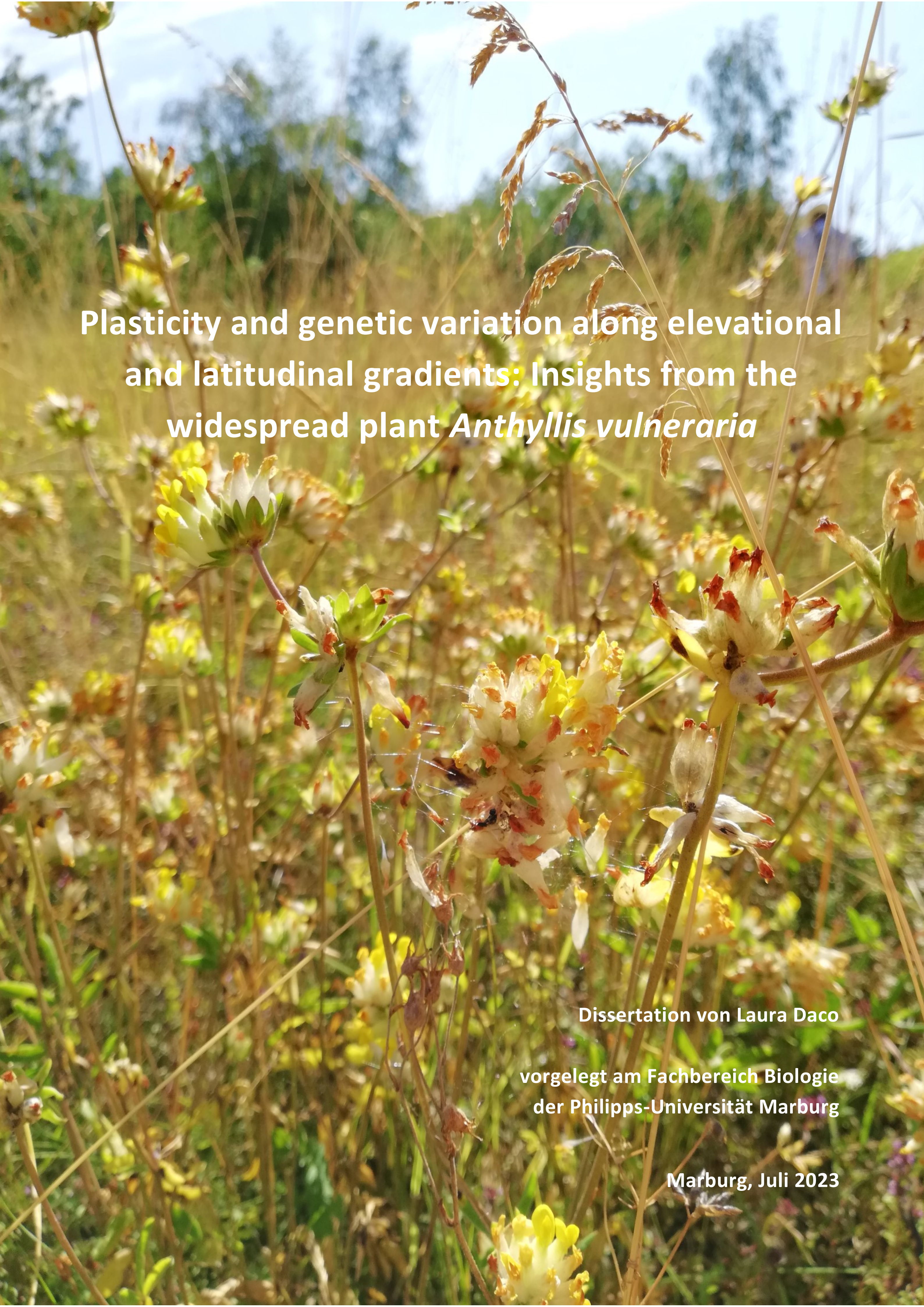

Plasticity and genetic variation along elevational and latitudinal gradients: Insights from the widespread plant *Anthyllis vulneraria*

Dissertation von Laura Daco

vorgelegt am Fachbereich Biologie
der Philipps-Universität Marburg

Marburg, Juli 2023

Plasticity and genetic variation along elevational and latitudinal gradients: Insights from the widespread plant *Anthyllis vulneraria*

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Zweitgutachter: Dr. Guy Colling

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DECLARATION OF AUTHOR’S CONTRIBUTIONS

This dissertation entitled “Plasticity and genetic variation along elevational and latitudinal gradients: Insights from the widespread plant *Anthyllis vulneraria*” was carried out at the Philipps-Universität Marburg under the supervision of Prof. Dr. Diethart Matthies and at the Musée national d’histoire naturelle Luxembourg under the supervision of Dr. Guy Colling.

Three independent scientific manuscripts are presented, which I prepared for submission and/or publication as lead author. The contributions of each co-author for each chapter are given as follows:

Chapter 1

Daco L, Colling G, Matthies D (2021) Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*. Oecologia 197(2), 537-549. https://doi.org/10.1007/s00442-021-05030-6

I organized and conducted field work and I collected data and samples and organized their storage. I analysed the data and prepared the illustrations. I wrote the first draft of the manuscript. All authors conceived the study, contributed to statistical analyses, improved earlier versions of the manuscript, and prepared the revisions.

Chapter 2

Daco L, Matthies D, Hermant S, Colling G (2022) Genetic diversity and differentiation of populations of *Anthyllis vulneraria* along elevational and latitudinal gradients. Ecology and Evolution 12(8), e9167. https://doi.org/10.1002/ece3.9167

I organized and conducted field work and I collected data and samples and organized their storage. I prepared the samples for genetic analyses and developed the protocol with the help of SH. SH processed the samples in the laboratory and prepared the genetic data for analyses. I analysed the data and prepared the illustrations. I wrote the first draft of the manuscript. LD, GC and DM conceived the study, contributed to statistical analyses, improved earlier versions of the manuscript, and prepared the revisions.

Chapter 3

Daco L, Colling G, Matthies D. Quantitative genetic differentiation and evolutionary potential in the widespread plant *Anthyllis vulneraria* along elevational and latitudinal gradients.

I prepared, organized and conducted field work and the common garden experiment. I collected data and samples and organized their storage. I analysed the data and prepared the illustrations. I wrote the first draft of the manuscript. All authors conceived the study, contributed to statistical analyses, improved earlier versions of the manuscript.

SUMMARY

The distribution and population characteristics of plants are largely influenced by the environmental conditions they encounter across their ranges. In response to environmental changes, populations can migrate to new locations, exhibit phenotypic plasticity and undergo evolutionary adaptation. Studies comparing population traits along gradients of elevation and latitude provide an opportunity to assess the influence of different environmental and historical factors on plant population characteristics. To investigate plant plasticity and evolutionary potential in response to environmental variation, I employed an integrative approach combining field surveys, population genetic studies, and experiments. The widespread plant *Anthyllis vulneraria* was selected as a model species because of its large elevational and latitudinal distribution in Europe. In particular, I studied across elevational and latitudinal gradients (1) the characteristics of habitats and populations of *A. vulneraria*, (2) the neutral molecular genetic diversity and differentiation of populations, (3) the quantitative genetic differentiation and the evolutionary potential of populations. The elevational gradient in the Alps ranged from 500 m to the elevational limit at 2500 m and the latitudinal gradient spanned 2400 km from Central Europe to the range limit in the North. The length of the two gradients was chosen to correspond to a change of c. 11.5 °C in annual mean temperature.

I explored the habitat characteristics of 40 populations of *A. vulneraria* along both the elevational and latitudinal gradients and studied their influence on population characteristics. Plant size and reproduction decreased, but plant density increased with elevation and latitude, indicating higher recruitment and demographic compensation among vital rates. The results support the view that demographic compensation may be common in widespread species. Temperature variation along both gradients was found to have the strongest effects on population characteristics, followed by differences in precipitation, solar radiation, and soil nutrients. The proportion of plants flowering, seed set and seed mass declined with latitude indicating resource limitation and reduced pollination in the North, while the strong variation in these traits along the elevational gradient was not related to elevation or other covarying environmental variables, but to local environmental variation in alpine habitats.

I examined the molecular genetic diversity and differentiation of the populations of *A. vulneraria* using microsatellites, which are considered to be neutral markers and unaffected by natural selection. Genetic diversity strongly declined and differentiation increased with latitude. These patterns are likely the outcome of serial founder effects during the northward colonization following glacier retreat after the last ice age. The genetic diversity and differentiation among populations of *A. vulneraria* was not related to elevation. This observation could be attributed to the higher gene flow facilitated by the much shorter elevational gradient. However, I found evidence of isolation by distance along both gradients, indicating restricted gene flow among populations along both gradients. Subarctic populations differed genetically from alpine populations indicating that the northern populations did not originate from high elevational ones in the Alps.

Plants grown from seeds originating from the studied populations were studied in a common garden experiment to assess the quantitative genetic differentiation and phenotypic plasticity of populations along the elevational and latitudinal gradients and to investigate their evolutionary potential. Most traits measured in the common garden exhibited clinal variation with elevation and latitude of origin indicating adaptive differentiation of traits along the gradients. Divergent selection was indicated by higher Q_{ST}-values (measuring quantitative genetic differentiation) than F_{ST}-values (measuring neutral molecular genetic differentiation) in some traits. Furthermore, I observed that the differences in performance between mothers and their progeny were smaller for populations from high elevations and latitudes, suggesting reduced plasticity of the measured traits in these populations. However, the evolvability of most traits did not vary consistently along the two gradients indicating that the evolutionary potential of peripheral populations was not reduced.

To conclude, this thesis has highlighted the importance of combining field surveys, population genetic studies, and ecological experiments to investigate plasticity and genetic variation in response to different environmental conditions. Gaining a deeper understanding of how plant populations adapt to changing environments is crucial for predicting their future responses to climate change. The results suggest that the abundant centre model (ACM) does not fully account for the observed population characteristics and genetic patterns in *A. vulneraria*. Other factors such as historical migration and local adaptation driven by varying selection pressures along the elevational and latitudinal gradients have also important effects in shaping the distribution of the species and its traits across different environments, and will shape its future responses to climate change. While the rather low phenotypic plasticity of the arctic and alpine populations of *A. vulneraria* alone may not be sufficient for them to persist, genetic variability in fitness-related traits together with gene flow may allow their adaptation to changing environmental conditions in the future.

ZUSAMMENFASSUNG

Die Verbreitung von Pflanzen und die Charakteristika ihrer Populationen werden stark von den Umweltbedingungen beeinflusst, denen sie ausgesetzt sind. Als Reaktion auf Umweltveränderungen können Populationen in neue Gebiete wandern, phänotypische Plastizität zeigen und sich evolutionär anpassen. Studien zum Vergleich von Populationsmerkmalen entlang von Höhen- und Breitengradienten bieten die Möglichkeit, den Einfluss verschiedener Umwelt- und historischer Faktoren auf die Merkmale von Pflanzenpopulationen zu bewerten. Zur Untersuchung der Plastizität und des evolutionären Potenzials einer Pflanze in Reaktion auf Umweltveränderungen habe ich einen integrativen Ansatz gewählt, der Feldstudien, populationsgenetische Studies und Experimente kombiniert. Die weit verbreitete Pflanze *Anthyllis vulneraria* wurde als Modellart ausgewählt, weil sie in Europa in verschiedenen Höhenlagen und über viele Breitengrade verbreitet ist. Insbesondere untersuchte ich entlang von Höhen- und Breitengradienten (1) die Merkmale der Lebensräume und Populationen von *A. vulneraria*, (2) die neutrale molekulargenetische Vielfalt und Differenzierung der Populationen, (3) die quantitative genetische Differenzierung und das evolutionäre Potenzial der Populationen. Der Höhengradient in den Alpen reichte von 500 m bis zur Höhengrenze auf 2500 m und der Breitengradient erstreckte sich über 2400 km von Mitteleuropa bis zur Verbreitungsgrenze im Norden. Die Länge der beiden Gradienten wurde so gewählt, dass sie einer ähnlichen Veränderung der Jahresmitteltemperatur von ca. 11,5 °C entsprach.

Ich untersuchte die Eigenschaften der Lebensräume von 40 Populationen von *A. vulneraria* entlang von Höhen- und Breitengradienten und untersuchte deren Einfluss auf die Eigenschaften der Populationen. Die Größe der Pflanzen und ihre Reproduktion nahmen entlang beider Gradienten ab, aber die Dichte der Pflanzen nahm zu, was auf eine höhere Rekrutierung und einen Ausgleich zwischen verschiedenen demographischen Prozessen hindeutet. Die Ergebnisse stützen die Ansicht, dass ein demographischer Ausgleich bei weit verbreiteten Arten häufig sein könnte. Es wurde festgestellt, dass Temperaturschwankungen die stärksten Auswirkungen auf verschiedene Populationsmerkmale entlang beider Gradienten haben, gefolgt von Unterschieden in Niederschlag, Sonneneinstrahlung und Bodennährstoffen. Der Anteil der blühenden Pflanzen, der Samenansatz und die Masse der Samen nahmen mit dem Breitengrad ab, was auf limitierende Ressourcen und reduzierte Bestäubung im Norden hindeutet, während die große Variation dieser Merkmale entlang des Höhengradienten nicht mit der Höhe oder damit korrelierten Umweltvariablen, sondern mit starken lokalen Unterschieden in den Umweltbedingungen in den alpinen Lebensräumen zusammenhing.

Ich untersuchte die molekulargenetische Vielfalt und Differenzierung der *A. vulneraria* Populationen anhand von Mikrosatelliten, die als neutrale Marker gelten, die von der natürlichen Selektion unbeeinflusst sind. Die genetische Vielfalt von Populationen nahm mit dem Breitengrad stark ab und die Differenzierung zwischen ihnen nahm zu. Diese Muster sind wahrscheinlich das Ergebnis von seriellen Gründereffekten während der Ausbreitung nach Norden infolge des Gletscherrückgangs nach der letzten Eiszeit. Die genetische Vielfalt und Differenzierung zwischen den Populationen von *A. vulneraria* hing nicht mit der Höhenlage zusammen. Diese Beobachtung könnte auf einen höheren Genfluss zurückzuführen sein, der durch den viel kürzeren Höhengradienten begünstigt wird. Ich fand jedoch Belege für eine Isolierung durch Entfernung entlang beider Gradienten, was auf einen eingeschränkten Genfluss zwischen den Populationen entlang beider Gradienten hindeutet. Die subarktischen Populationen unterschieden sich genetisch von den alpinen Populationen, was darauf hindeutet, dass die nördlichen Populationen nicht aus den hochgelegenen alpinen Populationen hervorgegangen sind.

Pflanzen, die aus Samen der untersuchten Populationen angezogen wurden, wurden in einem gemeinsamen Garten untersucht, um die quantitative genetische Differenzierung und phänotypische Plastizität dieser Populationen entlang von Höhen- und Breitengradienten zu bewerten und ihr evolutionäres Potenzial zu untersuchen. Die im Gemeinschaftsgarten gemessenen Merkmale wiesen eine klinale Variation mit der Höhe und dem Breitengrad des Herkunftsortes auf, was auf eine adaptive Differenzierung der Merkmale entlang der Gradienten hindeutet. Divergente Selektion wurde durch höhere Q_{ST}-Werte (Messung der quantitativen genetischen Differenzierung) als F_{ST}-Werte (Messung der neutralen molekulargenetischen Differenzierung) bei einigen Merkmalen angezeigt. Außerdem stellte ich fest, dass die Unterschiede in der Größe zwischen Müttern und ihren Nachkommen bei Populationen aus höheren Lagen und Breitengraden geringer waren, was auf eine geringere Plastizität in diesen Populationen schließen lässt. Die quantitative genetische Variation der meisten Merkmale nahm jedoch nicht entlang der beiden Gradienten ab, was darauf hindeutet, dass das Evolutionspotenzial in peripheren Populationen nicht verringert ist.

Zusammenfassend hat diese Arbeit deutlich gemacht, wie wichtig es ist, Felderhebungen, populationsgenetische Untersuchungen und ökologische Experimente zu kombinieren, um die Plastizität und genetische Variation als Reaktion von Arten auf unterschiedliche Umweltbedingungen zu untersuchen. Ein tieferes Verständnis dafür, wie sich Pflanzenpopulationen an veränderte Umweltbedingungen anpassen, ist entscheidend für die

Vorhersage ihrer künftigen Reaktion auf den Klimawandel. Die Ergebnisse deuten darauf hin, dass das „abundant centre model“ (ACM) die beobachteten Unterschiede in den Merkmalen der Populationen und die genetischen Muster bei *A. vulneraria* nicht vollständig erklären kann. Andere Faktoren wie historische Migration und lokale Anpassung, die durch einen unterschiedlichen Selektionsdruck entlang der Höhen- und Breitengradienten angetrieben werden, könnten eine bedeutendere Rolle bei der Verbreitung der Art und der Ausbildung der Merkmale der Art in verschiedenen Umwelten gespielt haben und ihre künftigen Reaktionen auf den Klimawandel beeinflussen. Während die geringere phänotypische Plastizität der arktischen und alpinen Populationen von *A. vulneraria* möglicherweise nicht ausreicht für ihr Überleben, könnte ihre genetische Variabilität in fitnessrelevanten Merkmalen ihre Anpassung an sich veränderte Umweltbedingungen ermöglichen.

GENERAL INTRODUCTION

Background

A major factor determining the distribution of plants is the variation of the environment with elevation and latitude (von Humboldt and Bonpland 1805; Woodward 1987; De Frenne et al. 2013). In response to environmental changes, populations can migrate to new locations, which involves the dispersal of propagules and successful establishment in previously unoccupied sites (Jump and Peñuelas 2005). With ongoing climate change, species are expected to migrate polewards or upwards (Parmesan and Yohe 2003; Root et al. 2003). When exposed to novel environmental conditions, plants can also exhibit phenotypic plasticity and undergo evolutionary adaptation. The importance of both plasticity and adaptive evolution for the response to environmental variation has been emphasized in addressing the challenges of climate change and planning conservation strategies (Davis and Shaw 2001; Atkin et al. 2006; Nicotra et al. 2010; Parmesan and Hanley 2015; Capblancq et al. 2020; Buckley and Kingsolver 2021).

The ability of plants to exhibit phenotypic plasticity is a crucial strategy for coping with environmental variability (Jump and Peñuelas 2005; Valladares et al. 2007). Through plasticity, plant species can adjust their response to different environmental conditions by expressing different reversible phenotypes (Piersma and Drent 2003; Atkin et al. 2006). However, there are limits to the capacity of both individuals and species to respond to environmental changes through phenotypic plasticity (de Jong 2005; Valladares et al. 2007). While plasticity enables plants to cope with short-term climate fluctuations within their lifetimes, it may not be sufficient to address the greater changes expected over longer time scales (Gutschick and BassiriRad 2003).

The occurrence of rapid climate change imposes new selection pressures on populations, potentially leading to evolutionary adaptation towards phenotypes that are better suited to the changing conditions (Jump and Peñuelas 2005). Adaptation to climate change can occur through the arrival and establishment of pre-adapted individuals, gene flow from warmer regions within the range of a species, or an increase in the frequency of pre-existing pre-adapted individuals (Jump and Peñuelas 2005). Consequently, maintaining genetic diversity is crucial for preserving the capacity of a species for evolutionary adaptation (Frankham et al. 2017). However, populations adapted to their current local conditions may not necessarily possess the ability to adapt to new conditions, resulting in a decline in fitness (Franks et al. 2014). Studying species with extensive and continuous distributions is essential for understanding the mechanisms of adaptation (Halbritter et al. 2013; Santamaría et al. 2003), because the fragmentation of populations resulting in reduced gene flow and genetic drift can mask clinal patterns in population traits. Studies conducted across environmental gradients can help to identify the relative importance of plasticity and adaptation for the changes observed along these gradients and can reveal selection pressures (Hoffmann and Sgrò 2011).

Temperature, as a critical climatic factor, plays a fundamental role in shaping plant physiology, and its effects can be readily investigated along gradients (Woodward 1987; De Frenne et al. 2013). In temperate regions, changes in temperature over thousands of meters in elevation are equivalent to latitudinal temperature changes over thousands of kilometres (Jump et al. 2009b). Therefore, populations distributed along gradients of elevation and latitude provide an excellent opportunity to examine the influence of temperature and other changing environmental conditions on plant populations. While previous studies have mainly focused on populations along either elevational or latitudinal gradients, there are few comparative studies examining population changes along both types of gradients (Siefert et al. 2015). Moreover, gradients offer the opportunity to test predictions of the abundant centre model of biogeography, which suggests that the environmental suitability for a species decreases from the centre of its distribution towards its range periphery (Brown 1984; Sagarin and Gaines 2002). Consequently, populations closer to the range limits are expected to have a lower performance and become scarcer, smaller, and more isolated compared to those at the centre of the range.

To investigate plasticity and the potential of adaptation in response to environmental variation in this thesis an integrative approach incorporating ecological and genetic methods was used. A combination of field surveys, population genetic studies, and ecological experiments was used to gain better insight into the potential impacts of environmental changes on plant populations. The importance of employing complementary methods to study local adaptation has been emphasized (De Kort et al. 2014; Lepais and Bacles 2014; Sork et al. 2013). Field studies provide detailed information on habitat variables and the suitability of sites for a species. They also offer insights into demographic processes in the populations of a species and fitness patterns across its range. Molecular genetic analyses provide information about genetic drift inbreeding and gene flow between populations, which is crucial knowledge to preserve the genetic diversity and evolutionary potential of species (Frankham et al. 2017). These analyses also serve as a foundation for comparing F_{ST} (neutral molecular genetic differentiation) and Q_{ST} (quantitative genetic differentiation) to detect adaptive differentiation due to selection across environmental gradients. To investigate quantitative genetic differentiation and phenotypic plasticity, individuals from different populations were grown in a common environment, allowing the quantification of phenotypic differences among them (Linhart and Grant 1996; Briggs and Walters 1997). Common garden experiments facilitate the study of the genetic foundations of complex traits and their evolutionary potential and can indicate whether plant populations are locally adapted to climate conditions (de Villemereuil et al. 2016).

Outline of the thesis

This thesis aims to examine the effects of elevation, latitude and covarying environmental variables like annual mean temperature on population characteristics, molecular and quantitative genetic variations of the widespread plant species *Anthyllis vulneraria*. *A. vulneraria* was selected as the model species due to its extensive distribution across elevations and latitudes in Europe. Populations were sampled along two large gradients to investigate local adaptation and evolutionary potential across a broad climatic range. Additionally, the study aimed to test various predictions derived from the abundant centre model of biogeography, which offers insights into how population traits change across the distributional range of a species.

The thesis consists of three studies:

Chapter 1 "Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*" investigates population and habitat characteristics of 40 *A. vulneraria* population along an elevational gradient in the Alps from 500 m to the elevational limit at 2500 m and a latitudinal gradient from Central Europe to the range limit in the North. Both gradients encompassed a similar range in annual mean temperature of c. 11.5 °C. The study analyses the correlations between habitat variables and elevation or latitude and examines the effects of elevation and latitude on population characteristics such as plant size, reproduction, population size, plant density, population structure, seed set, and seed mass. The study also explores the influence of the environmental variables temperature, precipitation, solar radiation, and soil nutrients on population characteristics.

This study was published as: Daco L, Colling G, Matthies D (2021). Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*. Oecologia 197(2), 537-549. https://doi.org/10.1007/s00442-021-05030-6

Chapter 2 "Genetic diversity and differentiation of populations of *Anthyllis vulneraria* along elevational and latitudinal gradients" focuses on the genetic diversity and differentiation of the same 40 *A. vulneraria* populations that were studied in Chapter 1. Using 17 microsatellite markers, the study investigates the genetic diversity within populations and the genetic differentiation among populations along the elevational and latitudinal gradients. The influence of gene flow, genetic drift, population size, geographical distance, and differences in elevation on the genetic distance between populations are also analysed.

This chapter was published as: Daco L, Matthies D, Hermant S, Colling G (2022). Genetic diversity and differentiation of populations of *Anthyllis vulneraria* along elevational and latitudinal gradients. Ecology and Evolution 12(8), e9167. https://doi.org/10.1002/ece3.9167

Chapter 3 "Quantitative genetic differentiation and evolutionary potential in the widespread plant *Anthyllis vulneraria* along elevational and latitudinal gradients" examines quantitative traits of progeny originating from plants from the 40 populations studied in chapters 1 and 2. Seeds from 20 families of each population were grown in a common garden in Luxembourg, under in general more benign conditions than in the populations of origin. The effects of elevation and latitude of origin on survival, fitness-related and phenological traits, and selfing ability were analysed to detect adaptive differentiation along the elevational and latitudinal gradients. Quantitative genetic differentiation (Q_{ST}) between populations was compared to neutral molecular genetic differentiation (F_{ST}) between populations to identify signals of diversifying selection in specific traits. The study also compares phenotypic measurements taken in the common garden with the ones taken on the mother plants in the field to assess the plastic responses of the plants. The evolvability of quantitative traits for plants from the populations along the elevational and latitudinal gradients was investigated to examine the evolutionary potential of peripheral populations.

CHAPTER 1

Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*

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LD, GC and DM conceived the study, analysed the data and prepared the manuscript; LD conducted field work and collected the data. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Widespread plants may provide natural models for how population processes change with temperature and other environmental variables and how they may respond to global change. Similar changes in temperature can occur along altitudinal and latitudinal gradients, but hardly any study has compared the effects of the two types of gradients. We studied populations of *Anthyllis vulneraria* along a latitudinal gradient from Central Europe to the range limit in the North and an altitudinal gradient in the Alps from 500 m to the altitudinal limit at 2500 m, both encompassing a change in annual mean temperature of c. 11.5 °C. Plant size and reproduction decreased, but plant density increased along both gradients, indicating higher recruitment and demographic compensation among vital rates. Our results support the view that demographic compensation may be common in widespread species in contrast to the predictions of the abundant centre model of biogeography. Variation in temperature along the gradients had the strongest effects on most population characteristics, followed by that in precipitation, solar radiation, and soil nutrients. The proportion of plants flowering, seed set and seed mass declined with latitude, while the large variation in these traits along the altitudinal gradient was not related to elevation and covarying environmental variables like annual mean temperature. This suggests that it will be more difficult to draw conclusions about the potential impacts of future climate warming on plant populations in mountains, because of the importance of small-scale variation in environmental conditions.

Keywords

Abundant centre model, climate change, environmental gradients, herbivory, seed set.

Introduction

Temperature is an important determinant of plant physiology and distribution (Woodward 1987; De Frenne et al. 2013). Climatic factors such as low temperatures and short growing seasons typically constrain plant growth and cause absolute limits to plant growth in arctic and alpine environments (Woodward 1987; Körner 2003; Halbritter et al. 2013). According to the altitude-for-latitude temperature model, similar changes in annual mean temperature of c. 5 °C occur over 1000 m of altitude and 1000 km of latitude in the temperate zone (Jump et al. 2009b). Studies of population traits along gradients of altitude and latitude with comparable changes in temperature provide an opportunity to study the influence of temperature and other environmental factors on plant population characteristics and structure. The study of natural variation in ecologically important traits is crucial to further increase our knowledge of the mechanisms involved in the distribution and abundance of plant species (Jump et al. 2009b).

Global warming is expected to affect the range and abundance of species in the future (Walther 2003; Thomas 2010; Swab et al. 2015). The global mean surface temperature is projected to increase during this century by up to 4.8 °C (IPCC 2014). An improved understanding of how climatic conditions influence plants is important for assessing the response of plants to global change and to conserve threatened plant species under changing climatic conditions. Plant species with populations occurring along strong gradients in altitude or latitude and corresponding strong changes in temperature may provide natural models for how population processes change with temperature (Halbritter et al. 2013).

However, altitudinal and latitudinal gradients differ in a number of factors including atmospheric pressure, precipitation, solar radiation, and soil nutrients (Körner 2007; De Frenne et al. 2013). The response of species across the two types of gradients may thus be expected to differ in potentially important aspects. While many studies have investigated different processes in plant populations along either altitudinal (Totland 2001; Pellissier et al. 2010; Rasmann et al. 2014) or latitudinal gradients (Sagarin et al. 2006; Moeller et al. 2017), there are hardly any studies that have compared the effect of the two types of gradients on populations of a single species (Siefert et al. 2015). Moreover, most studies did not try to disentangle the effects of temperature on plants from those of other environmental factors (De Frenne et al. 2013).

The aim of this study was to compare populations of *Anthyllis vulneraria* L. (Fabaceae) along two temperature gradients of c. 10 °C, a latitudinal gradient of c. 2400 km from Central Europe to northern Norway and three altitudinal gradients of c. 2000 m in the European Alps. We analysed climatic conditions using data from Worldclim (Fick and Hijmans 2017), soil nutrients and site productivity and investigated characteristics of the populations including their size and density, size-related plant traits, reproduction, pre-dispersal seed predation, and population structure. The altitudinal gradient spanned the species' range from lowland sites to populations at the altitudinal limit and the latitudinal gradient ranged from the geographical centre of the distribution to the northern distribution limit. These gradients allowed us to test some of the predictions of the abundant centre model (ACM; Brown 1984; Sagarin and Gaines 2002) of biogeography. The ACM predicts that the abundance and performance of species at the geographical periphery of their distribution area are lower than those at the centre of their range where environmental conditions are assumed to be most suitable (Abeli et al. 2014; Pironon et al. 2017).

We address the following questions: (1) How do population characteristics of *A. vulneraria* vary along the two gradients and do altitude and latitude have different effects? (2) Which environmental variables in addition to temperature are important predictors of population characteristics along altitudinal and latitudinal gradients? (3) Are characteristics of the populations along both gradients in line with the predictions of the ACM?

Material and methods

Study Species

Anthyllis vulneraria is a very polymorphic biennial or perennial occurring in nutrient-poor calcareous grasslands and screes all across Europe, the Mediterranean Basin and the Caucasus (GBIF.org 2019) from sea level up to 3000 m a.s.l. (Conert 1975). In total, 24 infraspecific taxa have been described in Europe (Cullen 1968). However, a phylogenetic study based on ITS sequences and chloroplast microsatellites showed that all subspecies of *A. vulneraria* clustered together and thus did not support the traditional taxonomic subdivisions based on morphology (Nanni et al. 2004). Moreover, AFLP variance in a study of eight taxa of *A. vulneraria* did not support recognizing intraspecific taxa of *A. vulneraria* at the species or subspecies level (Köster et al. 2008). In contrast to earlier studies which argued that subspecies do not interbreed due to the predominantly autogamous reproductive system (Couderc 1971; Couderc and Gorenflot 1978) more recent studies based on molecular markers found that there is considerable gene flow among and within populations (Honnay et al. 2006; Van Glabeke et al. 2007; Kesselring et al. 2015) indicating that the populations are not predominantly selfing. In the present study, we will therefore not distinguish between putative subspecies a priori to capture a large amount of variation over the whole study area. The basal leaves of *A. vulneraria* form rosettes and the flowers are grouped into flowerheads with four to more than 20 flowers. Plants usually start flowering in the second year after germination, but flowering can be delayed for up to eight years (Erschbamer and Retter 2004). Most plants are monocarpic but some may flower more than once (Sterk 1975). Flowers are pollinated by Hymenoptera (Couderc and Gorenflot 1978) and produce a single seed per fruit. An important specialist herbivore of *A. vulneraria* are the caterpillars of *Cupido minimus* (Lepidoptera) which feed on the developing seeds (Krauss et al. 2004). Although *A. vulneraria* is not considered to be endangered in most parts of its distribution, it has strongly declined in some areas in the last decades, e.g. in NE Germany (Jansen et al. 2019).

Altitudinal and latitudinal gradients

We studied the influence of environmental conditions on *A. vulneraria* in 40 populations along an altitudinal and a latitudinal gradient. The length of the two gradients was chosen to correspond to a change of 11.5 °C in annual mean temperature. The latitudinal gradient ranged from the centre of the distribution in Central Europe (46.4 °N) over 2400 km to the northern distribution limit in Scandinavia (68.1 °N) and the altitudinal gradient from valley populations in the Alps at 500 m to the altitudinal limit at 2500 m a.s.l. To account for potential regional climatic differences, the altitudinal gradient was replicated in three different alpine regions in France, Switzerland and Austria.

Field data

Based on information gathered from local botanists and the GBIF database (GBIF.org 2019), we sampled 20 *A. vulneraria* populations each along the altitudinal and the latitudinal gradients (Fig. 1; Appendix Table S1). A population was defined as a group of plants separated by at least 100 m from the next conspecific plants. During summer 2015, we recorded at each site the altitude above sea level, latitude and longitude with a GPS (eTrex 20, Garmin Ltd.), site exposition as absolute deviation from the north with a compass and inclination with a clinometer. Populations were sampled at a similar phenological state, i.e. southern and lowland populations were sampled first. In small populations, all plants of *A. vulneraria* were counted. In large populations, the population size was estimated from counts in parts of the total area. To estimate site productivity, we sampled in each population the above-ground biomass in two randomly chosen plots (25 cm x 25 cm) in which *A. vulneraria* was present. The vegetation was clipped at 5 cm above soil level, dried at 60 °C for 48 h to constant mass and weighed. At each site, we took at least three random samples of a total of at least 300 g of mineral soil. Soil organic carbon was determined by subtracting the inorganic carbon content measured with phosphoric acid from the total carbon content determined by dry combustion at 1200 °C with a Multi EA 4000 (Analytik Jena, Jena, Germany). Total nitrogen content was determined by dry combustion at 900 °C with an elemental analyzer (Vario MAX cube, Elementar, Langenselbold, Germany). The pH was measured in a 0.01 M solution of $CaCl_2$ with a SP2000 pH soil analyser (Skalar Analytical, Breda, Netherlands). Phosphorus (P_2O_5) and potassium

(K_2O) were extracted with a 0.05M calcium lactate and acetate solution and the contents were dosed by inductively coupled plasma optical emission spectrometry (Agilent 725 ICP-OES Radial, Santa Clara, USA).

In each population, we selected one plot of 1 m x 1 m in an area which appeared to have the highest density of *A. vulneraria* and two further plots located at random but with *A. vulneraria* present. In each plot, we counted the number of flowerheads of *A. vulneraria* and of flowering plants and calculated the mean number of flowerheads per plant and the mean per population. In the three plots, we determined the sum of the number of vegetative and of flowering *A. vulneraria* individuals and calculated the proportion of flowering plants per population.

In each population we collected all flowerheads from 20 fruiting plants along a 20 m long transect. We determined the height for each of these plants. In the laboratory, we determined for each mother plant the number of healthy seeds (green and large), fully developed but damaged seeds with clear signs of herbivory, and aborted seeds (small, brown or light green). We calculated seed set per population as [number of intact and damaged seeds collected / total number of seeds including aborted], and the proportion of damaged seeds as [sum of damaged

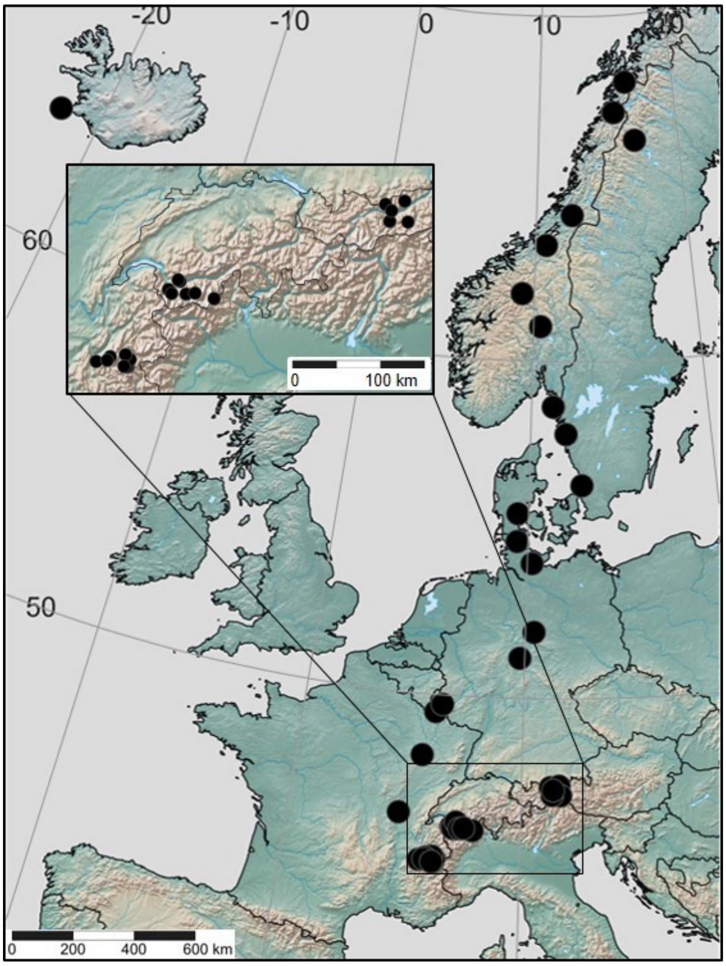

Fig. 1 Map of the *Anthyllis vulneraria* study sites in Europe. The inset shows the locations of the populations of the altitudinal gradient in France, Switzerland and Austria.

seeds / total number of seeds]. The presence of eggs of *Cupido minimus* in the collected flowerheads was recorded for each population. For each mother plant, the mean mass of healthy seeds was determined and then averaged to calculate mean seed mass per population.

Bioclimatic variables

For each population, we downloaded three climatic variables from the WorldClim database Version 2.0 (Fick and Hijmans 2017) in a 30 arc-seconds (1 km^2) resolution: annual mean temperature, annual precipitation, and solar radiation.

Statistical analyses

If not stated otherwise, all statistical analyses were carried out using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). To study the environmental changes with latitude and altitude, we analysed the relationships between altitude or latitude and annual mean temperature, annual precipitation, solar radiation, standing biomass, soil organic carbon content, nitrogen, phosphorus, and potassium content with linear regressions.

