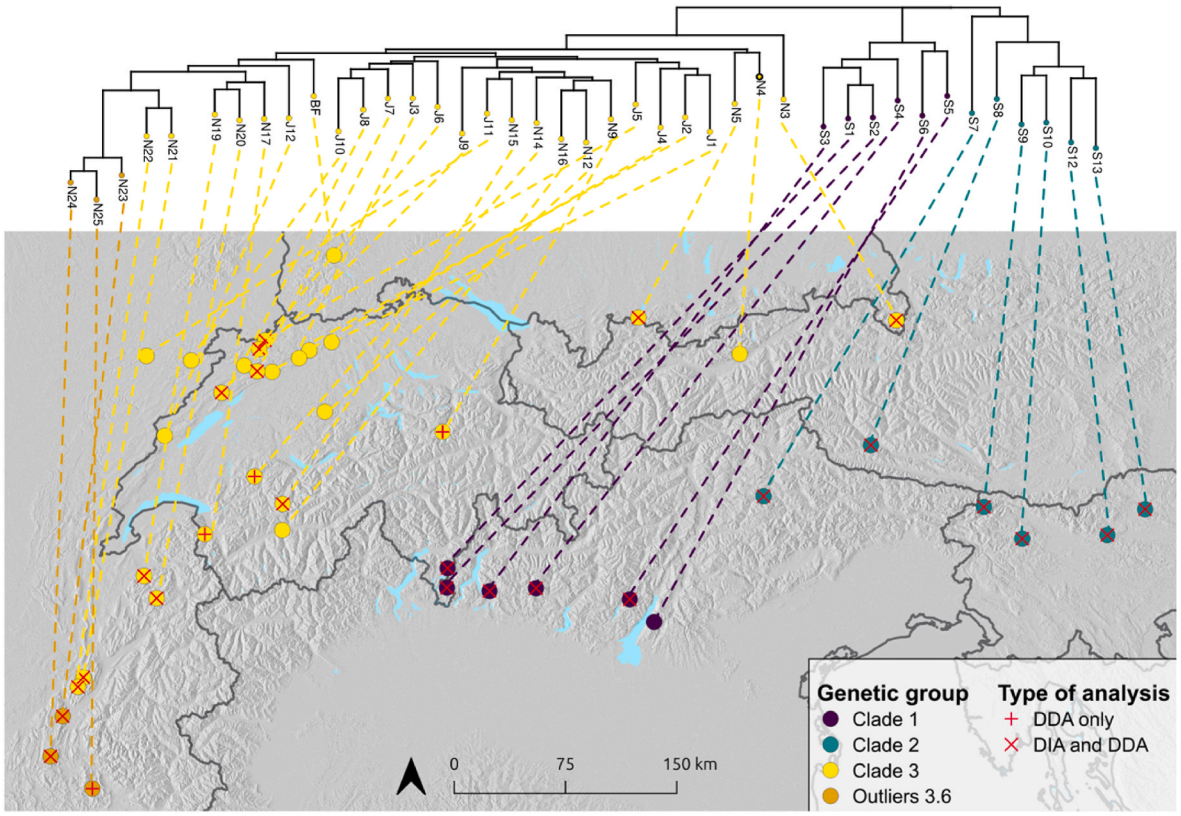

Fig. 1. Map of the sampling localities of the *P. auricula* complex across the European alpine arc. The phylogenetic tree above the map represents a Maximum Likelihood tree described in (Morelon et al., 2026) in our previous genetic study on this complex. Different colours represent the four different phylogeographic clades studied (see details in the text). Violet, green, yellow and brown colours represent clades 1, 2, 3 and 3.6 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

perform genetic and chemical analyses. Sampling was conducted under collection permits issued by the relevant local and regional authorities. Herbarium voucher specimens were prepared only at sites with sufficiently large populations to avoid detrimental impacts; in populations with low individual numbers, voucher material was replaced by high-resolution photographic documentation of sampled plants. Information on collectors, voucher specimens, and photographic records is provided in Table S1.

Immediately after collection, fresh leaves of each individual plant were carefully wrapped in tea bags and placed in plastic bags filled with Silica gel. Samples were let dry at 40 °C in a laboratory oven for a minimum of one week and stored at −40 °C before further analyses. Half of sampled leaf tissue was used for phylogeographic reconstruction and clade differentiation (Morelon et al., 2026). Using next-generation sequencing via double-digest restriction site associated DNA (ddRAD-seq) technique, the *P. auricula* complex was resolved into three monophyletic and geographically separated clades (Fig. 1). Clade 1 includes the Insubrian region and the Southern Swiss canton of Ticino. Clade 2 spans populations between the Adige valley in Italy and the Eastern Alps. Clade 3 comprises the northern and western Alps, together with the northern Jura and Nagelfluh regions. In addition, preliminary metabolomic analyses (see below) identified outlier individuals sampled from the Vercors and Dévoluy massifs. These individuals form a monophyletic branch with distinct genetic differentiation (Morelon et al., 2026) and were therefore retained as a separate subgroup within Clade 3, hereafter referred to as Outliers 3.6 (Fig. 1). Following the genetic discrimination of main genetic groups inside the *Primula auricula* complex, our final sampling contained 34 individuals from 6 localities inside Clade 1, 27 individuals from 6 localities inside Clade 2 and 132 individuals from 29 localities from Clade 3, including 18 samples from the Vercors and Dévoluy massif (Table S1). Raw demultiplexed genetic sequences are available at: Accession number: PRJNA1245062; https://www.ncbi.nlm.nih.gov/sra/PRJNA1245062.

2.2. Metabolomic analysis

For metabolomic profiling, 20 mg of the dried leaf material, added with 3 mm diameter tungsten beads, was ground to a fine powder using a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) for 4 min at 30 Hz. The leaf powder was added with 1000 µL of aqueous methanol (MeOH:H2O:Formic Acid; 80:19.5:05) and shaken for 4 min at 25 Hz. After centrifugation (25′000 G for 2.5 min), 150 µL of supernatant was transferred to HPLC vials for ultra-high-performance liquid chromatography (Acquity UPLC^{TM}I-Class, Thermo Fisher Scientific, Waltham, MA, USA) coupled to Synapt XS (Waters, milford, USA) quadrupole time-of-flight mass spectrometry. Chromatographic separation was performed on an Acquity UPLC HSS T3 column (100 × 2.1 mm, 1.8 µm; Waters). Quality control (QC) pools were made by mixing 10 µL of each sample. No clean-up procedure was applied before analysis.

We then performed two mass spectrometry (MS) experiments on the same leaf samples. First, the entire set of samples was analysed in full scan MS1 (data independent mode; DIA) to explore metabolome-wide variation. For this, the flow rate was set to 400 µL/min and maintained at 27 °C. A gradient with 0.05 % formic acid in water as mobile phase A and 0.05 % formic acid in acetonitrile as mobile phase B was applied: 0–40 % B in 7.0 min, 40–100 % B in 5.5 min, holding at 100 % B for 2.0 min, re-equilibration at 0 % B for 3.0 min. The injection volume was 0.2 µL. Both positive and negative ionization modes were tested for the QTOF analysis on representative samples. Given the number of samples, only negative ionization was used in further analysis since it provided richer chromatograms. Nitrogen compounds are less likely to be detected in negative ionization via the formation of (M-H)-or (M +

HCOO)-ions, but detection may still be possible in the case other specific functional groups such as carboxylic acids or alcohols are present in the molecules. Meanwhile, negative ionization mode increases the quality of flavonoids and saponins detection, in which richer chromatograms were retrieved and which represent the predominant specialized metabolites in *Primula*. The QTOF was operated in electrospray negative mode using the following spectrometric parameters: mass range 50–1200 Da, scan time 0.2 s, source temperature 140 °C, capillary voltage −1.0 kV, cone voltage −25 V, desolvation gas flow and temperature 1000 L/h and 500 °C, respectively, cone gas flow 250 L/h. A 50 ng/ml solution of the synthetic peptide leucine-enkephalin in water:acetonitrile:formic acid (50:50:0.1) was infused constantly into the mass spectrometer as internal reference to ensure accurate mass measurements (<2 ppm). QC samples were run 4 times before the batch and about every 30 samples during the batch. The whole system was controlled by Masslynx 4.2.

Second, we performed an MS experiment to investigate chemical superclass-level differences, using data-dependent acquisition (DDA) mode. This analysis was performed on a subset of the sampled localities, for a total 31 samples equally dispersed across the Alpine arc, including 5 samples from Clade 1, 6 samples from Clade 2 and 20 samples form Clade 3 (Fig. 1). The source parameters were the same as above, while the DDA parameters were as follows: mass range 50–2000 Da, MS1 scan time 0.1 s, MS2 scan time 0.05 s, top 7 MS/MS, deisotoping option activated, dynamic exclusion of 1.5 s after acquisition. An exclusion list of the 150 most intense background ions from a blank sample was created. A ramped collision energy dependent on the *m/z* ratio was used (Defossez et al., 2023).

