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Uncovering the evolution of elevational ecotypes in Alpine carnations

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Summary

Divergent selection pressures imposed by contrasting environmental conditions at opposite ends of environmental gradients can drive the evolution of populations that are adapted to local conditions. Elevational gradients in the Alps coincide with steep climatic gradients where plant populations experience divergent selection within a limited geographic scale. This feature makes alpine plants with a broad elevational range ideal for the study of evolution of local adaptation. In this thesis, we aimed to unravel the evolution of distinct locally adapted ecotypes of alpine carnations (*Dianthus* spp., Caryophyllaceae) in response to climate driven selection imposed by contrasting elevational habitats. As a study system, we used two perennial systems with an elevational distribution ranging from the colline to alpine belts in central Europe, *D. carthusianorum* and *D. sylvestris*. We used populations from low and high elevation growing in long-term reciprocal transplant experiments to study the evolutionary processes underlying ecotype formation by investigating performance across multiple fitness components and life stages of the perennial life cycle. Experiments for *D. sylvestris* were further combined with phenotypic selection analyses and a genome-wide association study based on a transplant of recombinant F2 crosses, which were used to examine the both contribution of divergent traits to adaptation and the fitness effect of alleles underlying these traits.

In **chapter I**, we first tested for local adaptation in *D. carthusianorum* by using data on performance in individual fitness components measured over a period of three years in the reciprocal transplant experiment. We found evidence of genotype by environment (GxE) interactions and fitness advantages of the local ecotype, though with extensive variation at different stages of the life cycle. We thus performed a complementary seedling recruitment experiment and integrated fitness over the course of the experiment through matrix population models. Population growth rates showed a strong signal of local adaptation in both elevational environments and further provided evidence of alternate life-history traits as determinants of plant fitness. The low elevation environment caused the local plants to express a faster life cycle characterized by high investment in early reproduction. Contrarily, fitness of the local plants in the high elevation ecotype was driven primarily by survival. The high elevation plants also reproduced more in the foreign environment, which caused them to exceed their physiological limit of resource allocation to reproduction and suffer a cost in terms of reduced post reproductive survival. **Chapter I** shows how selection imposed at the extremes of an elevational gradient drove ecotype formation in a perennial plant, highlighting the influence of trade-offs and phenotypic plasticity of life history traits as determinants of population performance under different environmental conditions.

In **chapter II**, we explored how selection acting through different fitness components of the perennial life cycle has driven ecotype formation in *D. sylvestris,* and we dissected the contribution of divergent traits to this process. Populations of *D. sylvestris* persisted in high elevation refugia during the Last Glacial Maximum and have subsequently colonized low

elevation habitats. We combined phenotypic and fitness data collected in a reciprocal transplant experiment over five years with phenotypic selection analyses on F2 crosses to unravel the contributions of adaptive traits to the responses to the contrasting environmental conditions and associated selection regimes. Our results revealed a strong genetic basis for plant size, plant height and flowering time, associated with elevational adaptation. The high elevation environment favored a conservative life history strategy characterized by a long life span and limited investment in reproduction. Consistently, selection acted towards early flowering to ensure completion of the reproductive cycle in the short alpine summer season. In contrast, the warmer low elevation environment favored a life history strategy characterized by high investment in early reproduction at the expense of a shorter life cycle, and thus plants achieving large size and maximized fecundity. Our results show that colonization of the warmer low elevation habitats proceeded through a shift in both phenotypic and life history traits linked to resource allocation in a high-energy environment with a longer reproductive season.

In **chapter III**, we leveraged results from chapter II to uncover the fitness effect of alleles underlying the traits that contributed to the adaptive divergence between the low and high elevation populations of *D. sylvestris*. We performed genome-wide association analyses and identified a polygenic genetic architecture underlying the studied adaptive traits. We found examples of both antagonistic pleiotropy and conditional neutrality describing the fitness effects of allelic variation at these loci. By dissecting separate fitness components, we revealed that alleles underlying successful reproduction at high elevation had a negative effect on fecundity, while this relationship turned positive at low elevation. These results suggest that the trade-off in resource allocation indicated in chapter II is accompanied by congruent signals at the level of the underlying genetic variants.

Zusammenfassung

Unterschiedlicher Selektionsdruck durch kontrastierende Umweltbedingungen an entgegengesetzten Enden von Umweltgradienten kann die Evolution von Populationen vorantreiben, die an die lokalen Bedingungen angepasst sind. Höhengradienten in den Alpen werden von steilen klimatischen Gradienten begleitet, bei denen Pflanzenpopulationen innerhalb einer begrenzten geografischen Skala divergierende Selektion erfahren. Dies macht alpine Pflanzen die auf verschiedenen Höhenlagen vorkommen ideal für die Untersuchung der Evolution lokaler Anpassungen. In dieser Arbeit untersuchten wir die Entwicklung verschiedener lokal angepasster Ökotypen alpiner Nelken (*Dianthus* spp., Caryophyllaceae) als Reaktion auf die klimatisch bedingte Selektion auf verschiedenen Höhenlagen. Als Studiensystem dienten uns zwei mehrjährige Pflanzenarten, die in Mitteleuropa von der collinen bis zur alpinen Stufe verbreitet sind: *D. carthusianorum* und *D. sylvestris*. Um die evolutionären Prozesse zu untersuchen, die der Bildung von Ökotypen zugrunde liegen, verwendeten wir Populationen aus niedrigen und hohen Lagen und führten Langzeitexperimente mit reziproker Transplantation durch. Dabei untersuchten wir die Leistung über mehrere Fitnesskomponenten und Lebensstadien des mehrjährigen Lebenszyklus. Die Experimente für *D. sylvestris* wurden darüber hinaus mit phänotypischen Selektionsanalysen und einer genomweiten Assoziationsstudie auf der Grundlage einer Transplantation von rekombinanten F2-Kreuzungen, sowohl den Beitrag divergenter Merkmale zur Anpassung als auch den Fitnesseffekt der diesen Merkmalen zugrunde liegenden Allele zu untersuchen.

In **Kapitel I** testeten wir zunächst die lokale Anpassung von *D. carthusianorum* anhand von Daten über die Leistung in einzelnen Fitnesskomponenten, die über einen Zeitraum von drei Jahren im Rahmen des reziproken Transplantationsexperiments gemessen wurden. Wir fanden Hinweise auf Wechselwirkungen zwischen Genotyp und Umwelt (GxE) und auf Fitnessvorteile des lokalen Ökotyps, allerdings mit erheblichen Abweichungen in verschiedenen Stadien des Lebenszyklus. Daher führten wir ein ergänzendes Experiment zur Rekrutierung von Sämlingen durch und integrierten die Fitness im Verlauf des Experiments durch Matrix-Populationsmodelle. Die Wachstumsraten der Populationen zeigten ein starkes Signal der lokalen Anpassung in beiden Höhenlagen und lieferten weitere Belege für alternative lebensgeschichtliche Merkmale als Determinanten der Pflanzenfitness. Die niedrig gelegene Umgebung veranlasste die lokalen Pflanzen zu einem schnelleren Lebenszyklus, der durch hohe Investitionen in die frühe Reproduktion gekennzeichnet war. Im Gegensatz dazu wurde die Fitness der einheimischen Pflanzen im hochgelegenen Ökotyp hauptsächlich durch das Überleben bestimmt. Die Pflanzen aus der höheren Lage vermehrten sich auch in der fremden Umgebung stärker, was sie dazu veranlasste ihre physiologische Grenze der Ressourcenallokation für die Fortpflanzung zu überschreiten und einen Preis in Form eines geringeren Überlebens nach der Fortpflanzung zu zahlen. **Kapitel I** zeigt, wie die Selektion an den Extremen eines Höhengradienten die Bildung von Ökotypen bei einer mehrjährigen Pflanze vorantreibt, und verdeutlicht den Einfluss von

Kompromissen und phänotypischer Plastizität der lebensgeschichtlichen Merkmale als Determinanten der Populationsleistung unter verschiedenen Umweltbedingungen.