The effects of altitude and latitude on population means of morphological and reproductive traits were tested with general and generalized linear models (GLMs) in R 3.5.2 (R Core Team 2018). These analyses investigated also whether mean plant performance declined continuously from the centre of the distribution to the range margins at high latitudes and altitudes as predicted by the abundant centre model. The GLMs for proportion data (e.g. proportion flowering, seed set, seeds damaged) were calculated with a logit link and a quasibinomial error distribution (see Crawley 2009). For the altitudinal gradient, the effects of the three regions within the Alps and the linear and quadratic effects of altitude as well as the interaction between the region and the linear and the quadratic terms were tested. The quadratic term was integrated to check for a potential mid-elevational maximum along the gradient. Similarly, linear and quadratic effects of latitude were also tested; however, the quadratic terms were never significant. Models were simplified by dropping non-significant terms ($P > 0.05$). For proportion data, McFadden's Pseudo r^2 was calculated as one minus the ratio between the log-likelihood of the model of interest and the log-likelihood of the null model. When region had a significant effect (only in the case of maximum density), the r^2 of the model for each region was calculated by correlating the predicted values from the overall model and the observed values. To study the possible effects of altitude or latitude, population size and maximum plant density on the presence of eggs of *Cupido minimus,* we carried out separate logistic regressions. Due to overdispersion, we used quasibinomial error distributions in the GLMs. We compared the strength of the relationships of mean values of population traits and altitude with the relationship of the same traits with latitude with *Z*-tests using the R-package cocor (Diedenhofen and Musch 2015).

We performed a principal component analysis (PCA) based on correlations with soil organic carbon, total nitrogen and phosphorus (P_2O_5) contents to characterize soil conditions at the study sites. The resulting PCA factor (PC Soil nutrients) explained 77.8 % of the total variance of the component variables and was used in multiple regressions as an explanatory environmental variable. Factor loadings were 0.96 for N_{tot}, 0.79 for P_2O_5, and 0.89 for C_{org}. To elucidate which environmental variables might be responsible for the observed relationships between certain population characteristics and latitude or altitude, we performed (generalized) linear models with normal or quasibinomial errors relating these characteristics to the following explanatory variables: annual mean temperature, annual precipitation, solar radiation, PC Soil nutrients, standing biomass (see Appendix Table S2). For each plant trait we calculated all possible models and their Akaike information criteria (AICc) or the quasi AICc (QAICc; for proportion data) using the function *dredge* of the R-package MuMIn (Barton 2019). To assess the importance of the individual predictors we averaged all possible models including these predictors (conditional average, Burnham and Anderson 2002) and derived importance values. In addition, we selected the model with the lowest AICc or QAICc. The r^2 of those models was calculated by correlating the predicted and the observed values for the Gaussian data and by calculating Mc Fadden's pseudo r^2 in the case of proportion data. We calculated standardized regression coefficients for the variables in these models. Standardized regression coefficients for the GLMs for proportion data were calculated using the latent-theoretical method (Grace et al. 2018).

In the analyses, data for population size, maximum plant density and mean number of flowerheads per plant were log-transformed prior to analysis to achieve normally distributed residuals and homoscedasticity. Population AAt5 was excluded from most of the analyses due to missing data (except for population size, max. plant density, seed set, seeds damaged and seed mass).

Results

Habitat characteristics and gradients

Most study sites had a southern exposition (median = 114.5 ° deviation from north) indicating that *A. vulneraria* prefers well-exposed habitats (Appendix Table S3). *Anthyllis vulneraria* was present at both level sites and slopes of up to 103 % inclination. Standing biomass was generally low (median 96.1 g m^{-2}) indicating little competition for light. Soils were slightly acidic to neutral (median pH = 7.1) and the nutrient content was generally low (medians: N_{tot} = 0.2 %, P_2O_5 < 3.0 mg/100 g soil, K_2O = 8.0 mg/100 g soil, C_{org} = 3.2 %). The range of annual mean temperatures at the study sites was similar for both the altitudinal (-0.4 °C to 10.8 °C) and the latitudinal gradients (-1.2 °C to 10.8 °C). Annual mean temperature decreased with both increasing altitude and latitude (Table 1, Appendix Fig. S1).

Several other environmental variables changed along the gradients of altitude and latitude (Table 1). With increasing altitude precipitation increased and standing biomass decreased, while with increasing latitude solar radiation, soil N, P and organic C decreased. Potassium (K) soil content did not vary along the gradients.

Table 1 Correlations between altitude and various habitat characteristics for the 20 altitudinal populations and latitude and various habitat characteristics for the 20 latitudinal gradient populations separately. (*) *P* < 0.1; * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001. [a]Sample size N = 19.

Habitat variable		Altitude	Latitude
		r	*r*
Annual mean temperature (°C)		-0.98 ***	-0.87 ***
Annual precipitation (mm)		0.63 **	0.28
Solar radiation (kJ m^{-2} day^{-1})	All sites	0.33	-0.95 ***
	France	0.99 ***	
	Switzerland	0.94 **	
	Austria	0.82 (*)	
Standing biomass (g m^{-2})		-0.40 (*) [a]	0.36
Organic carbon soil content (%)		0.06 [a]	-0.49 *
Nitrogen soil content (%)		0.14 [a]	-0.57 **
Phosphorus soil content (mg/100 g soil)		0.17 [a]	-0.48 *
K_2O soil content (mg/100 g soil)		-0.07 [a]	-0.33

Population characteristics and mean plant traits along the altitudinal and latitudinal gradients

The relationship between maximal plant density and altitude was best fitted by quadratic functions (Fig. 2a, Appendix Table S4). While in the Austrian and French Alps these functions indicated that plant density was highest at c. 1700 m, in Switzerland plant density increased up to 2500 m. However, the decline in density above 1800 m in Austria and above 2000 m in France was in both regions due to only a single population. Maximum plant density in the populations increased with latitude (Fig. 2b). In the 40 studied populations the number of plants and the maximum density were positively correlated ($r = 0.43$, $P < 0.01$). Population size varied along the altitudinal gradient from 80 - 30 000 individuals and along the latitudinal gradient from 50 - 10 000. The size of the populations increased significantly with altitude (Fig. 2c) but not with latitude (Fig. 2d). However, the difference in the strength of the relationships was not significant.

Plant height decreased with both altitude and latitude indicating that both gradients were limiting plant growth, but altitude was a much better predictor (Fig. 2e, f). In contrast, the proportion of flowering plants did not vary consistently with altitude (Fig. 2g), but strongly decreased with latitude (Fig. 2h). The number of flowerheads per plant decreased from c. nine in the low altitude to four in the high altitude populations (Fig. 3a) and from 17 in the Central European to five in the arctic populations (Fig. 3b). The mean number of flowerheads per plant was related to mean plant size in the 40 populations as it increased with plant height ($r = 0.52$, $P < 0.001$).

Seed set decreased with latitude (Fig. 3d), but there was no relationship with altitude (Fig. 3c). However, the difference between the two correlation coefficients was only significant at the 0.08 level. The proportion of seeds damaged by herbivory decreased with both altitude and latitude (Fig. 3e, f). We detected eggs of *Cupido minimus* in 27 of the 40 sites but did not find any significant relationships between the probability of their presence and altitude ($P = 0.96$), latitude ($P = 0.50$), population size (Alt.: $P = 0.42$; Lat.: $P = 0.10$) or density of *A. vulneraria* (Alt.: $P = 0.66$; Lat.: $P = 0.19$). There was no clear relationship between mean seed mass and altitude (Fig. 3g), while there was some support ($P = 0.068$) that seed mass decreased with latitude (Fig. 3h). The difference between the two correlation coefficients was only significant at the 0.07 level.

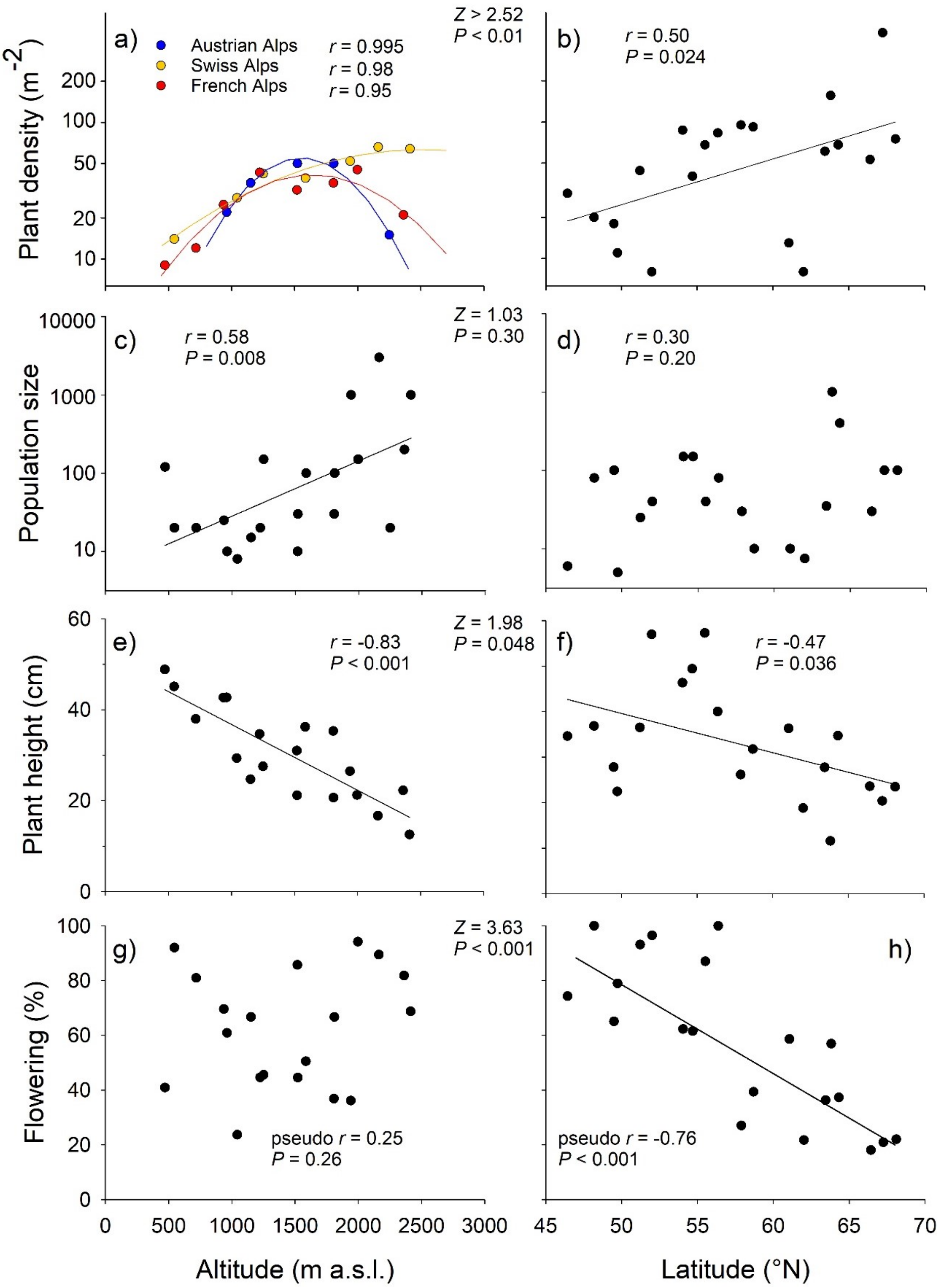

Fig. 2 Relationships between characteristics of populations of *A. vulneraria* with altitude and latitude. For the altitudinal gradient, the effects of the three regions within the Alps were significant only in the case of maximum density (panel a) resulting in distinct models for each region. The results of *Z*-tests shown in between the panels indicate the significance of differences in the two correlation coefficients showing the strength of the relationship between a population trait and altitude or latitude. The *Z*-tests between the correlation coefficients of maximum density and altitude or latitude were calculated for the three regions separately.

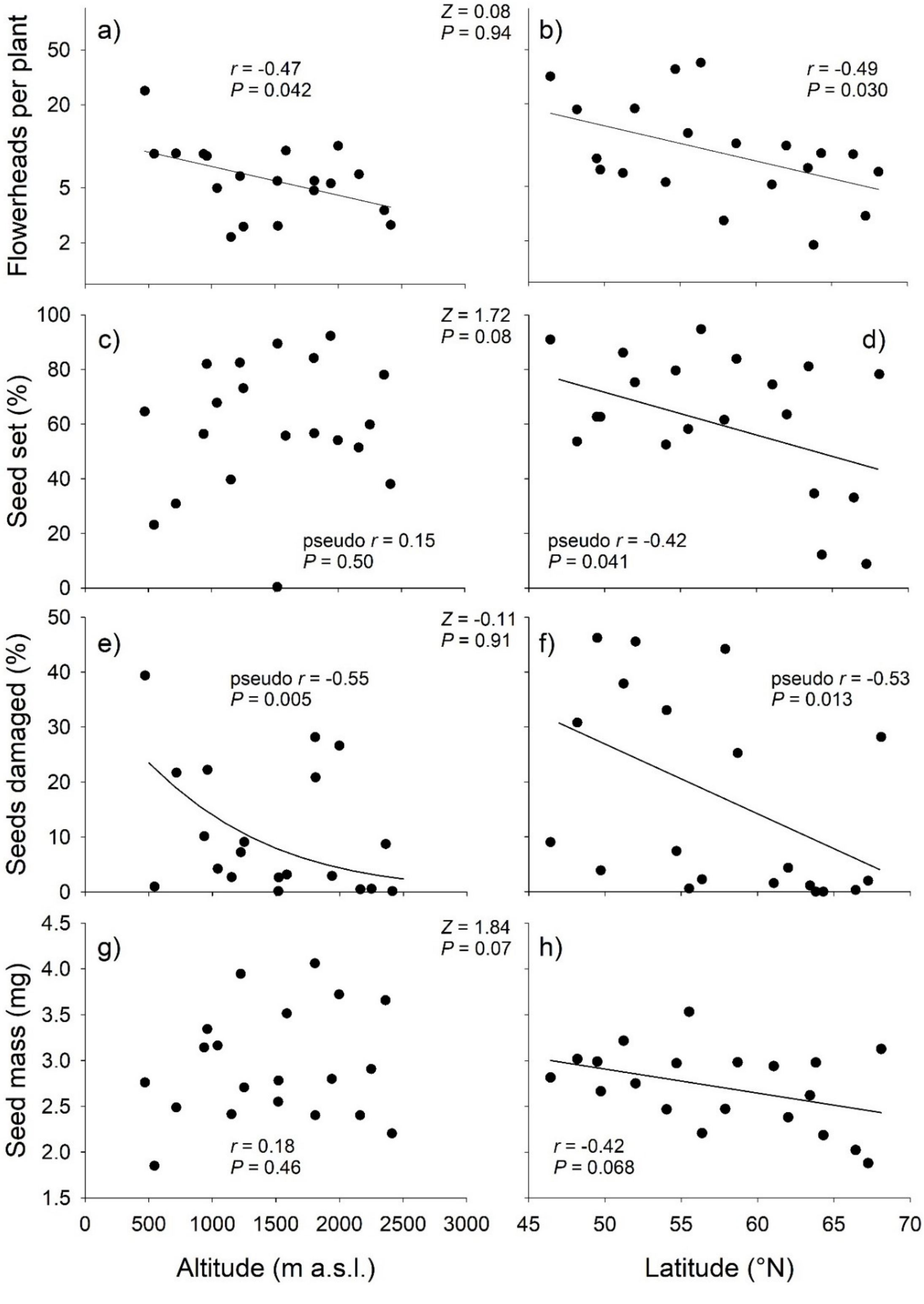

Fig. 3 Relationships between mean reproductive traits in populations of *A. vulneraria* with altitude and latitude. A regression line was added if $P < 0.1$. The results of *Z*-tests shown in between the panels indicate the significance of differences in the two correlation coefficients showing the strength of the relationship between a population trait and altitude or latitude.

Influences of habitat characteristics on mean population traits

Annual mean temperature was the environmental factor that was most important in the models for six out of eight mean population traits along the altitudinal gradient (Fig. 4, Appendix Table S5). Along the latitudinal gradient, temperature had also the highest importance values for most traits. Precipitation had overall the second highest importance. In the best models with the lowest AICc (Appendix Table S6) mean plant performance in terms of growth and reproduction declined in parallel with temperature along both gradients, but density and population size increased with lower temperatures in the higher regions of the Alps. Precipitation, soil nutrients and solar radiation were further environmental factors that influenced some plant traits, although mostly in addition to temperature while standing biomass was never part of the best model.

Discussion

We studied mean population traits of *A. vulneraria* along a latitudinal gradient that extended from the centre of the distribution of the species in Central Europe to its northern distributional margin, and along an altitudinal gradient that ranged from the lowlands to the altitudinal limit of the species in the Alps. Plant size, reproduction and density reacted similarly to changes in altitude and latitude and showed clinal variation, potentially allowing to assess the response of populations to predicted warming of the climate. However, some reproductive components like seed set and mass and the proportion of plants flowering responded differently to the two gradients and decreased with latitude but not with altitude indicating that the effects of climate warming on plant populations are likely to differ along the two gradients.

The abundant centre model predicts that all vital rates of a species like survival, reproduction, growth and recruitment are highest at the centre of the distribution of a species, where environmental conditions are assumed to be most suitable and decline towards the periphery (Brown 1984, Pironon et al. 2017). The reduced size and reproduction of *A. vulneraria* in peripheral northern and alpine populations were in line with the decline in plant performance towards the periphery predicted by the ACM (see e.g. also Jump and Woodward 2003; Angert 2006; Vaupel and Matthies 2012). However, only 43% of the studies reviewed by Pironon et al. (2017) found a significant decline of reproduction towards the periphery, while a further 21% found no clear pattern. In contrast to size and reproduction, the density of *A. vulneraria* increased along both the altitudinal and latitudinal gradients, indicating that recruitment must be much higher in peripheral high altitude and high latitude populations to more than

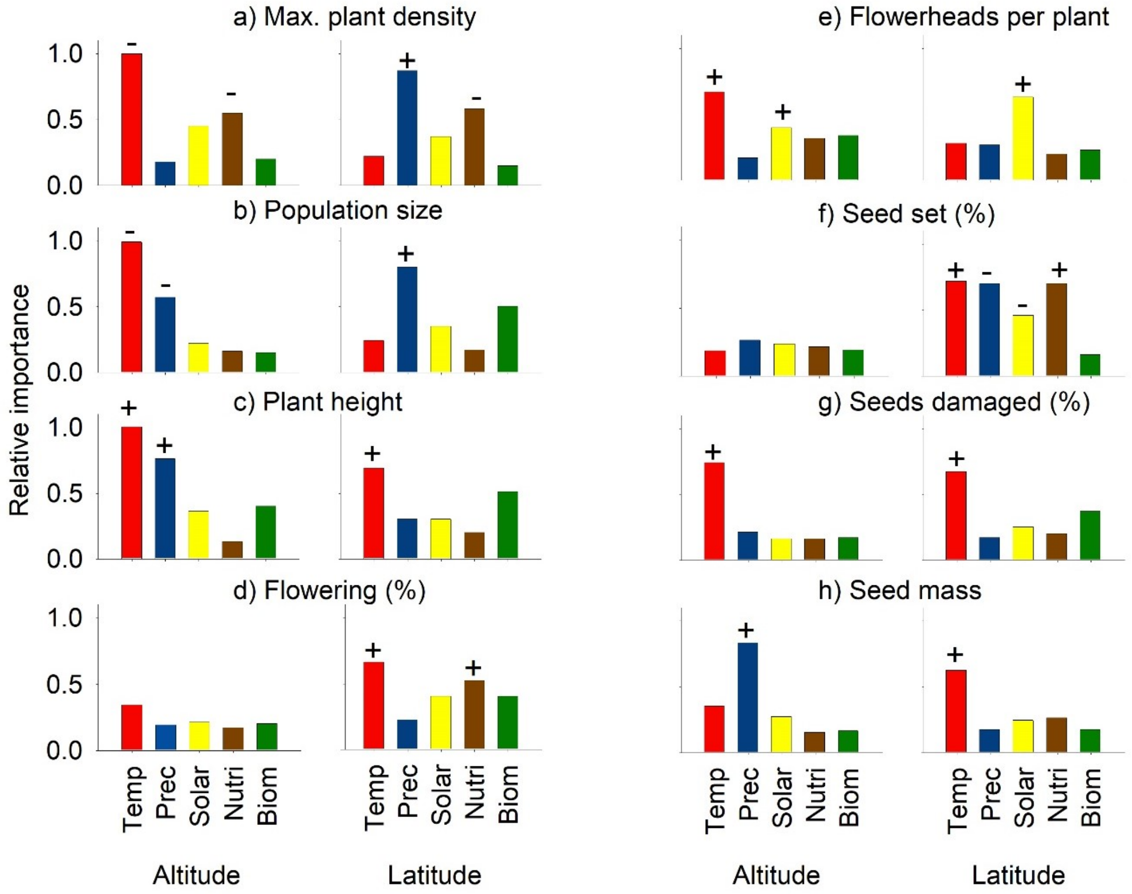

Fig. 4 The effects of annual mean temperature (Temp), annual precipitation (Prec), solar radiation (Solar), PC soil nutrients (Nutri), and standing biomass (Biom) on mean population characteristics of *Anthyllis vulneraria* along an altitudinal and a latitudinal gradient. Given are importance values from averaging over all possible models including these variables. For variables that are part of the best model with the lowest AICc (Appendix Table S6) the sign of the regression coefficient is shown: +, positive; -, negative effect. For model details see Appendix Table S5.

compensate for the reduced reproduction. The higher recruitment in peripheral populations could be the result of higher survival of young plants due to the higher precipitation and the lower intraspecific competition between the smaller plants in these populations (see also Villellas et al. 2013 for another short-lived species). The opposite trends in vital rates indicate the existence of demographic compensation between reproduction and recruitment in populations of *A. vulneraria.* Such compensatory changes in vital rates across the range are thought to allow species to occupy larger areas of distribution (Doak and Morris 2010; Villellas et al. 2015). Our results thus contribute to the increasing evidence that in contrast to the predictions of the ACM demographic compensation may be common in widespread species (Villellas et al. 2013, 2015; Peterson et al. 2018).

Plant size and density

The size of plants as measured by their height and their number of flowerheads decreased with both altitude and latitude. A similar pattern has been found in many other species (e.g. Woodward 1986; Weber and Schmid 1998; Hargreaves et al. 2015). In contrast, in some forest herbs plant height increased with latitude (De Frenne et al. 2011b; Acharya et al. 2017). For *A. vulneraria*, the results of the multiple regressions indicated that the reduced size of plants with increasing altitude and latitude is mainly due to the decrease in temperature. Temperature is an important constraint to growth and plant size is known to decrease with temperature and the resulting shorter growing season in many species (Körner 2003; De Frenne et al. 2013).

In contrast to plant size, plant density increased with both altitude and latitude. This could be an effect of reduced intraspecific competition due to the smaller size of plants. Furthermore, the density of *A. vulneraria* increased with lower soil nutrient levels along both gradients. *Anthyllis vulneraria* is a poor competitor (Sterk 1975) and negatively affected by high nutrient levels due to increased competition by other species (Ellenberg et al. 1992).

Seed set and seed mass

The lower seed set and reduced seed mass in high latitude populations of *A. vulneraria* could be due to several factors. First, plant size in *A. vulneraria* decreased with latitude and smaller plants often have lower seed set and smaller seeds due to resource limitation (Matthies 1990). In *A. vulneraria* the decrease in seed set with latitude was related to lower soil nutrients and temperatures, in line with the result of other studies (Moles and Westoby 2003; Wu et al. 2018). Second, the diversity and abundance of insect pollinators are reduced at high latitudes and dominated by Diptera (Elberling and Olesen 1999; Fulkerson et al. 2012). Because the flowers of *A. vulneraria* are exclusively pollinated by Hymenoptera (Couderc and Gorenflot 1978), seed set in the northern populations may have been lower due to a lack of pollen. Third, pollen limitation may result in increased self-fertilization and increased abortion of developing seeds and lower seed mass at higher latitudes.

While seed set and seed mass decreased with latitude, they did not vary consistently with altitude and temperature. As in other plant species (Körner et al. 1989; Guo et al. 2010) the size of plants of *A. vulneraria* declined with altitude, but this did not result in a decline of seed set and mass. Instead, both reproductive components were highly variable at all altitudes, confirming that local environmental variation may strongly influence reproduction in *A.*

vulneraria (Kesselring et al. 2015). Environmental factors may also interact with pollen availability to determine reproduction in alpine environments (Totland 2001). The few other studies that have investigated the relationship between seed set and altitude have found conflicting results (Totland and Birks 1996; Vaupel and Matthies 2012; Hargreaves et al. 2015). Similarly, studies that have investigated how mean seed mass varies with altitude in individual species found also no consistent pattern. In some species, seed mass increased (Holm 1994; Olejniczak et al. 2018), whereas in others it declined (Totland and Birks 1996; Pluess et al. 2005; Guo et al. 2010; Olejniczak et al. 2018), or showed no relationship with altitude (Holm 1994; Pluess et al. 2005; Vaupel and Matthies 2012; Olejniczak et al. 2018).

Seed predation

The seeds of *A. vulneraria* are attacked by several species of specific insect herbivores, e.g. *Cupido minimus*, *Hypera trilineata*, *Tychius* sp., *Bruchophagus* sp. (Sterk et al. 1982). Pre-dispersal seed predation decreased both along the latitudinal and the altitudinal gradient, although population size increased with altitude and plant density increased along both gradients. Larger and denser plant populations are more likely to be found by specific seed predators and to sustain viable populations of them (Kéry et al. 2001; Colling and Matthies 2004; Vaupel and Matthies 2012). In contrast to our results, the level of seed predation would thus have been expected to increase along the two gradients. The lower seed predation at high elevations and latitudes is most likely due to low temperatures and short summers that negatively affect larval development and abundance (Alonso 1999; Hodkinson 2005; Lee and Kotanen 2015). Our results are in line with those of many other studies that found a decrease in seed predation with increasing altitude (Alonso 1999; Giménez-Benavides et al. 2008; Buckley et al. 2019) and the few that studied effects of latitude (Vergeer and Kunin 2011; Lee and Kotanen 2015; but see Anstett et al. 2014). Our results indicate that the positive effects of increased density and larger population size of the host species *A. vulneraria* cannot compensate for the negative effects of the more severe weather conditions on the populations of the seed predators and support the notion of a general decline of the importance of insect herbivory with increasing latitude (Schemske et al. 2009).

Population structure

Anthyllis vulneraria is usually considered a biennial, which in the first year produces a rosette that in the second year flowers, fruits and then dies (Sterk 1975). In the current study, the proportion of flowering plants decreased strongly along the latitudinal gradient. A possible explanation for the high proportion of vegetative plants in the North could be the higher recruitment due to higher soil moisture, leading to an increase in the density of young vegetative plants in combination with a high mortality of vegetative plants over winter (Villellas et al. 2013). Alternatively, plants in the North may stay in a vegetative state for more than one year because flowering is delayed because of lower solar radiation, temperatures and nutrient availability (Lacey 1988; Lempe et al. 2005, but see Vergeer and Kunin 2011). This would increase the proportion of non-flowering plants over multiple growing and germination seasons as they accumulate in the population (Lacey 1988; Becker et al. 2006). In contrast, although similar environmental changes could be expected along the altitudinal gradient, at high altitudes both populations with a low and a high proportion of flowering plants were found. This indicates heterogeneity in local conditions (e.g. snow cover and time of snow melt) at a smaller scale than the Worldclim data that override the effects of general trends in climatic and edaphic conditions. Heterogeneity of small-scale habitat conditions is particularly high in alpine habitats (Körner 2007).

Conclusions

Our study of variation in population mean traits along two gradients from the centre of the distribution of *A. vulneraria* in Central Europe to its range margins in the subarctic North and at the high altitudes in the Alps only partially support the abundant centre model (ACM). The reduced size and reproduction of *A. vulneraria* in peripheral populations were in line with the decline in performance towards the range margins predicted by the ACM. However, the increase of recruitment and plant density towards the range limits contributes to the growing evidence that the general decline in vital rates towards the periphery assumed by the ACM is too simplistic (Abeli et al. 2014; Pironon et al. 2017). Instead, demographic compensation between vital rates, as between reduced reproduction and increased recruitment in *A. vulneraria*, may be an important factor contributing to the large area of distribution and wide altitudinal range of a species (Doak and Morris 2010). The plasticity of the life cycle might also provide some buffering for widespread species against negative effects of climate change (Doak and Morris 2010; Villellas et al. 2015; Peterson et al. 2018, but see Sheth and Angert 2018).

Population characteristics of *A. vulneraria* varied strongly along the altitudinal and latitudinal gradients and environmental conditions influenced mean population traits, population structure and demography. Changes in temperature along the gradients appeared to have by far the strongest effects on the populations, followed by those in other climate variables like precipitation and solar radiation, and in soil nutrients. Increasing altitude and latitude both reduced size-related traits of plants and seed predation, but only with latitude was there a clinal decline in the proportion of plants flowering, and in seed set and seed mass. Observed latitudinal patterns in population characteristics are thus of only limited value to predict changes with altitude. This indicates that it will be more difficult to draw conclusions about potential impacts of future climate warming on plant populations in mountains, because of large local variation in important traits not related to altitude and annual mean temperature, but to small-scale variation in environmental conditions (Scherrer and Körner 2010; Oldfather and Ackerly 2019) and potentially in plant-pollinator interactions (Totland and Birks 1996). However, the actual responses of species to predicted climate warming will also depend on genetic variation among populations and their phenotypic plasticity (Peterson et al. 2018). Our study did not allow to disentangle the effects of phenotypic plasticity, genetic variation and local adaptation. Common garden experiments and molecular genetic analyses will be necessary to identify the mechanisms involved.

CHAPTER 2

Genetic diversity and differentiation of populations of *Anthyllis vulneraria* along elevational and latitudinal gradients

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LD: Conceptualization (equal); data curation (supporting); formal analysis (supporting); funding acquisition (lead); investigation (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). DM: Conceptualization (equal); investigation (equal); supervision (supporting); validation (equal); writing –review and editing (equal). SH: Data curation (lead); formal analysis (lead). GC: Conceptualization (equal); investigation (equal); project administration (lead); supervision (lead); validation (equal); writing –review and editing (equal).

Abstract

The abundant centre model (ACM) predicts that the suitability of environmental conditions for a species decreases from the centre of its distribution towards its range periphery and consequently its populations will become scarcer, smaller and more isolated, resulting in lower genetic diversity and increased differentiation. However, little is known about whether genetic diversity shows similar patterns along elevational and latitudinal gradients with similar changes in important environmental conditions. Using microsatellite markers we studied the genetic diversity and structure of 20 populations each of *Anthyllis vulneraria* along elevational gradients in the Alps from the valleys to the elevational limit (2500 m), and along a latitudinal gradient (2500 km) from Central Europe to the range margin in northern Scandinavia. Both types of gradients corresponded to a 11.5 °C difference in mean annual temperature. Genetic diversity strongly declined and differentiation increased with latitude in line with the predictions of the ACM. However, as population size did not decline with latitude and genetic diversity was not related to population size in *A. vulneraria*, this pattern is not likely to be due to less favourable conditions in the North, but due to serial founder effects during the post-glacial recolonization process. Genetic diversity was not related to elevation, but we found significant isolation by distance along both gradients, although the elevational gradient was shorter by orders of magnitude. Subarctic populations differed genetically from alpine populations indicating that the northern populations did not originate from high elevational Alpine ones. Our results support the notion that postglacial latitudinal colonization over large distances resulted in a larger loss of genetic diversity than elevational range shifts. The lack of genetic diversity in subarctic populations may threaten their long-term persistence in the face of climate change, whereas alpine populations could benefit from gene flow from low-elevation populations.

Keywords

Abundant centre model, climate change, Pleistocene glaciation, isolation by distance, population size effect, founder effect

Introduction

The genetic diversity of populations and their differentiation is influenced both by contemporary evolutionary processes like gene flow, genetic drift, and natural selection and by the history of a species (Frankham et al. 2017). In small and isolated populations genetic variation is often strongly reduced and genetic divergence among populations increased because of reduced gene flow and stronger genetic drift (Fischer and Matthies 1998; Aguilar et al. 2008; Schlaepfer et al. 2018). Bottlenecks and founder effects may also have strong negative effects on genetic diversity (Frankham et al. 2017). Due to environmental gradients the processes that influence genetic diversity often vary across the distributional range of a species. The abundant centre model (ACM) predicts that the suitability of environmental conditions for a species decreases from the centre of its distribution to its range periphery and consequently its populations will become scarcer, smaller and more isolated towards the range limits (Brown 1984; Sagarin and Gaines 2002). Genetic consequences of the decrease in the number and size of populations towards the range periphery and their increasing isolation are predicted to be reduced genetic diversity within populations and increased genetic differentiation among populations due to increased genetic drift and reduced gene flow at the periphery of the distribution of a species (Eckert et al. 2008; Sexton et al. 2009; Hardie and Hutchings 2010; López-Delgado and Meirmans 2022). However, while a recent review found that only approximately half of the available studies supported these predictions (Pironon et al. 2017), a study of 91 North American native plants found strong support for the ACM (López-Delgado and Meirmans 2022).

The genetic diversity and population structure of plant species across its range may also be influenced by range shifts linked to Pleistocene climate oscillations (Harter et al. 2015), which had a major impact on the present distribution of plants (Hewitt 2000). Populations typically retained high levels of genetic diversity and allelic richness in the glacial refugia where they survived during the ice ages (Beatty and Provan 2011; López-Delgado and Meirmans 2022). With the retreat of the ice shields after climate warming, individuals from the surviving populations colonized the new suitable habitats. Postglacial colonization over long distances by serial founder events often resulted in a decline in genetic diversity. Thus, populations often have less genetic variation at higher latitudes and are genetically more differentiated than at lower latitudes (Hewitt 2004; Ehrich et al. 2007; López-Delgado and Meirmans 2022). However, northern populations in Europe may also have become established by a massive migration of cold-tolerant plants from the Central European tundra into the forelands of retreating ice shields during the window of opportunity before tree species migrated North. In this scenario one would expect that genetic diversity within populations would not decrease towards the northern periphery. An example is the artic-alpine species *Dryas octapetala* whose genetic diversity in Scandinavian populations is high and today’s arctic populations of the

European cluster are closely related to alpine populations indicating a common origin in the tundra south of the Scandinavian ice-shield (Skrede et al. 2006).

Important environmental conditions (e.g. temperature) that influence the suitability of habitats for a species may change along elevational gradients in similar ways as with latitude and influence the balance between drift and gene flow. However, there are also important differences between the changes in environmental conditions along the two types of gradients, including those in day length, irradiance, CO_2 partial pressure, and precipitation (Körner 2007). Moreover, elevational gradients are much shorter than latitudinal ones and thus gene flow between populations is more likely (Hahn et al. 2012; Halbritter et al. 2015). Four patterns of genetic diversity along elevational gradients have been found (Ohsawa and Ide 2008; Itino and Hirao 2016): (1) Populations at intermediate elevations have higher genetic diversity than populations at both higher and lower elevations, because conditions at intermediate (i.e. "central") elevations are optimal, whereas populations at the lower and upper elevation edges are more affected by restricted gene flow, genetic drift and founder effects, leading to reduced genetic diversity (Ohsawa et al. 2007; Herrera and Bazaga 2008; Byars et al. 2009; Meng et al. 2019). (2) Populations at low elevations are genetically most diverse (Premoli 2003; Quiroga and Premoli 2007), because conditions at low elevations are best and founder effects have occurred during upward range expansion. (3) Genetic diversity increases with elevation in species whose main habitats are in the alpine zone, or whose populations are negatively impacted by human activities at lower elevations (e.g. Reisch et al. 2005; Shi et al. 2011; Halbritter et al. 2015). Finally, genetic diversity may be unrelated to elevation due to extensive gene flow or random variation caused by strong local factors (Hahn et al. 2012; Halbritter et al. 2015). Reviews of studies on genetic diversity along elevational gradients have found no general patterns (Ohsawa and Ide 2008; Itino and Hirao 2016) and little is known about whether latitudinal and elevational gradients in environmental conditions have similar effects on the genetic structure and diversity of a plant species. A better understanding of patterns of genetic diversity and differentiation along these gradients is important, because genetic diversity will determine the potential of populations to adapt to ongoing global change (Jump et al. 2009a).

The effects of the two types of gradients should ideally be compared in species that have both a large latitudinal and elevation extension (Halbritter et al. 2015). We chose the kidney vetch *Anthyllis vulneraria* (Fabaceae) as a model species to study the patterns of genetic variability and differentiation as influenced by latitude and elevation, because it has an exceptionally wide geographic and elevational distribution in Europe. The large distribution of *A. vulneraria* allowed us to study general genetic patterns that cannot be detected in arctic-alpine species or in rare species with fragmented and isolated populations. We studied the genetic diversity and structure of *A. vulneraria* along two gradients chosen to correspond to a change of 11.5 °C in annual mean temperature: a latitudinal gradient of c. 2400 km from Central Europe to Iceland and northern Norway and three elevational gradients of c. 2000 m elevational difference in the European Alps (see also Daco et al. 2021). The latitudinal gradient ranged from the centre of the distribution of *A. vulneraria* to its northern range limit and the elevational gradient in the Alps from the valleys to the upper elevational edge of its distribution. We address the following specific questions: (1) Does the genetic diversity of *A. vulneraria* vary similarly along gradients of elevation and latitude? (2) Are patterns of genetic differentiation similar along the two types of gradients?