2.3. Metabolomic data preparation

Raw mass spectrometry data were centroided and converted to MzML format before processing by MZmine version v. 4.3 (Schmid et al., 2023). Noise values for MS1 and MS2 were defined at 1500 and 40 respectively, *m/z* tolerance to 0.015 and 15 ppm, S/N threshold to 30. Only compounds represented in at least 5 individuals from the total sampling were conserved for further analysis. Intensity threshold was set to 1500, peak duration range between 0.01 and 1 min, RT wavelength between 0.01 and 0.09. Minimum feature height was set to 1500 and coefficient area threshold to 50. After deconvolution, peak grouping was performed with the following settings: *m/z* tolerance: 0.015 or 15 ppm, RT:0.1, maximum charge: 2. Features were further annotated in SIRIUS by spectral matching against an *in silico* spectral database version of the Dictionary of Natural Product according to a previously described methodology (Scheubert et al., 2017). $(M- H)^-$ and $(M + Cl)^-$ ions in negative ionization mode were explored in the annotation. The following elements were allowed to compute the molecular formulas: hydrogen, oxygen and carbon (infinite number of atoms), nitrogen (up to 10 atoms), phosphorus (up to five atoms), and sulfur (up to five atoms). Metabolite structure prediction was made with CSI: FingerID (Dührkop et al., 2015) while chemical class prediction was made with CANOPUS (Dührkop et al., 2020) using the NPClassifier ontology (H. W. Kim et al., 2021). Based on this annotation, the metabolome was arranged at different hierarchical levels, where 'pathways' (e.g. terpenoids) represent the highest order level, followed by 'superclass' (e.g. triterpenoids) and 'classes' (e.g. limonoids). For the purpose of our study, we opted to study variation at the superclass level, as we found that the variation at the pathway level was too coarse, while variation at the class level was too uncertain. As such, only those molecules assigned to superclass with a probability score >70 % were retained.

2.4. Statistical analyses

All statistical analyses were performed in R v. 4.2.0 (R Core Team, 2022).

2.5. Whole metabolome variation across clades

First, for each sample of the DIA experiment, the Hill-Shannon diversity index was estimated for comparing phytochemical diversity across the four clades (Petrén et al., 2024). Clade differences were assessed with a one-way ANOVA with chemical diversity as a response variable and clades as a fixed factor. Second, a non-metric Multi-Dimensional Scaling analysis (NMDS) (*metaMDS* function from the 'vegan' package (Oksanen et al., 2019)) was performed on the Hellinger-scaled metabolomics dataset. Clade-level differences were estimated using a multivariate analysis of variance (PERMANOVA; n = 10,000 permutations), with the *adonis2* function in 'vegan' package. For both the NMDS and the PERMANOVA, the Bray-Curtis dissimilarity distance was calculated. Finally, we evaluated pairwise clade differences using the *pairwise. adonis2* function in the 'vegan' package.

2.6. Superclass level chemical differentiation across clades

The importance of each metabolomic group (i.e. superclasses retrieved from the NPClassifier ontology) contributing to divergence genetic groups was estimated by analysing loading values from the first and second axis obtained from Sparse partial least squares discriminant analysis (sPLS-DA) using the package 'mixOmics' in R (Rohart et al., 2017). For this, each model was tuned using the *tune. splsda* function on 50 replicates, using 5 folds and 10 components. The best number of components was selected, and the final model was evaluated using a background classification prediction and the predictive power on the first components, using the *background. predict* function. In each analysis, the significantly discriminant superclasses among at least the first or the second component of the model were determined with a Variable Importance Plot analysis (*vip* function from the 'vip' package (Greenwell et al., 2020). Sample classification was evaluated using a Clustered Image Map (*cim* function in 'mixOmic') over the identified important superclass to visualize if obvious patterns of metabolomic composition occurred among groups. To ensure reproducibility, seed 34 was set in the PLSDA tuning command. After model selection, the chemical features of the most discriminative superclasses of the first or the second components of the PLSDA model were summed by superclass for each individual feature. Differences among clades were evaluated using a Dunn post-hoc test with a Bonferroni correction as normality could not be assessed easily for all superclasses by the Shapiro-Wilkinson test (Table S2).

2.7. Phylogenetic versus climatic influence of the chemical superclasses across clades

To disentangle the relative contribution of the shared phylogenetic history versus the local climatic conditions of each population on the expression of the chemical superclasses, first, for each sampled locality, we estimated local average climatic conditions using 19 current bioclimatic variables, extracted from the CHELSA2 database (Karger et al., 2016) with the R package 'terra' (Hijmans et al., 2022). A principal component analysis (PCA) was performed to represent localities along the first two ordination axes, and a Redundancy Analysis (RDA) (*rda* in 'vegan') was performed to assess potential clade-level differences in climate. Next, we performed phylogenetic generalized least squares (PGLS) models with function *pgls* in the 'caper' package (Orme et al., 2013) to estimate the effect of climate on the chemical superclass. PGLS incorporates phylogenetic relationships to estimate the expected covariance in cross-species data. It assumes that closely related species exhibit more similar traits (in this case, superclasses of metabolites) due to their common ancestry, which results in similar residuals from the least squares regression line. By considering the anticipated covariance structure of these residuals, the method generates adjusted slope and intercept estimates that account for interspecies autocorrelation caused by phylogenetic relationships, assuming Brownian motion (Symonds et

Blomberg, 2014). For each individual superclass, we regressed the total amount of metabolites against the first and second axes of the climatic PCA as described above. The PGLS model summary provided the significance of the regression slope with the climatic coordinates, as well as the Pagel's lambda (λ), a phylogenetic parameter that indicates whether a superclass bear phylogenetic signal (i.e., λ = 1) or not (i.e., λ = 0).

3. Results

The complete list containing compounds annotations obtained in SIRIUS for the DDA analysis can be viewed in Table S3.

3.1. Whole metabolome variation across clades

We observed significant variation in overall phytochemical diversity across clades (Fig. 2A, $\mathbf{F}_{\text{Clades,Hill-Shannon diversity}} = 52.99$, $p < 0.001$), with Clade 2 exhibiting the highest mean phytochemical diversity (mean = 182), while Clade 3.6 displayed the lowest (mean = 101.34) (Fig. 2A). Yet, Clade 2 and Clade 3 were not statistically distinguishable based on their overall phytochemical diversity (Fig. 2A, p = 0.57). Moreover, NMDS ordination revealed distinct clustering patterns (Fig. 2B, $\mathbf{F}_{\text{Clades, Hellinger-scaled chemical matrix}} = 0.265$, p-value = 0.001), in which all pairwise comparisons showed significant differences between all clades ($p < 0.05$ for each comparison).

3.2. Superclass level chemical differentiation across clades

The partial least squares-discriminant analysis (PLS-DA) model showed enhanced predictive accuracy with a two-component configuration, as indicated by Bernoulli indices of 0.551 (component 1) and 0.493 (component 2) when using the maximum distance metric. Following hyperparameter optimization, the final model demonstrated improved performance with Bernoulli indices of 0.541 (component 1) and 0.405 (component 2). This optimized model allowed for robust discrimination of Clades 2 and 3 across both components (maximum distance ranging between 0.2 and 0.04 on components 1 and 2). However, the Outliers 3.6 group was effectively discriminated on component 2 alone (maximum distances of 1 and 0.38 for components 1 and 2, respectively). In contrast, Clade 1 showed poor discrimination across both components, with a maximum distance of 1 (Fig. 3A). Out of all the total, 15 discriminant superclasses of compounds were identified, including: aminosugars and aminoglycosides, carotenoids (C40 and C50), eicosanoids, fatty esters, flavonoids and isoflavonoids, nicotinic acid alkaloids, octadecanoids, peptides alkaloids, phenolic acids (C6–C1), phenylpropanoids (C6–C3), small peptides, triterpenoids and tyrosine alkaloids (Fig. 3B).

The comparative analysis of these superclasses revealed that Clade 2 produced significantly higher amounts of C40 carotenoids compared to Clade 1 (statistic = 2.743, p = 0.036), Clade 3 (statistic = −3.438, p = 0.004) and the Outliers 3.6 group (statistic = −2.743, p = 0.036). Importantly, C40 carotenoids were exclusively produced by Clade 2, which made this superclass the only discriminative chemical group separating Clade 1 from Clade 2 (Fig. 4). Clade 2 also exhibited higher production of peptide alkaloids and nicotinic acid alkaloids compared to the other groups. In the case of peptide alkaloids, significant differences were observed relative to Clade 3 (statistic = −2.763, p = 0.034) and Outlier 3.6 (statistic = −3.257, p = 0.007). For nicotinic acid alkaloids, significant differences were detected only when comparing Clade 2 and Outliers 3.6 (statistics = −2.75, p = 0.035). Conversely, Clade 3 showed higher production of fatty esters compared to Clade 1 (statistics = 2.669, p-value = 0.046) and Clade 2 (statistics = 2.892, p-value = 0.023), as well as elevated small peptide levels relative to Clade 2 (statistic = 1.623, p = 0.039). Outliers 3.6 displayed similar levels of fatty esters and small peptides to Clade 3. The Outliers 3.6 group exhibited a marked reduction in isoflavonoids and phenolic acids (C6–C1) relative to Clade 1 (statistic = −2.89 and −2.89, p = 0.023 for both comparisons), and Clade 3 (statistic = −3.39 and −2.87, p = 0.004 and 0.025 respectively). Aminosugars and aminoglycosides were also less abundant in Outliers 3.6, with a significant difference noted when compared to Clade 3 (statistic = −3.56, p = 0.002). Both Clade 3 and Outliers 3.6 produced