In **Kapitel II** untersuchten wir, wie die Selektion auf verschiedene Fitnesskomponenten des mehrjährigen Lebenszyklus die Bildung von Ökotypen bei *D. sylvestris* vorangetrieben hat und untersuchten den Beitrag divergenter Merkmale zu diesem Prozess. Populationen von *D. sylvestris* hielten sich während des letzten glazialen Maximums in hochgelegenen Refugien auf und haben anschließend Lebensräume in niedrigeren Lagen besiedelt. Wir kombinierten phänotypische und Fitnessdaten, die in einem fünfjährigen Experiment mit reziproker Transplantation erhoben wurden, mit phänotypischen Selektionsanalysen an F2-Kreuzungen, um die Beiträge adaptiver Merkmale zu den Reaktionen auf die unterschiedlichen Umweltbedingungen und die damit verbundenen Selektionsregime zu entschlüsseln. Unsere Ergebnisse zeigten eine starke genetische Grundlage für Pflanzengröße, Pflanzenhöhe und Blütezeit, die mit der Anpassung an die Höhenlage zusammenhängen. Die hochgelegene Umgebung begünstigte eine konservative Lebensstrategie, die durch eine lange Lebensspanne und begrenzte Investitionen in die Fortpflanzung gekennzeichnet ist. Die Selektion in höherer Lage begünstigte frühes Blühen, um den Abschluss des Reproduktionszyklus in der kurzen alpinen Sommersaison zu gewährleisten. Im Gegensatz dazu begünstigte die wärmere Umgebung in niedrigeren Höhenlagen eine Lebensstrategie, die durch hohe Investitionen in die frühe Reproduktion auf Kosten eines kürzeren Lebenszyklus gekennzeichnet ist, so dass die Pflanzen grösser werden und maximale Fruchtbarkeit erreichten. Unsere Ergebnisse zeigen, dass die Besiedlung der wärmeren Lebensräume in niedrigen Lagen durch eine Verschiebung sowohl der phänotypischen als auch der lebensgeschichtlichen Merkmale erfolgte, die mit der Ressourcenallokation in einer energiereichen Umgebung mit einer längeren Fortpflanzungssaison verbunden sind.

In **Kapitel III** haben wir die Ergebnisse aus Kapitel II genutzt, um die Fitnesseffekte der Allele aufzudecken, die den Merkmalen zugrunde liegen, die zur adaptiven Divergenz zwischen den Populationen von *D. sylvestris* in niedrigen und hohen Lagen beigetragen haben. Wir führten genomweite Assoziationsanalysen durch und identifizierten eine polygene genetische Architektur, die den untersuchten adaptiven Merkmalen zugrunde liegt. Wir fanden Beispiele sowohl für antagonistische Pleiotropie als auch für bedingte Neutralität, die die Fitnesseffekte der allelischen Variation an diesen Loci beschreiben. Durch die Zerlegung einzelner Fitnesskomponenten konnten wir feststellen, dass Allele die für eine erfolgreiche Reproduktion in höherer Lage verantwortlich sind, einen negativen Effekt auf die Fruchtbarkeit haben, während diese Beziehung in tieferer Lage positiv ist. Diese Ergebnisse deuten darauf hin, dass der in Kapitel II aufgezeigte Kompromiss bei der Ressourcenallokation von kongruenten Signalen auf der Ebene der zugrunde liegenden genetischen Varianten begleitet wird.

Introduction

The immense variation in the natural world has fascinated humanity since the antiquities (Krebs, 2004). The early theories on the origin of variation within and between species were plagued by mythological thinking, although a grain of evolutionary thought can be traced back to the ancient Greeks (Kocandrle, 2010). However, it was not until the discovery of natural selection by Darwin and Wallace more than 2000 years later that scientific theories on the origin of species and phenotypic variation began to take form (Wallace, 1858; Darwin, 1859). During the first half of the 20th century, seminal insights by early population geneticists such as Fischer, Haldane, and Wright linked the inheritance of quantitative traits to Mendelian genetics (Bowler, 2003). This effectively consolidated Mendelian genetics and the theory of natural selection, which allowed for the development of formal, testable hypotheses of the evolution of different life forms (Bowler, 2003). Since then, the field of evolutionary biology has matured and a plethora of studies performed during the last century have revealed insights into how natural selection shapes phenotypic variation and adaptation to contrasting environmental conditions. Consequently, we today have a foundational understanding of how evolutionary forces interact to shape the natural world. Phenotypes are an expression of the underlying genetic variation and the response to selection, hence, is ultimately a genetic phenomenon (Bomblies & Peichel, 2022; Wadgymar *et al.,* 2022). Indeed, divergent selection imposed by abiotic factors such as temperature and precipitation can drive the evolution of ecotypes adapted to their local conditions. Consequently, populations inhabiting alternate ends of steep ecological gradients varying in key climatic factors, provide an excellent opportunity to investigate adaptive divergence in response to alternate selection regimes. In this thesis, we aim to dissect how populations of two perennial carnations inhabiting opposite ends of an elevational gradient have adapted to their local environmental conditions by combining complementary lines of ecological evidence from a long term reciprocal transplant experiment.

Local adaptation

Studies of local adaptation have a long history in ecology and evolution. By conducting reciprocal transplant experiments of plant populations from an elevational gradient, Clausen *et al.,* (1940) produced some of the earliest evidence that populations can adapt to their local habitats. Their seminal work inspired the scientific community and an abundance of studies have since emerged, firmly establishing reciprocal transplant experiments as a sound ecological approach to test local adaptation (Johnson *et al.*, 2021; Wadgymar *et al.*, 2022). The essence of these experiments is that by transplanting individuals of populations from different habitats, one can draw inferences on their reciprocal performance in the alternate habitats. The outcome of these experiments is typically diagnosed using two criteria; the local vs. foreign criterion which states that populations should have higher performance in their "local" native habitat compared to "foreign" non-native populations, and perform better in their home habitat than when growing in the away habitat, i.e., the home vs. away criterion (Kawecki & Ebert, 2004). The local vs. foreign criterion is generally

regarded as the stronger indicator of local adaptation since it is not affected by possible quality differences between habitats.

Local adaptation is today known to be common across organisms and has been the topic of several meta-analyses and reviews in the 21st century (Wadgymar *et al.,* 2022). A meta-analysis by Leimu and Fischer (2008) covering local adaptation in plants showed that 71% of the studied systems fulfilled the local vs. foreign criterion. Hereford (2009) investigated performance trade-offs in both plants and animals and found extensive evidence of local adaptation accompanied by reduced performance in away habitats and Fraser *et al.,* (2011) and Sanford & Kelly (2011) found strong evidence of local adaptation in salmonoid fishes and across a wide array of marine invertebrates. These examples collectively show that local adaptation can be considered an essential property of evolutionary biology and fundamental to our understanding of nature.