Materials and methods

Study species

Anthyllis vulneraria L. (Fabaceae) is a diploid biennial to perennial herb of nutrient-poor calcareous grasslands and screes. Its distribution is exceptionally wide as it occurs from the North of the African continent across Europe to above 70 °N in Scandinavia and from sea level up to 3000 m a.s.l. (Conert 1975). *A. vulneraria* is not threatened in most parts of its distribution area but has become less common in certain geographical areas (e.g. Jansen et al. 2019). The flowers of *A. vulneraria* are grouped in heads and seed mass varies between 1.9 and 4.0 mg across the studied distribution range (Daco et al., 2021). *A. vulneraria* has been found to be auto- or xenogamous in different populations (Couderc 1971; Navarro 1999). Several subspecies of *A. vulneraria* have been described as the species is very polymorphic (Cullen 1968), but molecular genetic studies did not support the splitting into numerous subspecies (Nanni et al. 2004; Köster et al. 2008). In the present study, we did not differentiate between infraspecific taxa because we wanted to capture a large amount of genetic variation.

Sampling

We sampled 20 populations each of *A. vulneraria* along elevational and latitudinal gradients (Table 1). The latitudinal gradient ranged over 2000 km from the centre of the distribution of *A. vulneraria* in Central Europe to the northern distributional margin, and the elevational gradient extended from valley populations at 500 m a.s.l. to the populations at the elevational limit of the species in the Alps at 2500 m a.s.l. We defined a population as a group of plants that were at least 500 m from the next conspecific plant.

During summer 2015, we collected in each population leaves from 20 plants along a 20 m long transect and put them into separate paper bags. The leaf material was preserved in silica gel until DNA extraction. The local spatial reference of each sampled individual was recorded along the transects. At each site, we also recorded the elevation above sea level and the latitude and longitude with a handheld GPS (eTrex 20, Garmin Ltd.). In small populations (< 100) we recorded the number of plants to the nearest five, while in large populations we counted the plants in a part of the total area and extrapolated the number to the whole population area.

Genotyping using microsatellite markers

We extracted genomic DNA using a DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) starting from approximately 10 mg of dried material. Samples were genotyped at 17 microsatellite loci (AV2, AV3, AV7, AV8, AV10, AV12, AV14, AV23, AV-000290, AV-002128, AV-004868, AV-005692, AV-015354, AV-020270, AV-021012, AV-021224, AV-021803, for references see Van Glabeke et al. 2007 and Kesselring et al. 2013) in four multiplex reactions using the QIAGEN Multiplex PCR Kit (QIAGEN, Hilden, Germany).

Multiplex 1 contained loci AV23 (Van Glabeke et al. 2007), AV-000290 and AV-015354 (Kesselring et al. 2013). Multiplex 2 contained loci AV2, AV3, AV12 (Van Glabeke et al. 2007), AV-021012 and AV-021224 (Kesselring et al., 2013). Multiplex 3 contained loci AV7, AV8, AV10 (Van Glabeke et al. 2007) and AV-004868 (Kesselring et al. 2013). Multiplex 4 contained loci AV14 (Van Glabeke et al. 2007), AV-002128, AV-005692, AV-020270 and AV-021803 (Kesselring et al. 2013). We amplified each multiplex using the QIAGEN multiplex Kit (QIAGEN, Hilden, Germany). Each multiplex reaction contained 1 x QIAGEN multiplex master mix and 0.2 µM of each primer in a total volume of 6 µl.

The PCR conditions were: 5’ at 95 °C, 30 cycles of 30’’ at 95 °C, 90’’ at 53 °C (55 °C for Multiplex 1) and 30’’ at 72 °C and a last step of 30’ at 68 °C. Reactions were performed using a Mastercycler nexus (Eppendorf, Hamburg, Germany). PCR products were separated using an automated sequencer (3730xl DNA Analyzer, Applied Biosystems). The data were analysed using Geneious 11.1.5 (https ://www.geneious.com, Kearse et al. 2012).

To estimate the error rate, we extracted and genotyped 5% of the samples twice. The mean error rate per sample was calculated as the number of errors divided by the total number of analysed loci within replicated samples. We randomly chose one of the repeated samples to continue with the analyses.

Analysis of genetic diversity

All analyses unless otherwise stated were carried out using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA). Genetic diversity indices including number of multi-locus genotypes (N_G), number of private alleles (N_P), number of alleles (N_A), number of effective alleles (N_E), and observed and unbiased expected heterozygosity (H_O and uH_E, respectively) were estimated in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). Allelic richness (A_R) was calculated with the R-package PopGenKit 1.0 (Rioux Paquette 2012) and the inbreeding coefficient (F_{IS}, Weir and Cockerham, 1984) was calculated in FSTAT 2.9.4 (Goudet 2003). We used regression analysis to test for the effects of elevation and latitude on diversity measures and to test for the effect of population size on uH_E and F_{IS}. Population size was log-transformed prior to analysis.

We tested for the significance of heterozygote deficiency or excess (Hardy-Weinberg equilibrium) in the 40 populations using the Markov chain method in GENEPOP 4.7.3 (Rousset 2008) with 10 000 dememorisation steps, 500 batches and 10 000 subsequent iterations. The populations were tested for linkage disequilibrium among loci using an exact test based on a Markov chain method as implemented in GENEPOP. For both tests, the false discovery rate technique was used to eliminate false assignment of significance by chance (Verhoeven et al. 2005).

The software Microchecker (Van Oosterhout et al. 2004) was used to check for the presence of null alleles in each locus × population combination. Adjusted null allele frequencies were calculated with the software FreeNA (Chapuis and Estoup 2007). The adjusted allele frequencies were used to recalculate unbiased expected heterozygosity values.

Analysis of population differentiation

We used F_{ST} (Weir and Cockerham 1984) and G''_{ST} (Meirmans and Hedrick 2011) to measure genetic differentiation among the 40 studied populations and among the populations of the elevational and latitudinal gradients separately. In addition to F_{ST}, it has been recommended to use an alternative statistics like G''_{ST}, which is an unbiased estimator of population differentiation suitable to infer demographic history and migration (Meirmans and Hedrick

2011). Pairwise population F_{ST} and G''_{ST} and global F_{ST} and G''_{ST} were calculated with FSTAT 2.9.4 (Goudet 2003) and Genodive 3.0 (Meirmans 2020), respectively. Significance tests were based on 1000 (F_{ST}) and 999 permutations (G''_{ST}).

The partitioning of genetic variation within and among populations, and between the three mountain regions (France, Switzerland and Austria) was analysed with AMOVA as implemented in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). The significance of the results was tested with 999 permutations.

Mean pairwise G''_{ST} values for each population were calculated by averaging all pairwise G''_{ST} between a population and all other populations within each gradient. They represent a measure of population divergence (Yakimowski and Eckert 2008). We performed regression analyses to test for the effects of elevation and latitude on mean pairwise G''_{ST}-values. For the elevational gradient, the effects of the three Alpine regions, elevation and the interaction between region and elevation was tested in a general linear model. For the latitudinal gradient we tested whether the relationship between pairwise G''_{ST} and (log) geographic distance differed between southern (1 to 9) and northern populations (10 to 20) using a permutational GLM with 5000 permutations with the R-package lmPerm 2.1.0. (Wheeler and Torchiano 2016). To analyse the correlation between G''_{ST} and uH_E along both gradients the effects of gradient, uH_E and the interaction between gradient and uH_E were tested in a general linear model.

We examined the relationship between genetic (pairwise $G''_{ST}/(1- G''_{ST})$ and geographical distances for populations of the latitudinal gradient with a Mantel test (GenAlEx 6.5; 999 permutations) to test for isolation-by-distance (IBD). For the populations in the Alps, we analysed the relationship between pairwise genetic distances and both geographical distances and the differences in elevation with a linear model. *P*-values were derived from sequential permutation tests with 1000 permutations using lmPerm. We also tested whether the mean genetic distance between pairs of populations differed between populations north of 56 °N and those further south by relating the genetic distances to the geographic distances and population type using a permutational analysis of covariance with 1000 permutations using lmPerm.

Analysis of population genetic structure

We conducted a principal coordinate analysis (PCoA) based on pairwise G''_{ST}-values between populations. We fitted the two variables elevation and latitude on the ordination using the *envfit* function in the R-package vegan 2.5-7 (Oksanen et al. 2020).

We used STRUCTURE 2.3.4 (Pritchard et al. 2000) to analyse the genetic structure of the 40 *A. vulneraria* populations. To estimate the number of genetic clusters (*K*), we carried out ten independent runs with *K* = 1–20 with 10^6 Markov chain Monte Carlo (MCMC) iterations after a burn-in period of 10^5, using the model with correlated allele frequencies and assuming admixture. We decided on the most probable number of *K* based on the log probability of the data and their variability associated with each *K* (Pritchard et al. 2007; Gilbert et al. 2012) and the consistency with the PCoA. We used CLUMPAK (Kopelman et al. 2015) to summarize the runs and generate bar plots of cluster assignments.

Spatial genetic structure within populations

We carried out a spatial autocorrelation analysis with SPAGeDi 1.5d (Hardy and Vekemans 2002) using the kinship coefficient F_{ij} (Loiselle et al. 1995) and the local spatial coordinates of 744 individuals from 39 populations. Population A5 and five individuals from populations F3 and 10 had to be excluded from the dataset because the local coordinates had not been recorded. The intra-population distances were divided into ten distance classes each with a minimum of 27 637 pairs of individuals. The F_{ij} for each pair of individuals in each distance class was calculated and the significance levels of the means were obtained with permutation tests with 1000 permutations. Mean F_{ij} over pairs of individuals of *A. vulneraria* was plotted against mean distance for each class and the significance of the slope of that regression was obtained with a permutation test with 1000 permutations.

Results

Genetic diversity along the elevational and latitudinal gradients

We genotyped 768 individuals with 17 polymorphic microsatellite-markers to study the influence of the elevational and latitudinal gradients on genetic population structure and diversity. The estimated mean error rate was less than 1% per sample. We found 660 unique genotypes. Across the 40 populations, the 17 loci analysed yielded 209 alleles, with 4 – 26 (mean = 12.29) alleles per locus. The number of multi-locus genotypes (N_G) was generally high but was lower in the subarctic populations and was particularly low for the three northernmost populations (no. 18, 19 and 20; Table 1). We found at least one private allele in most elevational populations, but only in six latitude populations, and those were mostly located in the southern part of the gradient (Table 1). Two of the northernmost populations (no. 18 and 19) were

Table 1 Study sites, population size, number of samples, and genetic diversity indices for the 40 *A. vulneraria* populations sampled across elevational and latitudinal gradients.

Population	Country	Latitude (°N)	Longitude (°E)	Elevation (m a.s.l.)	N	n	N_G	N_P	N_A	N_E	A_R	uH_E	H_O	F_{IS}	P_{HWE}
Elevational gradient															
A1	Austria	47.3980	11.2661	961	100	20	20	2	4.53	2.75	3.82	0.55	0.59	-0.06	0.306
A2	Austria	47.4421	11.6501	1521	300	20	20	1	5.65	3.01	4.46	0.57	0.56	0.03	0.282
A3	Austria	47.1606	11.7149	1810	1000	19	19	1	3.24	1.99	2.78	0.39	0.37	0.06	0.129
A4	Austria	47.1690	11.3533	1151	150	19	19	4	3.82	2.24	3.38	0.47	0.42	0.11	**0.044**
A5	Austria	47.3127	11.3894	2250	200	19	19	3	5.12	3.02	4.34	0.57	0.55	0.03	0.230
S1	Switzerland	46.1338	7.0595	545	200	19	19	2	3.94	2.63	3.55	0.53	0.51	0.04	0.121
S2	Switzerland	46.0833	7.1265	1042	80	20	20	1	3.94	2.23	3.34	0.49	0.47	0.05	0.242
S3	Switzerland	46.0496	7.9564	2162	30000	20	20	2	4.06	2.47	3.37	0.50	0.40	0.20	**<0.001**
S4	Switzerland	46.2539	7.2734	1585	1000	20	20	0	5.53	3.24	4.50	0.63	0.58	0.08	0.063
S5	Switzerland	46.2735	7.2374	1250	1500	20	20	0	4.88	3.25	4.03	0.64	0.53	0.18	**0.004**
S6	Switzerland	46.0881	7.4067	1940	10000	20	20	1	5.24	3.13	4.26	0.59	0.54	0.08	**0.036**
S7	Switzerland	46.1081	7.5801	2413	10000	20	20	1	4.65	2.62	3.84	0.52	0.51	0.03	0.090
F1	France	45.0533	6.3892	2362	2000	20	20	1	3.71	2.01	2.96	0.40	0.32	0.21	**<0.001**
F2	France	45.0512	6.3533	1997	1500	17	17	1	4.24	2.59	3.71	0.52	0.39	0.26	**<0.001**
F3	France	45.1562	6.4237	1518	100	20	20	0	3.41	2.07	2.87	0.42	0.37	0.12	**0.018**
F4	France	45.2166	6.3250	1223	200	20	20	1	5.24	3.36	4.35	0.61	0.59	0.03	**0.007**
F5	France	45.0932	5.7804	471	1200	20	20	1	3.82	2.34	3.18	0.47	0.34	0.28	**<0.001**
F6	France	45.1735	6.0389	936	250	20	20	3	5.53	3.37	4.50	0.58	0.58	0.01	0.401
F7	France	45.1210	5.9852	717	200	10	10	0	3.88	2.45	3.80	0.56	0.56	0.00	0.574
F8	France	45.0598	6.3157	1807	300	19	19	0	4.29	2.68	3.64	0.50	0.46	0.07	**0.033**
Latitudinal gradient															
1	France	46.4368	4.7528	323	60	18	18	5	4.06	2.59	3.41	0.51	0.40	0.21	**<0.001**
2	France	48.1880	5.5534	443	800	19	19	1	4.06	2.67	3.54	0.52	0.46	0.11	0.610
3	Luxembourg	49.4956	5.9969	342	1000	20	20	2	4.41	2.78	3.78	0.57	0.54	0.04	0.109
4	Luxembourg	49.7314	6.2819	355	50	18	17	0	3.65	2.41	3.25	0.48	0.37	0.23	**<0.001**
5	Germany	51.2228	9.7610	442	250	20	19	1	4.00	2.69	3.48	0.54	0.47	0.15	**0.002**
6	Germany	52.0051	10.4075	191	400	20	20	0	3.71	2.37	3.23	0.48	0.47	0.03	0.949
7	Germany	54.0443	10.2290	32	1500	20	20	0	3.06	2.22	2.83	0.49	0.49	0.00	0.411
8	Germany	54.6873	9.4342	22	1500	20	20	0	3.59	2.30	3.05	0.51	0.43	0.16	**<0.001**
9	Denmark	55.5150	9.4244	42	400	20	20	0	3.88	2.41	3.32	0.50	0.52	-0.06	0.277
10	Sweden	56.3671	12.8002	81	800	15	15	0	1.94	1.30	1.77	0.18	0.15	0.15	0.189
11	Sweden	57.8892	11.9466	24	300	20	20	0	2.76	1.80	2.36	0.34	0.30	0.11	0.176
12	Sweden	58.6979	11.2199	5	100	18	18	0	3.06	1.85	2.70	0.36	0.31	0.13	0.107
13	Norway	61.0621	10.3971	438	100	20	20	1	1.88	1.44	1.76	0.23	0.18	0.21	0.716
14	Norway	62.0139	9.2074	483	75	20	9	0	1.47	1.27	1.30	0.15	0.02	0.88	**<0.001**
15	Norway	63.4409	10.6567	18	350	19	17	0	2.18	1.45	1.85	0.27	0.11	0.62	**<0.001**
16	Iceland	63.8164	-22.6970	20	10000	20	8	0	1.41	1.32	1.38	0.17	0.00	1.00	**<0.001**
17	Norway	64.3162	12.3475	168	4000	20	12	1	1.41	1.21	1.38	0.13	0.08	0.38	**0.008**
18	Sweden	66.4261	16.8501	453	300	20	2	0	1.00	1.00	1.00	0.00	0.00	NA	-
19	Norway	67.2511	15.4282	6	1000	20	1	0	1.00	1.00	1.00	0.00	0.00	NA	-
20	Norway	68.1022	16.3783	47	1000	19	2	0	1.06	1.06	1.06	0.06	0.06	NA	-

N population size, n sample size, N_G number of multi-locus genotype, N_P number of private alleles, N_A number of alleles, N_E number of effective alleles, A_R allelic richness, uH_E unbiased expected heterozygosity, H_O observed heterozygosity, F_{IS} inbreeding coefficient, P_{HWE} significance values of exact test for Hardy-Weinberg deviations (values in bold were significant before correction for multiple tests, values < 0.03 remain significant ($P < 0.05$) after adjusting for the false discovery rate) homozygous at every locus indicating that all individuals in the populations were fixed at the 17 microsatellite loci (N_A, N_E and A_R = 1; Table 1).

Expected heterozygosity (uH_E) per population varied from 0.39 - 0.65 (mean = 0.53) for the elevational gradient and from 0 - 0.57 (mean = 0.32) for the latitudinal gradient (Table 1). Genetic diversity (uH_E) did not decrease clearly with elevation (r = -0.19, P = 0.44; Fig. 1a) and also showed no optimum (quadratic regression, r^2 = 0.05, P = 0.34), but decreased strongly with latitude (r = -0.92, P < 0.001; Fig. 1b). Other measures of genetic diversity also decreased with latitude (N_A: r = -0.94; N_E: r = -0.94; A_R: r = -0.94; all P < 0.001), but not with elevation (N_A: r = 0.09, P = 0.72; N_E: r = -0.07, P = 0.77; A_R: r = -0.19, P = 0.94). However, closer inspection showed that genetic diversity (uH_E) was similarly high in populations from 46 °N to 56 °N (no. 1 to 10) and decreased strongly for the populations further north. Genetic diversity (uH_E) did not decrease with population size (Alt.: r = -0.01, P = 0.98; Lat.: r = -0.21, P = 0.39) indicating that there was no strong genetic drift in small populations (Fig. 2). In nearly all the populations, H_O was smaller than uH_E and ranged from 0.32 - 0.59 in the elevational and from 0 - 0.54 in the latitudinal gradient.

The inbreeding coefficient F_{IS} ranged from -0.06 to 0.28 in the elevational populations and from -0.06 to 1 (in populations where H_O = 0) in the populations along the latitudinal gradient (Table 1). In the most northern populations (no. 18, 19, 20), F_{IS} could not be calculated because nearly all the individuals of each population had identical multi-locus genotypes leading to very small uH_E. F_{IS} increased with latitude (r = 0.72, P < 0.001) but did not vary consistently with elevation (r = 0.19, P = 0.43).

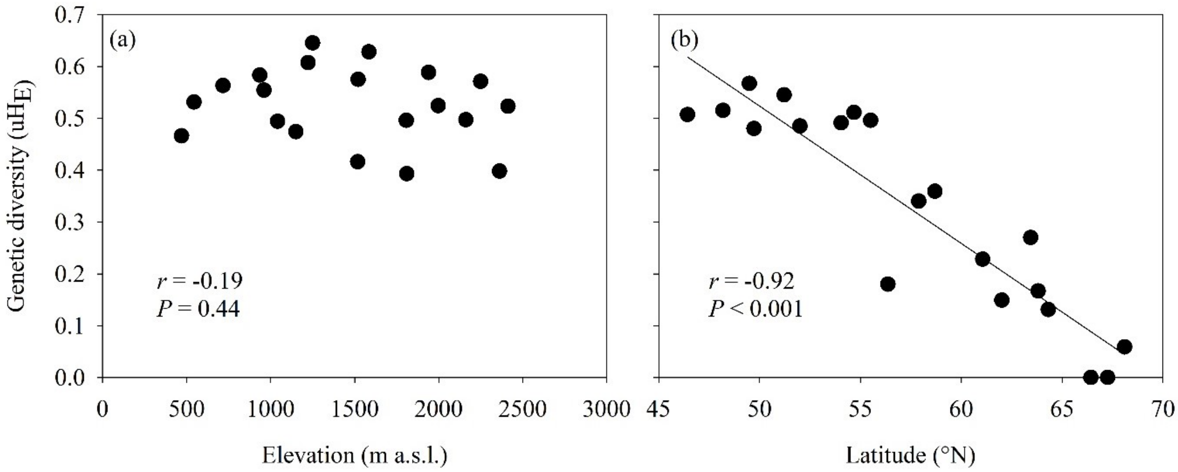

Fig. 1 The relationship between genetic diversity and (a) elevation for the populations along the elevational gradient and (b) latitude for the populations along the latitudinal gradient.

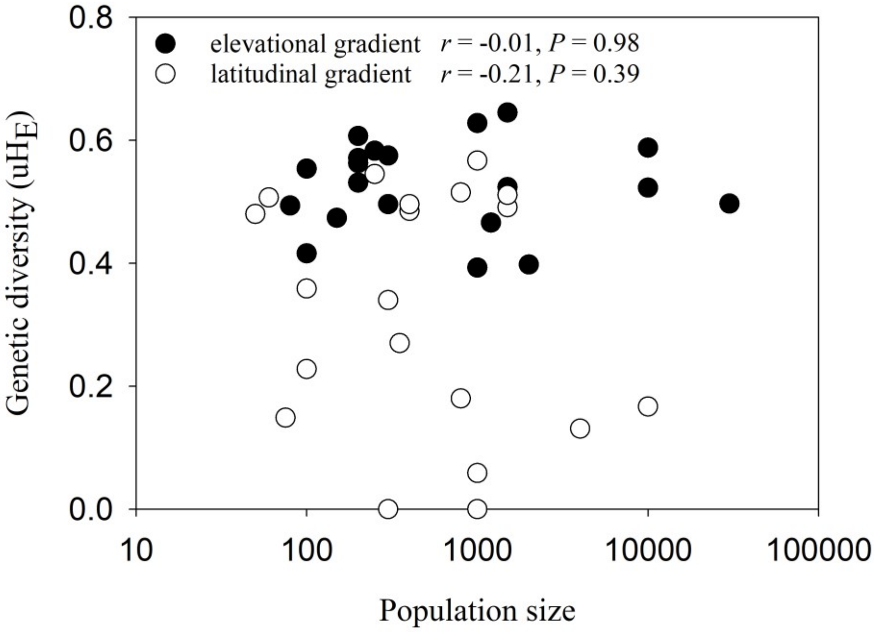

Fig. 2 The relationship between genetic diversity (uH$_E$) and size of the *A. vulneraria* populations along the elevational and latitudinal gradients.

We found significant deviations from Hardy-Weinberg equilibrium (HWE) for less than 10% of the allele × population combinations after correcting for the false discovery rate. Thirteen of the 17 loci significantly deviated from HWE in at least one population and 15 populations showed significant deviation from HWE (Table 1). Populations of the latitudinal gradient showed deviations from HWE from population 14 on northwards except in populations 18, 19, 20 where deviations from HWE could not be evaluated. No locus systematically deviated from HWE and no pairs of loci were systematically in linkage disequilibrium after correcting for multiple tests. All loci were therefore included in the subsequent analyses.

Null alleles were suggested in 64 locus × population combinations (9%), of which 17 were for locus AV23. However, adjusting the allele frequencies for the null alleles did not change the unbiased expected heterozygosity in any of the analysed populations (Mann-Whitney U test, $P > 0.81$). Therefore, all following analyses were performed with the original data set.

Population genetic structure

The first two axes of the PCoA explained 35.6% of the variation (Fig. 3). The populations from the Alps were placed close to each other along the first PCoA axis while all population north of 56 °N were placed to the right of the Central European populations. In particular, high elevation populations from the Alps and subarctic populations were widely separated from each other. Fitting of the two environmental gradients onto the ordination revealed that the genetic differentiation between populations was mainly correlated with the latitudinal gradient ($r^2 = 0.81$, $P < 0.001$ vs. $r^2 = 0.65$, $P < 0.001$ for elevation).

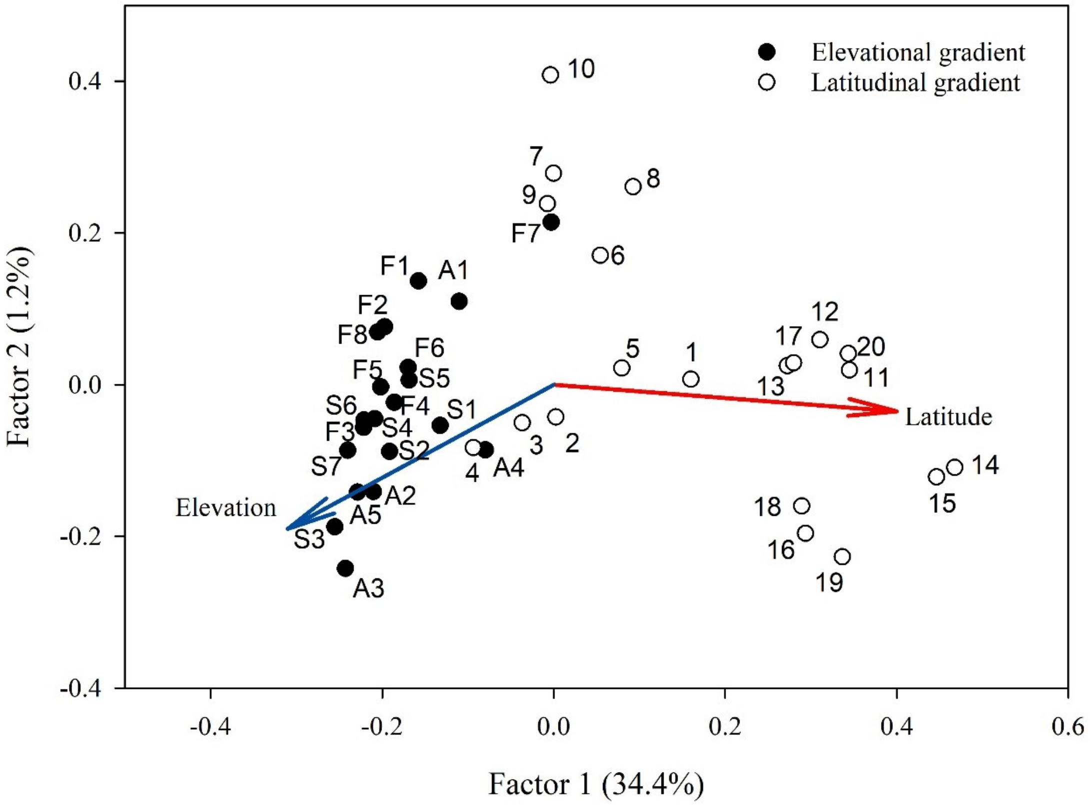

Fig. 3 Principal coordinate analysis (PCoA; first two factors) based on pairwise G''_{ST}-values between all studied populations of *Anthyllis vulneraria*. The elevational and latitudinal gradients were fitted as vectors onto the ordination. Population labels correspond to abbreviations used in Table 1.

In the STRUCTURE analysis the log probability of the data [ln P(D)] increased gradually and the value that also converged well across the ten independent runs was obtained for *K* = 7 (Appendix Fig. S1). However, the patterns for *K* = 6 and *K* = 7 were very similar and we therefore preferred the lower number of groups. Structuring the populations into six clusters grouped the ten most northern populations together (Fig. 4a, b), confirming their differentiation from the Central European ones. The main difference between the patterns for *K* = 6 and *K* = 7 was a further subdivision of the northern populations (Appendix Fig. S2) which was not consistent with the PCoA and probably spurious due to the very low genetic diversity of these populations. The ten Central European populations were divided into two groups of five populations consistent with their latitudinal positions along the gradient. In the Alps, two low-elevation populations (A1, F7) were grouped together with populations no. 6 to 10. High elevation Austrian and Swiss populations clustered together. Some admixture was present in the mid-elevations of the three mountain regions (Fig. 4b, c).

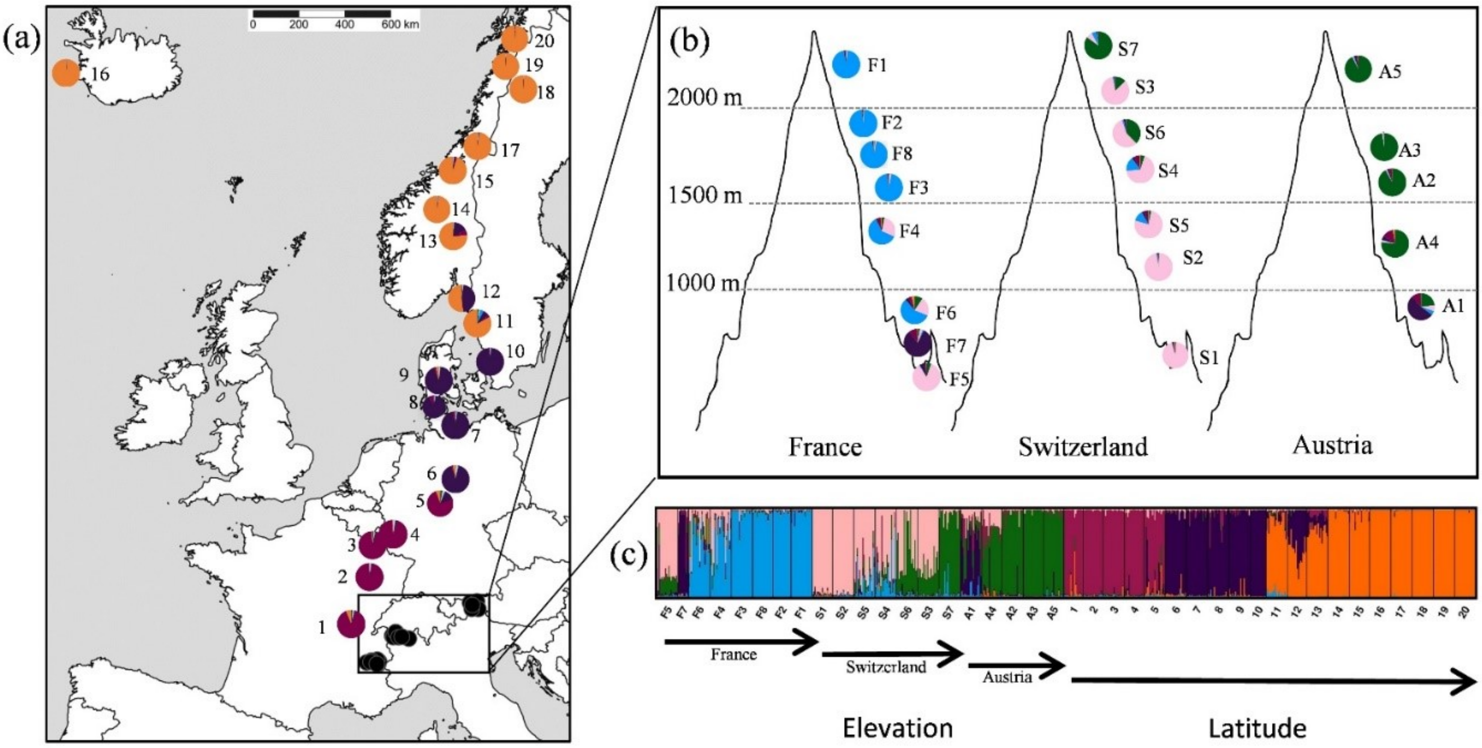

Fig. 4 Results of the STRUCTURE analysis for 40 populations sampled across elevational and latitudinal gradients assuming $K = 6$. Pie charts in (a) and (b) represent the proportion of individuals of each population assigned to the STRUCTURE clusters. In (c), each individual is represented by a vertical line, which is partitioned into a maximum of six coloured segments that represented an individual's estimated membership fractions in the six clusters. Vertical black lines separate the 40 different populations. Arrows represent increasing elevation along each of the three regions in the Alps and increasing latitude along the latitudinal gradient. For population labels see Table 1.

Population differentiation

We detected high levels of genetic differentiation among the 40 studied populations (global $F_{ST} = 0.36$ and $G''_{ST} = 0.63$). The genetic differentiation among populations was much higher across the latitudinal ($F_{ST} = 0.46$ and $G''_{ST} = 0.69$) than across the elevational gradient ($F_{ST} = 0.19$ and $G''_{ST} = 0.40$), indicating that the populations in the Alps are less differentiated from each other. AMOVA indicated that only a small proportion (4.8%) of the genetic variation was among the three mountain regions, the differentiation among populations within regions was much higher (15%). Most of the genetic variance was within populations (Table 2).

Table 2 Results of an AMOVA for the populations of *A. vulneraria* along the elevational gradient, partitioning the genetic variation between mountain regions and among and within populations.

Source of variation	*df*	Sum of squares	Variance components	Proportion of variation (%)	*P*-value
Among mountain regions	2	210.37	0.27	4.79	< 0.001
Among populations	17	625.89	0.85	15.04	< 0.001
Within populations	744	3361.14	4.52	80.16	< 0.001

Pairwise population F_{ST} and G''_{ST}-values between all populations are given in Table S1. Mean pairwise G''_{ST} of a population is a measure of genetic divergence and represents the genetic distinctness of that population from the other ones. Mean pairwise G''_{ST} per population within the elevational gradient did not vary with elevation (Fig. 5a) and did not vary among regions (elevation: $P = 0.90$, region: $P = 0.64$, interaction: $P = 0.32$). In contrast, mean pairwise G''_{ST} increased strongly with latitude (Fig. 5b) and was significantly higher for populations north of 56 °N than for southern populations ($P < 0.001$). Mean pairwise G''_{ST} decreased with increasing genetic diversity (uH_E) for both gradients (Fig. 6), indicating absence of migration-drift equilibrium. However, the relationships differed for the populations from the two gradients: Genetic distinctness of the latitudinal populations was much higher than that of the elevational populations (G''_{ST}: $P < 0.001$), but the slope of the relationship was less steep (interaction gradient type x uH_E : $P < 0.01$).

Among populations of the latitudinal gradient, genetic distance $G''_{ST}/(1-G''_{ST})$ was not related to geographical distance ($r = 0.07$, $P = 0.25$). However, after removing an outlier caused by the high mean G''_{ST} of northern populations, a pattern of isolation-by-distance (IBD) was detected for the latitudinal populations (Fig. 7a, $r = 0.21$, $P = 0.03$). The mean pairwise genetic distance between populations, adjusted for the effect of geographical distance, was much higher for the northern (lat. > 56 °N) than for the more southern populations (5.56 ± 0.53 vs. 1.16 ± 0.71, P < 0.001).

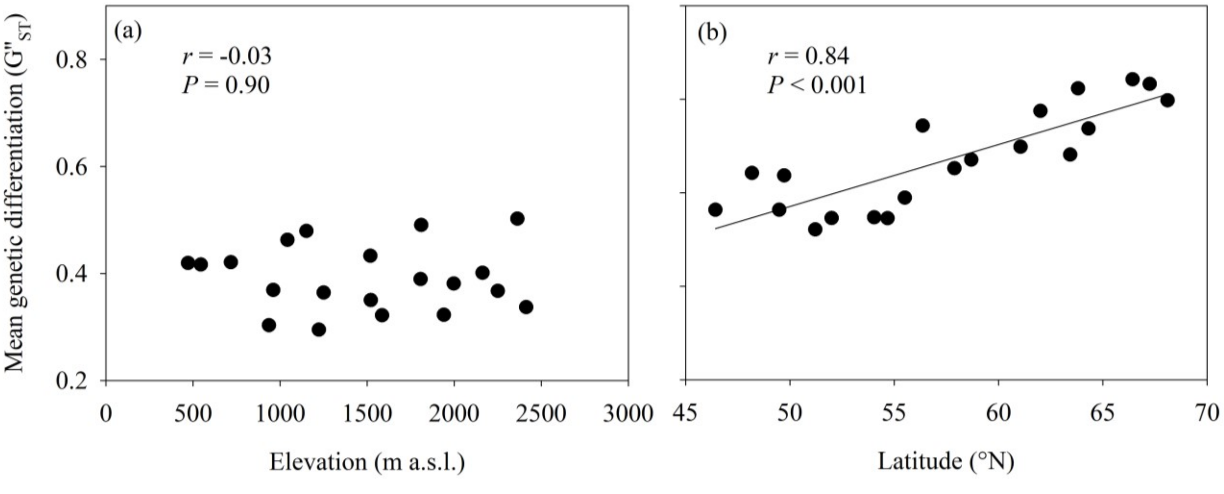

Fig. 5 The relationship between the mean genetic distance (G''_{ST}) of each population to all others and (a) elevation for the populations along the elevational gradient and (b) latitude for the populations along the latitudinal gradient.

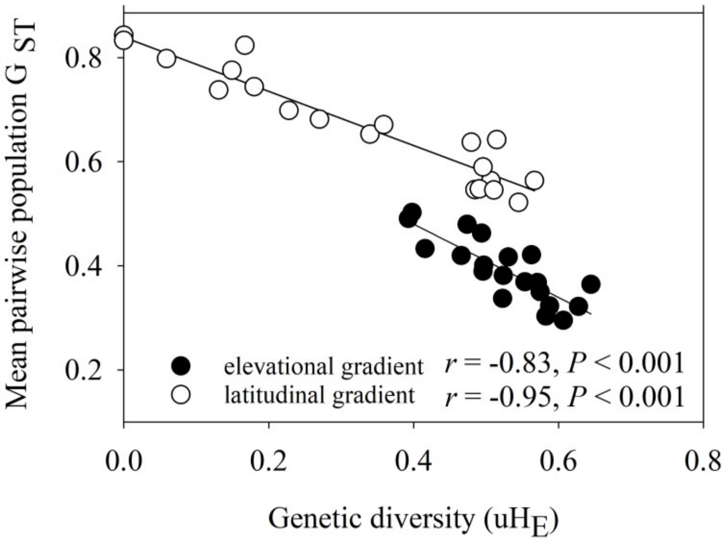

Fig. 6 The relationship between the mean genetic distance (G''_{ST}) of each population to all others and its genetic diversity (uH_E) for populations along the elevational and latitudinal gradient.

Genetic and geographical distances of the populations in the Alps were also related (Fig. 7b, *b* = 0.0008, *P* < 0.001). Moreover, adjusted for the effects of geographical distance, genetic differentiation between the populations in the Alps also increased with their difference in elevation (Fig. 7c, *b* = 0.129, *P* = 0.013). The effects of 1 km difference in elevation on the genetic distance between populations were similar to those of a difference of 161.3 km in horizontal distance indicating that the effects of vertical were much stronger than those of horizontal distance. However, the maximum elevational distance between populations was only 2 km.