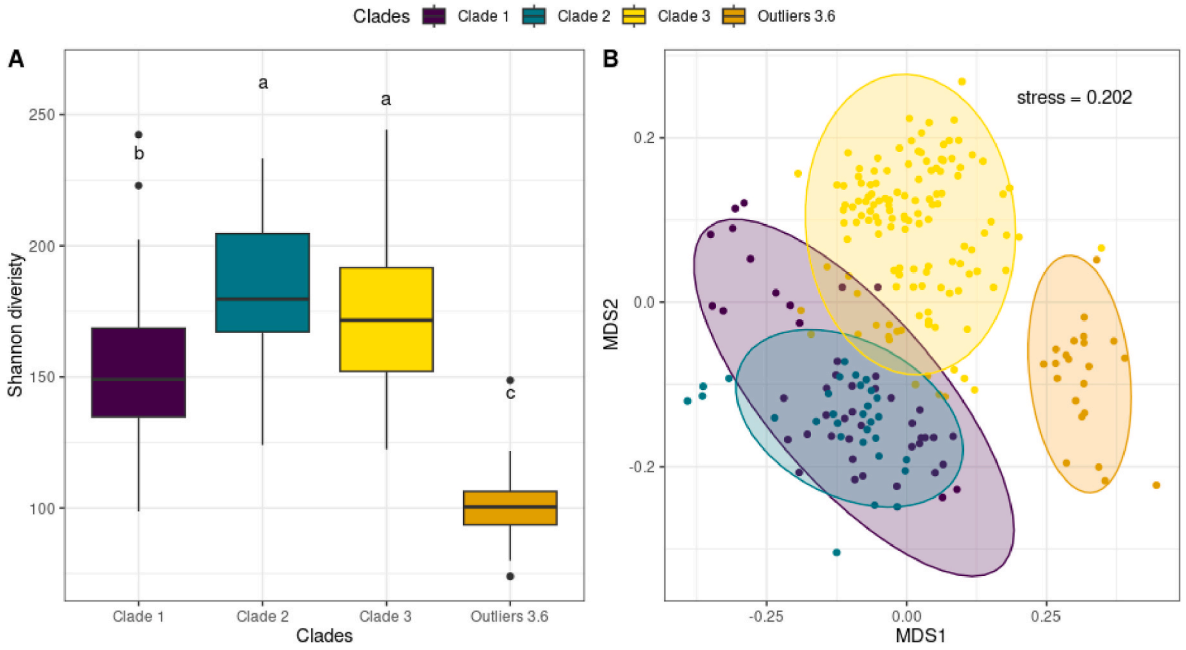

Fig. 2. Phytochemical diversity across *Primula auricula* genetic groups. A) Boxplots showing Hill-Shannon diversity indices calculated from the total metabolome of the *P. auricula* complex across the Alps. Different letters above boxes show significant differences among genetic clades ($p < 0.05$). **B)** Non-Metric Multidimensional Scaling Analysis (NMDS) plot of the entire metabolome of the *P. auricula* complex across the Alps. Samples are grouped into four clades based on phylogeographic reconstruction. Ellipses represent 95 % confidence intervals around samples belonging to each clade. For each plot, violet, green, yellow and brown colours represent clades 1, 2, 3 and 3.6 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

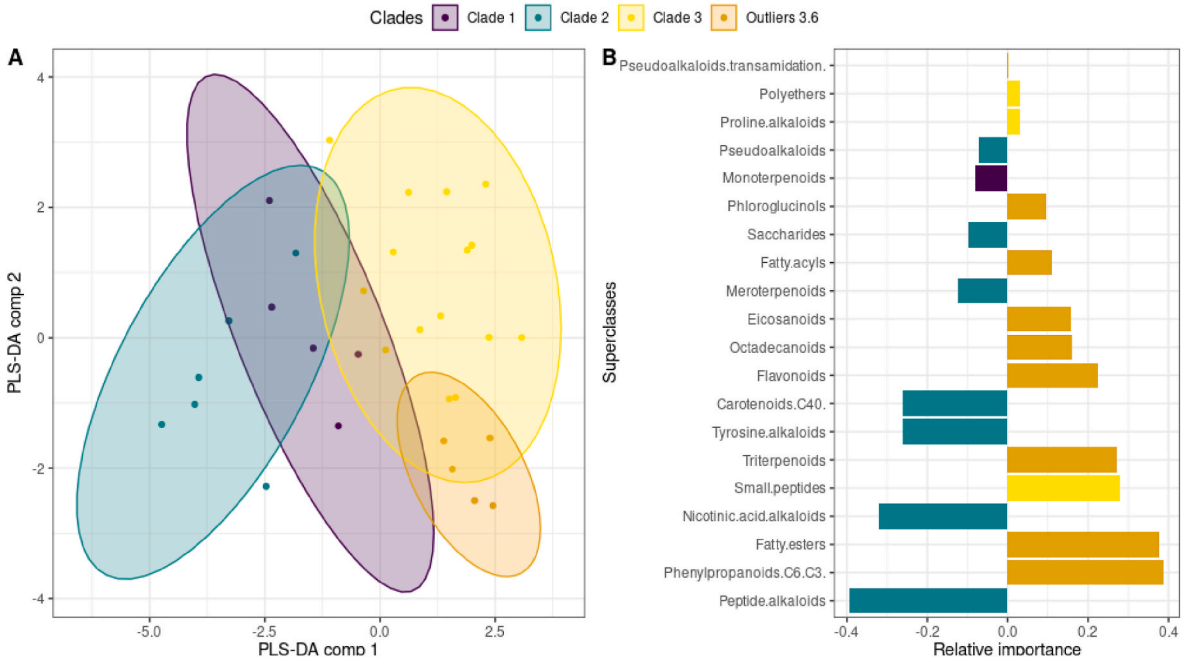

Fig. 3. Chemical discrimination between groups identified in the *P. auricula* complex. A) Two-dimensional Principal component analysis (PCoA) ordination for discriminating the four different phylogeographic clades based on the sparse Partial Least Square discriminant Analysis (sPLS-DA) optimization of the phytochemical features selection. B) shows the superclasses that most contribute to clade differentiation along the first sPLS-DA component. Violet, green, yellow and brown colours represent clades 1, 2, 3 and 3.6 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significantly higher levels of phenylpropanoids (C6–C3) than Clade 1 (statistic = 3.053 and 3.20, p = 0.014 and 0.008 respectively) and Clade 2 (statistic = 2.688, p = 0.043 for Outliers 3.6). Additionally, these groups demonstrated elevated levels of triterpenoids, small peptides and octadecanoids, although the differences were not statistically significant. Finally, while Outliers 3.6 exhibited reduced tyrosine alkaloids levels compared to other groups, this reduction was also not statistically significant (Fig. 4).

3.3. Phylogenetic versus climatic influence of the chemical superclasses across clades

The phylogenetic least squares (PGLS) analysis revealed that the residuals of several chemical superclasses conformed to a Brownian motion evolutionary model. Specifically, the absence of isoflavonoids (λ = 0.378, [0, 0.752]) and aminosugars and aminoglycosides (λ = 0.732, [0.210, 0.962]) in the Outliers 3.6 group, alongside the overexpression of phenylpropanoids (C6–C3) (λ = 1, [0.139, 1]) in both Clade 3 and Outliers 3.6 relative to Clade 1, as well as the unique production of C40 carotenoids (λ = 1, [0.507, 1]) by Clade 2 revealed a phylogenetic structure of the analysed chemistry (Fig. 5A, Table S4).

Finally, while clades did not show significant differences in terms of their average climatic niche ($\mathbf{F}_{\text{Clades, climatic variability of CHELSA2 conditions}}$ = 2.142, p = 0.093), we found that the octadecanoid superclass exhibited both a phylogenetic signal and a significant response to climatic variables, specifically along the first principal component (PC1) of the climatic PCA analysis (Fig. 5B, Fig. S1A). In other words, populations situated in regions with higher annual precipitations showed reduced octadecanoid production. In contrast, populations inhabiting areas with elevated mean daily maximum air temperatures during the warmest month or higher mean daily temperatures during the warmest quarter exhibited increased amounts of octadecanoid (Fig. 5B; t = −3.048, p = 0.006).

4. Discussion

This study provides novel insights into the interplay between phylogenetic divergence and chemical differentiation. By combining metabolomic analyses with phylogeographic data, we demonstrated that metabolomic diversity not only mirrors phylogenetic structure but also reflects the history of environmental adaptation and genetic drift in an alpine species complex such as *P. auricula* (Zhang, Comes, et Kadereit, 2004; Boucher et al., 2016a,b).

Does phytochemical diversity and composition mirror phylogenetic divergence within the *P. auricula* complex?

Our results indicate that the four genetic lineages of *P. auricula*, spread across the four corners of the Alps, are more similar in phytochemical diversity, within, than across clades. Accordingly, these results support the idea that phytochemical diversity bears - at least some extend - phylogenetic signal. In a large-scale study encompassing >400 species in the Alps, Defossez et al. (2021) highlighted a significant positive correlation between phytochemical richness and speciation events. Hence, phylogenetic signals of phytochemical diversity might also occur at smaller scales, such as within the *P. auricula* complex. Notably, in our study, the Outliers 3.6 group exhibited the lowest metabolomic diversity. This result might be explained by the fact that this group could have undergone genetic drift, maybe due to past bottlenecking. Multiple examples exist in the literature which demonstrate the effect of genetic diversity loss following bottleneck on the ability of plants to face pests and diseases. For example, Six et al. (2021) evaluated the impact of pine beetle damage on the genetic, chemical and phenotypic traits of *Pinus albicaulis*. While they observed significant differences in phytochemical diversity among tested populations, particularly in terpenoids emissions, these variations could not be directly linked to survivorship. Instead, the study revealed that pine beetle damage was disproportionately concentrated on a limited range of genotypes. Surviving trees exhibited higher genetic diversity compared to the overall population, including individuals that succumbed to the beetles. The reduction of genetic diversity after bottleneck and its impact is also well