Fitness and life-history evolution

Organismal performance is measured as fitness, which can be defined as the contribution of extant individuals to the gene pool of the next generation (Haldane, 1937; Orr, 2009; Grafen, 2020). Fitness is therefore a compound of the individual components of survival and reproduction and is usually very hard to quantify in nature. Indeed, it is commonly approximated through proxies that are known or assumed to be correlated with fitness (Acerenza, 2016; Younginger *et al.*, 2017). In plants, commonly used fitness proxies include seed count, survival, biomass and the ability to flower, this being a prerequisite condition for reproduction. Seed count, as an estimate of life time reproductive success, is generally considered a particularly strong fitness proxy, as it directly relates to the individual contribution to the next generation (Younginger *et al.,* 2017). A limitation, however, is that paternal reproductive success is indirectly measured through female reproductive success, which is primarily due to practical limitations. Moreover, the majority of studies investigating local adaptation in plants are done using annual or biannual systems, where life time reproductive success can be quantified within a single growing season (Gimenez-Benavides *et al.*, 2006; Ågren & Schemske, 2012). However, the majority of plant species are perennial, and the inference of local adaptation is complicated by the fact that different fitness proxies can provide different adaptive signals under contrasting environmental conditions (Kim & Donohue, 2013; Liancourt *et al.*, 2013; Ferris & Willis, 2018). Further, resources in nature are limited and investment in one fitness component may lead to reduced performance in others. Such trade-offs are pervasive across species and an essential part of the compound nature of fitness (Stearns, 1989; Obeso, 2002; Hamann *et al.*, 2021).

The shift from vegetative to reproductive growth and subsequent investment in offspring is a costly transition. Indeed, reproduction typically involves the re-allocation of resources from self-maintenance to reproductive structures. This involves investment in traits aimed

at ensuring successful fertilization, such as a large floral display and tall stalks, and direct investment in gametes (i.e., ovules and pollen) and resource storage in seeds to ensure viable offspring (Obeso, 2002; Shefferson *et al.*, 2003; Bogdanowicz *et al.*, 2011; Hamann *et al.*, 2021). This cost of reproduction is a well-known trade-off that can lead to reduced future reproduction, growth or survival. As this implies, specific life history traits, age- or size-specific reproduction bear direct effects on fitness and trade-offs, which can underlie the evolution of life history strategies optimized under the local environmental conditions (Stearns, 1989; Stearns, 1992; Laiolo & Obeso, 2017; Friedman, 2020). Depending on both abiotic and biotic factors of the local environment, alternative life history strategies may be favored. In environments with abundant resources, high competition and high extrinsic juvenile mortality, selection could favor short life-cycles and high levels of reproduction (Stearns, 1992; Forbis & Doak, 2004). Contrarily, a reduction in annual reproduction and an increased investment in self-maintenance could be favored in harsher environments, characterized by limiting factors such as a short growing season, unpredictable stresses, and low resource availability. Along environmental gradients, life-histories can thus be arranged along a continuum, expected to shift from faster life cycles with high reproductive investment in environments characterized by high resource availability to slower life cycles characterised by enhanced offspring quality vs. quantity, e.g., seed size vs. seed number (Paul-Victor & Turnbull, 2009; Suárez-Vidal *et al.*, 2017; Pers-Kamczyc *et al.*, 2022). The deleterious effects of environmental stochasticity in harsher environments could favor bet-hedging strategies, i.e., a reduction in variance in fitness at the expense of reduced mean fitness (Childs *et al.*, 2010; Vico *et al.*, 2016; Laiolo & Obeso, 2017). Due to these factors, locally adapted ecotypes across a heterogenous environment may evolve alternate life history strategies (Stearns, 1992; Goebl *et al.*, 2022) where the allocation of resources into one life stage may have effects on subsequent stages across the individual's life cycle. Hence, a corollary of divergent life history strategies is that alternate fitness components may become relevant for cumulative fitness across the life cycle of perennial plants. From a methodological point of view, this greatly complicates the inference of local adaptation, which requires long term studies and the use of integrated fitness estimates that account for temporal variation in the contribution of the separate fitness components.

Integrative fitness estimates and life-history analyses

Multiple methods have been developed to attain integrative fitness estimates (see e.g., Hargreaves *et al.*, 2014 for a meta analysis that utilizes different estimates across a range of species). These range from methods such as using the product of different performance fitness proxies (Konečná *et al.,* 2022), to inferences based on the number of years of successful reproduction (Favre *et al.,* 2016). More comprehensive methods rely on models for life-history analyses that unite all fitness components in a single analytical framework. One such methodology, which we employ in this thesis, is the aster modeling framework. Developed by Charles Geyer and Ruth Shaw in the mid 2000s (Geyer *et al.*, 2007), it has recently been increasingly applied in studies of ecology and evolution of wild populations

(e.g., Wadgymar *et al.*, 2017; Benning & Moeller, 2019; Wos *et al.*, 2022). Aster models are regression models where data on the separate fitness components of survival and reproduction, measured over multiple years, are modelled in a hierarchal graph structure. This structure consists of layers which, in turn, consist of separate nodes representing performance at subsequent time points. A typical three-layered structure is with the first layer representing survival, the second reproduction (e.g., flowering probability in plants) and the third reproductive output (e.g., seed count in plants). In this way, performance at the subsequent layers and nodes depends on performance at previous stages and the reproductive layer hence incorporates performance in the other fitness components. These models thus represent a useful approach of attaining an integrative individual fitness estimate at the level of individual members in a population.

Another approach for life-history analyses are demographic models which have been used for a long time by ecologists (Crone *et al.*, 2011). A particularly well developed method is the matrix population model which was developed in the mid 1940s and built on the importance of the life-cycle as a fundamental unit for the description of organisms. The overarching idea is that estimating performance in vital rates, i.e., age- or size-specific survival and reproduction, and subsequently implementing these estimates in a transition matrix provides a way to translate life-history data into quantitative estimates (Caswell, 2001). This modelling framework allows researchers to attain several useful parameters, such as the population growth rate, which can be used as an integrative fitness estimate (e.g., Caswell, 2001; Campbell & Waser, 2007; Samis & Eckert, 2009), elasticities, and stable age- or stage- distributions. Elasticities are the proportional sensitives of population growth rate to small perturbations in individual vital rates, and hence indicate which vital rates have the highest influence on population growth rate. The stable distributions provide estimates of the expected proportions of the population in each age- or stage-class at equilibrium. Additionally, by performing life table response experiments, matrix population models can decompose the effect of an experimental treatment into contributions of specific vital rates to the observed variation in population growth rate (Caswell, 1989). Matrix population models have been frequently used in conservation biology and there are today thousands of matrices for a large range of species across several kingdoms published in online databases (https://compadre-db.org/Data/Comadre) (Crone *et al.*, 2010). Yet, they are underutilized in adaptation studies (but see e.g., Peterson *et al.*, 2016; Goebl *et al.*, 2022), likely owing to the substantial data required to support the analyses. These ideally include data on multiple fitness components covering subsequent life stages, as well as an estimate of recruitment (i.e. seedling establishment), a life stage that is of critical importance but is rarely assessed in studies of adaptation (Kitajima & Fenner, 2000; Kim & Donohue, 2011).