Spatial genetic structure within populations

Spatial autocorrelation analysis within populations showed that mean kinship coefficients decreased with distance between plants in the populations (*b* = -0.00044, *P* < 0.001; Fig. 8). Plants growing less than 2 m from each other had a higher probability to be genetically related than plants separated by greater distances suggesting limited gene flow due to restricted pollinator movement and limited seed dispersal.

Discussion

Our results show that the patterns of genetic diversity and differentiation of the populations of the widespread plant species A*nthyllis vulneraria* differ between the elevational and latitudinal gradients. We observed a strong decline of genetic diversity with latitude down to only a single SSR-genotype in one of the most northern populations. In contrast, no pattern of genetic diversity in relation to elevation was observed in the Alps. Similarly, in the only comparable

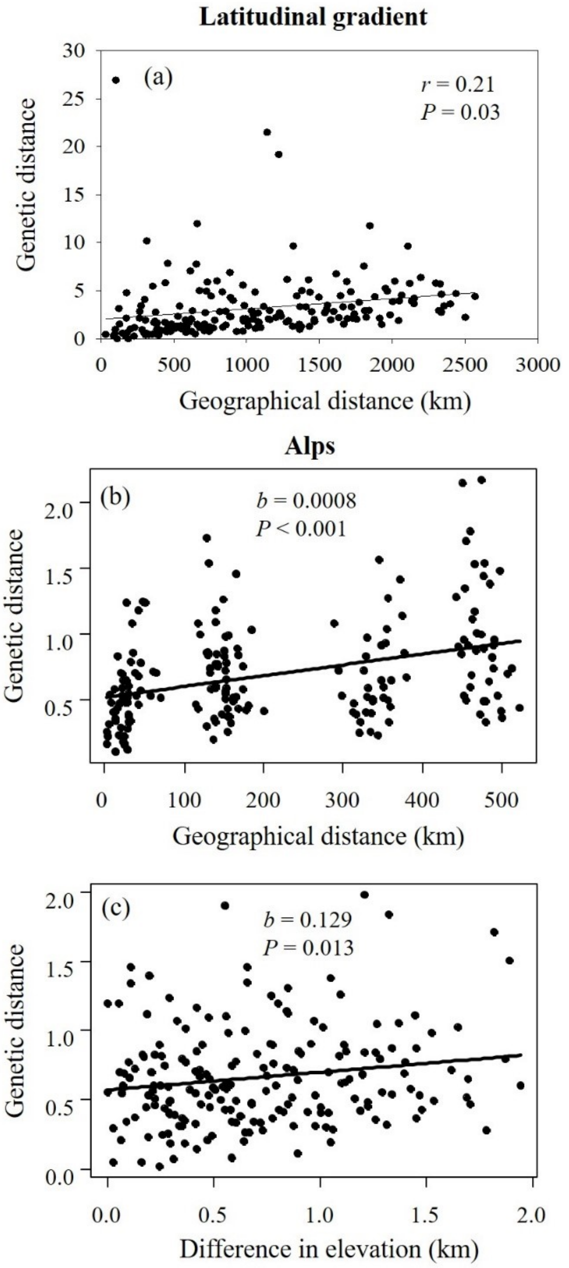

Fig. 7 (a) Relationship between pairwise genetic distances ($G''_{ST}/(1-G''_{ST})$) and geographical distances between *A. vulneraria* populations along the latitudinal gradient. An outlier was removed from the analysis (see text). (b, c) Partial residual plots of the relationship between pairwise genetic distances between populations in the Alps and (b) geographical distances, and (c) differences in elevation. *P*-values are derived from Mantel tests.

study of both gradients, genetic diversity of populations of *Plantago lanceolata* and *P. major* also decreased with latitude but not with elevation (Halbritter et al. 2015).

The decrease of genetic variation with latitude, the much stronger genetic differentiation among northern populations and the very few private alleles present in these populations are in line with the predictions of the abundant centre model (ACM). A decline of genetic diversity from the centre to the periphery of the distribution has been found in many species (Hirsch et al. 2015; Hirao et al. 2017; Gougherty et al. 2020; but see Ilves et al. 2016; Plenk et al. 2017; Casazza et al. 2021). However, the ACM assumes that the lower genetic diversity and stronger differentiation among peripheral populations is due to less favourable conditions which lead to

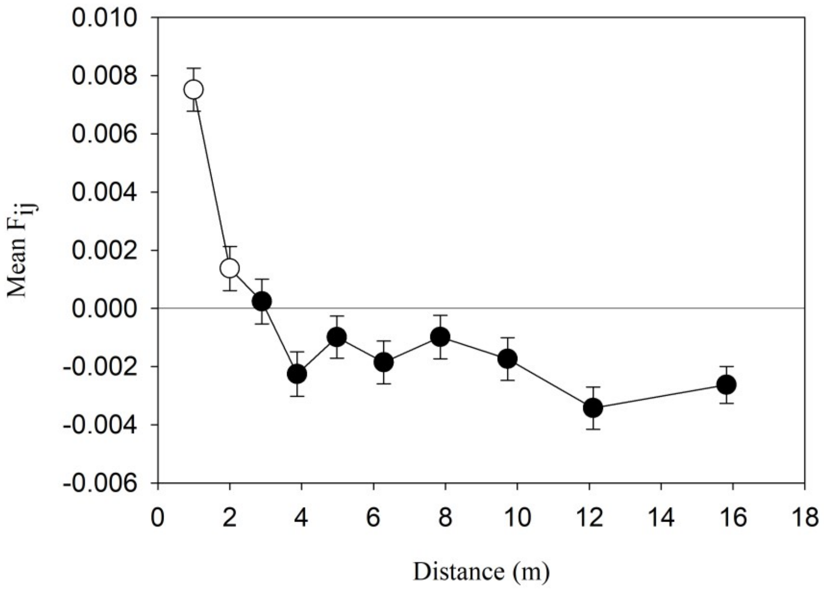

Fig. 8 Mean kinship coefficient between pairs of individuals in 39 populations of *Anthyllis vulneraria* for 10 distance classes containing each 27637 – 27642 pairs of individuals. Means ± 1 SD. The open symbols represent significant mean kinship coefficients ($P < 0.05$).

smaller and more isolated populations and subsequently to genetic erosion and strong differentiation (Eckert et al. 2008; Sexton et al. 2009; Hardie and Hutchings 2010). In contrast, in *A. vulneraria* the size of populations increased with latitude indicating favourable conditions in the north (Daco et al. 2021), and genetic variation was not related to current population size. This suggest that not current conditions resulting in fragmentation, but historical processes (colonization after the ice age) are responsible for the much lower genetic diversity of northern populations. Other short-lived plant species like *Arabidopsis thaliana* (Lewandowska-Sabat et al. 2010) or *Plantago coronopus* (Berjano et al. 2015), which have migrated north after the retreat of the ice sheet after the Last Glacial Maximum also showed such genetic patterns. A similar combination of founder effects followed by demographic expansion as in *A. vulneraria* has been suggested as the reason for the population structure of Scandinavian *Trollius europaeus* (Despres et al. 2002).

The decline of genetic diversity with increasing latitude was essentially restricted to populations situated north of 56 °N latitude (pop. no. 10 to 20). This limit corresponds to the southern limit of the ice-shield during the Younger Dryas period (Stroeven et al. 2016). Populations north of this latitude also formed a distinct cluster in the STRUCTURE and PCoA analyses. The decrease of genetic diversity and the increasing differentiation with latitude suggests that northern populations lost genetic diversity due to serial founder effects during the colonization of northern Europe after the ice age, producing genetically isolated populations with very low subsequent gene flow among them (Despres et al. 2002; Excoffier et al. 2009). The rare presence of the Scandinavian cluster in lowland populations in Central Europe are in line with the hypothesis that the Scandinavian populations were founded by random individuals from lowland Central European populations that migrated north after the retreat of the ice sheets. In contrast, our results do not support for *A. vulneraria* the scenario that arctic populations of species that also occur in the Alps were founded by alpine genotypes (Despres et al. 2002; Schönswetter et al. 2003; Albach et al. 2006; Skrede et al. 2006; Ehrich et al. 2007), as no high-elevation genotypes of the Alps were found in the Scandinavian populations of *A. vulneraria*. In contrast to studies that compared populations of arctic-alpine species, we were able to detect the importance of the serial founder effects during recolonization after the ice-age in *Anthyllis vulneraria*, because this species has a continuous distribution from the Alps to the arctic including populations in the lowlands.

The Central European *A. vulneraria* populations were separated into a southern (no. 1 to 5) and a northern (no. 6 to 10) subgroup in the STRUCTURE and the PCoA analyses. The geographical location of this separation is reflecting another genetic signature of the last ice age as this limit corresponds to the maximum extent of continental ice sheets during the Last Glacial Maximum (LGM) some 22 000 years BP (Stroeven et al. 2016). The STRUCTURE analysis indicated a similarity between high-elevation Swiss and Austrian populations whereas French high-elevation populations were separated. These results suggest that after the last glacial period, glacier forelands and alpine meadows were colonized from different refugia in France than in Austria and Switzerland.

The genetic variability of peripheral populations of *A. vulneraria* at the elevational limit in the Alps was similar to that of populations at lower elevations and similar to the mean value presented in the review of Nybom (2004). This is in contrast to the predictions of the ACM that genetic erosion in small populations would lead to reduced genetic diversity in high elevation populations. However, in *A. vulneraria* the size of populations actually increased with elevation in the Alps (Daco et al., 2021) and one might thus have even expected an increase of genetic diversity with elevation, but no relationship was found. No change of genetic diversity with elevation has also been found in several other studies (Pluess and Stöcklin 2004; see review of Ohsawa and Ide 2008; Hahn et al. 2012). A likely reason is gene flow among the populations along the short elevational gradient, which is supported by admixture in the mid-elevational populations of all three regions studied.

Another non-exclusive explanation would be that during the postglacial cold-tolerant genotypes rapidly colonized alpine habitats in glacier forelands when the ice shields retreated due to the short geographical distances from lowland to alpine environments. The alpine environment was to those cold-tolerant genotypes not ecologically marginal as predicted by the ACM but in fact corresponded largely to their ecological niche in the tundra of the lowlands. A third possible explanation would be that cold-tolerant plants survived locally in nunataks (see Schneeweiss and Schönswetter 2011) and colonized in post-glacial times glacier forelands and alpine meadows. However, in this case we would expect strong genetic differentiation among mountain regions and reduced genetic diversity in high elevation populations due to long-term isolation and low population sizes of the source populations in the isolated nunataks (Stehlik et al. 2002). Our results are not in line with the nunatak hypothesis as genetic differentiation among mountain regions was rather low and genetic diversity did not decrease with altitude.

Along both gradients, we found significant isolation by distance patterns, indicating that gene flow is restricted and strongest between geographically close populations. However, a vertical (elevational) distance of a certain length between populations in the Alps resulted in a much stronger genetic differentiation between populations than the same horizontal distance between populations of the latitudinal gradient. This could be due to phenological differences in flowering periods that may restrict cross-fertilization among populations at different elevations (Premoli 2003; Reisch et al. 2005; Yamagishi et al. 2005). However, overall the genetic differentiation between valley and alpine populations of *A. vulneraria* was much smaller than between Central European and subarctic populations along the same gradient in mean annual temperature (11.5 °C), because the elevational gradients were much shorter than the latitudinal gradient. The kinship analysis revealed that gene flow is even restricted over short distances within populations which may be due to restricted pollinator movement and seed dispersal.

Peripheral populations of *A. vulneraria* in northern Europe separated by a certain spatial distance were more differentiated genetically than populations in Central Europe, indicating lower gene flow between them. A possible reason are greater mean spatial distances between neighbouring populations in the North. Occurrence data from GBIF.org (2022) appear to support this, but might not be representative. A decline of population frequency towards the range periphery would be in line with the predictions of the ACM. The recent review of studies testing the ACM (Pironon et al. 2017) found that while there was only limited support for a general decline in population size, there was much stronger support for the prediction that the frequency of populations declines towards the range periphery.

Conclusions

Populations along the two gradients showed very different patterns of genetic diversity and genetic differentiation. While *A. vulneraria* maintained high amounts of genetic diversity in its Alpine and Central European populations, towards the North genetic diversity decreased strongly and genetic differentiation among populations increased due to serial founder effects during post-glacial recolonization. Our results support the notion that postglacial latitudinal colonization over large distances results in a larger loss of genetic diversity than elevational range shifts (Hewitt 1999; Ehrich et al. 2007). Subarctic populations differed genetically from alpine populations indicating that the subarctic populations did not originate from the high elevational alpine ones.

The consistently high genetic diversity, allelic richness and number of private alleles across the *A. vulneraria* populations from the three Alpine regions in comparison to the Scandinavian ones indicates that the alpine populations have a higher evolutionary potential. Responses of alpine and arctic populations to climate change are thus likely to differ. The lack of genetic diversity in subarctic populations may threaten their long-term persistence whereas in alpine populations gene flow from low-elevation populations along the short elevational gradient could permit admixture with genotypes originating from habitats with higher temperatures.

CHAPTER 3

Quantitative genetic differentiation and evolutionary potential in the widespread plant *Anthyllis vulneraria* along elevational and latitudinal gradients

Laura Daco, Guy Colling and Diethart Matthies

Abstract

The large distribution of a plant may be the result of phenotypic plasticity or of genetic differentiation of populations in different environments. We studied quantitative traits of the widespread plant *Anthyllis vulneraria* in a common garden. The plants originated from populations along an elevational gradient from the lowlands to the elevational limit of the species in the Alps, and from populations along a latitudinal gradient from the centre of the distribution of the species in Central Europe to its northern distributional margin. Traits measured in the common garden frequently showed clinal variation with elevation and latitude of origin indicating potential adaptive differentiation of traits along the gradients. Evidence for divergent selection was indicated by higher Q_{ST}-values (measuring quantitative genetic differentiation) than F_{ST}-values (measuring neutral molecular genetic differentiation) in some traits. The quantitative genetic diversity of most traits was only weakly correlated with the neutral molecular genetic diversity of populations. We also found that the ability to benefit from the benign conditions in the common garden declined with both increasing elevation and latitude of origin indicating lower plasticity, which may threaten survival under changing climatic conditions. In contrast, the evolvability of most traits did not vary consistently along the two gradients indicating that the evolutionary potential of peripheral populations was not reduced. The genetic variability in quantitative traits in arctic populations could allow them to adapt to changing environmental conditions although their genetic diversity was strongly reduced. Along the elevational gradient, evolutionary potential, the high molecular genetic diversity, and the short length of the gradient that favours gene flow may also allow genetic adaptation to new conditions. Our results support the growing evidence that there is no general relationship between variation in neutral molecular markers and variation in quantitative traits.

Keywords

Divergent selection, abundant centre model, climate change, quantitative genetic variation, environmental gradients, evolutionary potential

Introduction

Plant species with a wide distribution across elevational and latitudinal gradients can be plastic by expressing different reversible phenotypes and can differentiate genetically to adapt locally to the different conditions along these gradients (De Frenne et al. 2011a; Bakhtiari et al. 2019).

Adaptation consists of the evolution of a population towards a phenotype that better fits its present environment (Orr 2005), whereas plasticity acts as a buffer against environmental variation and can allow an individual plant to respond to different environments by changing its phenotype (Atkin et al. 2006; Nicotra et al. 2010). Adaptive differentiation in plants has frequently evolved in response to environmental changes associated with gradients in elevation (Gonzalo-Turpin and Hazard 2009; Vergeer and Kunin 2013; Zhang et al. 2019) and latitude (Santamaría et al. 2003; De Frenne et al. 2011a; Zhang et al. 2019). To study the extent of phenotypic plasticity and adaptive evolution is important for understanding and predicting the response of populations to a changing climate and for conserving the evolutionary potential of species (Franks et al. 2014). The ongoing changes in climate are particularly strong in alpine and arctic regions, where temperature increases more strongly than in other regions (Post et al. 2009; Pepin et al. 2015).

Strong gradients in elevation or latitude with associated changes in temperature and the length of the growth season provide ideal models to study plasticity and evolutionary changes in response to different environments (Halbritter et al. 2013). In the temperate zone, similar changes in annual mean temperature occur over a range of 1000 m in elevation vs. a range of 1000 km in latitude (Jump et al. 2009b). However, elevational and latitudinal gradients also exhibit distinct characteristics, such as different variations in atmospheric pressure, precipitation, solar radiation, and soil nutrients (Körner 2007; De Frenne et al. 2013; Daco et al. 2021). Consequently, the responses of species along these two types of gradients may differ significantly in various crucial aspects, but little is known about the different effects of environmental changes along both types of gradients on local plant populations (De Frenne et al. 2013).

Adaptation of species to different environments requires genetic diversity within species as the raw material of evolution (Jump et al. 2009a; Chung et al. 2023). The genetic variation within and between populations is influenced by mutations, natural selection, genetic drift, and gene flow. These processes can lead to genetic clines in allelic composition that are correlated with environmental gradients (Rossetto et al. 2011; Hahn et al. 2012; Daco et al. 2022). Population genetic studies have often solely considered neutral variation through the study of molecular markers. However, to detect variation in traits important for evolutionary responses to different environments and to assess the adaptive potential of populations, quantitative genetic approaches may be more appropriate (Kramer and Havens 2009; Pauls et al. 2013; Leamy et al. 2014; Diamond 2016; Hansen and Pélabon 2021; Teixeira and Huber 2021; Chung et al.

2023). A meta-analysis found only a weak correlation between molecular and quantitative genetic variation (Reed and Frankham 2001).

Quantitative genetic differentiation among populations can be studied in common gardens by quantifying the phenotypic differences among plants of different origins under the same environmental conditions (Linhart and Grant 1996; Briggs and Walters 1997). Growing plants in the same environment allows to study the genetic basis of phenotypic variation among and within populations. Genetic clines observed in common garden experiments typically align with the phenotypic clines observed in natural populations along environmental gradients, indicating a pattern of co-gradient variation (Kremer et al. 2014). By comparing traits measured for mother plants in the field and their progeny in the common garden, plastic responses to the new environmental conditions can be studied (Toczydlowski and Waller 2021).

To investigate possible divergent selection in specific quantitative traits one can compare the quantitative genetic differentiation (Q_{ST}) of traits with the differentiation in neutral molecular markers (F_{ST}) between populations. While F_{ST} reflects differentiation due to neutral genetic drift, Q_{ST} reveals both the consequences of drift and of different selection regimes due to environmental differences. Most Q_{ST} - F_{ST} studies found stronger differentiation in quantitative than molecular traits between populations suggesting that diversifying selection resulting in adaptation to environmental gradients is common (Frei et al. 2014; Evans et al. 2016; Walisch et al. 2015).

The study of quantitative genetic traits along gradients can also allow to test some of the predictions of the abundant centre model (ACM; Brown 1984; Sagarin and Gaines 2002) of biogeography. The ACM predicts that genetic variability within populations decreases and genetic differentiation among populations increases from the centre of its distribution toward its range periphery (Brown 1984; Pironon et al. 2017). Thus, range margins often represent the point beyond which adaptation is no longer possible because they lack selectively important alleles (Hargreaves et al. 2014; Volis et al. 2016). However, peripheral populations may also contain alleles important for the adaptation to changing environments due to selection under marginal conditions (Lesica and Allendorf 1995). Peripheral populations like alpine and arctic ones may face additional challenges, such as a scarcity of pollinators. In peripheral high latitude populations selection may favour the loss of incompatibility and a higher ability to self, allowing plants to reproduce even in the absence of reliable pollination services (García-Camacho and Totland 2009; Griffin and Willi 2014). However, there is conflicting evidence how pollinator dependence changes with elevation. Studies have found a negative, positive or no relationship between pollinator dependence and elevation (Eriksen et al. 1993; Arroyo et al. 2006; Wirth et al. 2010; Abdusalam and Li 2019; Xu et al. 2023).

The aim of this study was to investigate variation in quantitative traits and the evolutionary potential of plant populations sampled along elevational and latitudinal gradients. We chose the short-lived perennial plant *Anthyllis vulneraria* as a model species because of its wide geographical and elevational distribution across Europe. The two gradients were chosen to correspond to a similar change of 11.5 °C in annual mean temperature: an elevational gradient of c. 2000 m from lowland sites to the elevational limit in the European Alps and a latitudinal gradient of c. 2400 km from the centre of the distribution to its northern range limit in Scandinavia. In field populations of *A. vulneraria*, several phenotypic traits showed strong clinal variation along the two types of gradients (Daco et al. 2021) and molecular genetic diversity decreased with latitude (Daco et al. 2022). To study the variation of quantitative genetic traits along the same gradients, we grew plants from different populations and families in a common garden located at low elevation in the southern part of the latitudinal gradient. The choice of the lowland garden simulated an increase in mean annual temperature for most populations of origin along the elevational and latitudinal gradient to assess their potential to respond to climate change.

We address the following questions: (1) Do population mean traits show clinal variation along gradients of elevation and latitude? (2) Are population mean traits measured in the field and the common garden correlated? (3) Does the ability to take advantage of benign conditions in the common garden decrease with increasing elevation and latitude of the population of origin? (4) Is there evidence of diversifying selection for quantitative traits? (5) Does evolutionary potential in quantitative traits show clinal variation along the elevational and latitudinal gradients and is it positively correlated with molecular genetic variation?

Materials and methods

Study Species

Anthyllis vulneraria L. (Fabaceae) is a biennial to perennial herb of nutrient-poor calcareous grasslands and screes. It occurs all across Europe, the Mediterranean and the Caucasus from sea level up to 3000 m a.s.l. (Conert 1975). Its exceptionally wide geographic and elevational distribution in Europe makes it a good model species for studying patterns of genetic variation

(Daco et al. 2022). Seedlings of *A. vulneraria* develop a rosette in their first year and usually flower in their second year (Sterk 1975). Plant height and the number of flowerheads per plant decrease with elevation and latitude (Daco et al. 2021). *A. vulneraria* is a very polymorphic species and numerous infraspecific taxa have been described (Cullen 1968). However, we choose not to differentiate between subspecies because molecular genetic studies did not find support for this division (Nanni et al. 2004; Köster et al. 2008).

Sampling

We visited 20 populations of *Anthyllis vulneraria* along a 2400 km latitudinal gradient from the centre of its distribution in Central Europe (46.4 °N) to its northern distribution limit in Scandinavia (68.1 °N) and 20 populations along three elevational gradients in the French, Swiss and Austrian Alps from 500 m to the elevational limit at 2500 m a.s.l. (Table 1). The length of the two gradients was chosen to correspond to a change of 11.5 °C in annual mean temperature.

In summer 2015, we recorded at each site the elevation above sea level, latitude and longitude with a GPS (eTrex 20, Garmin Ltd.). In each population, we collected fruitheads from 20 plants along a 20 m transect and placed them in separate paper bags. To compare trait values in the field and in the common garden, for each mother plant we determined the height, the diameter of the rosette, the width of the longest leaflet, the number of stems, and the number of flowerheads. In the laboratory, we extracted all healthy seeds (i.e. green and large) from the fruitheads of each mother plant.

Cultivation in the common garden

In April 2016, ten seeds from each mother plant were scarified by rubbing them between sheets of sand paper, placed on moist filter paper in Petri dishes and kept at 20 °C in a greenhouse for germination. After five days, five seedlings (if available) per family (Table 1) were planted into square pots of 11 cm x 11 cm x 12 cm filled with a 3:1 mixture of low-nutrient soil (Substrat 1, Klasmann-Deilmann GmbH, Geeste, Germany) and sand. The plants were randomly placed outdoors in a common garden of the municipal park service of the city of Luxembourg. Plants were watered when necessary and re-randomized several times.

Measurements of quantitative traits

In July 2016, we recorded which of the initially 3207 plants had survived and recorded the following traits for each plant: number of leaves, diameter of the rosettes, and the width of their

Table 1 Populations of *A. vulneraria* sampled across elevational and latitudinal gradients.

Population	Country	Latitude °N	Longitude °E	Elevation m a.s.l.	N_{field}	N_{fam}	$N_{plants/family}$	N_{total}
Elevational gradient								
A1	Austria	47.3980	11.2661	961	100	20	4.4	87
A2	Austria	47.4421	11.6501	1521	300	20	4.8	95
A3	Austria	47.1606	11.7149	1810	1000	19	4.0	76
A4	Austria	47.1690	11.3533	1151	150	17	4.5	77
A5	Austria	47.3127	11.3894	2250	200	15	4.7	70
S1	Switzerland	46.1338	7.0595	545	200	18	4.4	80
S2	Switzerland	46.0833	7.1265	1042	80	16	4.2	67
S3	Switzerland	46.0496	7.9564	2162	30000	20	5.0	100
S4	Switzerland	46.2539	7.2734	1585	1000	20	4.7	93
S5	Switzerland	46.2735	7.2374	1250	1500	20	4.9	98
S6	Switzerland	46.0881	7.4067	1940	10000	20	4.8	96
S7	Switzerland	46.1081	7.5801	2413	10000	16	3.9	63
F1	France	45.0533	6.3892	2362	2000	20	4.9	97
F2	France	45.0512	6.3533	1997	1500	14	3.2	45
F3	France	45.1562	6.4237	1518	100	1	3.0	3
F4	France	45.2166	6.3250	1223	200	18	4.8	87
F5	France	45.0932	5.7804	471	1200	20	4.9	97
F6	France	45.1735	6.0389	936	250	20	4.9	97
F7	France	45.1210	5.9852	717	200	15	3.1	47
F8	France	45.0598	6.3157	1807	300	20	4.3	85
Latitudinal gradient								
1	France	46.4368	4.7528	323	60	20	5.0	99
2	France	48.1880	5.5534	443	800	20	5.0	99
3	Luxembourg	49.4956	5.9969	342	1000	16	3.1	49
4	Luxembourg	49.7314	6.2819	355	50	20	4.5	90
5	Germany	51.2228	9.7610	442	250	20	4.8	96
6	Germany	52.0051	10.4075	191	400	20	4.7	93
7	Germany	54.0443	10.2290	32	1500	20	5.0	99
8	Germany	54.6873	9.4342	22	1500	20	4.7	94
9	Denmark	55.5150	9.4244	42	400	20	4.9	98
10	Sweden	56.3671	12.8002	81	800	20	5.0	99
11	Sweden	57.8892	11.9466	24	300	13	3.5	45
12	Sweden	58.6979	11.2199	5	100	20	4.7	93
13	Norway	61.0621	10.3971	438	100	20	4.5	89
14	Norway	62.0139	9.2074	483	75	20	5.0	99
15	Norway	63.4409	10.6567	18	350	20	4.7	94
16	Iceland	63.8164	-22.6970	20	10000	18	3.4	62
17	Norway	64.3162	12.3475	168	4000	16	3.8	61
18	Sweden	66.4261	16.8501	453	300	17	4.3	73
19	Norway	67.2511	15.4282	6	1000	11	2.6	28
20	Norway	68.1022	16.3783	47	1000	20	4.4	87

N_{field} size of population of origin. N_{fam} number of seed families grown in the garden. $N_{plants/family}$ mean number of plants per family grown in the garden. N_{total} number of plants per population grown in the garden.

longest basal leaf. We measured leaf chlorophyll content with a chlorophyll meter (SPAD-502 Plus, Minolta, Osaka, Japan) and transformed the values obtained into actual chlorophyll concentrations using the formula for total chlorophyll content given by Richardson et al. (2002). One year later, in June 2017, we recorded the following traits for the 1043 surviving plants: plant height, date of opening of the first flower, number of flowering stems, total number of flowerheads and the number of flowerheads with open flowers. From each plant, we collected three flowerheads per plant and counted their number of flowers. We also collected the highest cauline leaf of each plant, placed them between wet paper towels in labelled envelopes in plastic bags and stored them at 5 °C. On the next day, the leaves were weighed to determine their fresh weight, placed in separate paper envelopes, pressed and dried with silica gel.

We weighed the dried leaves and scanned them at a resolution of 300 x 300 dpi together with a length standard. With the program ImageJ v. 1.51j8 (Schneider et al. 2012) we measured the area of the cauline leaves and calculated specific leaf area (SLA) as the ratio between leaf area and dry mass. Leaf dry-matter content (LDMC) was calculated as the ratio between dry and fresh weight. As a proxy for flowering phenology we calculated the proportion of heads flowering per population as the ratio between the sum of flowerheads with open flowers and the total number of flowerheads. Survival was calculated as the number of plants that survived per population divided by the total number of seedlings planted per population.

Pollination experiments

In June 2017, selfing-ability was tested on a subset of 223 plants from 27 populations. On each plant, an immature flowerhead was selected. One flower per flowerhead was marked with a permanent marker and the flowerhead protected by a bag of fine nylon mesh (mesh size ca. 0.1 mm) against pollinators. Once the flowers had opened, each flower was either left as a control for autonomous self-pollination or hand-pollinated with pollen from the same flowerhead by using a toothpick to gently transfer pollen to the receptive stigma. In August of the same year, the marked flowers were collected and the presence of developed seeds was determined.

Statistical analysis

We studied the effects of elevation and latitude on population means of traits with general and generalized linear models (GLMs) in R 4.2.2 (R Core Team 2022). The GLMs for proportion data (e.g. survival) were calculated with a logit link and a quasibinomial error distribution due to overdispersion (see Crawley 2009). For proportion data, McFadden's Pseudo r^2 was calculated as one minus the ratio between the log-likelihood of the model of interest and the log-likelihood of the null model. We also included seed mass in the model to check for potential maternal effects. For each population, survival was calculated as the number of plants that survived until summer of the second year / total number of plants, the proportion of heads flowering was calculated as number of heads flowering / total number of flowerheads, and the selfing ability as the sum of developed seeds / total number of flowers treated. The few plants that flowered in the first year (from populations F5 and S1) were excluded from the analyses of the variables "flowering onset" and "proportion of heads flowering". All statistical analyses were carried out using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, N.Y., USA) if not stated otherwise.

For the analysis of the selfing ability of the plants in the common garden, we tested separately for the data from the latitudinal and elevational gradients the effects of the gradient, of population identity and of pollination treatment on seed set with generalized linear models with a logit link. The effects of the gradients were tested against the variation among the populations, while the effects of treatment were tested against the residual deviance using quasi *F*-tests. No differences between the two treatments of autonomous and hand self-pollination were found in the latitude ($QF_{1,119} = 0.03$, $P = 0.86$) and the elevation dataset ($QF_{1,75} = 0.16$, $P = 0.66$) and data for the two treatments were therefore pooled per population.

For the studied populations along the two gradients, we performed bivariate correlations between the following mean traits determined for both plants in the field and in the common garden: height, rosette diameter, width of the longest leaf, number of stems and number of flowerheads. Based on these traits, pairwise Mahalanobis distances between both the field and the garden populations were calculated as a measure of quantitative genetic distances. Mahalanobis distances measure distance in multivariate space taking into account correlations among traits and are independent of the scale of the traits (Legendre and Legendre 1998). We compared the matrix of pairwise quantitative genetic distances for the traits measured in the field with the matrix of genetic distances calculated from the traits measured in the common garden for the same populations with a Mantel-test in GenAlEx 6.5 with 999 permutations (Peakall and Smouse 2006, 2012). Furthermore, as a measure of the plasticity of the studied populations in response to the conditions in the common garden, we calculated for each of the five traits the difference between the means for plants in the common garden and for plants in the field. We then related these trait differences to the elevational and latitudinal gradients by linear regressions.

To study the distribution of genetic variation in quantitative traits we calculated variance components for the traits measured in the common garden among populations (V_{pop}), among families within populations (V_{fam}) and among individuals within families (V_{error}) by restricted maximum likelihood with the *lmer* and *VarCorr* functions of the R-package lme4 v.1.1-31 (Bates et al. 2015). Q_{ST} was computed as V_{pop} / (4 V_{fam} + V_{pop}). We conservatively assumed that members of a family were full-sibs since the relationships between offspring of a mother plant was not known (Podolsky and Holtsford 1995; Jimenez-Ambriz et al. 2007). We calculated 95% confidence intervals for the Q_{ST} of each trait by jackknifing over populations (O'Hara and Merilä 2005). We calculated evolvability as the genetic coefficient of variation CV_{gen} =sqrt (2 V_{fam}) / mean.

Molecular genetic data based on microsatellites markers were available for all study populations (Daco et al. 2022). We calculated mean F_{ST} values over all loci and their respective 95% confidence intervals separately for both gradients by bootstrapping over loci in FSTAT 2.9.4. (Goudet 2003).

We examined the relationship between quantitative genetic distances (Mahalanobis distances) and pairwise elevational distances between populations along the elevational gradient, pairwise geographical distances between populations along the latitudinal gradient, and pairwise genetic distance (F_{ST}-values) between populations for both gradients separately with Mantel-tests (GenAlEx 6.5, 999 permutations). We also studied the relationship between Mahalanobis distances and elevational distances and between Mahalanobis distances and geographical distances while adjusting for the effect of neutral genetic distance (pairwise F_{ST}-values) with 999 permutations using the function *mantel.partial* in the R-package vegan 2.6-4 (Oksanen et al. 2022).

We studied the relationships between the following variables with simple regressions: (1) mean CV_{gen} per trait over populations of the elevational gradient vs. mean CV_{gen} per trait over populations of the latitudinal gradient, (2 CV_{gen} of a trait in a population vs. elevation, latitude, and uH_E of the population of origin, (3) mean CV_{gen} per trait over all populations vs. the Q_{ST} for that trait separately for the populations of the elevational and latitudinal gradient.

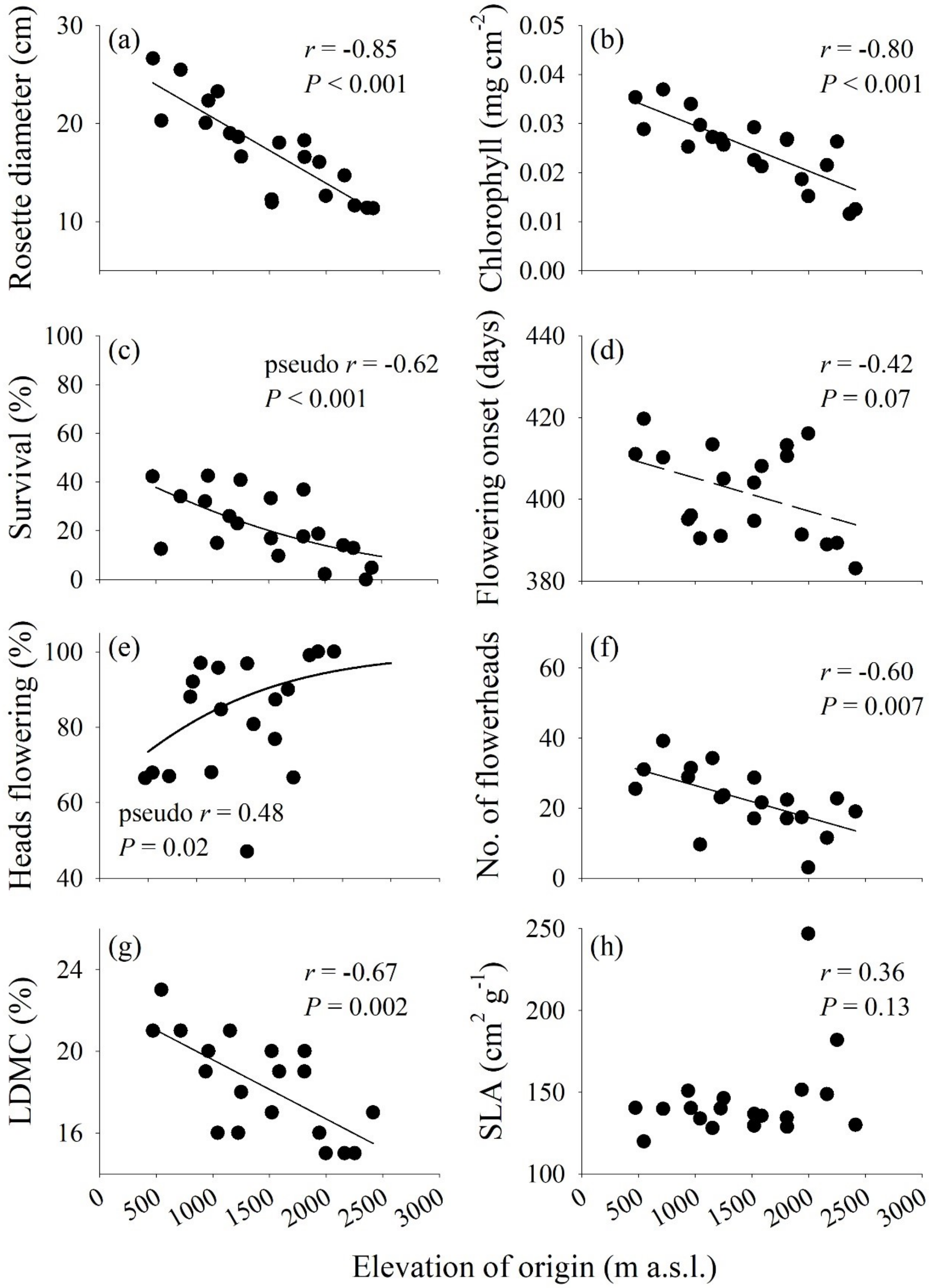

Fig. 1 The relationship between various traits in the common garden and the elevation of the populations of origin of *Anthyllis vulneraria* along the elevational gradient. (a) Diameter of first year rosettes, (b) chlorophyll content of first year leaves, (c) survival until summer of the second year, (d) time from planting until flowering, (e) mean proportion of heads with open flowers in mid-June of the second year, (f) mean number of flowerheads per plant, (g) leaf dry matter content (LDMC), (h) specific leaf area (SLA). Solid lines indicate relationships with $P < 0.05$, dashed lines those with ($P < 0.1$).