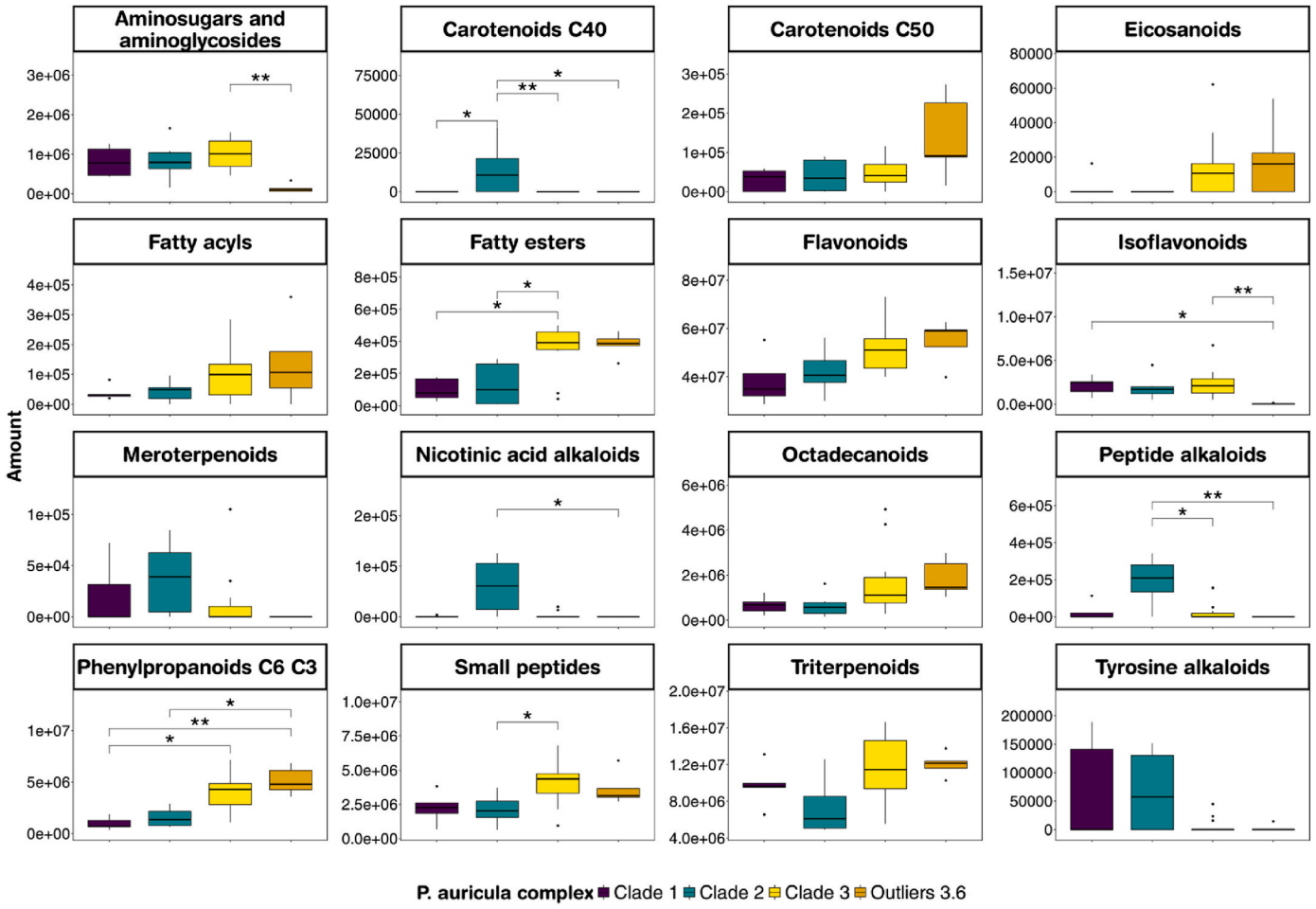

Fig. 4. Mean production of metabolic superclasses. Different panels show the most discriminating metabolic superclasses, and their average relative amounts among different clades of the *P. auricula* complex across the Alps. The significant p-values from Dunn's pairwise comparison test are displayed on individual boxplots (*: p≤0.05, **: p≤0.01). Violet, green, yellow and brown colours represent Clades 1, 2, 3 and 3.6 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

studied in domesticated plants. Szymański et al. (2020) evaluated the introgression impact of wild tomato accessions on modern cultivars. By combining gene-expression and metabolome functioning pathways in two introgressed cultivars, the authors highlighted that the domestication-driven bottleneck dramatically modified fruit ripening and pathogen resistance in plants. Particularly, the accumulation of pantothenic acid, which enhances fruit resistance to fungal attack, accumulated in both the wild tomato *Solanum pennelli* relative and in the introgressed cultivar, but was totally absent in the cultivated tomato *Solanum lycopersicum* which was particularly sensitive to fungal pathogens. Now, whether the Outliers 3.6 group has indeed undergone genetic erosion and whether it impacted its multitrophic interactions needs to be further studied with specific demographic models, something that should be addressed in future analyses.

Notwithstanding, our findings align with previous studies demonstrating that metabolomic traits can serve as robust indicators of genetic divergence, particularly in morphologically cryptic taxa. For instance, studies in *Swertia* (Kaur et al., 2019), *Viola* (Chervin et al., 2019), or *Heracleum* species (Ušjak et al., 2020), demonstrate the value of phytochemical markers in taxonomic resolution, including alpine genera such as *Primula* (Fico et al., 2007; Colombo et al., 2014; Holzbach et al., 2021) and*Gentiana* (Yuan et al., 2017). López-Álvarez et al. (2017) further showed that phytochemical composition can outperform morphological traits in differentiating *Brachypodium* species. Therefore, linking plant taxonomy and phytochemical variability in plants can allow deepening our understanding of recent and ongoing diversification events, within cryptic plant species.

4.1. *Are certain groups of metabolites consistently over- or under-expressed in specific clades?*

The observed patterns of metabolomic differentiation have far reaching implications for the ecological and evolutionary dynamics of *P. auricula*. Specialized metabolites mediate a wide range of interactions with biotic and abiotic factors, influencing plant fitness and survival (Rosenthal and Berenbaum, 2012; Karban et al., 2014; Salam et al., 2023). For example, the exclusive production of C40 carotenoids in Clade 2, which allows discriminating this group from the rest of the *P. auricula* complex in the Alps, including Clade 3, may enhance photoprotection while serving as a visual signal for pollinators. Such dual functions have been documented in other species, such as *Gentiana lutea*, where carotenoids not only protect against UV damage but also attract pollinators through flower pigmentation (Sobral et al., 2015, 2016; Veiga et al., 2016). Given that Clade 2 has also been historically differentiated by taxonomists from the lineages that extended across the northern and western Alps via their flower pigmentation (Zhang and Kadereit, 2004), carotenoids may serve as useful taxonomic markers, although their diagnostic value requires further fine-scale sampling (Somlyay and Bauer, 2010; Rudolf et al., 2018).

We also found phenylpropanoids and small peptides overexpressed in Clade 3 and 3.6. These compounds are associated with antioxidant activity and stress tolerance, traits particularly relevant in the rocky

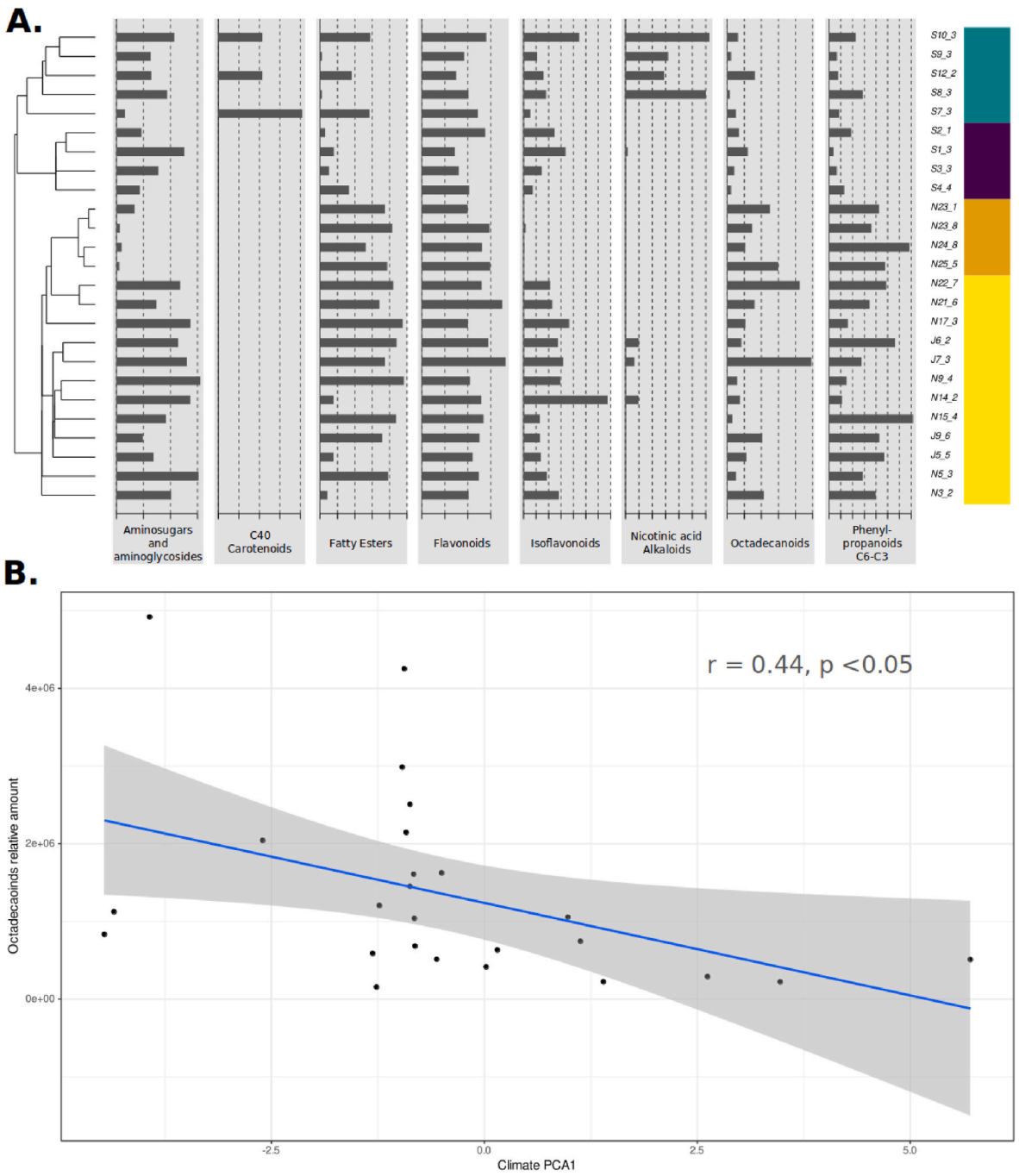

Fig. 5. Phylogenetic and climatic drivers of chemical production. Shown are **A)** a phylogenetic tree of the different *P. auricula* populations sampled across the alpine arc, and the associated relative amounts of different chemical superclasses that show significant levels of phylogenetic signal (Pagels' λ 0) in the residual variance of phylogenetic generalized least squares (pgls) model. **B)** Scatterplot and linear regression line (lm (x ~ Axis1)) of the discriminative superclasses of compounds which significantly discriminated among the first axis of the PCA on the climatic variables. Violet, green, yellow and brown colours represent clades 1, 2, 3 and 3.6 respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