Natural selection

Adaptive traits and natural selection

The evolution of local adaptation is driven by divergent selection acting on traits with distinct fitness effects across habitats. To unravel how adaptive divergence proceeds it is, therefore, crucial to identify the adaptive traits and dissect how they impact fitness under different environmental conditions (Conner & Hartl, 2004). There is a large literature providing evidence of the effect of selection on individual traits across varying trait complexities (Kingsolver *et al.*, 2001; Siepielski *et al.*, 2009; Kingsolver & Diamond, 2011). The effect of the total selection acting on a trait is a composite of direct selection acting on a focal trait and indirect selection acting through correlations with other traits. This complicates the dissection of the adaptive significance of individual traits as a relationship between a trait and fitness might be governed by indirect selection acting on a correlated trait. This complication can partly be statistically circumvented by regressing fitness on multiple traits simultaneously, i.e., multivariable regression (also called multivariate regression in much of the literature on phenotypic selection), which allows to account for phenotypic variation in correlated traits and thus facilitates the differentiation between direct selection and indirect selection (Lande & Arnold, 1983). Yet, in case traits are strongly associated with one another, even using multivariable regression is not always sufficient to disentangle direct from indirect selection (Ferris & Willis, 2018; Jordan, 1991; Hall & Willis, 2006). Another complicating factor is that directional and stabilizing selection can deplete genetic variation in phenotypic traits, and locally adapted populations are therefore expected to exhibit a low degree of phenotypic variation in adaptive traits. While these key aspects limit the utility of wild populations for phenotypic selection analyses, they can both be alleviated by using experimental recombinant crosses of populations originating from contrasting habitats. In hybrid crosses, trait associations shaped by natural selection simultaneously acting on multiple traits in the parental populations, are broken up by recombination. Hence, the phenotypes are disentangled from their population specific genetic background, allowing greater precision when investigating selection acting on individual phenotypic traits. Furthermore, experimental crossing can generate greater variation in the traits under investigation compared to the wild populations (Lexer *et al.*, 2003; Ferris & Willis, 2018).

Selective agents

Adaptive evolution is driven by selective agents, i.e., environmental factors that cause differences in fitness among organisms expressing different phenotypes (Wadgymar *et al.,* 2022). Identifying the selective agents that drive the evolution of local adaptation requires substantially more work than merely testing for local adaptation, as it necessitates careful manipulative field experiments (Wadgymar *et al.,*2022). Therefore, there is a relative lack of studies exploring this topic and consensus on the relative importance of specific environmental factors for the evolution of local adaptation across species is as of yet inconclusive. However, it is recognized that both abiotic and biotic selective agents can

impose the divergent selection pressure that drives the evolution of local adaptation (Hargreaves *et al.,* 2020; Wadgymar *et al.,* 2022; Briscoe Runquist *et al.*, 2020). On the abiotic side, particularly variations in temperature and precipitation impose strong selection pressure across study systems with prime examples from the literature including well studied model systems such as *Arabidopsis* (Leinonen *et al.*, 2009; Ågren & Schemske, 2012; Ellis *et al.*, 2021), *Mimulus* (Hall & Willis, 2006; Popovic & Lowry, 2020) and *Boechera* (Rushworth *et al.,* 2022) (MacTavish & Anderson, 2022). Indeed, physiological responses in key traits closely related to fitness, such as biomass accumulation, share a direct allometric relationship with ambient temperature (Weiner, 2004; Poorter *et al.*, 2011). Further, aspects such as nutrient availability, and light conditions are well known factors affecting the expression of adaptive traits (Cho *et al.*, 2017). For biotic agents, interactions with herbivores, pollinators or competitors can have a stark impact on fitness (Gomez *et al.*, 2009; Garrido *et al.*, 2012; Fukano *et al.*, 2020). Yet, there is limited inference on their direct contribution to the evolution of local adaptation (Hargreaves *et al.*, 2020; Wadgymar *et al.,* 2022).

Genetic architecture of adaptive traits and the fitness effect of the allelic variation

Local adaptation is determined by genotype by environment interactions, and specifically by the differential fitness effects of alleles underlying adaptive traits under different selection regimes (Orr, 2009; Bomblies & Peichel, 2022). A rich literature covering the genetic architecture underlying adaptive traits has developed, linked to the advent of NGS data, and today, we know that most adaptive traits are quantitative, resulting from a highly polygenic genetic architecture (see e.g., Bamba *et al.*, 2019 and Bomblies & Peichel, 2022, and references therein). Moreover, extensive pleiotropy is known to be involved in the genetic architecture of many complex traits (Visscher *et al.*, 2017). However, studies providing empirical estimates of the fitness effect of the allelic variation at adaptive loci are still limited to a few tractable study systems (e.g., threespined sticklebacks (*Gasterosteus aculeatus*), Barret *et al.*, 2008; *Mimulus guttatus*, Hall *et al.*, 2010; Boechera stricta, Leinonen *et al.*, 2013; and *Arabidopsis*, Oakley *et al.*, 2014). These studies provide empirical evidence of the two forms of fitness impact of the allelic variation predicted by theory, i.e., antagonistic pleiotropy, where alternative alleles at a specific locus are favored in different environments, or conditional neutrality, where an allele confers a fitness advantage in one environment but has a neutral effect in other environments (Savolainen *et al.*, 2013; Wadgymar *et al.*, 2022). Investigating which of these processes act on the genetic variation found at loci underlying adaptive traits is key to understanding the evolution of local adaptation. For diverging populations exhibiting a high degree of gene flow, antagonistic pleiotropy can contribute to local adaptation since it maintains polymorphism at an adaptive locus (Hall et al., 2010; Savolainen 2013; Wadgymar *et al.,* 2022). In case an allele displays conditional neutrality, it could spread across populations, thus leading to the evolution of a non-locally adapted generalist. On the other hand, conditional neutrality can underlie local adaptation if gene flow is weak and/or adaptation is controlled by multiple

loci, with alternative conditionally neutral effects between environments (Verhoeven *et al.*, 2004; Anderson *et al.*, 2013). Antagonistic pleiotropy and conditional neutrality are mutually exclusive at the level of a single locus, yet under adaptive divergence underlined by a polygenic genetic architecture, both are expected to simultaneously act across the genome (Anderson *et al.*, 2013; Hämälä & Savolainen, 2019; Wadgymar *et al.*, 2022).

Inference of the fitness effect of specific alleles is complicated by the fact that alleles do not act in isolation but their effects depend on the genetic background in which they occur (Chandler *et al.*, 2013). Hence, investigating the fitness effects of alleles, as for the phenotypic selection analyses, is ideally performed using recombinant crosses derived from locally adapted populations. By using the recombinants in transplant experiments, one can provide compelling evidence of the adaptive role of specific loci (Hall *et al.*, 2010; Ågren *et al.*, 2013; Anderson *et al.*, 2013; Wright *et al.*, 2021). Yet, few studies have been conducted thus far, likely because it requires large field experiments performed over multiple years (Barrett & Hoekstra, 2011; Wadgymar *et al.*, 2017).