Results

Effects of elevation and latitude of the population of origin on traits measured in the common garden

Overall, 3207 plants of *Anthyllis vulneraria* were grown from seeds and their fate and development followed over the course of two vegetation periods. The diameter of the rosettes (Fig.1a) and the chlorophyll content of the leaves (Fig. 1b) in the first summer decreased continuously with the elevation of the source population. Similarly, survival until summer of the second year also decreased with the elevation of the population of origin (Fig. 1c). Plants from higher elevations started to flower earlier than those from lower elevations (Fig. 1d) and in mid-June of the second year a greater proportion of their flowerheads were already flowering (Fig. 1d), both indicating an earlier phenology of alpine genotypes. Size-related traits like width of the longest leaflet and plant height were smaller in alpine plants (all $r < -0.67$, $P < 0.0014$), and the number of flowerheads also decreased with population elevation (Fig. 1f). The dry matter content (LDMC) of leaves decreased with population elevation (Fig. 1g), but specific leaf area (SLA) showed no clear pattern (Fig. 1h).

The mean trait values per population varied with increasing latitude of the origin of the populations in a similar way as with increasing elevation, as all signs of the correlations were the same for the populations from the two gradients (Fig. 2 a – h). However, except for the number of flowerheads the relationships with latitude were less strong than those with elevation. In particular, chlorophyll content (Fig. 2b) and survival (Fig. 2c) showed much weaker declines with increasing latitude than with increasing elevation of population origin. All these effects remained qualitatively the same when seed mass was included to account for potential maternal effects.

The proportion of flowers producing a fruit by selfing (selfing ability) increased with both elevation and latitude of the population of origin (Fig. 3a, b).

Comparison between traits measured both in the field and in the common garden

Several traits were measured both for the mother plants in the 40 field populations and for their progeny in the common garden. For populations of both gradients, the pairwise quantitative genetic distances (Mahalanobis distances) between populations based on mean traits of the progeny in the common garden were significantly correlated with the pairwise Mahalanobis distances between populations based on the same traits measured for the mother plants in the

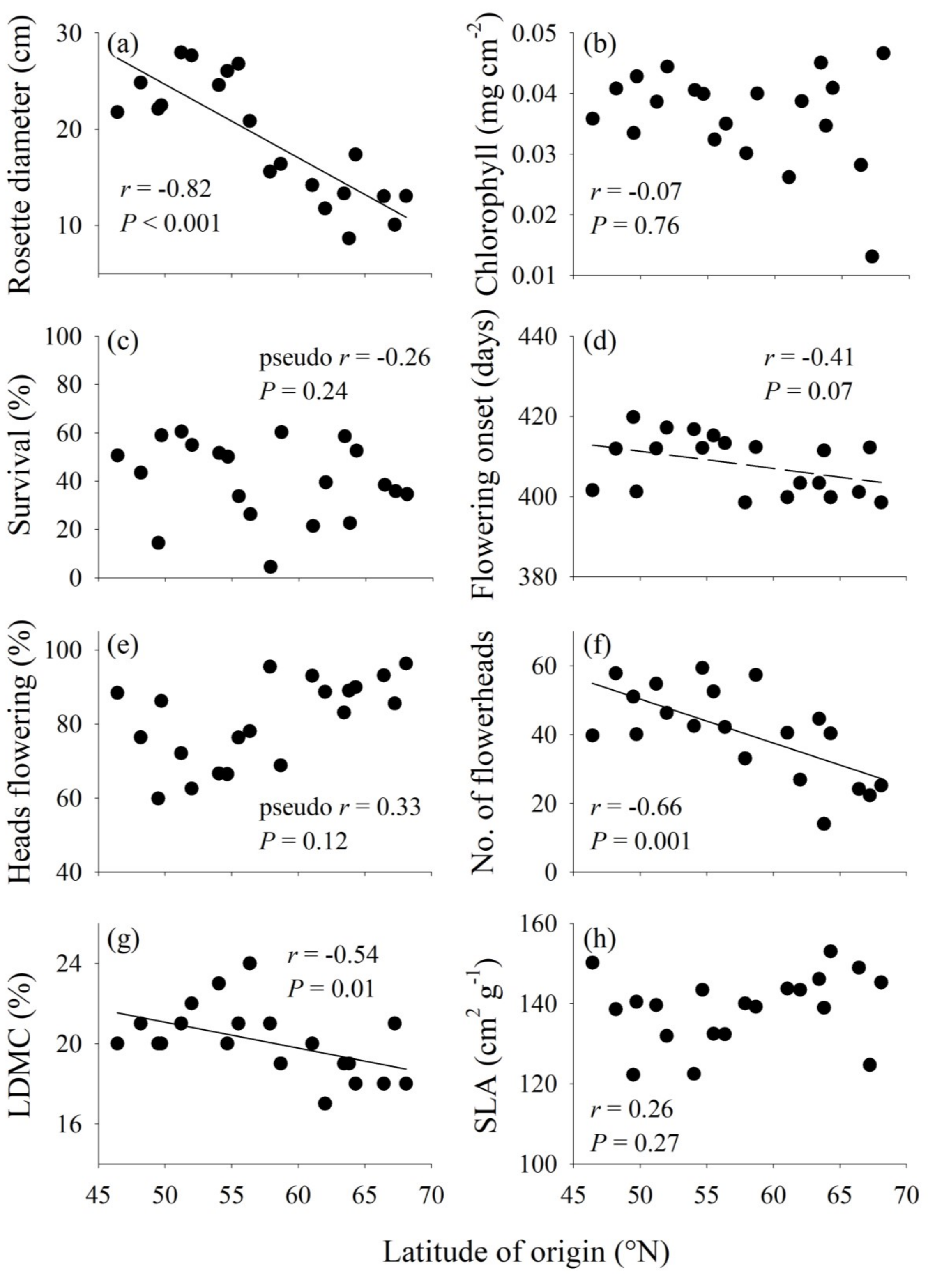

Fig. 2 The relationship between various traits in the common garden and the latitude of the populations of origin of *Anthyllis vulneraria* along the latitudinal gradient. (a) Diameter of first year rosettes, (b) chlorophyll content of first year leaves, (c) survival until summer of the second year, (d) time from planting until flowering, (e) mean proportion of heads with open flowers in mid-June of the second year, (f) mean number of flowerheads per plant, (g) leaf dry matter content (LDMC), (h) specific leaf area (SLA). Solid lines indicate relationships with $P < 0.05$, dashed lines those with ($P < 0.1$).

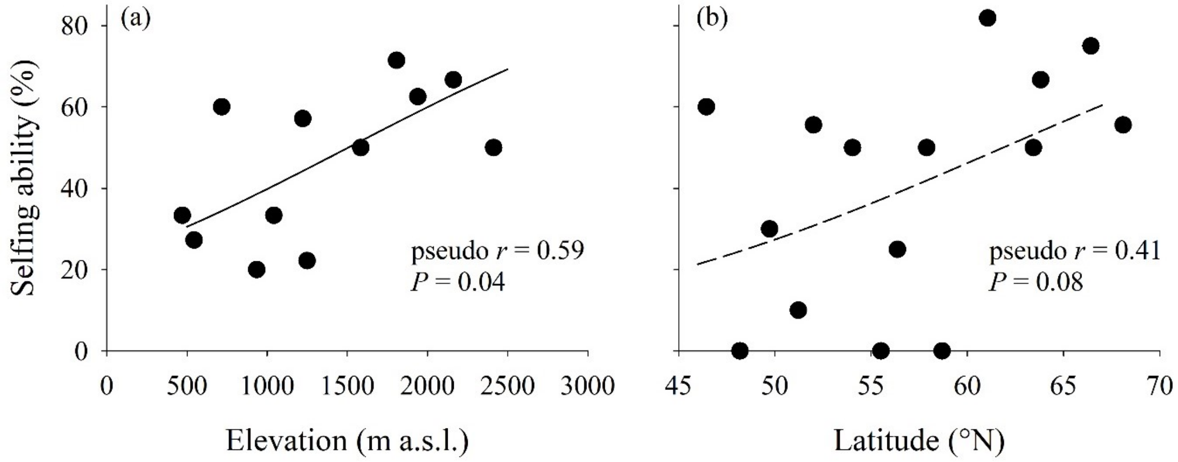

Fig. 3 Selfing ability (proportion of flowers producing a fruit by selfing) in the common garden of plants from populations (a) along the elevational gradient, and (b) along the latitudinal gradient. A solid lines indicates a relationship with $P < 0.05$, a dashed line that with ($P < 0.1$).

field (Fig. 4a, b). An analysis of the correlation between each trait measured for the populations in the field and the common garden indicated that for the elevational populations the positive correlations found between Mahalanobis distances were mainly due to relationships between the two height measurements and the two measurements of leaf size (Table 2a). Correlations between the Mahalanobis distances for the latitudinal populations were due to correlations between the same variables (height and leaflet size) and in addition due to the correlation between the mean number of flowerheads of the mother plants and the progeny (Table 2b).

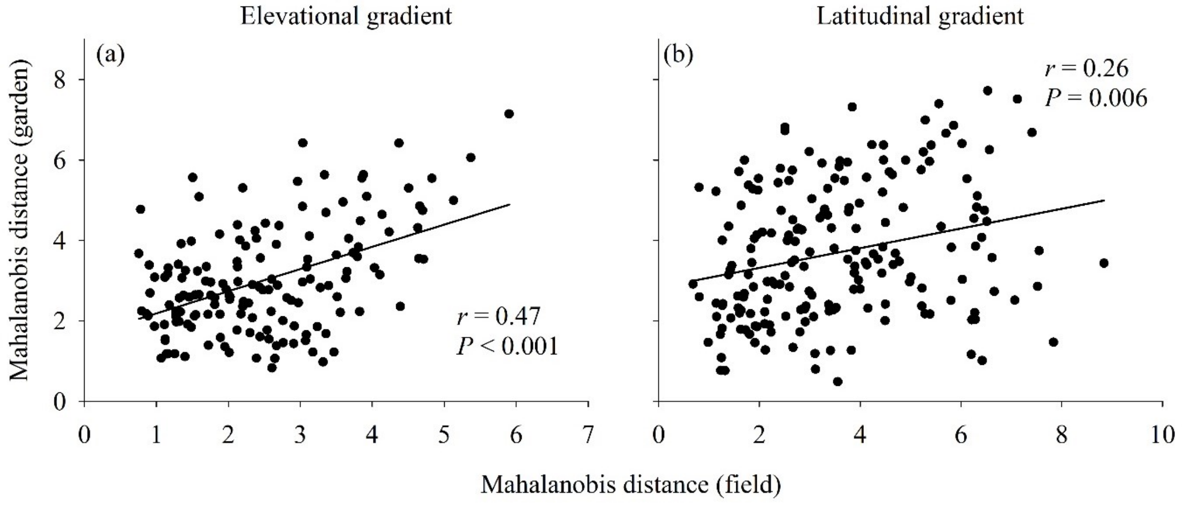

Fig. 4 Relationship between pairwise quantitative genetic distances (Mahalanobis distances) of traits measured in the common garden and of traits measured in the field for (a) the *A. vulneraria* populations of the elevational gradient and (b) the populations of the latitudinal gradient. *P*-values are derived from Mantel-tests.

Table 2 Correlations between traits measured both in the field and in the common garden on *A. vulneraria* plants (a) for the 20 elevational populations and (b) for the 20 latitudinal gradient populations separately. Note that diameter and width of longest leaflet were measured on adult plants in the field but on juvenile plants in the common garden.

	(a) Elevational gradient		(b) Latitudinal gradient	
Variable	*r*	*P*	*r*	*P*
Height	**0.67**	**< 0.01**	**0.77**	**< 0.001**
Diameter	-0.36	0.13	0.27	0.26
Width of longest leaflet	**0.51**	**0.03**	**0.73**	**< 0.001**
log No. of flowerheads	0.07	0.79	**0.60**	**0.005**
Stems per plant	-0.04	0.88	0.34	0.15

Mean plant size measured as rosette diameter or number of flowerheads per plant was generally much greater for the progeny in the common garden than the mother plants in the field populations, as indicated by the positive differences between performance of progeny and mother plants (Fig. 5a, b). These positive differences indicate more benign conditions for the *Anthyllis* plants in the common garden. However, plants from higher elevations and higher latitudes were less plastic and benefitted less from the more favourable conditions than those from lower elevations (Fig. 5a, c) and latitudes (Fig. 5b, d) in terms of rosette diameter and number of flowerheads.

Genetic differentiation among populations

The Q_{ST} values of individual traits for populations of the elevational and latitudinal gradient were correlated ($r = 0.67$, $P = 0.03$). Q_{ST} values were significantly higher than F_{ST} values for some of the measured traits for plants from populations of both gradients (Fig. 6) indicating divergent selection along the elevational and latitudinal gradients. This was true for rosette diameter and the date of onset of flowering. For the latitude populations Q_{ST} values for several traits were lower than the F_{ST} value, which was due to the much higher F_{ST} value. These traits included size-related traits like the number of leaves, stems and flowerheads as well as physiological traits like leaf chlorophyll, LDMC and SLA.

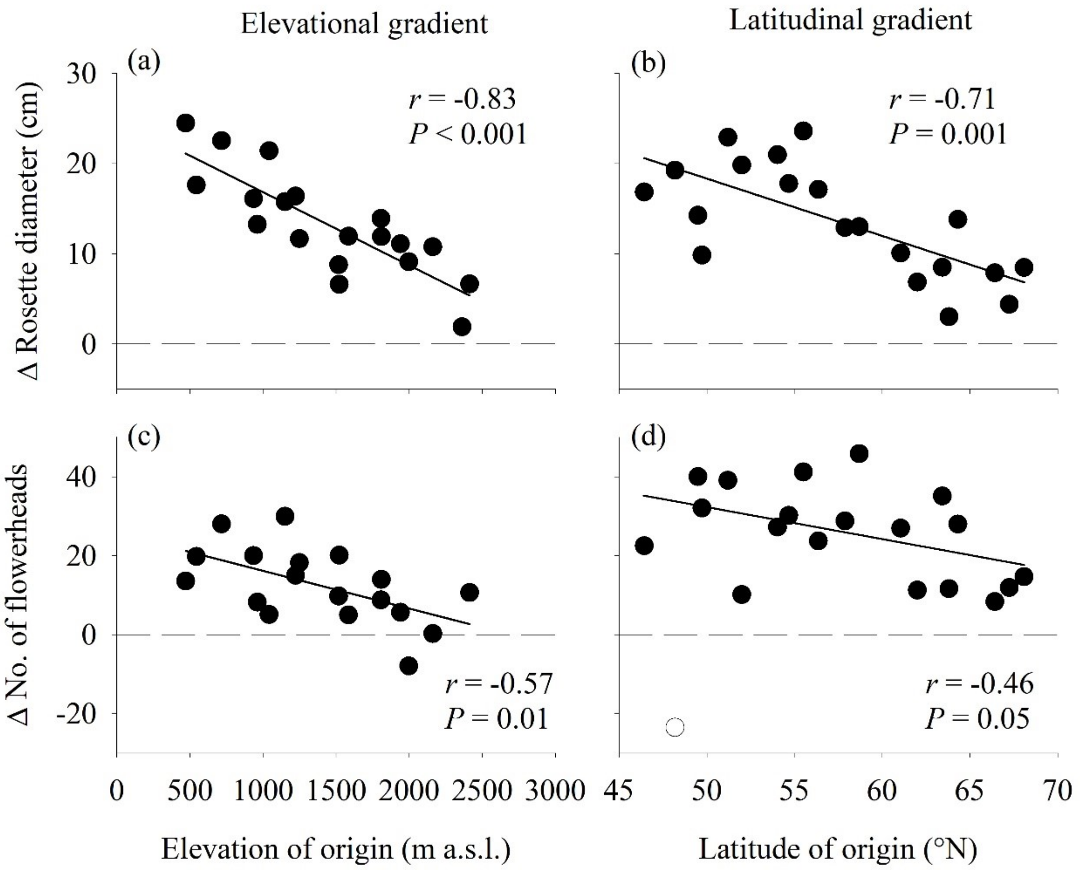

Fig. 5 Morphological plasticity of plants from populations of *A. vulneraria* along the elevational and the latitudinal gradients. (a, b) The difference between the rosette diameter of the progeny in the common garden and that of the mother plants in the field regressed against the position of a population along (a) the elevational gradient and (b) the latitudinal gradient. (c, d) The difference between the mean number of flowerheads of the progeny in the common garden and that of the mother plants in the field regressed against the position of a population along (c) the elevational gradient and (d) the latitudinal gradient. Dashed lines indicate Δ = 0. The population denoted by the open symbol in figure (d) was omitted from the regression analysis.

Pairwise quantitative (Mahalanobis distances) and elevational distances (Fig. 7a) or geographical distances (Fig. 7b) were significantly correlated. Pairwise quantitative (Mahalanobis distances) and molecular genetic distances (pairwise F_{ST} values) were only weakly correlated for populations of both the latitudinal gradient and the elevational gradient (Fig. 7c, d). The clear positive relationships between quantitative genetic distances and elevational or geographical distances remained even when the relationships were adjusted for the effects of molecular genetic distances (Fig. 7e, f). These patterns indicate that genetic drift is not sufficient to explain the differences in morphology among populations in the common garden, but that local adaptation and diversifying selection acts along both types of gradients.

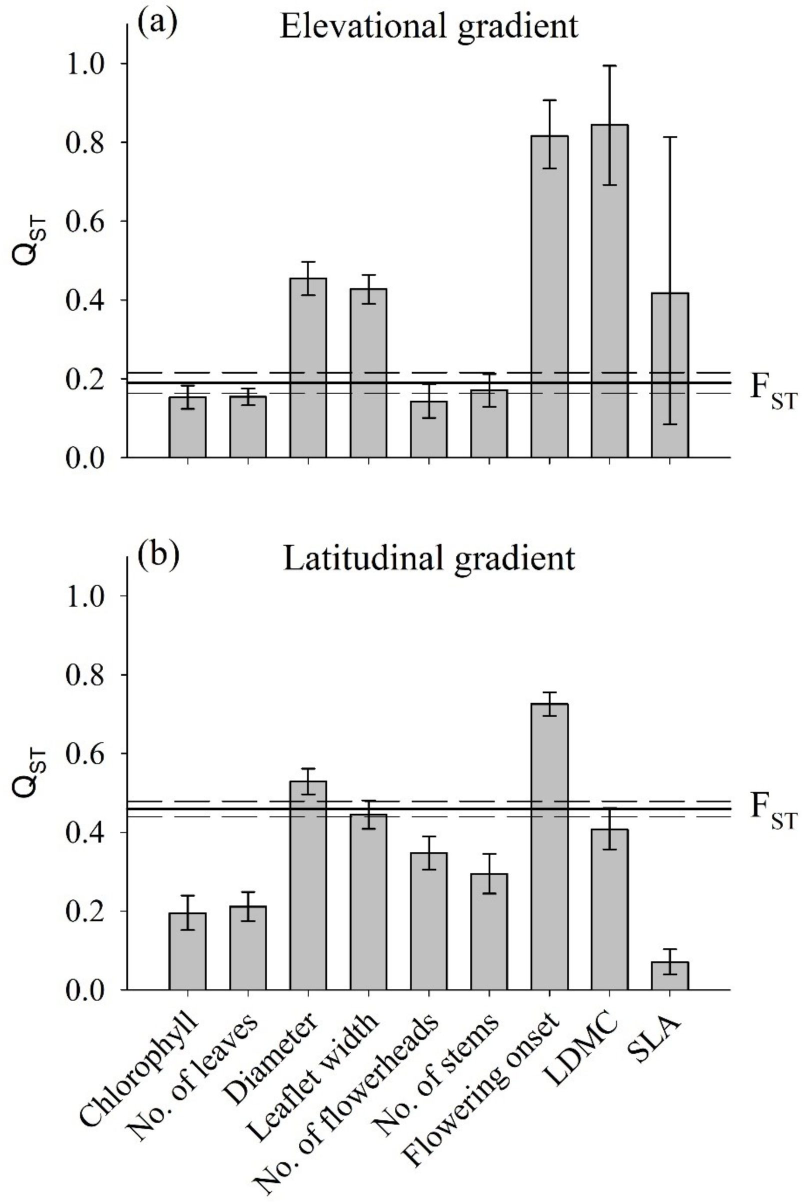

Fig. 6 Comparison between the quantitative genetic (Q_{ST}) and the molecular genetic differentiation (F_{ST}, from Daco et al. 2022) between populations of *Anthyllis vulneraria* (a) along the elevational and (b) the latitudinal gradient. Vertical lines show the lower limit of the 95% confidence intervals of the Q_{ST} and dashed lines show the confidence intervals of F_{ST}.

Quantitative genetic variation within populations

The mean evolvabilities per trait of the elevational gradient were highly correlated with the ones from the latitudinal gradient (Fig. 8). Relationships between quantitative genetic variation of traits in the populations and the position of a population along the elevational gradient were nearly all positive ($r = 0.14$ to $r = 0.43$), with exception of onset of flowering ($r = -0.25$, $P = 0.35$). The relationships between evolvability and latitude of a population were nearly all weak ($r = - 0.17$ to $r = 0.29$), with the exception of onset of flowering ($r = 0.54$, $P = 0.02$), leaf chlorophyll ($r = 0.47$, $P = 0.07$), and rosette diameter ($r = -0.40$, $P = 0.08$).

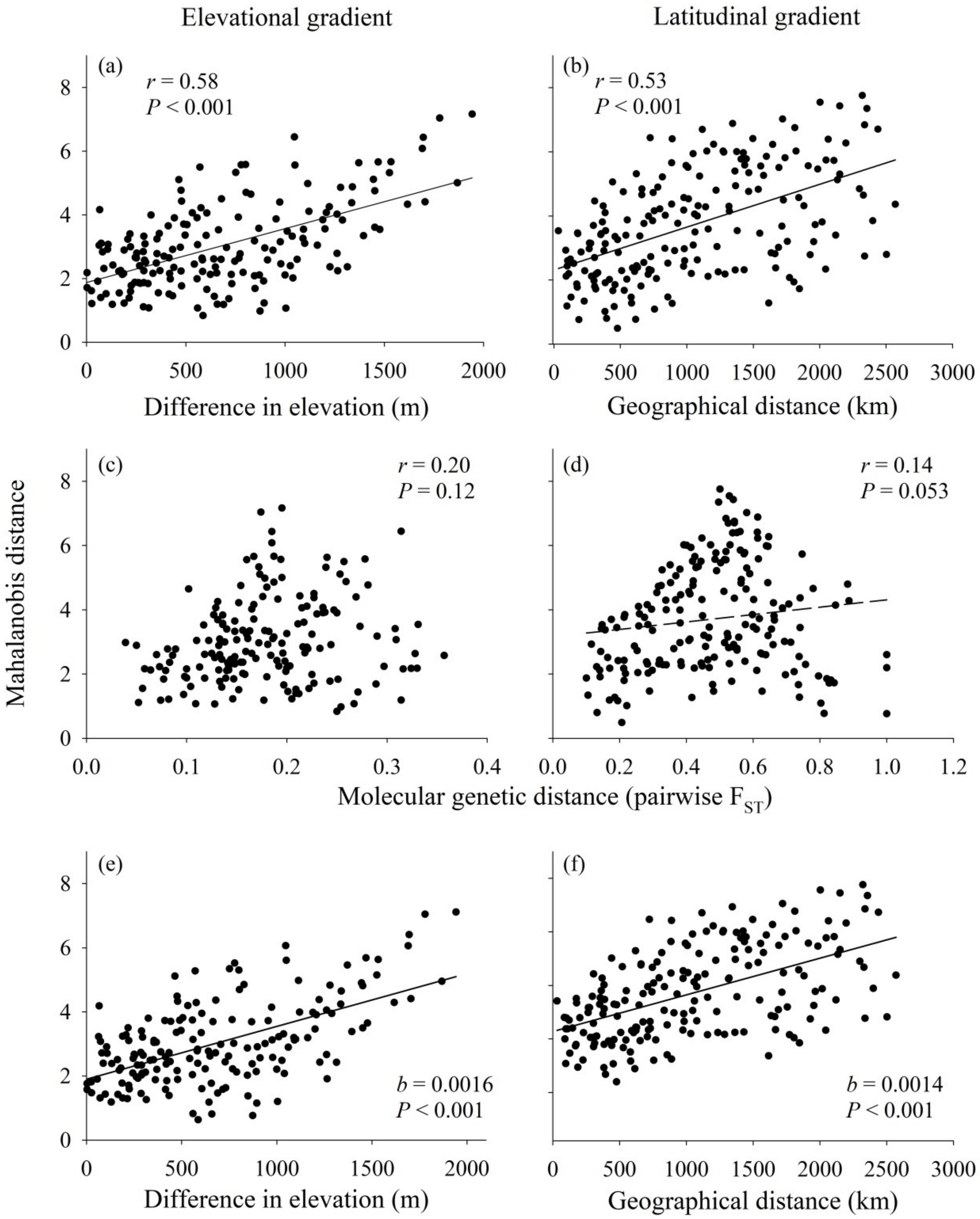

Fig. 7 Relationships of pairwise quantitative genetic distances (Mahalanobis distances) between populations of *Anthyllis vulneraria* and (a, e) pairwise elevational distances between populations along the elevational gradient (b, f) pairwise geographical distances between populations along the latitudinal gradient, and (c, d) pairwise molecular genetic distances (F_{ST}). Relationships in (e) and (f) were adjusted for the effects of pairwise molecular genetic distances (F_{ST}) between populations. *P*-values are derived from Mantel-tests. Solid lines indicate relationships with $P < 0.05$, dashed lines those with ($P < 0.1$).

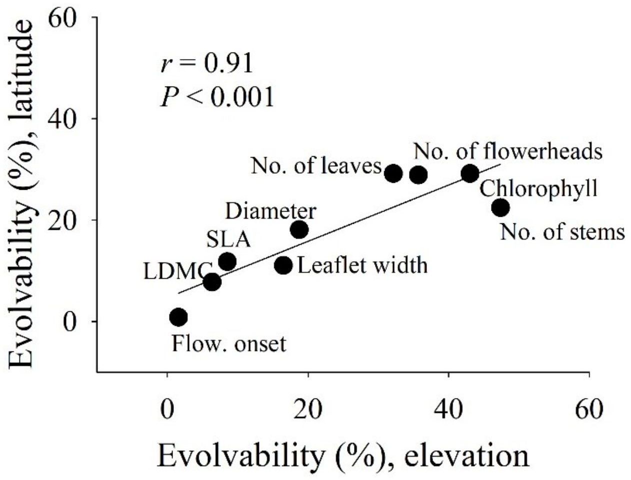

Fig. 8 Relationship between mean evolvability of a trait in the latitudinal and the elevational populations.

Only the evolvability (CV_{gen}) of chlorophyll per population was clearly correlated with molecular genetic variation (uH_E), and only for the populations of the latitudinal gradient ($r = -0.55$, $P = 0.03$; for all other traits and populations from both gradients: $|r| < 0.53$, $P > 0.06$). The mean evolvability of a trait in the populations decreased with increasing genetic differentiation between populations (Q_{ST}), both from the Alps and from the latitudinal gradient (Fig. 9a, b).

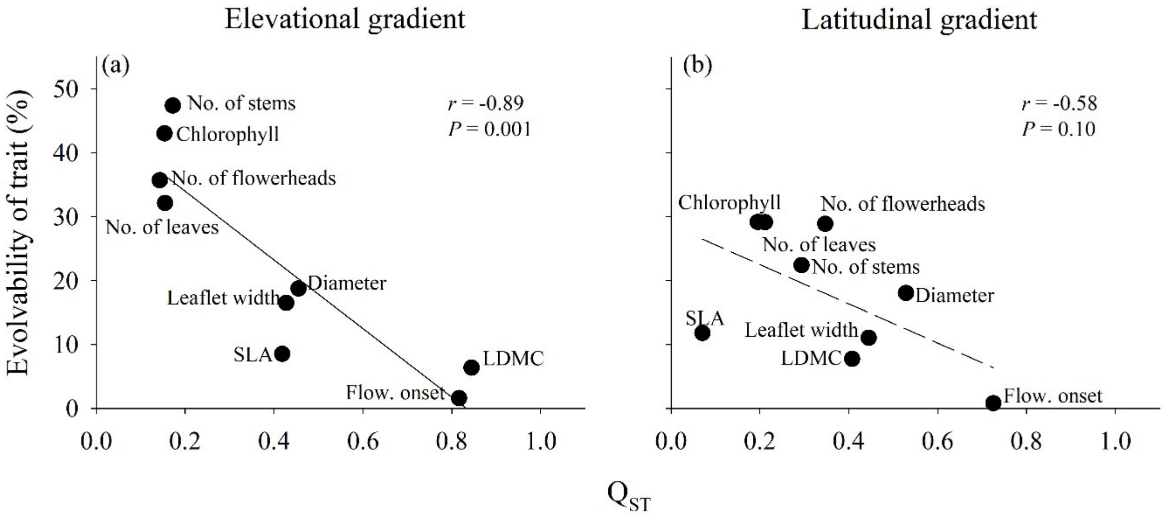

Fig. 9 Relationship between the mean evolvability of a trait and its quantitative genetic differentiation among populations (Q_{ST}) for the populations of (a) the elevational and (b) the latitudinal gradient.

Discussion

Plant species with a widespread distribution can respond with plasticity and/or genetic differentiation to different environments. We studied quantitative traits of plants of *Anthyllis vulneraria* in a common garden from populations along an elevational gradient that ranged from lowland populations to the elevational limit of the species in the Alps, and along a latitudinal gradient from the centre of the distribution of the species in Central Europe to its northern distributional margin. Several lines of evidence indicated divergent selection among populations of *A. vulneraria* along both gradients. Most population traits measured in the common garden showed clinal variation with elevation or latitude of origin suggesting selection and indicating potentially adaptive differentiation of traits along the gradients. A recent review (Halbritter et al. 2018) found that adaptation to elevation is common in plants and studies have also found local adaptation along latitudinal gradients (Joshi et al. 2001; Becker et al. 2006; De Frenne et al. 2011a). We found that the ability to benefit from the benign conditions in the common garden declined with both increasing elevation and latitude of the origin of the plants indicating lower plasticity. In contrast, the evolvability of most traits did not vary consistently along the two gradients indicating a considerable evolutionary potential even in peripheral populations.

Genetic differentiation among populations

Quantitative genetic distances between populations as measured by their Mahalanobis distances increased with elevational differences between populations in the Alps and geographical distances along the latitudinal gradient. These relationships remained when controlling for differences in neutral molecular genetic variation, indicating that quantitative genetic differentiation between populations was higher than expected by neutral processes, suggesting divergent selection along the elevational and latitudinal gradients. A similar pattern of quantitative genetic variation has been found in the plants *Primula sieboldii* (Yoshioka et al. 2007) and *Carduus defloratus* (Vaupel 2013).

The Mahalanobis distances between traits measured for mother plants in the field and their progeny in the common garden correlated strongly for both gradients, providing additional support that at least some differences in quantitative traits had a strong genetic component. However, a correlation between traits measured in the field and in the garden was only observed for plant height and the width of the longest leaf. This suggests either that the other traits do not have a strong genetic component or that a substantial plastic response masked the potential effects of selection.

Further evidence for selection was indicated by higher Q_{ST}-values (measuring quantitative genetic differentiation) than F_{ST}-values (measuring neutral molecular genetic differentiation) in traits like rosette diameter, leaflet width, time of flowering onset and LDMC in the populations from the elevational gradient, and in rosette diameter and time of flowering onset along the latitudinal gradient. These results are in line with those of most studies comparing Q_{ST} and F_{ST}, which found evidence for divergent selection (Merilä and Crnokrak 2001; De Kort et al. 2013; Leinonen et al. 2013). In contrast, for several traits of the latitudinal populations, F_{ST} was higher than Q_{ST}, which could be interpreted as the results of stabilizing selection. However, Q_{ST} values were mostly similar for the two gradients and the pattern of lower Q_{ST} than F_{ST} was due the high value of F_{ST} for the latitudinal populations, which was driven by the extremely strong differentiation between the arctic populations of *A. vulneraria* as a result of serial founder effects after the last ice age (Daco et al. 2022).

Clinal variation in quantitative traits

Plants from arctic and alpine populations flowered earlier than those from southern or lowland populations, and the proportion of heads flowering was higher at the time of measurement in alpine plants, indicating that they were further advanced in their life cycle in the common garden. A more rapid initiation of flowering in response to warm conditions is thought to be a crucial adaptation of plants from high elevations and latitudes to a shorter growing season which allows them to complete their reproductive cycle before the climatic conditions become unfavourable (Inouye and Wielgolaski 2003; Griffith and Watson 2005; Colautti and Barrett 2013). Northern populations have been found to flower earlier than southern ones in the herbs *Campanula rotundifolia* (Preite et al. 2015) and *Lythrum salicaria* (Olsson and Ågren 2002), but not in *Arabidopsis thaliana* (Stinchcombe et al. 2004). Similarly, flowering started earlier in high elevational populations of *Campanula rotundifolia* (Preite et al. 2015) and *Rhinanthus minor* (Ensing and Eckert 2019) in a common garden. In contrast, the start of flowering was delayed with increasing elevation of origin in other studies (Giménez-Benavides et al. 2007; Montesinos-Navarro et al. 2011; Hämälä et al. 2018).

Plants of *A. vulneraria* from arctic and alpine populations produced leaves with a lower LDMC than southern and lowland plants. Plants with a low LDMC allocate fewer resources to structural components, which allows the available resources to be used for rapid growth

(Wright et al. 2004; Grassein et al. 2015). This strategy to quickly exploit resources when they are available is found in fast-growing species at sites with short vegetation periods (Garnier et al. 2004; Polley et al. 2020) and could be an important adaptation for alpine and arctic plants (Körner 2016).

In *A. vulneraria* the ability to produce seeds by autonomous selfing increased with both elevation and latitude. An increased capacity for self-fertilization can be selected by conditions of chronic pollen limitation (Baker 1955; Cheptou 2012; Fulkerson et al. 2012; Hargreaves et al. 2015). It has been suggested that selfing rates generally increase with elevation to assure seed production, because pollination is limited in alpine environments due to the short vegetation period and the unfavourable conditions (Medan et al. 2002; Garcìa-Camacho and Totland 2009; Jiang and Xie 2020). The same argument has been made for populations at the northern range limit, and several studies have found plants from northern populations to be more self-compatible than those from southern populations (Ægisdóttir and Thórhallsdóttir 2006; Griffin and Willi 2014; Moeller et al. 2017; Yang et al. 2018). However, other studies did not find that plants are generally pollen-limited in alpine environments (Totland 2001; Giménez-Benavides et al. 2008; Xu et al. 2023) and that pollen limitation is higher at high latitudes (Koch et al. 2020; Dawson-Glass and Hargreaves 2022). Our study supports the notion of selection for a higher selfing ability in arctic and alpine environments.

In natural populations of *A. vulneraria* we found a decrease of plant size and number of flowerheads with elevation and latitude (Daco et al. 2021). We also found a decrease of these traits with elevation and latitude of origin in the common garden. A smaller size in cold environments is assumed to be adaptive for plants because closer to the ground the microclimate is warmer and they are protected from wind (Körner 2003; 2016; Halbritter et al. 2018). A decrease in fitness-related plant size traits with elevation or latitude was found in many other species in common garden settings (Olsson and Ågren 2002; Byars et al. 2007; De Frenne et al. 2013; Vitasse et al. 2014; Preite et al. 2015, but see Montesinos-Navarro et al. 2011).

The much lower survival of alpine populations indicates that alpine populations were maladapted to the conditions in the lowland common garden. A recent review on plant adaptations to elevation found that the environment limits the ability of genotypes to persist at strongly different elevations mainly through selection on survival (Halbritter et al. 2018). Similarly, another review of 38 studies on vascular plants found that individuals transplanted downwards showed lower survival than local individuals (Midolo and Wellstein 2020). The often low survival of alpine plants under lowland conditions has been linked to the higher respiration of alpine in comparison to lowland plants (Körner 2003) and a lower temperature optimum of photosynthesis (Billings and Mooney 1968). In *A. vulneraria*, the strongly reduced leaf chlorophyll content of plants from alpine populations may have contributed to their low survival in the common garden. Plants from high radiation environments like high elevation habitats have been found to show lower leaf chlorophyll concentrations than lowland plants in the field in *Hypericum perforatum* (Roblek et al. 2008). In contrast to the elevational gradient, we did not find a decrease in chlorophyll content with latitude. Other studies also found that alpine plants had lower chlorophyll concentrations than arctic ones when grown under common conditions (Mooney and Billings 1961; Tieszen and Bonde 1967). Chlorophyll content is often linked to nitrogen availability (Mohotti and Lawlor 2002) and the alpine populations of the legume *A. vulneraria* may have suffered in the common garden from a lack of nitrogen due to the absence of specific nitrogen-fixing rhizobia that occur at higher elevations (see Vollmann et al. 2011).

Since the common garden experiment was implemented with seeds collected in field populations, maternal effects could also have contributed to the observed clines. However, a strong influence of maternal effects is unlikely in the case of *A. vulneraria* because including seed mass in the analysis to account for maternal effects did not change the results qualitatively. Moreover, maternal effects are usually rare, weak and mostly observable in early life stages (Uller et al. 2013).

Phenotypic plasticity

To investigate if clines in plant traits were caused by plasticity or local adaptation, we compared phenotypic measurements taken in the common garden with the ones taken on the mother plants in the field. The size of the progeny from the southern and low elevation populations and the number of their flowerheads was much larger than that of their mother plants in the field. This indicates that conditions in the garden were more benign due to the absence of competition, watering of the plants, and higher nutrient levels. However, differences between the performance of progeny in the garden and that of their mother plants in the field were much smaller for populations from high elevations and latitudes, indicating that they were less plastic. The observed differences in traits between lowland and high elevation and between southern and high latitude populations are thus mainly due to genetic differences. In our experiment, environmental distances between the home site and the lowland common garden increased with increasing latitude and elevation. Our results are thus in contrast to those of Toczydlowski and Waller (2021) who found that the progeny of *Impatiens capensis* resembled their mothers less as the environmental distance between their home site and the common garden increased.