habitats where *P. auricula* thrives (Kim et al., 2021; Ortiz and Sansinenea, 2023). Phenylpropanoids, in particular, are known for their antioxidant properties, while small peptides can serve to coordinate plant response to biotic and abiotic stresses, providing efficient metabolic responses to herbivory, and to varying temperature or drought conditions (Scherrer and Körner, 2010; Kim et al., 2021; Ortiz and Sansinenea, 2023). The loss of this chemical composition in Outlier 3.6 suggests again that specific selective pressures are missing in these habitats (Pimpinelli et Piacentini, 2020; Sadgrove et al., 2020; Ramos et al., 2023), or that a genetic bottleneck has contributed to chemical diversity erosion. Additionally, peptides alkaloids and nicotinic acid alkaloids, which we observed to be overexpressed in Clade 2, are known to contribute to stress tolerance and herbivore defence (Baldwin and Schultz, 1983; Rosenthal and Berenbaum, 2012). Such variation in metabolomic strategies likely enhances clade-specific ecological resilience and may contribute to reproductive isolation. Reciprocal transplant experiments could help clarify how different metabolomes influence plant performance across environments. These findings also have implications under climate change, as alpine plants will face intensified abiotic stress and shifting biotic interactions (Scherrer and

Körner, 2010; Nomoto et al., 2024).

4.2. Are these chemical differences primarily associated with phylogenetic divergence or with environmental factors such as climatic variation?

Although phylogenetic structure was the strongest determinant of metabolomic differentiation, climatic factors also shaped certain traits, notably octadecanoids. This finding is consistent with research demonstrating that environmental gradients, such as temperature and precipitation, shape the production of secondary metabolites in plants (Isah, 2019; Waterman and Mole, 2019; Qaderi et al., 2023). Octadecanoids are central to stress signalling, and were upregulated in warmer or drier environments, consistent with their role in abiotic stress responses (Wasternack and Hause, 2013). At this stage, our coarse sampling design, which privileged collecting across the entire alpine arc over the fine-grained sampling of few populations, limits our ability to precisely link the expression of chemicals with the plants ability to cope with specific microclimatic conditions. Accordingly, for instance, as Clade 3 spans a wide geographic range, encompassing regions with distinct climatic conditions, individuals within this Clade 3 likely experience strong variation in microclimatic conditions, potentially leading to localized differences in secondary metabolite production (see Figure S1BC) (Luqman et al., 2023). Similar microclimate-driven chemical variation has been observed in *Quercus ilex* and *Rhododendron ferrugineum*, where populations across elevation gradients exhibited differential production of carotenoids and UV-absorbing pigments, reflecting adaptations to UV exposure and temperature extremes (Filella and Peñuelas, 1999). Further work combining high-resolution climatic monitoring with denser population sampling will be essential to disentangle these interactions.

Nonetheless, disentangling the relative contributions of genetic relatedness and environment to metabolomic variation remains a both technical as well as statistical challenge. First, clearly, the metabolome is the outer marker of the genomic makeup of a plant. Yet, environmental plasticity makes some chemotypes more expressed in one environment than in others, which is a reflection of the gene expression, measured using RNA-based transcriptomics, rather than gene presence, measured using DNA-based genomics (Macel et al., 2010). In this case, common garden experiments could be used to disentangle the effect of environment from genes in dictating phytochemical variation. Second, disentangling the multivariate climatic profile of a population (or individual) from its multivariate genetic makeup, as done in our study using phylogenetic-corrected linear regressions remains rather limited, and is often prone to type-I errors (Mazel et al., 2016; Swenson, 2019). Future work should thus integrate common garden experiments, to isolate the effects of genetic versus environmental influences on metabolomic profiles, with novel comparative genetic approaches in a multivariate framework (e.g. Svishcheva et al., 2022).

5. Conclusion and perspectives

Metabolomic profiling of the *P. auricula* complex across the Alps reveals its value as a tool for studying phenotypic differentiation and evolutionary processes. Our findings demonstrate that metabolomics can provide a low-cost, high-resolution complement to genomic approaches for investigating intraspecific diversity and demographic history.

Future research should explore how variations in metabolomic profiles between genetic groups of *P. auricula* influence multitrophic interactions with their environment. For instance, *Primula* species are primarily pollinated by Hymenoptera and Lepidoptera (Fisogni et al., 2011; Minuto et al., 2014), but specific pollinator preferences and their potential role in driving chemical co-evolution in *P. auricula* remain unstudied. Similarly, phytochemical defences may interact with herbivory pressures, shaping reproductive success and survival (Sasidharan et al., 2024). While large herbivores appear to avoid *P. auricula* due to its high concentrations of toxic saponins, field observations suggest molluscs as potential herbivores. Molluscs, with their limited dispersal ability and tendency toward endemism in rocky habitats (Dépraz et al., 2008), could contribute to localized co-evolutionary dynamics that influence the defensive metabolome of the plants. Investigating these multitrophic interactions could elucidate how biotic pressures drive both genomic and phytochemical diversification, potentially contributing to clade and species differentiation.

Further refinement in chemical characterization is also essential to understand qualitative differences among clades within similar structural groups of metabolites. The chemical annotation generated by tools such as Sirius provides a first, preliminary, framework for identifying clade-specific molecular structures. These networks suggest clear qualitative differentiation among clades, with certain compounds restricted to one or two clades. However, advanced techniques such as nuclear magnetic resonance (NMR) spectrometry are necessary to precisely identify and reference these compounds. A concerted effort is also needed to develop robust open-source chemical libraries to improve metabolomic identification and provide a comprehensive overview of the metabolome constitution (Gomes et al., 2024). For example such efforts should help clarify whether flavonoids and isoflavonoids, known to display specific patterns in *Primula* species from the section Auricula (Fico et al., 2007; Colombo et al., 2014; Holzbach et al., 2021) exhibit consistent variation across the three genetic groups studied here.

By linking phylogeography with metabolomics, this study highlights the role of genetic drift, isolation, and ecological pressures in shaping chemical diversity. The *P. auricula* complex, with its strong phylogeographic structure and cryptic morphology, provides a powerful model for testing metabolomic fingerprinting as a tool in evolutionary and conservation biology. Given the sensitivity of alpine plants to climate change (Paun et al., 2008; Boucher et al., 2016b; Schneeweiss et al., 2017), such approaches are particularly urgent. The relatively low cost of metabolomic analysis compared to genomic sequencing underscores its potential as a scalable approach for conservation in biodiversity hotspots. Integrating metabolomics with ecological and genomic perspectives will be critical for unravelling the complex processes shaping alpine biodiversity and for informing conservation strategies.

CRediT authorship contribution statement

Stéphanie Morelon: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gaëtan Glauser:** Writing – review & editing, Validation, Software, Resources, Methodology, Data curation, Conceptualization. **Emmanuel Defossez:** Writing – review & editing, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Jérémy Gauthier:** Writing – review & editing, Validation, Supervision, Methodology. **Jason Grant:** Validation, Resources, Project administration, Funding acquisition, Conceptualization. **Nadir Alvarez:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Conceptualization. **Sergio Rasmann:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Fundings

This research was supported by Swiss National Science Foundation (SNSF) grants 186227 to JG and 310030_204811 to SR.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Jason Grant reports financial support was provided by Swiss National Science Foundation. Sergio Rasmann reports financial support was provided by Swiss National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the regional environmental agencies of Swiss cantons (OFEV) and France (DREAL), the regional offices in Lombardy (Italy), in Tübingen district (RPT), Bayern district, Shwaben district as well as Protected areas directions (Berchtesgaden National park, Germany) who provided harvesting permits. The Inflora team (CH) and the Conservatoires Botaniques Français of Bourgogne-Franche-Comté (CBNFC) and Alpes (CBNA) provided precise plant coordinates. We are particularly grateful to Thomas Bulliard who both collected and participated to laboratory efforts, Asma Adam Abdi who offered valuable support in laboratory efforts; as well as Philippe and Laurent Juillerat, Mariama Diallo, Florian Boucher, Camille Voisin, Marion Deville-Cavellin, Adrian Möhl and Jérémy Guenat who collected additional plant material.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2026.105230.

Data availability

Data will be made available on request.

References

Aartsma, Y., Leroy, B., van der Werf, W., Dicke, M., Poelman, E.H., Bianchi, F.J., 2019. Intraspecific variation in herbivore-induced plant volatiles influences the spatial range of plant–parasitoid interactions. Oikos 128, 77–86.

Agrawal, A.A., 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294, 321–326.

Anacker, B.L., Strauss, S.Y., 2014. The geography and ecology of plant speciation: range overlap and niche divergence in sister species. Proc. R. Soc. B Biol. Sci. 281, 20132980.

Baldwin, I.T., Schultz, J.C., 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. Science 221, 277–279.