Methods to study the genetic architecture of adaptive traits

Broadly, methods for investigating the genetics of adaptation can be divided into two approaches, reverse and forward genetics (Bomblies & Peichel, 2022). While reverse genetics starts with a gene or genomic region identified as being under selection using "genome-scans" (e.g., Fst scans of populations across environments), forward genetics instead starts with identifying the loci underlying a phenotype and subsequently assess divergence of allele frequencies at such loci across environments (Tiffin & Ross-Ibarra, 2014; Hoban *et al.*, 2016). In this thesis, the starting point is phenotypic variation in presumably adaptive traits, and we hence follow the latter approach. The prime approaches under this framework are quantitative-trait-locus (QTL) analyses and genome-wide association studies (GWAS) (Tibbs Cortes *et al.*, 2021). QTL analyses are performed using linkage mapping populations, with a known pedigree, derived from individuals differing in a trait of interest. They are based on the premise that genetic markers linked to the QTL will co-segregate with the phenotype more often than expected. Because QTL analyses are based on experimental populations that have undergone very few rounds of recombination, they typically identify large linkage blocks. A prime benefit of this, that made QTL studies possible 14 years before the first GWAS (GWAS, Paterson *et al.*, 1988; QTL, Ozaki *et al.*, 2002), is that relatively limited genotyping is needed to support the analyses. With the advent of next generation sequencing methods, such as whole genome sequencing and reduced representation sequencing approaches, genotyping on a genome-wide scale became available for large scale use. This major technological advancement prompted the development of genome-wide association studies which enabled investigating the genetic architecture underlying quantitative adaptive traits also in non-model organisms. Indeed, major advantages of GWAS over QTL are the lack of need for controlled crosses, improved precision and ability to detect small effect loci. GWAS are based on the premise that by regressing phenotypic

variation in the trait(s) of interest on genotypes of genotyped loci, across the genome, one can identify loci (SNPs) whose allele frequencies systematically vary with the trait(s). These loci are then assumed to be a part of the genetic architecture of the trait(s) or in strong linkage with casual SNPs (e.g., Tibbs Cortes *et al.*, 2021 and references therein). While GWAS have been greatly successful across species and trait complexities (e.g., Tam *et al.*, 2019; Uffelmann *et al.*, 2021 and references therein), major complications which can lead to identifying false positives remain. Primarily these are related to spurious associations due to issues regarding multiple testing and population structure. Indeed, GWAS analyses imply performing up to millions of statistical tests, a prominent issue that has been the subject of multiple reviews, meta analyses and simulation studies (Sham & Purcell, 2014; Kaler *et al.*, 2019; Kaler & Purcell, 2019). Common methods to circumvent it have been developed, such as correcting the significance threshold by dividing it by the total number of tests, i.e., Bonferroni correction, or limiting the proportion of positive results that are expected to be false positives, i.e., FDR correction (Benjamini & Hochberg, 1995). Population structure can cause false positives due to variations in the degree of relatedness among individuals. Even if a GWAS is performed on a single population, some individuals in the population will be more closely related than others, and hence form subpopulations (Tibbs Cortes *et al.*, 2021). Since SNPs can be more common in different subpopulations, this can lead to false systemic associations with the phenotype of interest. To account for this, principal components analyses of the genetic variation in the sample or a relatedness matrix are added as covariates to the models. Once the complications of GWAS have been addressed and candidate SNPs have been identified, they can subsequently be used in downstream analyses to verify their effect (Ishigaki, 2022).

Phenotypic plasticity

Phenotypic changes can stem from the ability of a genotype to produce different phenotypes in response to environmental variation. This phenomenon, known as phenotypic plasticity, constitutes an alternative route to the genetic processes that underlies adaptation. Phenotypic plasticity is ubiquitous in nature, but its impact on the evolution of adaptation is less understood (Davidson *et al.,* 2011). The fitness consequences of the plastic response depend on whether phenotypes change in the direction of the local optimum (co-gradient) or further away from this (counter-gradient) (Ensing & Eckert 2019; Ghalambor *et al.,* 2007). Co-gradient plasticity can increase the environmental tolerance of an organism and, depending on the cost of maintaining the plastic phenotype, be favored by selection, for example when populations are exposed to variable environments with reliable cues and no single phenotype is superior in all environments (Ghalambor *et al.,* 2007). Considerable plasticity has been documented in multiple traits across a wide range of organisms but no consensus exists on the question of to what extent plasticity influences local adaptation (de Villemereuil *et al.,* 2018; Ensing & Eckert, 2019; Davidson *et al.,* 2011).

Elevational gradients as natural laboratories for plant adaptation studies

Elevational gradients constitute steep ecological gradients in abiotic environmental factors such as temperature, solar radiation and precipitation (Körner, 2003; Körner, 2007; Abbott & Brennan, 2014). Variation in these parameters can be substantial and comparable to differences otherwise observed over much larger geographic distances (Jump *et al.*, 2009). Consequently, despite the close proximity and opportunities for gene flow across populations occurring along elevation gradients, divergent selection can lead to the evolution of local adaptation mediated by both phenotypic and life-history traits. Indeed, as elevation increases, both plant biomass and stature tend to decrease, and phenological shifts in flowering time are frequently detected (Halbritter *et al.*, 2018). Further, as elevation increases, the life-history continuum tends to shift from faster life cycles with high reproductive investment towards slower life cycles characterised by longer life span and enhanced offspring quality at high elevation habitats (Laiolo & Obeso, 2017). Indeed, the relative abundance of annual compared to perennial life cycles tends to decrease as elevation increases (Körner, 2003; Arx *et al.,* 2006). Elevational gradients therefore constitute excellent natural laboratories for the study of ecotype formation as a response to different environmental conditions that occur within a short spatial range.

Study system

Dianthus carthusianorum and *Dianthus sylvestris* (Caryophyllaceae) are widespread perennial herbs that occur from the colline (~<1000 m.a.s.l.) to alpine (~>2000 m.a.s.l.) environments in the European Alps (Landolt & Urbanska, 1989; Bloch *et al.*, 2006). Populations along the elevational gradient of both species show substantial divergence in traits commonly linked to a physiological response to climate driven selection, such as plant size, plant height and flowering time and previous work in *D. carthusianorum* has identified divergent phenotypic selection acting between low and high elevation populations (Walther, 2020). Previous work on *D. sylvestris* has shown that during the last glacial maximum, its distribution range was restricted to Alpine refugia that were characterized by climatic conditions similar to present day high elevation habitats (Luqman *et al.*, 2022). The postglacial climatic shifts have since then driven a range expansion into warmer low elevation habitats, where populations experienced a suite of novel selection pressures.

Our study area is the region of Valais, a large east to west running valley in south western Switzerland that exhibits a particular topography including 4000 m mountains surrounding the main valley. Populations inhabiting alternate elevations experience dramatically different abiotic conditions and hence very different selection regimes, varying in aspects such as seasonal temperatures, precipitation regimes, and length of the growing season. The low elevation habitats are characterized by a particularly intense summer drought, whereas the climate at high elevation is typically alpine. The elevational gradients in Valais thus correspond to a particularly steep climatic gradient confined in a restricted geographic scale (Rigling *et al.*, 2012; Fior *et al*., unpublished). Populations of both our study species

inhabiting the lower and upper ends of the elevational gradients thus represent two closely related perennial systems that may have evolved different, species-specific, strategies to cope with the abiotic selection regimes mediated by the elevational gradient. Hence, this setting provides an excellent opportunity to dissect the impact of selection and the evolution of local adaptation in response to alternative abiotic environmental conditions.