Lower plasticity has been hypothesized to be an evolutionary response to adverse climate conditions (Hemborg and Després 2011), and arctic and alpine populations of *A. vulneraria* may thus show a cautious strategy of an early cessation of growth and a switch to reproduction even under benign environmental conditions. A reduced plasticity of rosette diameter and number of inflorescences was also found in *Ranunculus bulbosus* in high vs. low elevation populations (Frei et al. 2014).

Evolutionary potential of populations

The abundant centre model hypothesizes that genetic diversity within populations may decline and genetic differentiation among populations may increase from the centre of the distribution of a species towards the edge (Eckert et al. 2008; Pironon et al. 2017). However, we did not find a decrease neither of evolvability of quantitative traits nor of molecular genetic diversity with elevation (Daco et al. 2022). In contrast, in *A. vulneraria*, molecular genetic diversity strongly declined with latitude due to the loss of variability through serial founder effects (Daco et al. 2022). However, no such decline with latitude was observed for the evolvability of traits as a measure of quantitative genetic variation within populations, except for rosette diameter. In line with our results, northern populations of *Arabidopsis* species with low genetic diversity also presented strong evidence of adaptation and adaptive potential (Huber et al. 2014; Hämälä et al. 2018; Takou et al. 2021). A possible explanation for the lack of a relationship between the genetic variation in molecular markers and quantitative traits is that quantitative traits in contrast to molecular markers are affected by selection (McKay et al. 2001; Kingsolver et al. 2012; Estoup et al. 2016). Moreover, molecular markers are discrete traits strongly affected by bottlenecks, whereas quantitative traits integrate effects and interactions of multiple genes, which are much less prone to the negative effects of bottlenecks (Reed and Frankham 2001; Estoup et al. 2016). Polygenic quantitative traits are more likely to accumulate variability due to mutations and should thus sustain more genetic variability at small population sizes and recover genetic variability more rapidly after a bottleneck than discrete molecular markers (Estoup et al. 2016). The evolutionary potential of arctic *A. vulneraria* populations may thus have been preserved despite serial founder effects leading to very low genetic diversity in neutral markers (Daco et al. 2022).

Conclusions

We found clinal variation in quantitative traits measured in *Anthyllis vulneraria* in a common garden in plants originating from populations along large elevational and latitudinal gradients. The observed variations were similar along the two types of gradients although the relationships with latitude were weaker. The correlation between measurements in the field and in the garden indicated that a large part of the clinal variation in phenotypic traits had a genetic basis. The genetic differentiation among populations along the gradients allows *A. vulneraria* to occupy a wide climatic niche and questions the existence of the many subspecies which have been described (Cullen 1968).

Alpine and arctic regions are particularly affected by ongoing changes in climate with higher increases in temperature than in other regions (Pepin et al. 2015). The lower plasticity of plants from alpine and arctic than lowland and southern populations may jeopardize their future survival. The alpine populations are particularly sensitive to changing conditions as their survival in the lowland garden was very low. However, their genetic diversity and the short length of the elevational gradient favouring gene flow among alpine and lowland populations could allow genetic adaptation to new conditions (Gonzalo-Turpin and Hazard 2009). In contrast, high latitude populations did not show lower survival in the common garden. Their evolutionary potential in quantitative traits could allow them to locally adapt to changing environmental conditions although arctic populations had a very reduced molecular genetic diversity (Daco et al. 2022). Our results confirm that the variation in neutral molecular markers is a poor predictor of variation in quantitative traits and is of limited usefulness to determine the adaptive potential of populations (Reed and Frankham 2001; Teixeira and Huber 2021; Chung et al. 2023). Since we did not find a decline of evolutionary potential from the centre to the periphery of the gradients, our results also support the growing evidence that the abundant centre model does not capture the complexity of predicting the suitability of a species to its environment (Pironon et al. 2017).

GENERAL DISCUSSION

Environmental variations, such as those found across gradients of elevation and latitude, play a crucial role in determining the distribution of species, their population characteristics, and their ability to survive (Woodward 1987; De Frenne et al. 2013). In response to new environmental conditions, plants can exhibit phenotypic plasticity by expressing reversible phenotypes (Piersma and Drent 2003; Jump and Peñuelas 2005; Atkin et al. 2006; Valladares et al. 2007). They can also undergo evolutionary changes and adapt towards phenotypes that are better suited to the local conditions (Jump and Peñuelas 2005). Conserving genetic variation is essential to preserve a species' capacity for evolutionary adaptation (Frankham et al. 2017). In this dissertation, I studied populations of a widespread plant species (*Anthyllis vulneraria*) along an extensive latitudinal gradient from Central Europe to the Arctic and an elevational gradient of 2000 m in the European Alps to examine plasticity and genetic variation of populations and how they may respond to changing environmental conditions. In both studied gradients, mean annual temperature decreased by c. 11.5 °C.

Clinal variations along elevational and latitudinal gradients

Previous research has predominantly focused on studying populations along either elevational (Totland 2001; Pellissier et al. 2010; Rasmann et al. 2014) or latitudinal gradients (Sagarin et al. 2006; Moeller et al. 2017), often assuming that changes along elevation can serve as a proxy for changes with latitude or ongoing climate change. However, the effects of the two types of gradients should preferably be compared and species that have both large latitudinal and elevation distributions are well suited for this approach (Halbritter et al. 2015). Even if similar changes in annual mean temperature can be measured over 1000 m in elevation and 1000 km in latitude (Jump et al. 2009b), elevational and latitudinal gradients diverge in a number of aspects including atmospheric pressure, precipitation, solar radiation, and soil nutrients (Körner 2007; De Frenne et al. 2013). Consequently, the response of species across these gradients may vary in crucial characteristics.

To study the clinal variations in population traits along the elevational and latitudinal gradients, I explored the impact of various habitat characteristics correlating with elevation and latitude. Temperature variations were found to have the strongest effects on population characteristics along both gradients, followed by differences in precipitation, solar radiation, and soil nutrients. Along both the elevational and latitudinal gradients, there were clines in plant size, reproduction, and density of *A. vulneraria* populations. However, while the density of *A. vulneraria* populations increased along both gradients, the size and reproduction of the plants exhibited a decline. This contrasting trend between reproduction and recruitment indicated a compensation between vital rates in populations of *A. vulneraria* along the gradients called demographic compensation, which is known to play a role in shaping the distribution of widespread species across a broad climatic range. Furthermore, there was a consistent decrease in seed predation along both the elevational and latitudinal gradients, which could be attributed to the influence of lower temperatures on the development and abundance of herbivores. In contrast, seed set and seed mass exhibited distinct responses along the two gradients. The decline in these reproductive variables with latitude could be due to changing habitat characteristics such as decreasing soil nutrients and temperatures, decreasing diversity and abundance of pollinators, or increasing pollen limitation. On the other hand, seed set and mass did not exhibit a consistent pattern with elevation, displaying high variability instead, which is likely a result of local environmental heterogeneity or stochasticity in pollen availability.

Regarding population structure I only found a cline along the latitudinal gradient, where the proportion of flowering plants decreased, suggesting different life cycle strategies between high-latitude and high-elevation populations of this plant species previously described as mainly biennial. In northern populations, higher levels of precipitation may explain the increased recruitment that, in combination with a high mortality of young plants during winter, resulted in a higher proportion of vegetative plants. Another possible explanation is that flowering in northern populations experienced a delay due to plants remaining vegetative for more than one year, indicating the absence of a strict biennial pattern. The lack of consistent variations in the proportion of flowering plants along the elevational gradient suggests that these populations grow in very heterogeneous habitats that vary on a small geographical scale. Alpine habitats generally exhibit high levels of heterogeneity in small-scale habitat conditions (Körner 2007).

Using microsatellite markers, which are considered to be neutral and unaffected by natural selection, I examined the presence of clines in molecular genetic diversity and differentiation of the *A. vulneraria* populations. The observed genetic patterns differed between the two gradients. Along the latitudinal gradient, genetic diversity decreased, while differentiation among populations increased. These patterns are likely the outcome of serial founder effects during the northward colonization following glacier retreat after the last ice age. This is supported by the identification of distinct genetic clusters in populations along the latitudinal gradient. Populations located north of the ice shield's limit during the Last Glacial Maximum and the Younger Dryas formed separate clusters. Due to the migration toward northern regions, the newly established populations in the North experienced genetic isolation, resulting in limited gene flow that led to a strong genetic differentiation. In contrast to the latitudinal gradient, the genetic diversity and differentiation among populations of *A. vulneraria* did not exhibit clinal patterns along the elevational gradient. This observation could be attributed to the higher gene flow facilitated by the much shorter elevational gradient and supported by the presence of admixture in populations situated at intermediate elevations. However, despite the disparity in gradient length, I observed evidence of isolation by distance in both the elevational and latitudinal gradients indicating restricted gene flow among populations along both gradients.

Clinal variations with elevation or latitude of origin were also found for population means of quantitative traits of *A. vulneraria* plants grown in a common garden from seeds originating from the studied populations. Arctic and alpine populations exhibited earlier flowering and a higher proportion of flowering heads compared to southern or lowland populations, indicating their advancement in the reproductive cycle in the common garden. This early flowering is believed to be an important adaptation to cope with shorter growing seasons in high elevations and latitudes (Inouye and Wielgolaski 2003; Griffith and Watson 2005; Colautti and Barrett 2013). Additionally, plants from arctic and alpine populations displayed lower leaf dry matter content (LDMC) compared to plants from southern and lowland populations. Plants with low LDMC values allocate fewer resources to structural components, which allows for rapid growth and a shorter life cycle. Furthermore, I found that the capacity for self-fertilization increased with both elevation and latitude in *A. vulneraria*, suggesting an adaptation to conditions of limited pollination in alpine and arctic environments. Plants from alpine populations of *A. vulneraria* exhibited lower survival rates in the common garden than plants originating from low elevations. Possible explanations for this maladaptation to the common garden conditions include higher respiration and a lower temperature optimum of photosynthesis in alpine plants. The reduced leaf chlorophyll content in alpine populations of the legume *A. vulneraria* may also have contributed to their low survival in the common garden, potentially indicating nitrogen limitation due to the incompatibility with local rhizobia. I also found that the evolutionary potential in the measured quantitative traits was not reduced towards the periphery of the gradient since the evolvability of most traits did not vary consistently with elevation or latitude.

To determine whether the observed clinal variations in plant traits were due to plasticity or local adaptation we compared the phenotypic measurements of plants grown in the common garden with those of their mother plants in the field. The differences in performance between progeny and mothers were smaller for populations from high elevations and latitudes, suggesting reduced plasticity and a strong genetic component in these populations. Correlations between traits measured in the common garden and on their mother plants in the field also supported the strong genetic component of some quantitative traits. Furthermore, quantitative genetic distances between populations increased with elevational and latitudinal distances confirming the presence of genetic differentiation between populations and indicating divergent selection along these gradients. Also indicating divergent selection in both gradients were the higher values of quantitative genetic differentiation (Q_{ST}) compared to molecular genetic differentiation (F_{ST}) found for many traits along the elevational gradient and for some traits along the latitudinal gradient.

Considerations regarding the abundant centre model

The abundant centre model (ACM) has been explored through the analysis of population patterns along elevational and latitudinal gradients in many studies (Vaupel and Matthies 2012; Villellas et al. 2013; Casazza et al. 2021). The population characteristics observed along both types of gradients in this thesis have also been examined within the framework of the ACM. I studied the gradients from the centre of the distribution of the *A. vulneraria* in Central Europe to its northern edge and from the lowlands to the elevational limit of the species in the Alps. According to the ACM, vital rates and genetic diversity are expected to decrease, while genetic differentiation among populations increases from the centre to the periphery of a species' distribution (Brown 1984; Sagarin and Gaines 2002). These patterns are supposed to be due to less suitable environmental conditions at the periphery, leading to smaller, more isolated and genetically depauperate populations. However, I mostly found no support for these predictions. Contrary to expectations, population sizes and density did not decrease towards the periphery in both the elevational and latitudinal gradients. In fact, they increased with elevation and latitude. Although there was a decline in plant size and reproduction, which is in line with the predictions of the ACM, a more detailed analysis revealed the significant role of demographic compensation, by compensating the decreased reproduction with an increased recruitment. Such compensatory changes in vital rates across the range are thought to allow species to occupy large areas of distribution (Doak and Morris 2010). Similarly, the decline in molecular genetic diversity and the increase in molecular genetic differentiation observed along the latitudinal gradient were in line with the ACM. However, these patterns are likely a result of the historical migration process after the last ice age. Furthermore, measurements on plants in the common garden experiment confirmed that there was no decline in quantitative genetic variability from the centre to the periphery of the distribution of *A. vulneraria* since evolvability did not decrease with elevation or latitude. The predictions of the ACM were only partially confirmed in peripheral populations in the North, which exhibited reduced seed set and seed mass. However, these patterns were not observed in populations at the elevational limit of the species in the Alps. Overall, the findings suggest that the ACM does not fully explain the observed population characteristics and genetic patterns in *A. vulneraria*. Other factors such as historical migration and local adaptation may have played more significant roles in shaping the species' distribution and traits across different environments.

Potential responses to climate change

Climate plays a fundamental role in shaping the distribution of plants (Woodward 1987; De Frenne et al. 2013). Gaining a deeper understanding of how plant populations respond to changing environmental conditions is crucial for predicting their future responses to climate change and taking measures in species conservation management. The effects of changing climatic conditions on plants can be explored by studying populations distributed along environmental gradients like those found across different elevations and latitudes (Halbritter et al. 2013). The results of the studies along elevational and latitudinal gradients in this thesis can thus be used to explore how climate change may affect populations of the widespread species *A. vulneraria*.

I found that temperature plays an essential role in the distribution and physiology of *A. vulneraria*, and that changes in temperature will have strong effects on plant populations. However, populations along the elevational and latitudinal gradients will face different challenges with climate change. Some population characteristics in the field showed distinct patterns along the two types of gradients, suggesting that climate change may have different effects on *A. vulneraria* populations along the elevational and latitudinal gradients. Consequently, latitudinal patterns in population characteristics have a limited value for predicting changes with elevation (Jump et al. 2009b). The proportion of plants flowering, seed set and seed mass declined with latitude, whereas the considerable variation in these traits along the elevational gradient was not associated with elevation or other covarying environmental factors like annual mean temperature. This suggests that drawing conclusions about the potential impacts of future climate warming on mountain populations will be more challenging due to the significance of small-scale environmental variation (Scherrer and Körner 2010; Oldfather and Ackerly 2019). However, the observed differences in population structure and resulting in potential plasticity in life cycle strategies between high-latitude and high-elevation populations may provide some resilience against the adverse effects of climate change (Doak and Morris 2010; Villellas et al. 2015; Peterson et al. 2018, but see Sheth and Angert 2018).

I found distinct patterns of genetic diversity and genetic differentiation among the *A. vulneraria* populations in Alpine and Scandinavian regions. The alpine populations exhibited consistently high genetic diversity, allelic richness, and a greater number of private alleles, suggesting a higher evolutionary potential compared to the Scandinavian populations. These results confirm that responses to climate change may differ between alpine and subarctic populations. The limited neutral molecular genetic diversity of subarctic populations raises concerns for their long-term persistence in the face of climate change. On the other hand, the alpine populations have the potential to benefit from gene flow from low-elevation populations along the shorter elevational gradient, allowing for admixture with genotypes originating from warmer habitats (Tanto Hadado et al. 2010; Rossetto et al. 2011). This genetic exchange may contribute to the adaptive potential of the alpine populations in response to changing climatic conditions.

I found that the variation in neutral molecular markers is of limited usefulness to determine the adaptive potential of *A. vulneraria* populations because the relationship between the genetic variation in neutral molecular markers and that of most quantitative traits was weak. The evolutionary potential of high latitude populations had been preserved despite the very low genetic diversities in neutral markers because no decline of evolutionary potential in quantitative traits with latitude was found. Thus, the evolutionary potential in quantitative traits could allow northern populations to locally adapt to changing environmental conditions. Similar to the arctic populations, there was no decline in evolutionary potential in quantitative traits in the alpine populations, allowing genetic adaptation to new conditions as well. However, alpine populations might be more sensitive to changing conditions as they had a very low survival in the lowland garden. Plants from higher elevations and latitudes exhibited lower levels of phenotypic plasticity and derived less benefit from the more favourable conditions in the common garden compared to lower elevations and latitudes of origin which may threaten their survival in the short term under rapidly changing climatic conditions (Radersma et al. 2020).

Perspectives

While this work has shed light on key aspects of genetic variation and plasticity in quantitative genetic traits, further research is needed to enhance our comprehension of the mechanisms driving adaptation to environmental changes. Reciprocal transplant experiments, which involve planting individuals from different origins both at their home sites and other sites, are ideal experimental setups to determine whether their traits vary across different environmental conditions and to test for local adaptation (Becker et al. 2006; Franks et al. 2014). In reciprocal transplant experiments, local adaptation is shown by an enhanced fitness in the local populations in comparison to the immigrant ones (Kawecki and Ebert 2004). Thus, to further investigate if local adaptation in populations of *A. vulneraria* could increase or decrease their performance in the face of global change and to predict how populations may respond to rapid climate change, conducting reciprocal transplant experiments throughout the species' distribution would be highly valuable.

Climate change is also likely to increase the frequency of drought (Mukherjee et al. 2018; AghaKouchak et al. 2021). To investigate the effects of drought and explore the potential for adaptation, an experiment with seedlings from Central European lowland populations, high elevation and high latitude populations of *A. vulneraria* could be conducted in which the plants are subjected to different levels of drought and performance and physiological traits are recorded. This experiment would allow to gain insights into differences in plastic responses to drought and genetic variation in drought resistance between high elevation/latitude and lowland populations. Furthermore, this experiment could investigate the evolutionary potential of populations to respond to increased drought periods and whether genotypes exist at higher elevations and higher latitudes that are preadapted to the predicted drier conditions (Gugger et al. 2015; Hamann et al. 2018).

In addition to common garden experiments, genomics studies might provide a complementary approach to further our understanding of local adaptation (de Villemereuil et al. 2016). By using genomic analyses, one could identify specific loci that are non-neutral and potentially associated with adaptive traits and that show correlations in allele frequencies with environmental gradients (Pardo-Diaz et al. 2014; Ahrens et al. 2018). These markers could offer a means of detecting adaptation and provide valuable insights into the genetic architecture of complex traits, helping to understand the contribution of genetic variation to phenotypic variation.

Furthermore, the results of this study could initiate further investigations into the demographic characteristics of *Anthyllis vulneraria*, particularly with respect to its life strategies. Although the species is generally considered biennial (Sterk 1975), this study revealed variation in its life cycle among the populations along the elevational and latitudinal gradients. To gain a better understanding of these differences, demographic studies could investigate whether these populations exhibit divergent lifespans and survival strategies in response to varying climatic conditions across their range.

REFERENCES

Abdusalam A, Li QJ (2019) Elevation-related variation in the population characteristics of distylous *Primula nivalis* affects female fitness and inbreeding depression. Plant Diversity 41:250–257. https://doi.org/10.1016/j.pld.2019.06.004

Abeli T, Gentili R, Mondoni A, Orsenigo S, Rossi G (2014) Effects of marginality on plant population performance. J Biogeogr 41:239–249. https://doi.org/10.1111/jbi.12215

Acharya KP, De Frenne P, Brunet J, Chabrerie O, Cousins SAO, Diekmann M, Hermy M, Kolb A, Lemke I, Plue J, Verheyen K, Graae BJ (2017) Latitudinal variation of life-history traits of an exotic and a native impatiens species in Europe. Acta Oecol 81:40–47. https://doi.org/10.1016/j.actao.2017.05.002

Ægisdóttir HH, Thórhallsdóttir TE (2006) Breeding system evolution in the Arctic: a comparative study of *Campanula uniflora* in Greenland and Iceland. Arctic, Antarctic, and Alpine Research 38:305–312. https://doi.org/10.1657/1523-0430(2006)38[305:BSEITA]2.0.CO;2

AghaKouchak A, Mirchi A, Madani K, Di Baldassarre G, Nazemi A, Alborzi A, Anjileli H, Azarderakhsh M, Chiang F, Hassanzadeh E, Huning LS, Mallakpour I, Martinez A, Mazdiyasni O, Moftakhari H, Norouzi H, Sadegh M, Sadeqi D, Van Loon AF, Wanders N (2021) Anthropogenic Drought: Definition, Challenges, and Opportunities. Reviews of Geophysics 59:e2019RG000683. https://doi.org/10.1029/2019RG000683

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17:5177–5188. https://doi.org/10.1111/j.1365-294X.2008.03971.x

Ahrens CW, Rymer PD, Stow A, Bragg J, Dillon S, Umbers KDL, Dudaniec RY (2018) The search for loci under selection: trends, biases and progress. Molecular Ecology 27:1342–1356. https://doi.org/10.1111/mec.14549

Albach DC, Schoenswetter P, Tribsch A (2006) Comparative phylogeography of the *Veronica alpina* complex in Europe and North America. Molecular ecology 15:3269–3286. https://doi.org/10.1111/j.1365-294X.2006.02980.x

Alonso C (1999) Variation in herbivory by *Yponomeuta mahalebella* on its only host plant *Prunus mahaleb* along an elevational gradient. Ecological Entomology 24:371–379. https://doi.org/10.1046/j.1365-2311.1999.00211.x

Angert AL (2006) Demography of central and marginal populations of monkeyflowers (*Mimulus cardinalis* and *M. lewisii*). Ecology 87:2014–2025. https://doi.org/10.1890/0012-9658(2006)87[2014:DOCAMP]2.0.CO;2

Anstett DN, Naujokaitis-Lewis I, Johnson MTJ (2014) Latitudinal gradients in herbivory on *Oenothera biennis* vary according to herbivore guild and specialization. Ecology 95:2915–2923. https://doi.org/10.1890/13-0932.1

Arroyo MTK, Muñoz MS, Henríquez C, Till-Bottraud I, Pérez F (2006) Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above-tree-line species in the high Andes of Chile. Acta Oecologica 30:248–257. https://doi.org/10.1016/j.actao.2006.05.006

Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. Journal of experimental botany 57:267–281. https://doi.org/10.1093/jxb/erj029

Baker HG (1955) Self-compatibility and establishment after “long-distance” dispersal. Evolution 9:347–349. https://doi.org/10.2307/2405656

Bakhtiari M, Formenti L, Caggìa V, Glauser G, Rasmann S (2019) Variable effects on growth and defense traits for plant ecotypic differentiation and phenotypic plasticity along elevation gradients. Ecology and Evolution 9:3740–3755. https://doi.org/10.1002/ece3.4999

Barton K (2019) MuMIn: multi-model inference. R package version 1.43.6. https://CRANR-project.org/package=MuMIn

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using **lme4**. J Stat Soft 67. https://doi.org/10.18637/jss.v067.i01

Beatty GE, Provan J (2011) Comparative phylogeography of two related plant species with overlapping ranges in Europe, and the potential effects of climate change on their intraspecific genetic diversity. BMC Evolutionary Biology 11:29. https://doi.org/10.1186/1471-2148-11-29

Becker U, Colling G, Dostal P, Jakobsson A, Matthies D (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. Oecologia 150:506–518. https://doi.org/10.1007/s00442-006-0534-9

Berjano R, Villellas J, García MB, Terrab A (2015) Phylogeography reveals latitudinal population structure in the common herb *Plantago coronopus*. Bot J Linn Soc 179:618–634. https://doi.org/10.1111/boj.12350

Billings WD, Mooney HA (1968) The ecology of arctic and alpine plants. Biological reviews 43:481–529. https://doi.org/10.1111/j.1469-185X.1968.tb00968.x

Briggs D, Walters SM (1997) Plant variation and evolution, 3. ed. Cambridge Univ. Press, Cambridge

Brown JH (1984) On the relationship between abundance and distribution of species. Am Nat 124:255–279. https://doi.org/10.1086/284267

Buckley J, Widmer A, Mescher MC, Moraes CMD (2019) Variation in growth and defence traits among plant populations at different elevations: Implications for adaptation to climate change. Journal of Ecology 107:2478–2492. https://doi.org/10.1111/1365-2745.13171

Buckley LB, Kingsolver JG (2021) Evolution of thermal sensitivity in changing and variable climates. Annual Review of Ecology, Evolution, and Systematics 52:563–586. https://doi.org/10.1146/annurev-ecolsys-011521-102856

Burnham KP, Anderson DR (2002) Model selection and multimodal inference: a practical information-theoretic approach. Springer, New York

Byars SG, Papst W, Hoffmann AA (2007) Local adaptation and cogradient selection in the alpine plant, *Poa hiemata*, along a narrow altitudinal gradient. Evolution 61:2925–2941. https://doi.org/10.1111/j.1558-5646.2007.00248.x

Byars SG, Parsons Y, Hoffmann AA (2009) Effect of altitude on the genetic structure of an Alpine grass, *Poa hiemata*. Annals of Botany 103:885–899. https://doi.org/10.1093/aob/mcp018

Capblancq T, Fitzpatrick MC, Bay RA, Exposito-Alonso M, Keller SR (2020) Genomic prediction of (mal)adaptation across current and future climatic landscapes. Annual Review of Ecology, Evolution, and Systematics 51:245–269. https://doi.org/10.1146/annurev-ecolsys-020720-042553

Casazza G, Macrì C, Dagnino D, Guerrina M, Juin M, Minuto L, Thompson JD, Baumel A, Médail F (2021) When ecological marginality is not geographically peripheral: exploring genetic predictions of the centre-periphery hypothesis in the endemic plant *Lilium pomponium*. PeerJ 9:e11039. https://doi.org/10.7717/peerj.11039

Chapuis M-P, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24:621–631. https://doi.org/10.1093/molbev/msl191

Cheptou P-O (2012) Clarifying Baker's Law. Annals of Botany 109:633–641. https://doi.org/10.1093/aob/mcr127

Chung MY, Merilä J, Li J, Mao K, López-Pujol J, Tsumura Y, Chung MG (2023) Neutral and adaptive genetic diversity in plants: An overview. Front Ecol Evol 11:1116814. https://doi.org/10.3389/fevo.2023.1116814

Colautti RI, Barrett SCH (2013) Rapid adaptation to climate facilitates range expansion of an invasive plant. Science 342:364–366. https://doi.org/10.1126/science.1242121

Colling G, Matthies D (2004) The effects of plant population size on the interactions between the endangered plant *Scorzonera humilis*, a specialised herbivore, and a phytopathogenic fungus. Oikos 105:71–78. https://doi.org/10.1111/j.0030-1299.2004.12783.x

Conert HJ (1975) Leguminosae-Tropaelaceae. In: Illustrierte Flora von Mittel-Europa, Band IV, Teil 3, Dicotyledones 2, Hegi. Paul Parey, Berlin, pp 1114–1750

Couderc H (1971) Etude expérimentale de la reproduction de l'*Anthyllis vulneraria* L. Bulletin de la Société Botanique de France 118:359–373. https://doi.org/10.1080/00378941.1971.10838912

Couderc H, Gorenflot R (1978) Adaptation de la fleur entomophile de l'*Anthyllis vulneraria* L. à l'autogamie. Bulletin de la Société Botanique de France 125:369–378. https://doi.org/10.1080/00378941.1978.10839462

Crawley MJ (2009) The R book, Reprinted with corrections. John Wiley & Sons, Chichester, England

Cullen J (1968) *Anthyllis* L. In: Flora Europaea, Tutin T.G. et al. Cambridge University Press, Cambridge, UK, pp 177–182

Daco L, Colling G, Matthies D (2021) Altitude and latitude have different effects on population characteristics of the widespread plant *Anthyllis vulneraria*. Oecologia 197:537–549. https://doi.org/10.1007/s00442-021-05030-6

Daco L, Matthies D, Hermant S, Colling G (2022) Genetic diversity and differentiation of populations of *Anthyllis vulneraria* along elevational and latitudinal gradients. Ecology and Evolution 12. https://doi.org/10.1002/ece3.9167

Davis MB, Shaw RG (2001) Range shifts and adaptive responses to quaternary climate change. Science 292:673–679. https://doi.org/10.1126/science.292.5517.673

Dawson-Glass E, Hargreaves AL (2022) Does pollen limitation limit plant ranges? Evidence and implications. Philosophical Transactions of the Royal Society B: Biological Sciences 377:20210014. https://doi.org/10.1098/rstb.2021.0014

De Frenne P, Brunet J, Shevtsova A, Kolb A, Graae BJ, Chabrerie O, Cousins SA, Decocq G, De SCHRIJVER A, Diekmann M, Gruwez R, Heinken T, Hermy M, Nilsson C, Stanton S, Tack W, Willaert J, Verheyen K (2011a) Temperature effects on forest herbs assessed by warming and transplant experiments along a latitudinal gradient. Global Change Biology 17:3240–3253. https://doi.org/10.1111/j.1365-2486.2011.02449.x

De Frenne P, Graae BJ, Kolb A, Shevtsova A, Baeten L, Brunet J, Chabrerie O, Cousins SAO, Decocq G, Dhondt R, Diekmann M, Gruwez R, Heinken T, Hermy M, Öster M, Saguez R, Stanton S, Tack W, Vanhellemont M, Verheyen K (2011b) An intraspecific application of the leaf-height-seed ecology strategy scheme to forest herbs along a latitudinal gradient. Ecography 34:132–140. https://doi.org/10.1111/j.1600-0587.2010.06399.x

De Frenne P, Graae BJ, Rodríguez-Sánchez F, Kolb A, Chabrerie O, Decocq G, Kort H, Schrijver A, Diekmann M, Eriksson O, Gruwez R, Hermy M, Lenoir J, Plue J, Coomes DA, Verheyen K (2013) Latitudinal gradients as natural laboratories to infer species' responses to temperature. J Ecol 101:784–795. https://doi.org/10.1111/1365-2745.12074

de Jong G (2005) Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. New Phytologist 166:101–118. https://doi.org/10.1111/j.1469-8137.2005.01322.x

De Kort H, Vandepitte K, Bruun HH, Closset-Kopp D, Honnay O, Mergeay J (2014) Landscape genomics and a common garden trial reveal adaptive differentiation to temperature across Europe in the tree species *Alnus glutinosa*. Molecular Ecology 23:4709–4721. https://doi.org/10.1111/mec.12813

De Kort H, Vandepitte K, Honnay O (2013) A meta-analysis of the effects of plant traits and geographical scale on the magnitude of adaptive differentiation as measured by the difference between Q_{ST} and F_{ST}. Evolutionary Ecology 27:1081–1097. https://doi.org/10.1007/s10682-012-9624-9

de Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I (2016) Common garden experiments in the genomic era: new perspectives and opportunities. Heredity 116:249–254. https://doi.org/10.1038/hdy.2015.93

Després L, Loriot S, Gaudeul M (2002) Geographic pattern of genetic variation in the European globeflower *Trollius europaeus* L. (Ranunculaceae) inferred from amplified fragment length polymorphism markers. Molecular Ecology 11:2337–2347. https://doi.org/10.1046/j.1365-294X.2002.01618.x

Diamond SE (2016) Evolutionary potential of upper thermal tolerance: biogeographic patterns and expectations under climate change. Annals of the New York Academy of Sciences 1389:5–19. https://doi.org/10.1111/nyas.13223

Diedenhofen B, Musch J (2015) cocor: A comprehensive solution for the statistical comparison of correlations. PLoS ONE 10:e0121945. https://doi.org/10.1371/journal.pone.0121945

Doak DF, Morris WF (2010) Demographic compensation and tipping points in climate-induced range shifts. Nature 467:959–962. https://doi.org/10.1038/nature09439

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. Mol Ecol 17:1170–1188. https://doi.org/10.1111/j.1365-294X.2007.03659.x

Ehrich D, Gaudeul M, Assefa A, Koch MA, Mummenhoff K, Nemomissa S, Consortium I, Brochmann C (2007) Genetic consequences of Pleistocene range shifts: contrast between the

Arctic, the Alps and the East African mountains. Molecular Ecology 16:2542–2559. https://doi.org/10.1111/j.1365-294X.2007.03299.x

Elberling H, Olesen JM (1999) The structure of a high latitude plant-flower visitor system: the dominance of flies. Ecography 22:314–323. https://doi.org/10.1111/j.1600-0587.1999.tb00507.x

Ellenberg H, Weber HE, Düll R, Wirth V, Werner W, Paulißen D (1992) Zeigerwerte von Pflanzen in Mitteleuropa. Scripta Geobotanica 18:1–248

Ensing DJ, Eckert CG (2019) Interannual variation in season length is linked to strong co-gradient plasticity of phenology in a montane annual plant. New Phytologist 224:1184–1200. https://doi.org/10.1111/nph.16009

Eriksen B, Molau U, Svensson M (1993) Reproductive strategies in two arctic *Pedicularis* species (Scrophulariaceae). Ecography 16:154–166. https://doi.org/10.1111/j.1600-0587.1993.tb00067.x

Erschbamer B, Retter V (2004) How long can glacier foreland species live? Flora - Morphology, Distribution, Functional Ecology of Plants 199:500–504. https://doi.org/10.1078/0367-2530-00178

Estoup A, Ravigné V, Hufbauer R, Vitalis R, Gautier M, Facon B (2016) Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51–72. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Evans LM, Kaluthota S, Pearce DW, Allan GJ, Floate K, Rood SB, Whitham TG (2016) Bud phenology and growth are subject to divergent selection across a latitudinal gradient in *Populus angustifolia* and impact adaptation across the distributional range and associated arthropods. Ecol Evol 6:4565–4581. https://doi.org/10.1002/ece3.2222

Excoffier L, Foll M, Petit RJ (2009) Genetic consequences of range expansions. Annual Review of Ecology, Evolution and Systematics 40:481. https://doi.org/10.1146/annurev.ecolsys.39.110707.173414

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302–4315. https://doi.org/10.1002/joc.5086

Fischer M, Matthies D (1998) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). Am J Bot 85:811–819. https://doi.org/10.2307/2446416

Frankham R, Ballou JD, Ralls K, Eldridge M, Dudash MR, Fenster CB, Lacy RC, Sunnucks P (2017) Genetic management of fragmented animal and plant populations. Oxford University Press

Franks SJ, Weber JJ, Aitken SN (2014) Evolutionary and plastic responses to climate change in terrestrial plant populations. Evol Appl 7:123–139. https://doi.org/10.1111/eva.12112

Frei ER, Hahn T, Ghazoul J, Pluess AR (2014) Divergent selection in low and high elevation populations of a perennial herb in the Swiss Alps. Alp Botany 124:131–142. https://doi.org/10.1007/s00035-014-0131-1

Fulkerson JR, Whittall JB, Carlson ML (2012) Reproductive ecology and severe pollen limitation in the polychromic tundra plant, *Parrya nudicaulis* (Brassicaceae). PLoS ONE 7:e32790. https://doi.org/10.1371/journal.pone.0032790

García-Camacho R, Totland Ø (2009) Pollen limitation in the alpine: a meta-analysis. Arctic, Antarctic, and Alpine Research 41:103–111. https://doi.org/10.1657/1523-0430-41.1.103

Garnier E, Cortez J, Billès G, Navas M-L, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellmann A, Neill C, Toussaint J-P (2004) Plant functional markers capture ecosystem properties during secondary succession. Ecology 85:2630–2637. https://doi.org/10.1890/03-0799

GBIF.org (2019) GBIF Occurrence Download https://doi.org/10.15468/dl.1n1pfa

GBIF.org (2022) GBIF Occurrence Download https://doi.org/10.15468/dl.jm9r9a.