Barton, N.H., 2020. On the completion of speciation. Philos. Trans. R. Soc. B 375, 20190530.

Baudière, A., Serve, L., 1971. Recherches sur les teneurs en carbone organique des sols de haute montagne dans le bassin méditerranéen occidental. Bull. Soc. Botaniq. France, 118(sup2), 147–170.

Baumel, A., Roquet, C., Lavergne, S., Smyčka, J., Garraud, L., Abdulhak, S., Dentant, C., Mouly, A., Vuillemenot, M., Crémel, K., others, 2023. Evolutionary distinctiveness with incomplete isolation of the narrow endemic alpine plant Saxifraga delphinensis ravaud. Alpine Bot. 1–15.

Benton, M.J., 2009. The red queen and the court jester: species diversity and the role of biotic and abiotic factors through time. science 323, 728–732.

Berenbaum, M.R., 2002. Postgenomic chemical ecology: from genetic code to ecological interactions. J. Chem. Ecol. 28, 873–896.

Boucher, F.C., Casazza, G., Szövényi, P., Conti, E., 2016a. Sequence capture using RAD probes clarifies phylogenetic relationships and species boundaries in Primula sect. Auricula. Mol. Phylogenet. Evol. 104, 60–72. https://doi.org/10.1016/j.ympev.2016.08.003.

Boucher, F.C., Dentant, C., Ibanez, S., Capblancq, T., Boleda, M., Boulangeat, L., Smyčka, J., Roquet, C., Lavergne, S., 2021. Discovery of cryptic plant diversity on the rooftops of the Alps. Sci. Rep. 11, 11128. https://doi.org/10.1038/s41598-021-90612-w.

Boucher, F.C., Zimmermann, N.E., Conti, E., 2016b. Allopatric speciation with little niche divergence is common among alpine Primulaceae. J. Biogeogr. 43, 591–602. https://doi.org/10.1111/jbi.12652.

Carcaillet, C., Brun, J.-J., 2000. Changes in landscape structure in the northwestern Alps over the last 7000 years: Lessons from soil charcoal. J. Veg. Sci. 11 (5), 705–714.

Chae, L., Kim, T., Nilo-Poyanco, R., Rhee, S.Y., 2014. Genomic signatures of specialized metabolism in plants. Science 344, 510–513.

Chervin, J., Talou, T., Audonnet, M., Dumas, B., Camborde, L., Esquerré-Tugayé, M.-T., Roux, C., Cabanac, G., Marti, G., 2019. Deciphering the phylogeny of violets based on multiplexed genetic and metabolomic approaches. Phytochemistry 163, 99–110. https://doi.org/10.1016/j.phytochem.2019.04.001.

Colombo, P.S., Flamini, G., Christodoulou, M.S., Rodondi, G., Vitalini, S., Passarella, D., Fico, G., 2014. Farinose alpine Primula species: phytochemical and morphological investigations. Phytochemistry 98, 151–159.

Colombo, P.S., Flamini, G., Rodondi, G., Giuliani, C., Santagostini, L., Fico, G., 2017. Phytochemistry of European Primula species. Phytochemistry 143, 132–144.

Cunningham, S.A., Summerhayes, B., Westoby, M., 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. Ecol. Monogr. 69, 569–588.

De Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879–886.

Defossez, E., Bourquin, J., von Reuss, S., Rasmann, S., Glauser, G., 2023. Eight key rules for successful data-dependent acquisition in mass spectrometry-based metabolomics. Mass Spectrom. Rev. 42, 131–143.

Defossez, E., Pitteloud, C., Descombes, P., Glauser, G., Allard, P.-M., Walker, T.W., Fernandez-Conradi, P., Wolfender, J.-L., Pellissier, L., Rasmann, S., 2021. Spatial and evolutionary predictability of phytochemical diversity. Proc. Natl. Acad. Sci. 118, e2013344118.

Dépraz, A., Cordellier, M., Hausser, J., Pfenninger, M., 2008. Postglacial recolonization at a snail's pace (trochulus villosus): confronting competing refugia hypotheses using model selection. Mol. Ecol. 17, 2449–2462. https://doi.org/10.1111/j.1365-294X.2008.03760.x.

Dührkop, K., Nothias, L.F., Fleischauer, M., Ludwig, M., Hoffmann, M.A., Rousu, J., Dorrestein, P.C., Böcker, S., 2020. Classes for the Masses: Systematic Classification of Unknowns Using Fragmentation Spectra. BioRxiv.

Dührkop, K., Shen, H., Meusel, M., Rousu, J., Böcker, S., 2015. Searching molecular structure databases with tandem mass spectra using CSI: fingerid. Proc. Natl. Acad. Sci. 112, 12580–12585.

Evergetis, E., Haroutounian, S.A., 2020. Volatile systematics: a novel biochemical interpretation of essential oil compounds enhances their chemophenetic significance. Biochem. Systemat. Ecol. 92, 104087.

Fico, G., Rodondi, G., Flamini, G., Passarella, D., Tomé, F., 2007. Comparative phytochemical and morphological analyses of three Italian Primula species. Phytochemistry 68, 1683–1691. https://doi.org/10.1016/j.phytochem.2007.04.019.

Filella, I., Peñuelas, J., 1999. Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of Quercus ilex and Rhododendron ferrugineum in the Mediterranean region. Plant Ecol. 145, 157–165.

Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613–635. https://doi.org/10.1111/mec.14486.

Fisogni, A., Cristofolini, G., Podda, L., Galloni, M., 2011. Reproductive ecology in the endemic Primula apennina Widmer (Primulaceae). Plant Biosyst. - Int. J. Deal. Asp. Plant Biol. 145, 353–361. https://doi.org/10.1080/11263504.2011.563514.

Frei, E.S., Scheepens, J., Armbruster, G.F., Stöcklin, J., 2012. Phenotypic differentiation in a common garden reflects the phylogeography of a widespread Alpine plant. J. Ecol. 100, 297–308.

Galtier, N., 2019. Delineating species in the speciation continuum: a proposal. Evol. Appl. 12, 657–663. https://doi.org/10.1111/eva.12748.

García-Ramos, G., Huang, Y., 2013. Competition and evolution along environmental gradients: patterns, boundaries and sympatric divergence. Evol. Ecol. 27, 489–504. https://doi.org/10.1007/s10682-012-9614-y.

Gatehouse, J.A., 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytol. 156, 145–169.

Ghalambor, C.K., McKay, J.K., Carroll, S.P., Reznick, D.N., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct. Ecol. 21, 394–407.

Gomes, P.W.P., Mannochio-Russo, H., Schmid, R., Zuffa, S., Damiani, T., Quiros-Guerrero, L.-M., Caraballo-Rodríguez, A.M., Zhao, H.N., Yang, H., Xing, S., Charron-Lamoureux, V., Chigumba, D.N., Sedio, B.E., Myers, J.A., Allard, P.-M., Harwood, T. V., Tamayo-Castillo, G., Kang, K.B., Defossez, E., Koolen, H.H.F., da Silva, M.N., E Silva, C.Y.Y., Rasmann, S., Walker, T.W.N., Glauser, G., Chaves-Fallas, J.M., David, B., Kim, H., Lee, K.H., Kim, M.J., Choi, W.J., Keum, Y.-S., de Lima, E.J.S.P., de Medeiros, L.S., Bataglion, G.A., Costa, E.V., da Silva, F.M.A., Carvalho, A.R.V., Reis, J.D.E., Pamplona, S., Jeong, E., Lee, K., Kim, G.J., Kil, Y.-S., Nam, J.-W., Choi, H., Han, Y.K., Park, S.Y., Lee, K.Y., Hu, C., Dong, Y., Sang, S., Morrison, C.R., Borges, R.M., Teixeira, A.M., Lee, S.Y., Lee, B.S., Jeong, S.Y., Kim, K.H., Rutz, A., Gaudry, A., Bruelhart, E., Kappers, I.F., Karlova, R., Meisenburg, M., Berdaguer, R., Tello, J.S., Henderson, D., Cayola, L., Wright, S.J., Allen, D.N., Anderson-Teixeira, K. J., Baltzer, J.L., Lutz, J.A., McMahon, S.M., Parker, G.G., Parker, J.D., Northen, T.R., Bowen, B.P., Pluskal, T., van der Hooft, J.J.J., Carver, J.J., Bandeira, N., Pullman, B. S., Wolfender, J.-L., Kersten, R.D., Wang, M., Dorrestein, P.C., 2024. plantMASST - community-driven chemotaxonomic digitization of plants. https://doi.org/10.1101/2024.05.13.593988.

Greenwell, B.M., Boehmke, B.C., Gray, B., 2020. Variable importance plots-An introduction to the VIP package. R J. 12 (1), 343.

Gugerli, F., Englisch, T., Niklfeld, H., Tribsch, A., Mirek, Z., Ronikier, M., Zimmermann, N.E., Holderegger, R., Taberlet, P., 2008. Relationships among levels of biodiversity and the relevance of intraspecific diversity in conservation – a project synopsis. Perspect. Plant Ecol. Evol. Systemat. 10, 259–281. https://doi.org/10.1016/j.ppees.2008.07.001.

He, X., Cao, J.-J., Zhang, W., Li, Y.-Q., Zhang, C., Li, X.-H., Xia, G.-H., Shao, J.-W., 2022. Integrative taxonomy of herbaceous plants with narrow fragmented distributions: a case study on Primula merrilliana species complex. J. Systemat. Evol. 60, 859–875. https://doi.org/10.1111/jse.12726.

Hernández-Hernández, T., Miller, E.C., Román-Palacios, C., Wiens, J.J., 2021. Speciation across the tree of l ife. Biol. Rev. 96, 1205–1242.