Aims and approach

In this thesis, we aim to unravel both the ecological and genetic aspects of the evolution of elevational ecotypes in response to climate driven selection at opposing ends of the elevational gradient. we aim to investigate adaptive signals across the perennial life cycle of the alpine carnations by combining analyses on multiple different individual fitness components and integrative fitness estimates. Further, we aim to dissect elevational divergence in life-history traits, investigate the impact of phenotypic plasticity on the adaptation process and identify the contribution of adaptive traits. Through a GWAS we intend to identify loci underlying these traits in *D. sylvestris* and assess the fitness effect of the allelic variation contributing to the adaptive response in the alternate elevational environments. To approach these goals, we used six populations of each of our two study species that were collected in low and high elevation habitats. In 2015, we germinated the seedlings in a greenhouse and used them set up a reciprocal transplant experiment with two replicate low and high elevation sites, representative of the lower and upper ends of the elevational distribution of our study species. We further implemented germination experiments in the same transplant sites to achieve empirical data on the transition from seed to seedling. Finally, we produced recombinant F2 crosses of *D. sylvestris* and transplanted them to one low and one high elevation transplant site. We collected data on phenotypic traits and fitness over multiple years and genotyped the F2 crosses using RAD-seq.

Chapter I – Adaptation to elevation in a perennial plant is mediated by alternative life history traits and fitness trade-offs

In chapter I, we aimed to test for local adaptation to elevation, and the contribution of life history traits, trade-offs and phenotypic plasticity to the adaptive response of *D. carthusianorum*. We collected data on performance in individual fitness proxies across three-years of the reciprocal transplant experiment and used both analyses of individual fitness proxies and integrative fitness estimates of population growth rate.

Chapter II – Trait evolution linked to climatic shifts contributes to adaptation in an alpine carnation

In chapter II, we aimed to dissect the mechanisms underlying the evolution of distinct ecotypes of *D. sylvestris* following population expansion into novel habitats by estimating The impact of the environment on population fitness and identifying how adaptive traits contributed to the evolution of local adaptation. We combined tests for local adaption using

integrative fitness estimates obtained from population growth rate models based on fitness data collected over five years with complementary analyses of phenotypic selection performed on recombinant crosses growing in the same transplant sites as the wild populations.

Chapter III - The fitness effects of allelic variation underlying adaptive traits in an alpine plant mediate divergence between elevational ecotypes

In chapter III, we aimed to uncover the fitness effects of the allelic variation underlying the adaptive traits that drove the evolution of the distinct ecotypes of *D. sylvestris* as identified in chapter II. we combined uni- and multi-variate GWA approaches and tested the compound fitness effect of the polygenic genetic architecture underlying elevational adaptation. By combining the use of both an integrative fitness estimate with analyses on individual fitness proxies, we could make inferences of the compound allelic effect on separate fitness components linked to differential resource allocation.

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Adaptation to elevation in a perennial plant is mediated by alternative life history traits and fitness trade-offs

Unpublished manuscript co-authored by:
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Author contributions

SF and AW designed and set up the reciprocal transplant experiment. AP designed and set up the establishment experiment. AP an UW collected the field data. AP performed all analyses. AP wrote the manuscript, which all authors revised.

Abstract

Natural selection mediated by contrasting environmental conditions can drive local adaptation and lead to the evolution of divergent ecotypes. Plant species growing along elevational gradients provide prime examples of ecotype formation. Dissecting the adaptation process is particularly challenging in perennial systems where natural selection acts across multiple stages of the life cycle that are linked by trade-offs between subsequent fitness components. Here, we test for local adaptation and dissect the impact of selection on subsequent stages of the life cycle in populations of an alpine carnation (*Dianthus carthusianorum)* originating from opposite ends of the species' elevational range in the European Alps. By integrating fitness estimated over three years in a reciprocal transplant experiment through matrix population models and analyses of individual fitness components, we assess the contribution of different life stages to adaptation, and identify life history traits that are adaptive under the climatic regimes at contrasting elevations. We found strong genotype-by-environment interactions for fitness. Selection on survival significantly affected the foreign ecotype in both environments, and adaptation to low elevation was driven by high investment in fecundity of young individuals, in contrast to a conservative strategy maximizing self-maintenance at high elevation. The alternative life history traits favored between elevational environments are subject to strong phenotypic plasticity in the foreign ecotype in the direction of selection. Such co-gradient environmental response could facilitate the persistence of local populations in a warming climate. Our study reveals the key role of divergent life history traits as determinants of elevational ecotypes in a perennial plant, and highlights the importance of multi-year experiments to achieve a comprehensive understanding of fitness components driving population responses to environmental selection.

Keywords

Natural selection, fitness trade-off, life history traits, matrix population models, phenotypic plasticity , reciprocal transplant experiment, stable age distribution

Introduction

The process of adaptation is driven by spatially divergent natural selection acting on populations occurring across heterogeneous environments (Savolainen *et al.*, 2013). Local adaptation relies on individuals carrying traits that maximize performance under local conditions, resulting in ecotype formation and phenotypic divergence (Kawecki & Ebert, 2004; Leimu & Fischer, 2008; Hereford, 2009). Adaptation in plants is well studied owing to their amenability to reciprocal transplant experiments, where the performance of alternative ecotypes and the contribution of phenotypic traits to adaptation can be tested. Plant performance is commonly approximated by fitness proxies such as survival, flowering or reproductive success (Gimenez-Benavides *et al.*, 2007; Ågren & Schemske, 2012). In annual species, the proportional contribution of a genotype to the next generation can be represented by its progeny, as this captures life-time reproductive success following the combined effects of selection on life history traits expressed in one growing season. In perennial species, on the other hand, plant performance depends on selection acting at subsequent steps of a life cycle that spans over multiple years, during which plants go through different developmental stages, each providing a complementary component of life-time fitness (Gonzalo-Turpin & Hazard, 2009; Kim & Donohue, 2011). This convolutes the impact of selection, and ecotypes may evolve different life history traits that optimize trade-offs across their life cycle (Stearns, 1992). Dissecting the impact of selection acting through alternative fitness components across the life cycle is key to understand of the evolution of locally adapted ecotypes.

Life history traits of perennial plants, such as age- or size-specific reproduction and longevity, characterize the investment of individuals in different fitness components, that ultimately determine their life-time fitness (Stearns, 1992; Laiolo & Obeso, 2017). The expression of these life history traits depends on the individual's resource status, with selection favoring combinations that maximize life-time performance. Life history theory predicts interactions between investment in separate fitness components. These may be positive in resource-rich environments, where maximization of one fitness component is reflected in others. In many natural environments, however, resources may be limited and their allocation is associated with fitness trade-offs. A classic example is investment into reproduction that is often costly in terms of reduced future survival and growth (Obeso, 2002; Sletvold & Ågren, 2015; De Gasperin *et al.*, 2019; Hamann *et al.*, 2021). The cost of reproduction trade-off can vary across the life span of perennial plants and can be exacerbated in low energy input environments (Stearns, 1992; Acerenza, 2016). Contrasting selection pressure on different life history traits can lead to the evolution of divergent strategies, which can be optimized and pertinent for population persistence (Stearns, 1992; Körner, 2003; Childs *et al.*, 2010). Further, biomass has a pervasive influence on performance in individual fitness components across the life-cycle, which highlights the relevance of resource allocation to growth and reproduction for life-time fitness (Laiolo & Obeso, 2017; Younginger *et al.*, 2017). Characterizing the adaptive role of life history traits is

complicated by the need for field trials that capture selection at subsequent stages of the life cycle, which should be modelled in an integrative framework to assess their specific contributions to fitness.