Gilbert KJ, Andrew RL, Bock DG, Franklin MT, Kane NC, Moore J-S, Moyers BT, Renaut S, Rennison DJ, Veen T (2012) Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. Molecular ecology 21:4925–4930. https://doi.org/10.1111/j.1365-294X.2012.05754.x

Giménez-Benavides L, Escudero A, Iriondo JM (2007) Reproductive limits of a late-flowering high-mountain Mediterranean plant along an elevational climate gradient. New Phytologist 173:367–382. https://doi.org/10.1111/j.1469-8137.2006.01932.x

Giménez-Benavides L, Escudero A, Iriondo JM (2008) What shapes the altitudinal range of a high mountain Mediterranean plant? Recruitment probabilities from ovule to seedling stage. Ecography 31:731–740. https://doi.org/10.1111/j.0906-7590.2008.05509.x

Gonzalo-Turpin H, Hazard L (2009) Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species *Festuca eskia*. Journal of Ecology 97:742–751. https://doi.org/10.1111/j.1365-2745.2009.01509.x

Goudet J (2003) Fstat (ver. 2.9.4), a program to estimate and test population genetics parameters. Available from http://www.unil.ch/izea/softwares/fstat.html Updated from Goudet [1995]

Gougherty AV, Chhatre VE, Keller SR, Fitzpatrick MC (2020) Contemporary range position predicts the range-wide pattern of genetic diversity in balsam poplar (*Populus balsamifera* L.). J Biogeogr 47:1246–1257. https://doi.org/10.1111/jbi.13811

Grace JB, Johnson DJ, Lefcheck JS, Byrnes JEK (2018) Quantifying relative importance: computing standardized effects in models with binary outcomes. Ecosphere 9:e02283. https://doi.org/10.1002/ecs2.2283

Grassein F, Lemauviel-Lavenant S, Lavorel S, Bahn M, Bardgett RD, Desclos-Theveniau M, Laîné P (2015) Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species. Annals of Botany 115:107–115. https://doi.org/10.1093/aob/mcu233

Griffin PC, Willi Y (2014) Evolutionary shifts to self-fertilisation restricted to geographic range margins in North American *Arabidopsis lyrata*. Ecol Lett 17:484–490. https://doi.org/10.1111/ele.12248

Griffith TM, Watson MA (2005) Stress avoidance in a common annual: reproductive timing is important for local adaptation and geographic distribution. Journal of Evolutionary Biology 18:1601–1612. https://doi.org/10.1111/j.1420-9101.2005.01021.x

Gugger S, Kesselring H, Stöcklin J, Hamann E (2015) Lower plasticity exhibited by high- versus mid-elevation species in their phenological responses to manipulated temperature and drought. Ann Bot mcv155. https://doi.org/10.1093/aob/mcv155

Guo H, Mazer SJ, Du G (2010) Geographic variation in seed mass within and among nine species of *Pedicularis* (Orobanchaceae): effects of elevation, plant size and seed number per fruit. Journal of Ecology 98:1232–1242. https://doi.org/10.1111/j.1365-2745.2010.01688.x

Gutschick VP, BassiriRad H (2003) Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. New Phytologist 160:21–42. https://doi.org/10.1046/j.1469-8137.2003.00866.x

Hahn T, Kettle CJ, Ghazoul J, Frei ER, Matter P, Pluess AR (2012) Patterns of genetic variation across altitude in three plant species of semi-dry grasslands. PLoS ONE 7:e41608. https://doi.org/10.1371/journal.pone.0041608

Halbritter AH, Alexander JM, Edwards PJ, Billeter R (2013) How comparable are species distributions along elevational and latitudinal climate gradients? Global Ecology and Biogeography 22:1228–1237. https://doi.org/10.1111/geb.12066

Halbritter AH, Billeter R, Edwards PJ, Alexander JM (2015) Local adaptation at range edges: comparing elevation and latitudinal gradients. J Evol Biol 28:1849–1860. https://doi.org/10.1111/jeb.12701

Halbritter AH, Fior S, Keller I, Billeter R, Edwards PJ, Holderegger R, Karrenberg S, Pluess AR, Widmer A, Alexander JM (2018) Trait differentiation and adaptation of plants along elevation gradients. J Evol Biol 31:784–800. https://doi.org/10.1111/jeb.13262

Hämälä T, Mattila TM, Savolainen O (2018) Local adaptation and ecological differentiation under selection, migration, and drift in *Arabidopsis lyrata*. Evolution 72:1373–1386. https://doi.org/10.1111/evo.13502

Hamann E, Kesselring H, Stöcklin J (2018) Plant responses to simulated warming and drought: a comparative study of functional plasticity between congeneric mid and high elevation species. Journal of Plant Ecology 11:364–374. https://doi.org/10.1093/jpe/rtx023

Hansen TF, Pélabon C (2021) Evolvability: A quantitative-genetics perspective. Annu Rev Ecol Evol Syst 52:153–175. https://doi.org/10.1146/annurev-ecolsys-011121-021241

Hardie DC, Hutchings JA (2010) Evolutionary ecology at the extremes of species' ranges. Environ Rev 18:1–20. https://doi.org/10.1139/A09-014

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol Ecol Notes 2:618–620. https://doi.org/10.1046/j.1471-8286.2002.00305.x

Hargreaves AL, Samis KE, Eckert CG (2014) Are species' range limits simply niche limits writ large? A review of transplant experiments beyond the range. The American Naturalist 183:157–173. https://doi.org/10.1086/674525

Hargreaves AL, Weiner JL, Eckert CG (2015) High-elevation range limit of an annual herb is neither caused nor reinforced by declining pollinator service. J Ecol 103:572–584. https://doi.org/10.1111/1365-2745.12377

Harter DEV, Jentsch A, Durka W (2015) Holocene re-colonisation, central-marginal distribution and habitat specialisation shape population genetic patterns within an Atlantic European grass species. Plant Biol J 17:684–693. https://doi.org/10.1111/plb.12269

Hegi G (1975) Illustrierte Flora von Mittel-Europa, Band IV, Teil 3, Dicotyledones 2. Teil, Leguminosae-Tropaelaceae, Verlag Paul Parey Berlin und Hamburg 1114–1750

Hemborg ÅM, Després L (2011) Floral phenotypic plasticity as a buffering mechanism in the globeflower–fly mutualism. Plant Ecol 212:1205–1212. https://doi.org/10.1007/s11258-011-9898-y

Herrera C, Bazaga P (2008) Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. Heredity 100:275–285. https://doi.org/10.1038/sj.hdy.6801072

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quaternary. Phil Trans R Soc Lond 359:183–195. https://doi.org/10.1098/rstb.2003.1388

Hewitt GM (1999) Post-glacial re-colonization of European biota. Biological Journal of the Linnean Society 68:87–112. https://doi.org/10.1111/j.1095-8312.1999.tb01160.x

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913. https://doi.org/10.1038/35016000

Hirao AS, Watanabe M, Tsuyuzaki S, Shimono A, Li X, Masuzawa T, Wada N (2017) Genetic diversity within populations of an arctic–alpine species declines with decreasing latitude across the Northern Hemisphere. Journal of Biogeography 44:2740–2751. https://doi.org/10.1111/jbi.13085

Hirsch H, Wagner V, Danihelka J, Ruprecht E, Sánchez-Gómez P, Seifert M, Hensen I (2015) High genetic diversity declines towards the geographic range periphery of *Adonis vernalis*, a Eurasian dry grassland plant. Plant Biol J 17:1233–1241. https://doi.org/10.1111/plb.12362

Hodkinson ID (2005) Terrestrial insects along elevation gradients: species and community responses to altitude. Biological Reviews 80:489–513. https://doi.org/10.1017/S1464793105006767

Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. Nature 470:479–485. https://doi.org/10.1038/nature09670

Holm S-O (1994) Reproductive patterns of *Betula pendula* and *B. pubescens* coll. along a regional altitudinal gradient in northern Sweden. Ecography 17:60–72. https://doi.org/10.1111/j.1600-0587.1994.tb00077.x

Honnay O, Coart E, Butaye J, Adriaens D, Van Glabeke S, Roldán-Ruiz I (2006) Low impact of present and historical landscape configuration on the genetics of fragmented *Anthyllis vulneraria* populations. Biological Conservation 127:411–419. https://doi.org/10.1016/j.biocon.2005.09.006

Huber CD, Nordborg M, Hermisson J, Hellmann I (2014) Keeping it local: evidence for positive selection in Swedish *Arabidopsis thaliana*. Molecular Biology and Evolution 31:3026–3039. https://doi.org/10.1093/molbev/msu247

Ilves A, Metsare M, Seliškar A, García MB, Vassiliou L, Pierce S, Tatarenko I, Tali K, Kull T (2016) Genetic diversity patterns of the orchid *Anacamptis pyramidalis* at the edges of its distribution range. Plant Syst Evol 302:1227–1238. https://doi.org/10.1007/s00606-016-1328-0

Inouye DW, Wielgolaski FE (2003) High altitude climates. In: Schwartz MD (ed) Phenology: An Integrative Environmental Science. Springer Netherlands, Dordrecht, pp 195–214

IPCC (2014) Climate change 2014: Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change. Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.), Geneva, Switzerland

Itino T, Hirao AS (2016) Plant genetic diversity and plant–pollinator interactions along altitudinal gradients. In: Kudo G (ed) Structure and Function of Mountain Ecosystems in Japan. Springer Japan, Tokyo, pp 63–88

Jansen F, Bonn A, Bowler DE, Bruelheide H, Eichenberg D (2019) Moderately common plants show highest relative losses. Conserv Lett e12674. https://doi.org/10.1111/conl.12674

Jiang X, Xie Y (2020) Meta-analysis reveals severe pollen limitation for the flowering plants growing in East Himalaya-Hengduan Mountains region. BMC Ecol 20:53. https://doi.org/10.1186/s12898-020-00322-6

Jiménez-Ambriz G, Petit C, Bourrié I, Dubois S, Olivieri I, Ronce O (2007) Life history variation in the heavy metal tolerant plant *Thlaspi caerulescens* growing in a network of contaminated and noncontaminated sites in southern France: role of gene flow, selection and phenotypic plasticity. New Phytologist 173:199–215. https://doi.org/10.1111/j.1469-8137.2006.01923.x

Joshi J, Schmid B, Caldeira MC, Dimitrakopoulos PG, Good J, Harris R, Hector A, Huss-Danell K, Jumpponen A, Minns A, Mulder CPH, Pereira JS, Prinz A, Scherer-Lorenzen M, Siamantziouras A-SD, Terry AC, Troumbis AY, Lawton JH (2001) Local adaptation enhances performance of common plant species. Ecol Letters 4:536–544. https://doi.org/10.1046/j.1461-0248.2001.00262.x

Jump AS, Marchant R, Peñuelas J (2009a) Environmental change and the option value of genetic diversity. Trends in Plant Science 14:51–58. https://doi.org/10.1016/j.tplants.2008.10.002

Jump AS, Mátyás C, Peñuelas J (2009b) The altitude-for-latitude disparity in the range retractions of woody species. Trends Ecol Evol 24:694–701. https://doi.org/10.1016/j.tree.2009.06.007

Jump AS, Peñuelas J (2005) Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010–1020. https://doi.org/10.1111/j.1461-0248.2005.00796.x

Jump AS, Woodward FI (2003) Seed production and population density decline approaching the range-edge of *Cirsium* species. New Phytol 160:349–358. https://doi.org/10.1046/j.1469-8137.2003.00873.x

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Letters 7:1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kéry M, Matthies D, Fischer M (2001) The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*:

Interaction between a rare gentian and a butterfly. J Ecol 89:418–427. https://doi.org/10.1046/j.1365-2745.2001.00550.x

Kesselring H, Armbruster GFJ, Hamann E, Stöcklin J (2015) Past selection explains differentiation in flowering phenology of nearby populations of a common alpine plant. Alp Botany 125:113–124. https://doi.org/10.1007/s00035-015-0157-z

Kesselring H, Hamann E, Stöcklin J, Armbruster GFJ (2013) New Microsatellite Markers for *Anthyllis vulneraria* (Fabaceae), Analyzed with Spreadex Gel Electrophoresis. Applications in Plant Sciences 1:1300054. https://doi.org/10.3732/apps.1300054

Kingsolver JG, Diamond SE, Siepielski AM, Carlson SM (2012) Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. Evol Ecol 26:1101–1118. https://doi.org/10.1007/s10682-012-9563-5

Koch V, Zoller L, Bennett JM, Knight TM (2020) Pollinator dependence but no pollen limitation for eight plants occurring north of the Arctic Circle. Ecology and Evolution 10:13664–13672. https://doi.org/10.1002/ece3.6884

Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Molecular Ecology Resources 15:1179–1191. https://doi.org/10.1111/1755-0998.12387

Körner C (2003) Alpine plant life: functional plant ecology of high mountain ecosystems ; with 47 tables, 2. ed. Springer, Berlin

Körner C (2007) The use of 'altitude' in ecological research. Trends in Ecology & Evolution 22:569–574. https://doi.org/10.1016/j.tree.2007.09.006

Körner C (2016) Plant adaptation to cold climates. F1000Res 5:F1000 Faculty Rev-2769. https://doi.org/10.12688/f1000research.9107.1

Körner C, Neumayer M, Menendez-Riedl SP, Smeets-Scheel A (1989) Functional morphology of mountain plants. Flora 182:353–383. https://doi.org/10.1016/S0367-2530(17)30426-7

Köster E, Bitocchi E, Papa R, Pihu S (2008) Genetic structure of the *Anthyllis vulneraria* L. s. l. species complex in Estonia based on AFLPs. Open Life Sciences 3:442–450. https://doi.org/10.2478/s11535-008-0033-6

Kramer AT, Havens K (2009) Plant conservation genetics in a changing world. Trends in Plant Science 14:599–607. https://doi.org/10.1016/j.tplants.2009.08.005

Krauss J, Steffan-Dewenter I, Tscharntke T (2004) Landscape occupancy and local population size depends on host plant distribution in the butterfly *Cupido minimus*. Biological Conservation 120:355–361. https://doi.org/10.1016/j.biocon.2004.03.007

Kremer A, Potts BM, Delzon S (2014) Genetic divergence in forest trees: understanding the consequences of climate change. Functional Ecology 28:22–36. https://doi.org/10.1111/1365-2435.12169

Lacey EP (1988) Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (Apiaceae). Ecology 69:220–232. https://doi.org/10.2307/1943178

Leamy LJ, Lee C, Cousins V, Mujacic I, Manzaneda AJ, Prasad K, Mitchell-Olds T, Song B (2014) Large-scale adaptive divergence in *Boechera fecunda,* an endangered wild relative of *Arabidopsis*. Ecol Evol 4:3175–3186. https://doi.org/10.1002/ece3.1148

Lee Y, Kotanen PM (2015) Differences in herbivore damage and performance among *Arctium minus* (burdock) genotypes sampled from a geographic gradient: a common garden experiment. Biol Invasions 17:397–408. https://doi.org/10.1007/s10530-014-0737-7

Legendre P, Legendre L (1998) Numerical ecology, 2nd English ed. Elsevier, Amsterdam ; New York

Leinonen T, McCairns RJS, O'Hara RB, Merilä J (2013) Q_{ST}–F_{ST} comparisons: evolutionary and ecological insights from genomic heterogeneity. Nat Rev Genet 14:179–190. https://doi.org/10.1038/nrg3395

Lempe J, Balasubramanian S, Sureshkumar S, Singh A, Schmid M, Weigel D (2005) Diversity of flowering responses in wild *Arabidopsis thaliana* strains. PLOS Genetics 1:e6. https://doi.org/10.1371/journal.pgen.0010006

Lepais O, Bacles CFE (2014) Two are better than one: combining landscape genomics and common gardens for detecting local adaptation in forest trees. Molecular Ecology 23:4671–4673. https://doi.org/10.1111/mec.12906

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conservation Biology 9:753–760. https://doi.org/10.1046/j.1523-1739.1995.09040753.x

Lewandowska-Sabat AM, Fjellheim S, Rognli OA (2010) Extremely low genetic variability and highly structured local populations of *Arabidopsis thaliana* at higher latitudes. Molecular Ecology 19:4753–4764. https://doi.org/10.1111/j.1365-294X.2010.04840.x

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. Annual Review of Ecology and Systematics 27:237–277. https://doi.org/10.1146/annurev.ecolsys.27.1.237

Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). American Journal of Botany 82:1420–1425. https://doi.org/10.1002/j.1537-2197.1995.tb12679.x

López-Delgado J, Meirmans PG (2022) History or demography? Determining the drivers of genetic variation in North American plants. Molecular Ecology 31:1951–1962. https://doi.org/10.1111/mec.16230

Matthies D (1990) Plasticity of reproductive components at different stages of development in the annual plant *Thlaspi arvense* L. Oecologia 83:105–116. https://doi.org/10.1007/BF00324641

McKay JK, Bishop JG, Lin J-Z, Richards JH, Sala A, Mitchell-Olds T (2001) Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. Proc R Soc Lond B 268:1715–1721. https://doi.org/10.1098/rspb.2001.1715

Medan D, Montaldo NH, Devoto M, Maniese A, Vasellati V, Roitman GG, Bartoloni NH (2002) Plant-pollinator relationships at two altitudes in the Andes of Mendoza, Argentina. Arctic, Antarctic, and Alpine Research 34:233–241. https://doi.org/10.1080/15230430.2002.12003490

Meirmans PG (2020) GENODIVE version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. Mol Ecol Resour 1755–0998.13145. https://doi.org/10.1111/1755-0998.13145

Meirmans PG, Hedrick PW (2011) Assessing population structure: F_{ST} and related measures. Molecular Ecology Resources 11:5–18. https://doi.org/10.1111/j.1755-0998.2010.02927.x

Meng H, Wei X, Jiang M (2019) Contrasting elevational patterns of genetic variation in *Euptelea pleiospermum* along mountains at the core and edges of its latitudinal range. Plant Ecol 220:13–28. https://doi.org/10.1007/s11258-018-0899-y

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. Journal of Evolutionary Biology 14:892–903. https://doi.org/10.1046/j.1420-9101

Midolo G, Wellstein C (2020) Plant performance and survival across transplant experiments depend upon temperature and precipitation change along elevation. J Ecol 108:2107–2120. https://doi.org/10.1111/1365-2745.13387

Moeller DA, Runquist RDB, Moe AM, Geber MA, Goodwillie C, Cheptou P-O, Eckert CG, Elle E, Johnston MO, Kalisz S, Ree RH, Sargent RD, Vallejo-Marin M, Winn AA (2017) Global biogeography of mating system variation in seed plants. Ecology Letters 20:375–384. https://doi.org/10.1111/ele.12738

Mohotti AJ, Lawlor DW (2002) Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. Journal of Experimental Botany 53:313–322. https://doi.org/10.1093/jexbot/53.367.313

Moles AT, Westoby M (2003) Latitude, seed predation and seed mass. Journal of Biogeography 30:105–128. https://doi.org/10.1046/j.1365-2699.2003.00781.x

Montesinos-Navarro A, Wig J, Xavier Pico F, Tonsor SJ (2011) *Arabidopsis thaliana* populations show clinal variation in a climatic gradient associated with altitude. New Phytologist 189:282–294. https://doi.org/10.1111/j.1469-8137.2010.03479.x

Mooney HA, Billings WD (1961) Comparative physiological ecology of Arctic and Alpine populations of *Oxyria digyna*. Ecological Monographs 31:1–29. https://doi.org/10.2307/1950744

Mukherjee S, Mishra A, Trenberth KE (2018) Climate Change and Drought: a Perspective on Drought Indices. Curr Clim Change Rep 4:145–163. https://doi.org/10.1007/s40641-018-0098-x

Nanni L, Ferradini N, Taffetani F, Papa R (2004) Molecular phylogeny of *Anthyllis* spp. Plant Biology 6:454–464. https://doi.org/10.1055/s-2004-820968

Navarro L (1999) Reproductive biology of *Anthyllis vulneraria* subsp. *vulgaris* (Fabaceae) in northwestern Iberian Peninsula. Nordic Journal of Botany 19:281–287. https://doi.org/10.1111/j.1756-1051.1999.tb01113.x

Nicotra AB, Atkin OK, Bonser SP, Davidson AM, Finnegan EJ, Mathesius U, Poot P, Purugganan MD, Richards CL, Valladares F, van Kleunen M (2010) Plant phenotypic plasticity in a changing climate. Trends in Plant Science 15:684–692. https://doi.org/10.1016/j.tplants.2010.09.008

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13:1143–1155. https://doi.org/10.1111/j.1365-294X.2004.02141.x

O'Hara RB, Merilä J (2005) Bias and precision in Q_{ST} estimates: problems and some solutions. Genetics 171:1331–1339. https://doi.org/10.1534/genetics.105.044545

Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. Global Ecol Biogeography 17:152–163. https://doi.org/10.1111/j.1466-8238.2007.00357.x

Ohsawa T, Tsuda Y, Saito Y, Sawada H, Ide Y (2007) Altitudinal genetic diversity and differentiation of *Quercus crispula* in the Chichibu Mountains, central Japan. International Journal of Plant Sciences 168:333–340. https://doi.org/10.1086/510413

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Szoecs E, Wagner H (2020). vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, Caceres MD, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette M-H, Cunha ER, Smith T, Stier A, Braak CJFT, Weedon J (2022) vegan: Community Ecology Package. R package version 2.6-4. https://CRAN.R-project.org/package=vegan

Oldfather MF, Ackerly DD (2019) Microclimate and demography interact to shape stable population dynamics across the range of an alpine plant. New Phytologist 222:193–205. https://doi.org/10.1111/nph.15565

Olejniczak P, Czarnoleski M, Delimat A, Majcher BM, Szczepka K (2018) Seed size in mountain herbaceous plants changes with elevation in a species-specific manner. PLoS ONE 13:e0199224. https://doi.org/10.1371/journal.pone.0199224

Olsson K, Ågren J (2002) Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. Journal of Evolutionary Biology 15:983–996. https://doi.org/10.1046/j.1420-9101.2002.00457.x

Orr HA (2005) The genetic theory of adaptation: a brief history. Nat Rev Genet 6:119–127. https://doi.org/10.1038/nrg1523

Pardo-Diaz C, Salazar C, Jiggins CD (2015) Towards the identification of the loci of adaptive evolution. Methods in Ecology and Evolution 6:445–464. https://doi.org/10.1111/2041-210X.12324

Parmesan C, Hanley ME (2015) Plants and climate change: complexities and surprises. Annals of Botany 116:849–864. https://doi.org/10.1093/aob/mcv169

Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42. https://doi.org/10.1038/nature01286

Pauls SU, Nowak C, Bálint M, Pfenninger M (2013) The impact of global climate change on genetic diversity within populations and species. Mol Ecol 22:925–946. https://doi.org/10.1111/mec.12152

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pellissier L, Fournier B, Guisan A, Vittoz P (2010) Plant traits co-vary with altitude in grasslands and forests in the European Alps. Plant Ecol 211:351–365. https://doi.org/10.1007/s11258-010-9794-x

Pepin N, Bradley RS, Diaz HF, Baraer M, Caceres EB, Forsythe N, Fowler H, Greenwood G, Hashmi MZ, Liu XD, Miller JR, Ning L, Ohmura A, Palazzi E, Rangwala I, Schöner W, Severskiy I, Shahgedanova M, Wang MB, Williamson SN, Yang DQ (2015) Elevation-dependent warming in mountain regions of the world. Nature Climate Change 5:424–430. https://doi.org/10.1038/nclimate2563

Peterson ML, Doak DF, Morris WF (2018) Both life-history plasticity and local adaptation will shape range-wide responses to climate warming in the tundra plant *Silene acaulis*. Glob Change Biol 24:1614–1625. https://doi.org/10.1111/gcb.13990

Piersma T, Drent J (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology & Evolution 18:228–233. https://doi.org/10.1016/S0169-5347(03)00036-3

Pironon S, Papuga G, Villellas J, Angert AL, García MB, Thompson JD (2017) Geographic variation in genetic and demographic performance: new insights from an old biogeographical paradigm. Biol Rev 92:1877–1909. https://doi.org/10.1111/brv.12313

Plenk K, Bardy K, Höhn M, Thiv M, Kropf M (2017) No obvious genetic erosion, but evident relict status at the westernmost range edge of the Pontic-Pannonian steppe plant *Linum flavum* L. (Linaceae) in Central Europe. Ecol Evol 7:6527–6539. https://doi.org/10.1002/ece3.2990

Pluess AR, Schütz W, Stöcklin J (2005) Seed weight increases with altitude in the Swiss Alps between related species but not among populations of individual species. Oecologia 144:55–61. https://doi.org/10.1007/s00442-005-0047-y

Pluess AR, Stöcklin J (2004) Population genetic diversity of the clonal plant *Geum reptans* (Rosaceae) in the Swiss Alps. American Journal of Botany 91:2013–2021. https://doi.org/10.3732/ajb.91.12.2013

Podolsky RH, Holtsford TP (1995) Population structure of morphological traits in *Clarkia dudleyana*. I. Comparison of F_{ST} between allozymes and morphological traits. Genetics 140:733–744. https://doi.org/10.1093/genetics/140.2.733

Polley H, Yang C, Wilsey B, Fay P (2020) Spectrally derived values of community leaf dry matter content link shifts in grassland composition with change in biomass production. Remote Sensing in Ecology and Conservation 6. https://doi.org/10.1002/rse2.145

Post E, Forchhammer MC, Bret-Harte MS, Callaghan TV, Christensen TR, Elberling B, Fox AD, Gilg O, Hik DS, Høye TT, Ims RA, Jeppesen E, Klein DR, Madsen J, McGuire AD, Rysgaard S, Schindler DE, Stirling I, Tamstorf MP, Tyler NJC, Van Der Wal R, Welker J, Wookey PA, Schmidt NM, Aastrup P (2009) Ecological dynamics across the Arctic associated with recent climate change. Science 325:1355–1358. https://doi.org/10.1126/science.1173113

Preite V, Stöcklin J, Armbruster GFJ, Scheepens JF (2015) Adaptation of flowering phenology and fitness-related traits across environmental gradients in the widespread *Campanula rotundifolia*. Evol Ecol 29:249–267. https://doi.org/10.1007/s10682-015-9754-y

Premoli AC (2003) Isozyme polymorphisms provide evidence of clinal variation with elevation in *Nothofagus pumilio*. Journal of heredity 94:218–226. https://doi.org/10.1093/jhered/esg052

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959. https://doi.org/10.1093/genetics/155.2.945

Pritchard JK, Wen X, Falush D (2007) Documentation for STRUCTURE software: Version 2. Available at http://pritch.bsd.uchicago.edu/software

Quiroga MP, Premoli AC (2007) Genetic patterns in *Podocarpus parlatorei* reveal the long-term persistence of cold-tolerant elements in the southern Yungas. Journal of Biogeography 34:447–455. https://doi.org/10.1111/j.1365-2699.2006.01613.x

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://wwwR-project.org/

R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://wwwR-project.org/

Radersma R, Noble DWA, Uller T (2020) Plasticity leaves a phenotypic signature during local adaptation. Evolution Letters 4:360–370. https://doi.org/10.1002/evl3.185

Rasmann S, Pellissier L, Defossez E, Jactel H, Kunstler G (2014) Climate-driven change in plant–insect interactions along elevation gradients. Functional Ecology 28:46–54. https://doi.org/10.1111/1365-2435.12135

Reed D, Frankham R (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1103. https://doi.org/10.1111/j.0014-3820.2001.tb00629.x

Reisch C, Anke A, Röhl M (2005) Molecular variation within and between ten populations of *Primula farinosa* (Primulaceae) along an altitudinal gradient in the northern Alps. Basic and Applied Ecology 6:35–45. https://doi.org/10.1016/j.baae.2004.09.004

Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist 153:185–194. https://doi.org/10.1046/j.0028-646X.2001.00289.x

Rioux Paquette S (2012) PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets. R package version 1.0.

Roblek M, Germ M, Trošt Sedej T, Gaberščik A (2008) Morphological and biochemical variations in St. John's wort, *Hypericum perforatum* L., growing over altitudinal and UV-B radiation gradients. Periodicum biologorum 110:257–262

Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. Nature 421:57–60. https://doi.org/10.1038/nature01333

Rossetto M, Thurlby KA, Offord CA, Allen CB, Weston PH (2011) The impact of distance and a shifting temperature gradient on genetic connectivity across a heterogeneous landscape. BMC Evolutionary Biology 11:126. https://doi.org/10.1186/1471-2148-11-126

Rousset F (2008) genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8:103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Sagarin RD, Gaines SD (2002) The 'abundant centre' distribution: to what extent is it a biogeographical rule? Ecol Lett 5:137–147. https://doi.org/10.1046/j.1461-0248.2002.00297.x

Sagarin RD, Gaines SD, Gaylord B (2006) Moving beyond assumptions to understand abundance distributions across the ranges of species. Trends in ecology & evolution 21:524–530. https://doi.org/10.1016/j.tree.2006.06.008

Santamaría L, Figuerola J, Pilon JJ, Mjelde M, Green AJ, De Boer T, King RA, Gornall RJ (2003) Plant performance across latitude: The role of plasticity and local adaptation in an aquatic plant. Ecology 84:2454–2461. https://doi.org/10.1890/02-0431

Schemske DW, Mittelbach GG, Cornell HV, Sobel JM, Roy K (2009) Is there a latitudinal gradient in the importance of biotic interactions? Annu Rev Ecol Evol Syst 40:245–269. https://doi.org/10.1146/annurev.ecolsys.39.110707.173430

Scherrer D, Körner C (2010) Infra-red thermometry of alpine landscapes challenges climatic warming projections. Global Change Biology 16:2602–2613. https://doi.org/10.1111/j.1365-2486.2009.02122.x

Schlaepfer DR, Braschler B, Rusterholz H-P, Baur B (2018) Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. Ecosphere 9:e02488. https://doi.org/10.1002/ecs2.2488

Schneeweiss GM, Schönswetter P (2011) A re-appraisal of nunatak survival in arctic-alpine phylogeography. Molecular Ecology 20:190–192. https://doi.org/10.1111/j.1365-294X.2010.04927.x

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. https://doi.org/10.1038/nmeth.2089

Schönswetter P, Paun O, Tribsch A, Niklfeld H (2003) Out of the Alps: colonization of Northern Europe by East Alpine populations of the glacier buttercup *Ranunculus glacialis* L.(Ranunculaceae). Molecular ecology 12:3373–3381. https://doi.org/10.1046/j.1365-294X.2003.01984.x

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. Annu Rev Ecol Evol Syst 40:415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Sheth SN, Angert AL (2018) Demographic compensation does not rescue populations at a trailing range edge. PNAS 115:2413–2418. https://doi.org/10.1073/pnas.1715899115

Shi M-M, Michalski SG, Chen X-Y, Durka W (2011) Isolation by elevation: genetic structure at neutral and putatively non-neutral loci in a dominant tree of subtropical forests, *Castanopsis eyrei*. PLoS One 6:e21302. https://doi.org/10.1371/journal.pone.0021302

Siefert A, Lesser MR, Fridley JD (2015) How do climate and dispersal traits limit ranges of tree species along latitudinal and elevational gradients? Global Ecology and Biogeography 24:581–593. https://doi.org/10.1111/geb.12287

Skrede I, Eidesen PB, Portela RP, Brochmann C (2006) Refugia, differentiation and postglacial migration in arctic-alpine Eurasia, exemplified by the mountain avens (*Dryas octopetala* L.): Phylogeography of *Dryas octopetala* in Eurasia. Molecular Ecology 15:1827–1840. https://doi.org/10.1111/j.1365-294X.2006.02908.x

Sork VL, Aitken SN, Dyer RJ, Eckert AJ, Legendre P, Neale DB (2013) Putting the landscape into the genomics of trees: approaches for understanding local adaptation and population responses to changing climate. Tree Genetics & Genomes 9:901–911. https://doi.org/10.1007/s11295-013-0596-x

Stehlik I, Schneller JJ, Bachmann K (2002) Immigration and in situ glacial survival of the low-alpine *Erinus alpinus* (Scrophulariaceae). Biol J Linn Soc 77:87–103. https://doi.org/10.1046/j.1095-8312.2002.00094.x

Sterk AA (1975) Demographic studies of *Anthyllis vulneraria* L. in the Netherlands. Acta botanica neerlandica 24:315–337

Sterk AA, Van Duijkeren A, Hogervorst J, Verbeek EDM (1982) Demographic studies of *Anthyllis vulneraria* L. in the Netherlands: II. Population density fluctuations and adaptations to arid conditions, seed populations, seedling mortality, and influence of the biocenosis on demographic features. Acta Botanica Neerlandica 31:11–40. https://doi.org/10.1111/j.1438-8677.1982.tb01590.x

Stinchcombe JR, Weinig C, Ungerer M, Olsen KM, Mays C, Halldorsdottir SS, Purugganan MD, Schmitt J (2004) A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. Proceedings of the National Academy of Sciences 101:4712–4717. https://doi.org/10.1073/pnas.0306401101

Stroeven AP, Hättestrand C, Kleman J, Heyman J, Fabel D, Fredin O, Goodfellow BW, Harbor JM, Jansen JD, Olsen L, Caffee MW, Fink D, Lundqvist J, Rosqvist GC, Strömberg B, Jansson KN (2016) Deglaciation of Fennoscandia. Quaternary Science Reviews 147:91–121. https://doi.org/10.1016/j.quascirev.2015.09.016

Swab RM, Regan HM, Matthies D, Becker U, Bruun HH (2015) The role of demography, intra-species variation, and species distribution models in species' projections under climate change. Ecography 38:221–230. https://doi.org/10.1111/ecog.00585

Takou M, Hämälä T, Koch EM, Steige KA, Dittberner H, Yant L, Genete M, Sunyaev S, Castric V, Vekemans X, Savolainen O, Meaux JD (2021) Maintenance of adaptive dynamics and no detectable load in a range-edge outcrossing plant population. Molecular Biology and Evolution 38:1820–1836. https://doi.org/10.1093/molbev/msaa322

Tanto Hadado T, Rau D, Bitocchi E, Papa R (2010) Adaptation and diversity along an altitudinal gradient in Ethiopian barley (*Hordeum vulgare* L.) landraces revealed by molecular analysis. BMC Plant Biol 10:121. https://doi.org/10.1186/1471-2229-10-121

Teixeira JC, Huber CD (2021) The inflated significance of neutral genetic diversity in conservation genetics. Proc Natl Acad Sci USA 118:e2015096118. https://doi.org/10.1073/pnas.2015096118

Thomas CD (2010) Climate, climate change and range boundaries. Divers Distrib 16:488–495. https://doi.org/10.1111/j.1472-4642.2010.00642.x

Tieszen LL, Bonde EK (1967) The influence of light intensity on growth and chlorophyll in arctic, subarctic, and alpine populations of *Deschampsia caespitosa* and *Trisetum spicatum*. Series in Biology 39

Toczydlowski RH, Waller DM (2021) Plastic and quantitative genetic divergence mirror environmental gradients among wild, fragmented populations of *Impatiens capensis*. Am J Bot ajb2.1782. https://doi.org/10.1002/ajb2.1782

Totland Ø (2001) Environment-dependent pollen limitation and selection on floral traits in an alpine species. Ecology 82:2233–2244. https://doi.org/10.1890/0012-9658(2001)082[2233:EDPLAS]2.0.CO;2

Totland Ø, Birks HJB (1996) Factors influencing inter-population variation in *Ranunculus acris* seed production in an alpine area of southwestern Norway. Ecography 19:269–278. https://doi.org/10.1111/j.1600-0587.1996.tb01254.x

Uller T, Nakagawa S, English S (2013) Weak evidence for anticipatory parental effects in plants and animals. Journal of evolutionary biology 26:2161–2170. https://doi.org/10.1111/jeb.12212

Valladares F, Gianoli E, Gómez JM (2007) Ecological limits to plant phenotypic plasticity. New Phytol 176:749–763. https://doi.org/10.1111/j.1469-8137.2007.02275.x

Van Glabeke S, Coart E, Honnay O, Roldán-Ruiz I (2007) Primer note: Isolation and characterization of polymorphic microsatellite markers in *Anthyllis vulneraria*. Molecular Ecology Notes 7:477–479. https://doi.org/10.1111/j.1471-8286.2006.01625.x

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Vaupel A (2013) The ecology and genetics of central and peripheral populations of *Carduus defloratus*. Philipps-Universität Marburg

Vaupel A, Matthies D (2012) Abundance, reproduction, and seed predation of an alpine plant decrease from the center toward the range limit. Ecology 93:2253–2262. https://doi.org/10.1890/11-2026.1

Vergeer P, Kunin WE (2011) Life history variation in *Arabidopsis lyrata* across its range: effects of climate, population size and herbivory. Oikos 120:979–990. https://doi.org/10.1111/j.1600-0706.2010.18944.x

Vergeer P, Kunin WE (2013) Adaptation at range margins: common garden trials and the performance of *Arabidopsis lyrata* across its northwestern European range. New Phytol 197:989–1001. https://doi.org/10.1111/nph.12060

Verhoeven KJF, Simonsen KL, McIntyre LM (2005) Implementing false discovery rate control: increasing your power. Oikos 108:643–647. https://doi.org/10.1111/j.0030-1299.2005.13727.x

Villellas J, Doak DF, García MB, Morris WF (2015) Demographic compensation among populations: what is it, how does it arise and what are its implications? Ecol Lett 18:1139–1152. https://doi.org/10.1111/ele.12505

Villellas J, Ehrlén J, Olesen JM, Braza R, García MB (2013) Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36:136–145. https://doi.org/10.1111/j.1600-0587.2012.07425.x

Vitasse Y, Lenz A, Kollas C, Randin CF, Hoch G, Körner C (2014) Genetic vs. non-genetic responses of leaf morphology and growth to elevation in temperate tree species. Functional ecology 28:243–252. https://doi.org/10.1111/1365-2435.12161

Volis S, Ormanbekova D, Yermekbayev K, Song M, Shulgina I (2016) The conservation value of peripheral populations and a relationship between quantitative trait and molecular variation. Evol Biol 43:26–36. https://doi.org/10.1007/s11692-015-9346-3

Vollmann J, Walter H, Sato T, Schweiger P (2011) Digital image analysis and chlorophyll metering for phenotyping the effects of nodulation in soybean. Computers and Electronics in Agriculture 75:190–195. https://doi.org/10.1016/j.compag.2010.11.003

von Humboldt A, Bonpland A (1805) Essai sur la géographie des plantes; accompagné d'un tableau physique des régions équinoxiales, fondé sur des mesures exécutées, depuis le dixième degré de latitude boréale jusqu'au dixième degré de latitude australe, pendant les années 1799, 1800, 1801, 1802 et 1803. Levrault, Schoell et Cie, Paris

Walisch TJ, Colling G, Bodenseh M, Matthies D (2015) Divergent selection along climatic gradients in a rare central European endemic species, *Saxifraga sponhemica*. Ann Bot 115:1177–1190. https://doi.org/10.1093/aob/mcv040

Walther G-R (2003) Plants in a warmer world. Perspect Plant Ecol Evol Syst 6:169–185. https://doi.org/10.1078/1433-8319-00076

Weber E, Schmid B (1998) Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. Am J Bot 85:1110–1121. https://doi.org/10.2307/2446344

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370. https://doi.org/10.2307/2408641

Wheeler B, Torchiano M (2016) lmPerm: permutation tests for linear models. R package version 2.1.0.