Hewitt, G., 2000. The genetic legacy of the Quaternary ice ages. Nature 405, 907–913. https://doi.org/10.1038/35016000.
Hijmans, R.J., Bivand, R., Forner, K., Ooms, J., Pebesma, E., Sumner, M.D., 2022. Package 'Terra.' Maint. Vienna Austria.
Holzbach, B., Reuter, V., Bacher, M., Schinnerl, J., Brecker, L., Rosenau, T., Valant-Vetschera, K., 2021. Flavonoid diversification in different leaf compartments of Primula auricula (Primulaceae). Biochem. Systemat. Ecol. 98, 104310. https://doi.org/10.1016/j.bse.2021.104310.
Isah, T., 2019. Stress and defense responses in plant secondary metabolites production. Biol. Res. 52.
Jobbagy, E.G., Jackson, R.B., 2001. The distribution of soil nutrients with depth: global patterns and the imprint of plants. Biogeochemistry 53, 51–77.
Kadereit, J.W., Abbott, R.J., 2021. Plant speciation in the Quaternary. Plant Ecol. Divers. 14, 105–142.
Karban, R., Wetzel, W.C., Shiojiri, K., Ishizaki, S., Ramirez, S.R., Blande, J.D., 2014. Deciphering the language of plant communication: volatile chemotypes of sagebrush. New Phytol. 204, 380–385. https://doi.org/10.1111/nph.12887.
Karger, D.N., Conrad, O., Böhner, J., Kawohl, T., Kreft, H., Soria-Auza, R.W., Zimmermann, N.E., Linder, H.P., Kessler, M., 2016. CHELSA Climatologies at High Resolution for the Earth's Land Surface Areas version 1.0.
Kaur, P., Pandey, D.K., Gupta, R., Dey, A., 2019. Assessment of genetic diversity among different population of five Swertia species by using molecular and phytochemical markers. Ind. Crops Prod. 138, 111569.
Kergunteuil, A., Humair, L., Maire, A.-L., Moreno-Aguilar, M.F., Godschalx, A., Catalán, P., Rasmann, S., 2020. Tritrophic interactions follow phylogenetic escalation and climatic adaptation. Sci. Rep. 10, 2074.
Kergunteuil, A., Humair, L., Münzbergová, Z., Rasmann, S., 2019. Plant adaptation to different climates shapes the strengths of chemically mediated tritrophic interactions. Funct. Ecol. 33, 1893–1903.
Kim, H.W., Wang, M., Leber, C.A., Nothias, L.-F., Reher, R., Kang, K.B., van der Hooft, J. J.J., Dorrestein, P.C., Gerwick, W.H., Cottrell, G.W., 2021. NPClassifier: a deep neural network-based structural classification tool for natural products. J. Nat. Prod. 84, 2795–2807. https://doi.org/10.1021/acs.jnatprod.1c00399.
Kim, J.S., Jeon, B.W., Kim, J., 2021. Signaling peptides regulating abiotic stress responses in plants. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.704490.
Lande, R., Shannon, S., 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 434–437.
Liu, M.-L., Shang, Q.-H., Cheng, Y.-J., Wang, N., Sa, W., Li, B.-G., Li, Z.-H., 2023. Drivers of intraspecific differentiation of an alpine cold-tolerant herb, Notopterygium oviforme: roles of isolation by distance and ecological factors. J. Systemat. Evol. 61, 383–398. https://doi.org/10.1111/jse.12844.
López-Álvarez, D., Zubair, H., Beckmann, M., Draper, J., Catalán, P., 2017. Diversity and association of phenotypic and metabolomic traits in the close model grasses Brachypodium distachyon, B. stacei and B. hybridum. Ann. Bot. 119, 545–561.
Luqman, H., Wegmann, D., Fior, S., Widmer, A., 2023. Climate-induced range shifts drive adaptive response via spatio-temporal sieving of alleles. Nat. Commun. 14, 1080. https://doi.org/10.1038/s41467-023-36631-9.
Macel, M., van Dam, N.M., Keurentjes, J.j.B., 2010. Metabolomics: the chemistry between ecology and genetics. Mol. Ecol. Resour. 10, 583–593. https://doi.org/10.1111/j.1755-0998.2010.02854.x.
Martinidou, E., Palmieri, L., Sordo, M., Masuero, D., Ourda, M., Delucchi, L., Fusani, P., Tremml, V., Poulopoulou, I., Gauly, M., others, 2023. Assessment of the chemical and genetic variability among accessions of Cicerbita alpina (L.) Wallr., an alpine plant with anthelmintic properties. Front. Plant Sci. 14, 1269613.
Martucci, M.E.P., De Vos, R.C., Carollo, C.A., Gobbo-Neto, L., 2014. Metabolomics as a potential chemotaxonomical tool: application in the genus Vernonia Schreb. PLoS One 9, e93149.
Mazel, F., Davies, T.J., Georges, D., Lavergne, S., Thuiller, W., Peres-Neto, P.R., 2016. Improving phylogenetic regression under complex evolutionary models. Ecology 97, 286–293.
Médail, F., Diadema, K., 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. J. Biogeogr. 36, 1333–1345.
Minuto, L., Guerrina, M., Roccotiello, E., Roccatagliata, N., Mariotti, M.G., Casazza, G., 2014. Pollination ecology in the narrow endemic winter-flowering Primula allionii (Primulaceae). J. Plant Res. 127, 141–150.
Moreira, X., Abdala-Roberts, L., Galmán, A., Francisco, M., de la Fuente, M., Butrón, A., Rasmann, S., 2018. Assessing the influence of biogeographical region and phylogenetic history on chemical defences and herbivory in Quercus species. Phytochemistry 153, 64–73.
Morelon, S., Juillerat, P., Bilat, J., Mottaz, H., Bulliard, T., Boucher, F.C., Juillerat, L., Grant, J., Rasmann, S., Gauthier, J., Alvarez, N., 2026. The role of peripheral regions in shaping the phylogeography of the bear's ear complex across the Alps. J. Biogeogr. 53 (1), e70125. https://doi.org/10.1111/jbi.70125.
Morlon, H., Andréoletti, J., Barido-Sottani, J., Lambert, S., Perez-Lamarque, B., Quintero, I., Senderov, V., Veron, P., 2024. Phylogenetic insights into diversification. Annu. Rev. Ecol. Evol. Syst. 55.
Nevado, B., Atchison, G.W., Bridges, E.L., Orzell, S., Filatov, D., Hughes, C.E., 2024. Pleistocene diversification of unifoliolate-leaved Lupinus (Leguminosae: Papilionoideae) in Florida. Mol. Ecol. 33, e17232. https://doi.org/10.1111/mec.17232.
Nomoto, H., Fior, S., Alexander, J., 2024. Competitors alter selection on alpine plants exposed to experimental climate change. Evol. Lett. 8, 114–127. https://doi.org/10.1093/evlett/qrad066.
Nosil, P., 2008. Ernst Mayr and the integration of geographic and ecological factors in speciation. Biol. J. Linn. Soc. 95, 26–46. https://doi.org/10.1111/j.1095-8312.2008.01091.x.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., 2019. Package 'Vegan.' Community Ecol. Package Version 2. others.
Orians, C.M., 2000. The effects of hybridization in plants on secondary chemistry: implications for the ecology and evolution of plant–herbivore interactions. Am. J. Bot. 87, 1749–1756.
Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., Pearse, W., 2013. The caper package: comparative analysis of phylogenetics and evolution in R. R Package Version 5, 1–36.
Ortiz, A., Sansinenea, E., 2023. Phenylpropanoid derivatives and their role in plants' health and as antimicrobials. Curr. Microbiol. 80, 380.
Paun, O., Schönswetter, P., Winkler, M., Consortium, I., Tribsch, A., 2008. Historical divergence vs. contemporary gene flow: evolutionary history of the calcicole Ranunculus alpestris group (Ranunculaceae) in the European Alps and the Carpathians. Mol. Ecol. 17, 4263–4275. https://doi.org/10.1111/j.1365-294X.2008.03908.x.
Peters, K., Blatt-Janmaat, K.L., Tkach, N., van Dam, N.M., Neumann, S., 2023. Untargeted metabolomics for integrative taxonomy: Metabolomics, DNA marker-based sequencing, and phenotype bioimaging. Plants 12, 881.
Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135.
Petrén, H., Anaia, R.A., Aragam, K.S., Bräutigam, A., Eckert, S., Heinen, R., Jakobs, R., Ojeda-Prieto, L., Popp, M., Sasidharan, R., others, 2024. Understanding the chemodiversity of plants: quantification, variation and ecological function. Ecol. Monogr. e1635.
Pimpinelli, S., Piacentini, L., 2020. Environmental change and the evolution of genomes: transposable elements as translators of phenotypic plasticity into genotypic variability. Funct. Ecol. 34, 428–441. https://doi.org/10.1111/1365-2435.13497.
Qaderi, M.M., Martel, A.B., Strugnell, C.A., 2023. Environmental factors regulate plant secondary metabolites. Plants 12, 447.
Ramos, Y.J., Gouvêa-Silva, J.G., de Brito Machado, D., Felisberto, J.S., Pereira, R.C., Sadgrove, N.J., de Lima Moreira, D., 2023. Chemophenetic and chemodiversity approaches: new insights on modern study of plant secondary metabolite diversity at different spatiotemporal and organizational scales. Rev. Bras. Farmacogn. 33, 49–72. https://doi.org/10.1007/s43450-022-00327-w.
Reyer, C.P., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., De Lorenzi, F., Dury, M., Gloning, P., Abou Jaoudé, R., others, 2013. A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. Glob. Change Biol. 19, 75–89.
Rogivue, A., Graf, R., Parisod, C., Holderegger, R., Gugerli, F., 2018. The phylogeographic structure of Arabis alpina in the Alps shows consistent patterns across different types of molecular markers and geographic scales. Alpine Bot. 128, 35–45. https://doi.org/10.1007/s00035-017-0196-8.
Rohart, F., Gautier, B., Singh, A., Lê Cao, K.-A., 2017. mixOmics: an R package for 'omics feature selection and multiple data integration. PLoS Comput. Biol. 13, e1005752.
Rosenthal, G.A., Berenbaum, M.R., 2012. Herbivores: Their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes. Academic press.
Rudolf, A., Vreš, B., Dakskobler, I., 2018. Sites of rare form of auricula (Primula auricula var. tolminensis nom. prov.) in the southern Julian Alps/Rastišča redke oblike lepega jegliča (Primula auricula var. tolminensis nom. prov.) v južnih Julijskih Alpah. Folia Biol. Geol. 59, 75–91.
Sadgrove, N.J., Padilla-González, G.F., Telford, I.R.H., Greatrex, B.W., Jones, G.L., Andrew, R., Bruhl, J.J., Langat, M.K., Melnikovova, I., Fernandez-Cusimamani, E., 2020. Prostanthera (Lamiaceae) as a 'Cradle of Incense': chemophenetics of Rare Essential Oils from Both New and Forgotten Australian 'Mint Bush' species. Plants 9. https://doi.org/10.3390/plants9111570.
Salam, U., Ullah, S., Tang, Z.-H., Elateeq, A.A., Khan, Y., Khan, J., Khan, A., Ali, S., 2023. Plant metabolomics: an overview of the role of primary and secondary metabolites against different environmental stress factors. Life 13, 706.
Sasidharan, R., Grond, S.G., Champion, S., Eilers, E.J., Müller, C., 2024. Intraspecific plant chemodiversity at the individual and plot levels influences flower visitor groups with consequences for germination success. Funct. Ecol.
Scherrer, D., Körner, C., 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. Glob. Change Biol. 16, 2602–2613.
Scheubert, K., Hufsky, F., Petras, D., Wang, M., Nothias, L.-F., Dührkop, K., Bandeira, N., Dorrestein, P.C., Böcker, S., 2017. Significance estimation for large scale metabolomics annotations by spectral matching. Nat. Commun. 8, 1494.
Schneeweiss, G.M., Winkler, M., Schönswetter, P., 2017. Secondary contact after divergence in allopatry explains current lack of ecogeographical isolation in two hybridizing alpine plant species. J. Biogeogr. 44, 2575–2584. https://doi.org/10.1111/jbi.13071.
Schönswetter, P., Stehlik, I., Holderegger, R., Tribsch, A., 2005. Molecular evidence for glacial refugia of mountain plants in the European Alps. Mol. Ecol. 14, 3547–3555.
Schönswetter, P., Tribsch, A., Stehlik, I., Niklfeld, H., 2004. Glacial history of high alpine Ranunculus glacialis (Ranunculaceae) in the European Alps in a comparative phylogeographical context. Biol. J. Linn. Soc. 81, 183–195.
Six, D.L., Trowbridge, A., Howe, M., Perkins, D., Berglund, E., Brown, P., Hicke, J.A., Balasubramanian, G., 2021. Growth, chemistry, and genetic profiles of whitebark pine forests affected by climate-driven Mountain pine beetle outbreaks. Front. For. Glob. Change 4. https://doi.org/10.3389/ffgc.2021.671510.
Schmid, R., Heuckeroth, S., Korf, A., Smirnov, A., Myers, O., Dyrlund, T.S., Bushuiev, R., Murray, K.J., Hoffmann, N., Lu, M., Sarvepalli, A., Zhang, Z., Fleischauer, M., Dührkop, K., Wesner, M., Hoogstra, S.J., Rudt, E., Mokshyna, O., Brungs, C., Ponomarov, K., Mutabdžija, L., Damiani, T., Pudney, C.J., Earll, M., Helmer, P.O., Fallon, T.R., Schulze, T., Rivas-Ubach, A., Bilbao, A., Richter, H., Nothias, L.-F.,