Matrix population models (MPMs) offer a suitable analytical framework to obtain estimates of population growth rates by integrating temporally varying performance and trade-offs across multiple fitness proxies (Caswell, 2001). When applied to multi-year reciprocal transplant experiments, MPMs provide an integrative estimate of population fitness suitable to compare performance of alternative ecotypes, with life-table response experiments (LTRE) indicating vital rates with the strongest contribution to adaptation in each environment (Caswell, 1989). The effects of specific vital rates on population growth are returned as elasticities, and age classes that compose each ecotype are estimated as stable age distributions (Caswell, 2001). Together, these estimators can provide complementary descriptors of life history traits characterizing the responses of ecotypes to different environments. Even though MPMs are a powerful tool for life history analyses, they remain underutilized in adaptation studies (e.g., Peterson *et al.*, 2016; Goebl *et al.,* 2022) because they require comprehensive datasets that ideally include estimates of seedling establishment, which is of critical importance for plant fitness but is rarely assessed in reciprocal transplant experiments (Kitajima & Fenner, 2000; Kim & Donohue, 2011).

Understanding the contributions of life history traits to plant performance is particularly important for systems that are predicted to face substantial environmental change, such as alpine species (Anderson & Song, 2020). Plant populations occurring along elevational gradients typically experience contrasting environmental conditions, and commonly show evidence for local adaptation (Halbritter *et al.*, 2018). Ongoing warming is exacerbated in mountain ranges and altered selection through both abiotic and biotic factors may threaten the persistence of alpine plant species (Nomoto & Alexander, 2021). Life history traits often vary along elevational gradients which suggests that alternative strategies may be advantageous under different environmental conditions (Laiolo & Obeso, 2017). While this variation may have a genetic basis, it may also result from the ability of genotypes to produce different phenotypes in different environments, i.e., phenotypic plasticity (Price *et al.*, 2003; von Arx *et al.*, 2006; Ghalambor *et al.*, 2007; Acasuso-Rivero *et al.*, 2019). Considerable plasticity has been documented in life history traits across a wide range of organisms (Davidson *et al.*, 2011; Palacio-Lopez *et al.*, 2015; de Villemereuil *et al.*, 2018; De Gasperin *et al.*, 2019; Ensing & Eckert, 2019), and while there is no consensus on to what extent it influences adaptation, plasticity can provide an immediate response to changing conditions and support transient population persistence (Nicotra *et al.*, 2010).

Here we use data from a reciprocal transplant experiment that was established to test for adaptation of high and low elevation ecotypes in the alpine carnation *Dianthus carthusianorum* to the contrasting environmental conditions at their elevations of origin and

to assess the contributions of life history traits, trade-offs and phenotypic plasticity to adaptation. Using populations from the central European Alps growing in replicated low- and high-elevation transplant sites, we tested for ecotype by environment interactions driven by selection on individual fitness proxies at subsequent stages of the life cycle, and combine this evidence with an experimental test of seedling establishment to generate MPMs. Using this experimental framework, we ask: 1) Has environmental variation driven the evolution of elevational ecotypes, and which stages of the life cycle impact adaptation? 2) How do life history traits affect plant performance under contrasting environments, and do they imply fitness trade-offs that contribute to the adaptation process? 3) How does environmental variation alter the expression of life history traits, and is such response in line with strategies expressed by the adapted ecotypes? We hypothesize that climatic variation has driven adaptation along elevational gradients mediated by life history traits as a result of differential trade-offs involved in plant performance. We further hypothesize that phenotypic plasticity contributes to population response expressed across elevation, thus influencing the ability to respond to rapid climatic change.

Materials and methods

Study system

Dianthus carthusianorum L. (Caryophyllaceae) is native to Europe where it occurs from Spain in the west, Belgium in the north, to Ukraine in the east, and Italy, Greece and Turkey in the south (GBIF 2022). The species is widespread and grows on dry and nutrient poor grasslands and rocky slopes and at elevations from sea level up to the alpine zone above 2’600 m asl in the Alps (GBIF 2022). *D. carthusianorum* is a short-lived herbaceous perennial with a woody taproot and a basal rosette of grass-like leaves. Upon flowering, it produces one to several mostly unramified generative stalks, each with a single terminal inflorescence consisting of up to fifteen pink to purple flowers (Garcke, 1972). The main pollinators are diurnal butterflies (Bloch *et al.*, 2006). *D. carthusianorum* is gynodioecious, self-compatible and primarily outcrossing, but geitonogamous self-pollination occurs at a low rate in hermaphrodites (Walther *et al.,* 2022).

Our study was performed in the in the Upper Rhône Valley (Valais, Switzerland) in the Western Alps (Figure 1). The Upper Rhône Valley is a major east-west oriented alpine valley with a continental climate (Braun-Blanquet, 1961). Erosion during Pleistocene glaciations generated a high relief landscape with peaks over 4000 m (Sternai *et al*., 2013), resulting in pronounced climatic gradients between dry and warm conditions at low elevation, and cool and wet conditions at higher elevation. In September 2015, we established two common gardens each at low and high elevation (~900 and 2100 m, respectively). We established reciprocal transplant experiments of elevational ecotypes represented by three low (i.e., < 900 m) and three high (i.e., > 2000 m) elevation populations sampled at opposite ends of the elevational gradient in neighboring valleys and representative of the distribution of *D.*

carthusianorum in the colline and lower alpine belts. Each site was equipped with a weather station (DS3 IP66, SensorScope, Lausanne, Switzerland). Over the course of the experiment (2015-2018), our weather stations measured a mean annual temperature gradient ranging from 9.9 to 14.2 °C and a mean annual precipitation gradient ranging from 5.0 to 6.3 kg m^2 during the growing seasons in our common gardens (see below), thus evidencing contrasting climatic conditions. The comparison with parameters inferred from CHELSA database (Karger *et al.,* 2017) indicates that the transplant sites effectively capture the climate experienced by the populations at their original sites (Figure 1).