Wirth LR, Graf R, Gugerli F, Landergott U, Holderegger R (2010) Lower selfing rate at higher altitudes in the alpine plant *Eritrichium nanum* (Boraginaceae). American Journal of Botany 97:899–901. https://doi.org/10.3732/ajb.0900297

Woodward FI (1986) Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. Oecologia 70:580–586. https://doi.org/10.1007/BF00379908

Woodward FI (1987) Climate and plant distribution. Cambridge University Press, Cambridge, UK

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J, Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee W, Lusk C, Midgley JJ, Navas M-L, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R (2004) The worldwide leaf economics spectrum. Nature 428:821–827. https://doi.org/10.1038/nature02403

Wu H, Meng H, Wang S, Wei X, Jiang M (2018) Geographic patterns and environmental drivers of seed traits of a relict tree species. For Ecol Manage 422:59–68. https://doi.org/10.1016/j.foreco.2018.04.003

Xu Y-W, Sun L, Ma R, Gao Y-Q, Sun H, Song B (2023) Does pollinator dependence decrease along elevational gradients? Plant Diversity S2468265923000513. https://doi.org/10.1016/j.pld.2023.03.006

Yakimowski SB, Eckert CG (2008) Populations do not become less genetically diverse or more differentiated towards the northern limit of the geographical range in clonal *Vaccinium stamineum* (Ericaceae). New Phytologist 180:534–544. https://doi.org/10.1111/j.1469-8137.2008.02582.x

Yamagishi H, Allison TD, Ohara M (2005) Effect of snowmelt timing on the genetic structure of an *Erythronium grandiflorum* population in an alpine environment. Ecological Research 20:199–204. https://doi.org/10.1007/s11284-004-0032-7

Yang M-L, Wang L-L, Zhang G-P, Meng L-H, Yang Y-P, Duan Y-W (2018) Equipped for migrations across high latitude regions? Reduced spur length and outcrossing rate in a biennial *Halenia elliptica* (Gentianaceae) with mixed mating system along a latitude gradient. Front Genet 9:223. https://doi.org/10.3389/fgene.2018.00223

Yoshioka Y, Honjo M, Iwata H, Ninomiya S, Ohsawa R (2007) Pattern of geographical variation in petal shape in wild populations of *Primula sieboldii* E. Morren. Plant Species Biol 22:87–93. https://doi.org/10.1111/j.1442-1984.2007.00180.x

Zhang M, Suren H, Holliday JA (2019) Phenotypic and genomic local adaptation across latitude and altitude in *Populus trichocarpa*. Genome Biology and Evolution 11:2256–2272. https://doi.org/10.1093/gbe/evz151

APPENDIX

Chapter 1

Table S1 *Anthyllis vulneraria* study sites.

Population	Country	Latitude (°N)	Longitude (°E)	Altitude (m a.s.l.)
Altitudinal gradient				
AFr1	France	45.05330	6.38920	2362
AFr2	France	45.05119	6.35326	1997
AFr3	France	45.15615	6.42366	1518
AFr4	France	45.21658	6.32499	1223
AFr5	France	45.09324	5.78040	471
AFr6	France	45.17351	6.03890	936
AFr7	France	45.12095	5.98519	717
AFr8	France	45.05978	6.31574	1807
ACh1	Switzerland	46.13377	7.05947	545
ACh2	Switzerland	46.08331	7.12650	1042
ACh3	Switzerland	46.04960	7.95643	2162
ACh4	Switzerland	46.25389	7.27336	1585
ACh5	Switzerland	46.27354	7.23742	1250
ACh6	Switzerland	46.08813	7.40669	1940
ACh7	Switzerland	46.10807	7.58007	2413
AAt1	Austria	47.39796	11.26613	961
AAt2	Austria	47.44211	11.65014	1521
AAt3	Austria	47.16064	11.71487	1810
AAt4	Austria	47.16901	11.35334	1151
AAt5	Austria	47.31265	11.38936	2250
Latitudinal gradient				
L1	France	46.43682	4.75282	323
L2	France	48.18803	5.55341	443
L3	Luxembourg	49.49562	5.99690	342
L4	Luxembourg	49.73139	6.28194	355
L5	Germany	51.22280	9.76097	442
L6	Germany	52.00507	10.40749	191
L7	Germany	54.04430	10.22901	32
L8	Germany	54.68729	9.43418	22
L9	Denmark	55.51500	9.42435	42
L10	Sweden	56.36709	12.80019	81
L11	Sweden	57.88923	11.94657	24
L12	Sweden	58.69792	11.21994	5
L13	Norway	61.06211	10.39708	438
L14	Norway	62.01385	9.20743	483
L15	Norway	63.44094	10.65668	18
L16	Iceland	63.81644	-22.69699	20
L17	Norway	64.31624	12.34754	168
L18	Sweden	66.42609	16.85014	453
L19	Norway	67.25107	15.42817	6
L20	Norway	68.10215	16.37830	47

Table S2 Pairwise correlations between the predictor variables used in the general and generalized linear models for both the altitudinal and latitudinal gradients. (*) $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

	Annual mean temperature *r*		Annual precipitation *r*	Solar radiation *r*		Standing biomass *r*
Altitude						
Annual precipitation	-0.628	**				
Solar radiation	-0.317		0.284			
Standing biomass	0.334		-0.087	0.002		
PC Soil nutrients	-0.103		0.130	0.057		-0.203
Latitude						
Annual precipitation	0.044					
Solar radiation	0.815	***	-0.258			
Standing biomass	-0.324		-0.021	-0.456	*	
PC Soil nutrients	0.539	*	0.040	0.666	**	-0.271

Table S3 Medians and ranges of habitat characteristics for the 40 studied *A. vulneraria* populations.

Habitat characteristic	Median	Range		
Exposure (° deviating from north)	114.5	0.0	-	177.0
Slope (%)	24.0	0.0	-	102.7
Standing biomass (g m^{-2})	96.1	23.0	-	334.2
pH	7.1	4.7	-	7.6
P_2O_5 (mg [100 g soil]$^{-1}$)	3.0	1.0	-	10.0
K_2O (mg [100 g soil]$^{-1}$)	8.0	2.0	-	40.0
C_{org} (%)	3.2	0.2	-	17.5
N_{tot} (%)	0.2	0.0	-	1.1
Annual mean temperature (°C)	6.1	-1.2	-	10.8
Annual precipitation (mm)	1037.5	413.0	-	1462.0

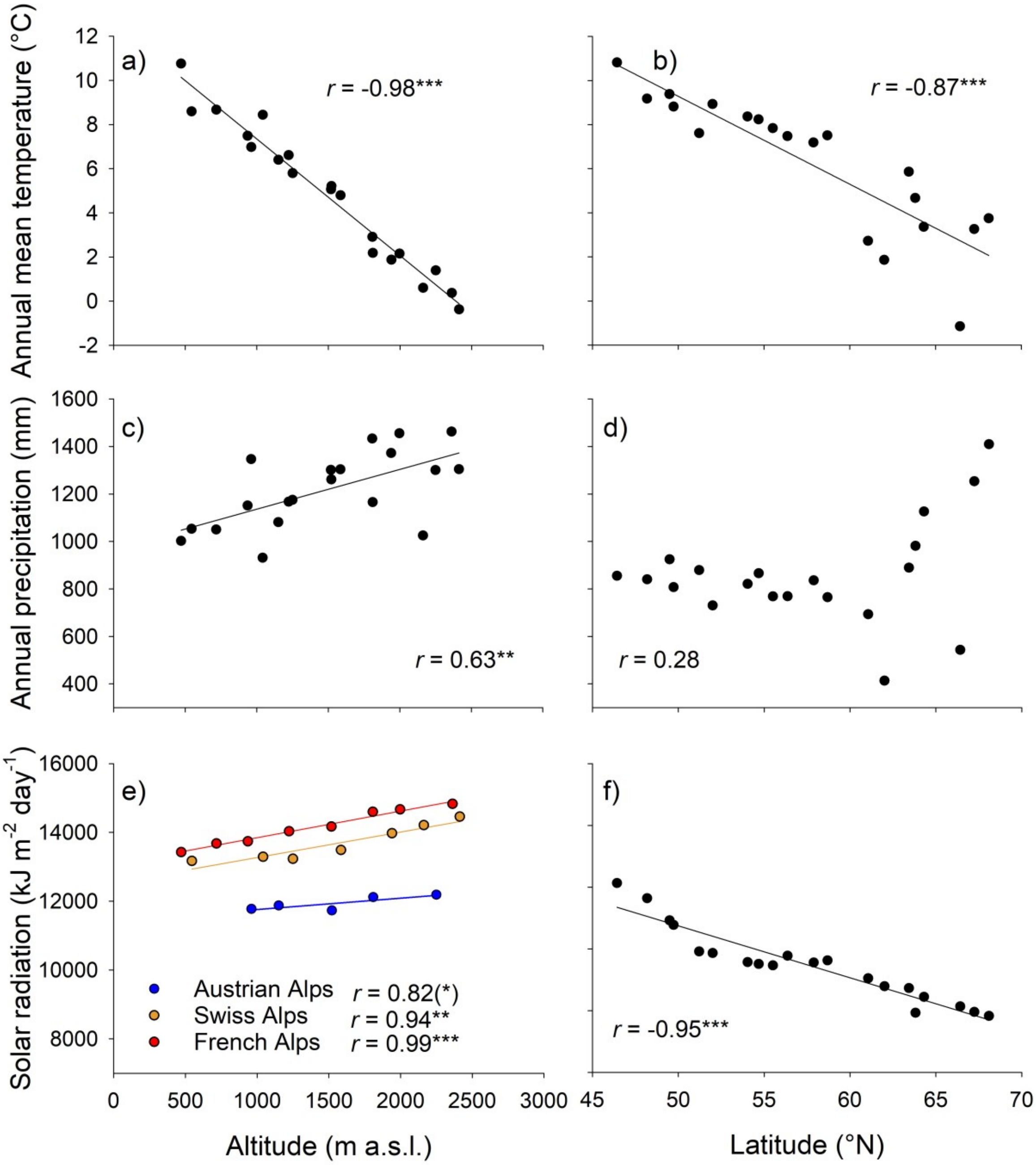

Fig. S1 Relationship between various habitat characteristics of *A. vulneraria* populations and (a, c, e) altitude for the populations along the altitude gradient and (b, d, f) latitude for the populations along the latitudinal gradient.

Table S4 Analysis of variance of the effects of the three regions within the Alps and the linear and quadratic effects of altitude on the maximal plant density of the populations of the altitudinal gradient. r^2 of the overall model = 0.94.

Max. plant density (m^{-2})	df	*F*	*P*
Region	2	13.2	0.001
Altitude linear	1	57.4	<0.001
Altitude quadratic	1	59.9	<0.001
Region x Alt. linear	2	8.7	0.005
Region x Alt. quadratic	2	11.6	0.002
Error	11		

Table S5 Results of model averaging of the effects of five explanatory variables on various population characteristics along the altitudinal and latitudinal gradients. Model averaging of all possible models and importance calculation was done using the R-package MuMIn. n: number of populations included in the analyses.

Dependent variable	Intercept	Explanatory variable	Importance	Estimate	Standard error
Altitude (n = 19)					
Max. plant density	2.301	Annual mean temperature	1.00	-0.06107	0.01422
		PC Soil nutrients	0.55	-0.06304	0.03313
		Solar radiation	0.45	-0.00007	0.00004
		Standing biomass	0.20	-0.00048	0.00054
		Annual precipitation	0.18	-0.00024	0.00031
Population size	4.581	Annual mean temperature	0.99	-0.18888	0.05458
		Annual precipitation	0.57	-0.00183	0.00096
		Solar radiation	0.22	0.00014	0.00014
		PC Soil nutrients	0.16	0.06067	0.11628
		Standing biomass	0.15	-0.00074	0.00184
Plant height	-18.640	Annual mean temperature	1.00	3.14337	0.62758
		Annual precipitation	0.76	0.02335	0.00986
		Standing biomass	0.40	0.02837	0.01749
		Solar radiation	0.36	0.00216	0.00141
		PC Soil nutrients	0.13	-0.41895	1.21362
Prop. flowering	0.223	Annual mean temperature	0.34	-0.10809	0.07935
		Solar radiation	0.21	0.00016	0.00022
		Standing biomass	0.20	0.00208	0.00338
		Annual precipitation	0.19	-0.00086	0.00160
		PC Soil nutrients	0.17	0.04993	0.22627
Flowerheads per plant	0.074	Annual mean temperature	0.67	0.04241	0.02033
		Solar radiation	0.40	0.00010	0.00006
		Standing biomass	0.34	-0.06627	0.05016
		PC Soil nutrients	0.32	0.00109	0.00079
		Annual precipitation	0.17	-0.00003	0.00048
Prop. seed set	0.541	Annual precipitation	0.26	0.00195	0.00177
		Solar radiation	0.24	-0.00028	0.00029
		PC Soil nutrients	0.21	0.21317	0.26602
		Standing biomass	0.19	-0.00177	0.00314
		Annual mean temperature	0.18	-0.03240	0.08476
Prop. seeds damaged	-3.219	Annual mean temperature	0.74	0.26420	0.12110
		Annual precipitation	0.21	0.00057	0.00369
		Standing biomass	0.17	-0.00078	0.00405
		Solar radiation	0.16	-0.00009	0.00036
		PC Soil nutrients	0.16	-0.02075	0.39170
Seed mass	-0.092	Annual precipitation	0.83	0.00231	0.00099
		Annual mean temperature	0.35	0.06893	0.05750
		Solar radiation	0.27	0.00016	0.00015
		Standing biomass	0.16	0.00052	0.00183
		PC Soil nutrients	0.15	-0.03694	0.12351

Latitude (n = 20)					
Max. plant density	1.341	Annual precipitation	0.87	0.00111	0.00040
		PC Soil nutrients	0.58	-0.26629	0.13404
		Solar radiation	0.37	-0.00017	0.00014
		Annual mean temperature	0.22	0.02906	0.06075
		Standing biomass	0.15	-0.00024	0.00197
Population size	2.207	Annual precipitation	0.80	0.00142	0.00056
		Standing biomass	0.50	0.00431	0.00244
		Solar radiation	0.35	-0.00025	0.00022
		Annual mean temperature	0.24	0.06261	0.08813
		PC Soil nutrients	0.17	-0.03557	0.23627
Plant height	40.272	Annual mean temperature	0.69	2.36622	1.35573
		Standing biomass	0.51	-0.09715	0.05568
		Annual precipitation	0.30	-0.01613	0.01392
		Solar radiation	0.30	-0.00296	0.00602
		PC Soil nutrients	0.20	-2.84218	4.96278
Prop. flowering	-3.557	Annual mean temperature	0.66	0.27155	0.12482
		PC Soil nutrients	0.52	0.90605	0.51620
		Solar radiation	0.40	0.00070	0.00052
		Standing biomass	0.40	0.00793	0.00497
		Annual precipitation	0.22	-0.00080	0.00114
Flowerheads per plant	0.111	Solar radiation	0.63	0.00015	0.00008
		Annual mean temperature	0.28	0.02761	0.04028
		Annual precipitation	0.27	-0.00038	0.00037
		Standing biomass	0.23	-0.00148	0.00174
		PC Soil nutrients	0.20	0.04763	0.15840
Prop. seed set	4.500	Annual mean temperature	0.72	0.24958	0.14977
		PC Soil nutrients	0.70	1.08700	0.60205
		Annual precipitation	0.70	-0.00249	0.00136
		Solar radiation	0.46	-0.00074	0.00058
		Standing biomass	0.16	-0.00384	0.00617
Prop. seeds damaged	-3.060	Annual mean temperature	0.67	0.28989	0.15632
		Standing biomass	0.37	-0.01380	0.00937
		Solar radiation	0.25	0.00010	0.00047
		PC Soil nutrients	0.20	0.37806	0.54226
		Annual precipitation	0.17	0.00098	0.00190
Seed mass	2.415	Annual mean temperature	0.62	0.06845	0.03673
		PC Soil nutrients	0.26	0.15930	0.16620
		Solar radiation	0.24	0.00002	0.00015
		Annual precipitation	0.17	0.00009	0.00045
		Standing biomass	0.17	-0.00029	0.00207

Table S6 Standardized regression coefficients for general and generalized linear models of the relationship between population characteristics of *Anthyllis vulneraria* along an altitudinal and a latitudinal gradient and habitat characteristics. Standardized regression coefficients for binomial models for proportions are calculated with the latent-theoretical method (Grace et al. 2018). t-values are given for variables with a gaussian distribution and z-values for the ones with a quasibinomial distribution. The best model was chosen using the lowest AICc for the variables with a gaussian distribution and using the lowest QAICc for the variables with a quasibinomial distribution using the R-package MuMIn. Only dependent variables are presented for which the best model contained at least one explanatory variable in addition to the intercept. Total r^2 is indicated for the models with a gaussian distribution, McFadden's pseudo r^2 for the ones with a quasibinomial one. Annual mean temperature is highlighted in bold face. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Dependent variable	Explanatory variable	Coefficient	t/z-value	*P*
Altitude (n = 19)				
Log Max. plant density ($r^2 = 0.62$***)	**Annual mean temperature**	-0.76	-4.90	< 0.001
	PC Soil nutrients	-0.29	-1.85	0.083
Log Population size ($r^2 = 0.58$***)	**Annual mean temperature**	-0.94	-4.57	< 0.001
	Annual precipitation	-0.40	-1.92	0.073
Plant height ($r^2 = 0.69$***)	**Annual mean temperature**	1.06	6.65	< 0.001
	Annual precipitation	0.39	2.48	0.025
Log Flowerheads per plant ($r^2 = 0.35$**)	**Annual mean temperature**	0.63	2.83	0.012
	Solar radiation	0.41	1.83	0.086
Prop. seeds damaged (pseudo $r^2 = 0.34$)	**Annual mean temperature**	0.40	2.57	0.020
Seed mass ($r^2 = 0.27$*)	Annual precipitation	0.52	2.53	0.022
Latitude (n = 20)				
Log Max. plant density ($r^2 = 0.49$***)	Annual precipitation	0.58	3.33	0.004
	PC Soil nutrients	-0.43	-2.47	0.024
Log Population size ($r^2 = 0.28$*)	Annual precipitation	0.53	2.67	0.016
Plant height ($r^2 = 0.24$*)	**Annual mean temperature**	0.49	2.38	0.028
Prop. flowering (pseudo $r^2 = 0.57$)	**Annual mean temperature**	0.33	2.44	0.026
	PC Soil nutrients	0.28	1.95	0.068
Log Flowerheads per plant ($r^2 = 0.28$*)	Solar radiation	0.53	2.64	0.017
Prop. seed set (pseudo $r^2 = 0.59$)	**Annual mean temperature**	0.53	2.98	0.009
	Annual precipitation	-0.35	-2.89	0.011
	Solar radiation	-0.57	-2.53	0.023
	PC Soil nutrients	0.47	2.56	0.022
Prop. seeds damaged (pseudo $r^2 = 0.30$)	**Annual mean temperature**	0.46	2.36	0.030
Seed mass ($r^2 = 0.23$*)	**Annual mean temperature**	0.48	2.31	0.033

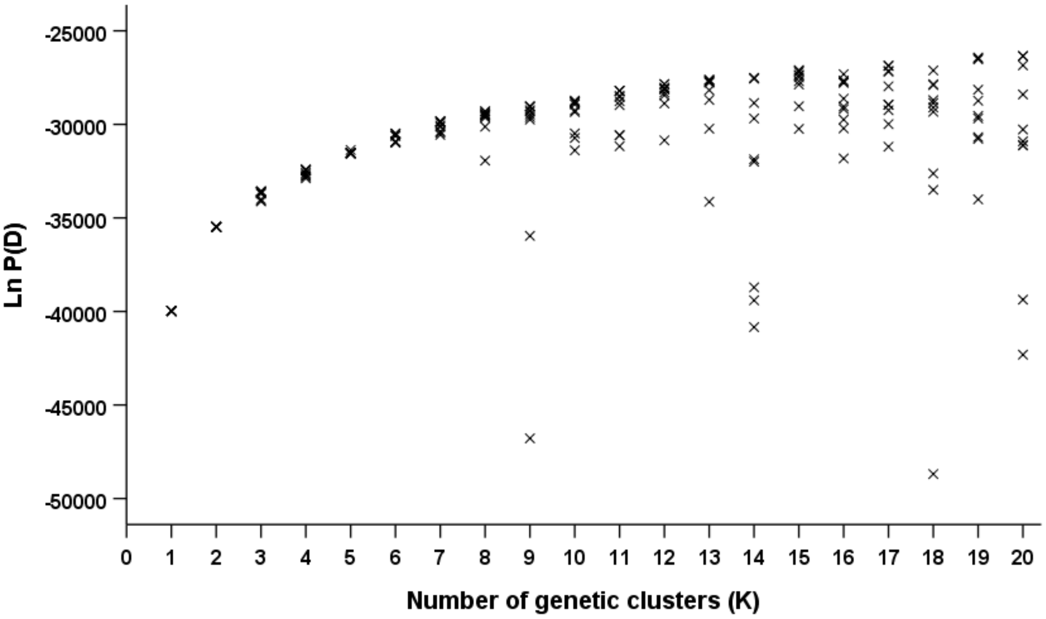

Fig. S1 Inference of the number of genetic clusters *K* resulting from the STRUCTURE analysis of 40 *A. vulneraria* populations from the elevational and latitudinal gradients using a model with correlated allele frequencies and assumed admixture. Log probability of the data [ln P(D)] of ten independent runs for each number of genetic clusters inferred using STRUCTURE.

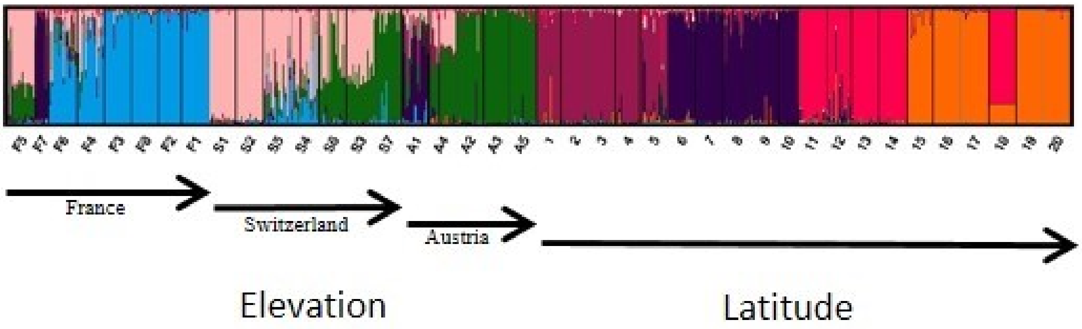

Fig. S2 Results of the STRUCTURE analysis for 40 populations sampled across elevational and latitudinal gradients assuming *K* = 7. Each individual is represented by a vertical line, which is partitioned into a maximum of seven coloured segments that represented an individual's estimated membership fractions in the seven clusters. Vertical black lines separate the 40 different populations. Arrows represent increasing elevation along each of the three regions in the Alps and increasing latitude along the latitudinal gradient. For population labels see Table 1.

Table S1 Pairwise F_{ST} (below diagonal) and G''_{ST} (above diagonal) between populations of *Anthyllis vulneraria*.

	AAt1	AAt2	AAt3	AAt4	AAt5	ACh1	ACh2	ACh3	ACh4	ACh5	ACh6	ACh7	AFr1	AFr2	AFr3	AFr4	AFr5	AFr6	AFr7	AFr8	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
AAt1	0	0.34	0.55	0.30	0.31	0.33	0.46	0.45	0.35	0.36	0.31	0.33	0.51	0.40	0.46	0.33	0.34	0.24	0.23	0.42	0.46	0.49	0.32	0.47	0.30	0.35	0.27	0.25	0.33	0.53	0.62	0.63	0.67	0.81	0.74	0.80	0.70	0.82	0.83	0.79
AAt2	0.15	0	0.23	0.40	0.15	0.42	0.46	0.25	0.28	0.38	0.24	0.22	0.59	0.45	0.45	0.31	0.33	0.27	0.42	0.47	0.48	0.50	0.31	0.40	0.43	0.47	0.47	0.56	0.54	0.69	0.74	0.71	0.71	0.85	0.76	0.82	0.76	0.85	0.84	0.85
AAt3	0.29	0.12	0	0.56	0.27	0.55	0.59	0.31	0.50	0.56	0.35	0.33	0.68	0.58	0.60	0.50	0.46	0.50	0.61	0.60	0.67	0.69	0.57	0.59	0.59	0.60	0.63	0.71	0.71	0.79	0.83	0.79	0.86	0.93	0.86	0.88	0.83	0.93	0.90	0.91
AAt4	0.15	0.19	0.31	0	0.34	0.40	0.60	0.53	0.45	0.48	0.43	0.33	0.69	0.58	0.56	0.46	0.43	0.45	0.50	0.63	0.55	0.59	0.39	0.56	0.44	0.48	0.56	0.49	0.53	0.76	0.68	0.70	0.72	0.82	0.75	0.85	0.77	0.85	0.85	0.79
AAt5	0.14	0.06	0.14	0.16	0	0.39	0.50	0.32	0.29	0.36	0.18	0.16	0.63	0.52	0.49	0.36	0.36	0.37	0.43	0.54	0.55	0.53	0.37	0.45	0.48	0.50	0.51	0.53	0.52	0.74	0.73	0.74	0.75	0.86	0.80	0.87	0.78	0.86	0.89	0.86
ACh1	0.15	0.19	0.30	0.20	0.18	0	0.32	0.39	0.37	0.42	0.37	0.41	0.63	0.46	0.51	0.31	0.37	0.36	0.44	0.45	0.58	0.55	0.44	0.45	0.39	0.44	0.51	0.48	0.48	0.70	0.71	0.66	0.70	0.83	0.77	0.82	0.74	0.84	0.85	0.78
ACh2	0.22	0.21	0.33	0.31	0.24	0.16	0	0.44	0.33	0.37	0.43	0.49	0.65	0.47	0.52	0.29	0.50	0.39	0.51	0.47	0.61	0.61	0.49	0.48	0.48	0.57	0.57	0.61	0.59	0.74	0.79	0.77	0.78	0.88	0.81	0.86	0.79	0.90	0.87	0.86
ACh3	0.21	0.12	0.17	0.27	0.15	0.19	0.22	0	0.35	0.43	0.30	0.34	0.59	0.46	0.50	0.30	0.36	0.34	0.53	0.45	0.61	0.56	0.46	0.40	0.48	0.54	0.58	0.64	0.60	0.74	0.77	0.74	0.81	0.89	0.83	0.83	0.82	0.89	0.87	0.86
ACh4	0.14	0.11	0.24	0.20	0.12	0.16	0.14	0.15	0	0.16	0.21	0.24	0.47	0.35	0.35	0.15	0.39	0.21	0.35	0.32	0.52	0.45	0.31	0.39	0.44	0.47	0.45	0.48	0.41	0.67	0.68	0.74	0.68	0.83	0.75	0.81	0.76	0.85	0.85	0.83
ACh5	0.14	0.15	0.27	0.21	0.14	0.17	0.16	0.18	0.06	0	0.19	0.29	0.47	0.36	0.42	0.22	0.44	0.28	0.34	0.37	0.48	0.46	0.32	0.47	0.43	0.48	0.42	0.44	0.41	0.67	0.67	0.67	0.71	0.83	0.76	0.80	0.77	0.83	0.86	0.83
ACh6	0.14	0.10	0.18	0.20	0.08	0.16	0.20	0.14	0.08	0.07	0	0.09	0.54	0.41	0.45	0.24	0.35	0.31	0.31	0.42	0.54	0.51	0.35	0.45	0.45	0.46	0.40	0.50	0.39	0.64	0.73	0.68	0.72	0.87	0.77	0.80	0.78	0.86	0.85	0.82
ACh7	0.15	0.10	0.18	0.16	0.07	0.20	0.24	0.16	0.10	0.12	0.04	0	0.54	0.41	0.39	0.29	0.39	0.30	0.40	0.44	0.55	0.57	0.33	0.46	0.48	0.47	0.47	0.53	0.46	0.69	0.73	0.72	0.74	0.88	0.79	0.84	0.78	0.87	0.86	0.84
AFr1	0.26	0.30	0.41	0.39	0.33	0.34	0.36	0.32	0.23	0.23	0.27	0.29	0	0.19	0.38	0.35	0.59	0.33	0.48	0.24	0.69	0.64	0.58	0.65	0.58	0.53	0.52	0.55	0.55	0.68	0.77	0.78	0.80	0.90	0.84	0.83	0.79	0.91	0.88	0.90
AFr2	0.18	0.20	0.32	0.29	0.23	0.22	0.23	0.22	0.15	0.15	0.18	0.20	0.10	0	0.25	0.21	0.47	0.16	0.41	0.10	0.61	0.56	0.44	0.52	0.48	0.48	0.42	0.51	0.49	0.65	0.74	0.69	0.75	0.87	0.80	0.83	0.74	0.88	0.85	0.85
AFr3	0.24	0.23	0.36	0.31	0.25	0.27	0.28	0.27	0.17	0.20	0.23	0.20	0.23	0.13	0	0.27	0.56	0.25	0.53	0.27	0.64	0.60	0.46	0.53	0.55	0.58	0.57	0.62	0.56	0.77	0.77	0.81	0.78	0.90	0.82	0.82	0.78	0.89	0.88	0.87
AFr4	0.14	0.13	0.25	0.21	0.15	0.13	0.13	0.13	0.06	0.08	0.10	0.13	0.18	0.09	0.13	0	0.37	0.13	0.32	0.19	0.47	0.42	0.32	0.35	0.34	0.43	0.42	0.46	0.41	0.63	0.68	0.71	0.65	0.83	0.72	0.76	0.74	0.82	0.81	0.79
AFr5	0.17	0.16	0.26	0.23	0.17	0.19	0.26	0.19	0.18	0.19	0.17	0.19	0.33	0.24	0.31	0.17	0	0.37	0.44	0.44	0.57	0.61	0.50	0.51	0.48	0.40	0.47	0.50	0.49	0.65	0.76	0.70	0.78	0.88	0.80	0.86	0.79	0.90	0.88	0.84
AFr6	0.10	0.11	0.25	0.21	0.16	0.16	0.18	0.15	0.08	0.11	0.13	0.14	0.17	0.07	0.13	0.05	0.17	0	0.33	0.17	0.47	0.44	0.31	0.39	0.35	0.41	0.36	0.42	0.42	0.59	0.65	0.66	0.70	0.81	0.71	0.78	0.71	0.82	0.83	0.79
AFr7	0.10	0.18	0.33	0.24	0.19	0.20	0.25	0.25	0.14	0.13	0.13	0.18	0.26	0.19	0.28	0.13	0.22	0.14	0	0.42	0.38	0.43	0.32	0.43	0.32	0.17	0.01	0.16	0.14	0.37	0.58	0.58	0.60	0.79	0.63	0.75	0.62	0.81	0.78	0.71
AFr8	0.20	0.22	0.33	0.32	0.25	0.22	0.24	0.22	0.14	0.16	0.19	0.22	0.13	0.05	0.15	0.09	0.23	0.08	0.20	0	0.63	0.58	0.49	0.54	0.51	0.50	0.46	0.52	0.48	0.66	0.74	0.76	0.75	0.88	0.81	0.82	0.74	0.89	0.85	0.86
L1	0.22	0.22	0.37	0.28	0.25	0.28	0.30	0.30	0.22	0.20	0.24	0.26	0.38	0.29	0.34	0.21	0.29	0.21	0.18	0.31	0	0.50	0.32	0.44	0.33	0.43	0.43	0.44	0.52	0.70	0.49	0.56	0.60	0.67	0.60	0.81	0.65	0.73	0.78	0.69
L2	0.23	0.23	0.38	0.30	0.24	0.26	0.30	0.28	0.19	0.19	0.23	0.27	0.35	0.27	0.32	0.18	0.31	0.20	0.20	0.29	0.25	0	0.36	0.38	0.42	0.57	0.51	0.52	0.59	0.47	0.38	0.41	0.42	0.52	0.41	0.54	0.51	0.59	0.65	0.85
L3	0.14	0.13	0.29	0.19	0.16	0.20	0.23	0.22	0.12	0.13	0.15	0.15	0.30	0.20	0.23	0.13	0.24	0.13	0.14	0.23	0.15	0.16	0	0.31	0.30	0.46	0.39	0.43	0.45	0.42	0.31	0.34	0.34	0.49	0.38	0.52	0.44	0.50	0.57	0.58
L4	0.23	0.19	0.33	0.29	0.21	0.22	0.24	0.20	0.17	0.20	0.21	0.23	0.36	0.26	0.29	0.16	0.26	0.18	0.21	0.28	0.22	0.19	0.15	0	0.36	0.50	0.51	0.56	0.54	0.48	0.38	0.41	0.42	0.58	0.47	0.58	0.53	0.64	0.66	0.65
L5	0.14	0.19	0.31	0.21	0.21	0.18	0.23	0.23	0.18	0.18	0.20	0.23	0.31	0.22	0.28	0.14	0.23	0.15	0.14	0.24	0.16	0.20	0.13	0.18	0	0.35	0.35	0.30	0.43	0.38	0.28	0.30	0.33	0.45	0.35	0.50	0.44	0.52	0.54	0.52
L6	0.17	0.22	0.33	0.25	0.24	0.22	0.29	0.28	0.21	0.21	0.21	0.23	0.30	0.24	0.32	0.19	0.21	0.19	0.08	0.26	0.22	0.29	0.22	0.26	0.17	0	0.21	0.29	0.26	0.33	0.35	0.32	0.42	0.53	0.39	0.50	0.40	0.61	0.58	0.53
L7	0.13	0.22	0.35	0.29	0.24	0.25	0.29	0.29	0.20	0.18	0.18	0.23	0.29	0.21	0.31	0.19	0.24	0.17	0.00	0.23	0.21	0.25	0.19	0.26	0.17	0.11	0	0.23	0.20	0.21	0.37	0.33	0.42	0.55	0.41	0.54	0.47	0.64	0.61	0.56
L8	0.12	0.26	0.39	0.25	0.24	0.23	0.30	0.32	0.21	0.18	0.22	0.26	0.30	0.24	0.33	0.20	0.25	0.19	0.08	0.26	0.21	0.25	0.20	0.28	0.14	0.14	0.12	0	0.28	0.30	0.29	0.30	0.38	0.49	0.41	0.54	0.45	0.56	0.62	0.56
L9	0.16	0.25	0.39	0.27	0.24	0.24	0.30	0.30	0.18	0.17	0.18	0.23	0.31	0.24	0.30	0.19	0.25	0.20	0.07	0.24	0.26	0.29	0.21	0.27	0.21	0.13	0.10	0.14	0	0.34	0.38	0.37	0.43	0.56	0.43	0.53	0.49	0.64	0.61	0.57
L10	0.32	0.41	0.55	0.50	0.45	0.44	0.47	0.47	0.38	0.37	0.37	0.43	0.47	0.41	0.53	0.37	0.42	0.35	0.26	0.42	0.45	0.74	0.70	0.74	0.63	0.52	0.34	0.48	0.54	0	0.83	0.77	0.77	0.92	0.86	0.91	0.87	0.96	0.95	0.91
L11	0.34	0.40	0.52	0.40	0.40	0.40	0.46	0.45	0.35	0.34	0.39	0.41	0.49	0.42	0.48	0.36	0.45	0.35	0.34	0.43	0.28	0.67	0.57	0.64	0.51	0.60	0.63	0.50	0.66	0.60	0	0.49	0.63	0.60	0.67	0.83	0.73	0.78	0.83	0.75
L12	0.34	0.38	0.49	0.41	0.39	0.37	0.44	0.42	0.37	0.33	0.36	0.40	0.48	0.39	0.50	0.37	0.41	0.35	0.33	0.43	0.32	0.73	0.64	0.71	0.54	0.55	0.57	0.53	0.65	0.56	0.32	0	0.74	0.62	0.70	0.81	0.74	0.80	0.85	0.73
L13	0.41	0.43	0.59	0.47	0.46	0.44	0.50	0.52	0.39	0.40	0.43	0.46	0.55	0.47	0.53	0.38	0.50	0.41	0.40	0.48	0.38	0.67	0.56	0.65	0.53	0.65	0.66	0.61	0.67	0.61	0.45	0.53	0	0.76	0.68	0.83	0.73	0.83	0.85	0.83
L14	0.53	0.54	0.68	0.57	0.56	0.55	0.59	0.60	0.51	0.49	0.55	0.58	0.66	0.59	0.65	0.51	0.61	0.51	0.56	0.60	0.45	0.78	0.77	0.83	0.69	0.77	0.81	0.74	0.83	0.78	0.46	0.47	0.62	0	0.68	0.87	0.80	0.88	0.89	0.82
L15	0.43	0.44	0.58	0.47	0.46	0.46	0.50	0.51	0.41	0.41	0.43	0.48	0.56	0.49	0.54	0.40	0.51	0.41	0.39	0.50	0.36	0.68	0.66	0.75	0.60	0.64	0.67	0.68	0.70	0.66	0.47	0.48	0.51	0.54	0	0.69	0.60	0.85	0.59	0.66
L16	0.51	0.51	0.64	0.58	0.55	0.53	0.57	0.55	0.49	0.47	0.49	0.55	0.59	0.55	0.58	0.47	0.59	0.48	0.53	0.55	0.54	0.82	0.82	0.85	0.77	0.75	0.81	0.82	0.79	0.75	0.62	0.60	0.66	0.74	0.54	0	0.86	0.92	0.79	0.88
L17	0.46	0.50	0.62	0.54	0.53	0.50	0.55	0.57	0.47	0.48	0.51	0.53	0.58	0.52	0.58	0.49	0.56	0.47	0.48	0.51	0.45	0.75	0.68	0.77	0.64	0.56	0.67	0.63	0.68	0.74	0.53	0.58	0.54	0.70	0.48	0.69	0	0.91	0.85	0.89
L18	0.60	0.60	0.75	0.66	0.62	0.62	0.67	0.66	0.58	0.56	0.60	0.63	0.73	0.67	0.71	0.57	0.69	0.58	0.67	0.68	0.55	0.80	0.71	0.84	0.72	0.82	0.86	0.77	0.86	0.89	0.64	0.66	0.74	0.81	0.74	0.84	0.83	0	1.00	0.98
L19	0.60	0.61	0.73	0.66	0.64	0.63	0.66	0.65	0.58	0.58	0.60	0.64	0.71	0.65	0.70	0.57	0.67	0.59	0.67	0.65	0.60	0.86	0.80	0.86	0.74	0.77	0.81	0.83	0.82	0.88	0.69	0.71	0.76	0.83	0.52	0.72	0.74	1.00	0	0.96
L20	0.56	0.61	0.73	0.59	0.63	0.57	0.66	0.65	0.58	0.57	0.58	0.63	0.72	0.64	0.70	0.57	0.63	0.56	0.63	0.64	0.55	0.64	0.78	0.85	0.68	0.69	0.74	0.73	0.74	0.85	0.61	0.63	0.71	0.80	0.63	0.80	0.82	1.00	1.00	0

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ERKLÄRUNG

Ich versichere, dass ich meine Dissertation

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Luxemburg, den 14.07.2023

Laura Daco