Wang, M., Orešič, M., Weng, J.-K., Böcker, S., Jeibmann, A., Hayen, H., Karst, U., Dorrestein, P.C., Petras, D., Du, X., Pluskal, T., 2023. Integrative analysis of multimodal mass spectrometry data in MZmine 3. Nat. Biotechnol. 41, 447–449. https://doi.org/10.1038/s41587-023-01690-2.

Smyčka, J., Roquet, C., Renaud, J., Thuiller, W., Zimmermann, N.E., Lavergne, S., 2017. Disentangling drivers of plant endemism and diversification in the European Alps – a phylogenetic and spatially explicit approach. Perspect. Plant Ecol. Evol. Systemat. 28, 19–27. https://doi.org/10.1016/j.ppees.2017.06.004.

Sobral, M., Losada, M., Veiga, T., Guitián, Javier, Guitián, José, Guitián, P., 2016. Flower color preferences of insects and livestock: effects on Gentiana lutea reproductive success. PeerJ 4, e1685.

Sobral, M., Veiga, T., Domínguez, P., Guitián, J.A., Guitián, P., Guitián, J.M., 2015. Selective pressures explain differences in flower color among Gentiana lutea populations. PLoS One 10, e0132522.

Somlyay, L., Bauer, N., 2010. Nomenclatural and taxonomic notes on two eastern taxa of the Primula auricula complex. Biologia (Bratisl.) 65, 784–788.

Svishcheva, G.R., Tiys, E.S., Elgaeva, E.E., Feoktistova, S.G., Timmers, P.R., Sharapov, S. Z., Axenovich, T.I., Tsepilov, Y.A., 2022. A novel framework for analysis of the shared genetic background of correlated traits. Genes 13, 1694.

Swenson, N.G., 2019. Phylogenetic Ecology: a History, Critique, and Remodeling. University of Chicago Press.

Symonds, M.R.E., Blomberg, S.P., 2014. A primer on phylogenetic generalised least squares. In: Garamszegi, L.Z. (Ed.), Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology: Concepts and Practice. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 105–130. https://doi.org/10.1007/978-3-662-43550-2_5.

Szymański, J., Bocobza, S., Panda, S., Sonawane, P., Cárdenas, P.D., Lashbrooke, J., Kamble, A., Shahaf, N., Meir, S., Bovy, A., Beekwilder, J., Tikunov, Y., Romero de la Fuente, I., Zamir, D., Rogachev, I., Aharoni, A., 2020. Analysis of wild tomato introgression lines elucidates the genetic basis of transcriptome and metabolome variation underlying fruit traits and pathogen response. Nat. Genet. 52, 1111–1121. https://doi.org/10.1038/s41588-020-0690-6.

Theodoridis, S., Nogués-Bravo, D., Conti, E., 2019. The role of cryptic diversity and its environmental correlates in global conservation status assessments: Insights from the threatened bird's-eye primrose (Primula farinosa L.). Divers. Distrib. 25 (9), 1457–1471.

Troisi, J., Cavallo, P., Colucci, A., Pierri, L., Scala, G., Symes, S., Jones, C., Richards, S., 2020. Metabolomics in genetic testing. Adv. Clin. Chem. 94, 85–153.

Ušjak, L., Niketić, M., Drobac, M., Petrović, S., 2020. Chemosystematic evaluation of leaf and flower essential oils of eight Heracleum taxa from Southeastern Europe. Plant Systemat. Evol. 306, 4.

Veiga, T., Guitián, J., Guitian, P., Guitian, J., Munilla, I., Sobral, M., 2016. Flower colour variation in the montane plant Gentiana lutea L.(Gentianaceae) is unrelated to abiotic factors. Plant Ecol. Divers. 9, 105–112.

Voisin, C., Dentant, C., Rioux, D., Boucher, F.C., 2023. Introgression of an isolated Primula lineage suggests the existence of a glacial refugium in the Écrins range (Southwestern French Alps). Alpine Bot. 133, 21–33. https://doi.org/10.1007/s00035-022-00288-9.

Walker, T.W., Alexander, J.M., Allard, P.-M., Baines, O., Baldy, V., Bardgett, R.D., Capdevila, P., Coley, P.D., David, B., Defossez, E., 2022. Functional traits 2.0: the power of the metabolome for ecology. others J. Ecol.

Walker, T.W., Schrodt, F., Allard, P.-M., Defossez, E., Jassey, V.E., Schuman, M.C., Alexander, J.M., Baines, O., Baldy, V., Bardgett, R.D., 2023. Leaf metabolic traits reveal hidden dimensions of plant form and function. others Sci. Adv. 9. eadi4029.

Wasternack, C., Hause, B., 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. Ann. Bot. 111, 1021–1058.

Waterman, P.G., Mole, S., 2019. Extrinsic factors influencing production of secondary metabolites in plants. In: Insect-Plant Interactions. CRC press, pp. 107–134.

Yuan, H.-Y., Kwaku, O.R., Pan, H., Han, J.-X., Yang, C.-R., Xu, M., 2017. Iridoid glycosides from the genus Gentiana (Gentianaceae) and their chemotaxonomic sense. Nat. Prod. Commun. 12, 1934578X1701201035.

Záveská, E., Kirschner, P., Frajman, B., Wessely, J., Willner, W., Gattringer, A., Hülber, K., Lazić, D., Dobeš, C., Schönswetter, P., 2021. Evidence for glacial refugia of the forest understorey species Helleborus niger (Ranunculaceae) in the Southern as well as in the Northern limestone alps. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.683043.

Zhang, L.-B., Comes, H.P., Kadereit, J.W., 2004. The temporal course of Quaternary diversification in the European high mountain endemic Primula sect. Auricula (Primulaceae). Int. J. Plant Sci. 165, 191–207.

Zhang, L.-B., Kadereit, J.W., 2004. Classification of Primula sect. Auricula (Primulaceae) based on two molecular data sets (ITS, AFLPs), morphology and geographical distribution. Bot. J. Linn. Soc. 146, 1–26.