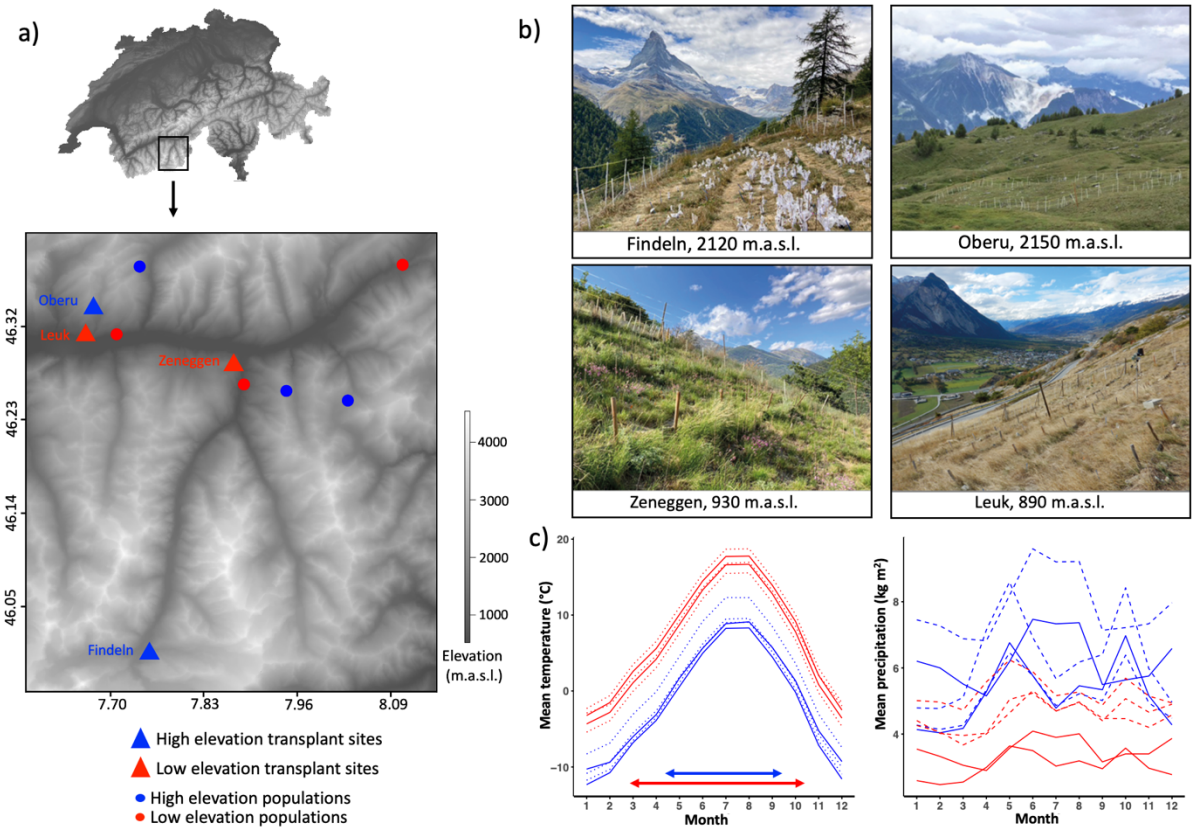

Figure 1. Map of Switzerland and the study area in the Upper Rhône Valley and climatic conditions at the transplant sites and sites from which *D. carthusianorum* populations originate. a) Map of Switzerland. The inlet shows the locations of the sampled *D. carthusianorum* populations and the transplant sites. Red and blue colors indicate low and high elevation locations, respectively. Circles and triangles indicate the locations of the wild sampled populations and the transplant sites at low and high elevations, respectively. b) Photos of the low and high elevation transplant sites. c) Mean monthly temperature and precipitation at our low (red) and high (blue) elevation transplant sites (solid lines) and the sites where wild populations were originally collected (dotted lines). Estimates are the monthly averages calculated over the timespan 1981-2004 using the Chelsa high-resolution dataset (Karger *et al.,* 2017). The red and blue arrow indicate the length of the growing season in the low and high elevation transplant sites, respectively.

Transplant design

Prior to the experiment (2012 and 2014), we collected seeds from 20-39 naturally occurring plants (i.e., families) in each of six focal populations. In summer 2015, we germinated the seeds and grew seedlings over a period of three months in a greenhouse at ETH research station Lindau-Eschikon (Switzerland) in peat moss based soil (Klasmann Deilmann Gmbh) under a 12-hour day/night cycle, with temperatures set to 20 and 18°C during the day and night, respectively, and a relative humidity of 50-60%. In fall, we transplanted ~500 seedlings into each site, representative of the sampled families (i.e., 127-135 maternal families per site; Table S1, Table S2) in eight randomized blocks separated by approximately 1 m. Each block contained 72 individuals that were separated by 25 cm. As the winter season following establishment of the transplant experiment was exceptionally warm and dry, we watered seedlings twice a week until mid-January to help them survive. Individuals that died before the first winter were attributed to transplant shock and excluded from the analyses. The sites were fenced to exclude large herbivores commonly present in the study area, such as deer, cattle, and marmots. Throughout the experiment, we trimmed the surrounding vegetation to avoid focal plants from being overgrown.

Assessment of vital rates

We monitored vital rates over three growing seasons (2016-2018), encompassing the vegetative and three reproductive transitions. At the low sites, we defined the start and end of each growing season as the first and last date of 6-day windows with mean daily temperatures above 10º C. Because the weather stations had to be removed during winter at the high sites to prevent damage from snow, we defined the start of the growing season based on the observed start of vegetative growth, which approximately corresponded to three weeks after snowmelt. The growing seasons ranged from March to November at the low elevation sites, with flowering occurring typically in June, and from late May to October at the high elevation sites, with flowering primarily in July (Figure 1). We recorded survival at the start and end of each growing season and visited each site twice a week in 2016 and once a week in 2017 and 2018 to record individuals that flowered. We bagged individual inflorescences in organza bags for seed collection after flowers had wilted. In the laboratory we later extracted seeds from capsules by carefully separating the chaff and undeveloped seeds and quantified seed output as the average number of seeds from two independent runs of an elmor C3 High Sensitive Seed Counter (Elmor Ltd, Schwyz, Switzerland). We estimated the size of the basal rosette at the start and end of each growing season from high resolution pictures of each plant (Nikon D810; 7360×4912 pixels) including a reference standard, and computing the mean of orthogonal diameters measured in image J v.2.0 (Schindelin *et al.,* 2012). This estimate of plant size constitutes a suitable proxy for biomass as evidenced by its correlation with plant dry weight (Figure S1). Data for plant size were not collected at the end of the second year.

Seedling establishment

We assessed the successful establishment of seeds of all six focal populations growing in each transplant site for implementation in matrix population models. From the seed harvested in 2017 at each site, we selected ~200 seeds for each of the populations, represented by fruiting plants belonging to up to five families originally collected in the wild populations. At the high elevation sites seeds from one low and two high elevation populations only could be included in the experiment due to low seed production by individuals from the missing populations. In fall 2018, we sowed ~10 seeds per family in the same sites in which they were produced in peat moss soil (Klasmann Deilmann Gmbh) contained in biodegradable pots of 10x8 cm. We placed 96-165 pots per site in the ground after random assignment to positions within the blocks of the experiment left empty by dead individuals. Plants that germinated successfully and were alive at the end of the 2019 growing period were considered established.

Statistical analyses

Separate vital rates

We assessed elevational adaptation from separate vital rates including flowering probability, seed output, plant size and survival. For each vital rate, we tested ecotype by environment interactions between elevational ecotypes and the transplant environments, as well as for differential performance of the alternative ecotypes growing within each environment (local vs foreign criterion) and for the effect of the environment on each ecotype (home vs away criterion for local adaptation, Kawecki & Ebert, 2004). We fitted separate generalized linear mixed effect models for flowering probability, seed output and survival at subsequent stages of the life cycle. In each model, we implemented the vital rate as response variable and ecotype, transplant environment and their interaction as predictors, using a binomial error distribution for the categorical variables (i.e., flowering probability and survival), and a zero-inflated Poisson error distribution for the count variable (i.e., seed output). We analysed plant size using the same model structure as above but implemented in linear mixed effect models with a Gaussian error distribution and we log transformed the data to improve distribution of the residuals. In all analyses, we implemented maternal families nested within population and block nested within site as random effects. We implemented mixed effect models using the R package lme4 v1.1 (Bates *et al.*, 2015), assessed significance levels of interactions using likelihood ratio tests with the package lmerTest v.3.3 (Kuznetsova *et al*., 2017). We obtained estimates, significance levels and confidence intervals of the contrasts between groups with the emmeans package (Lenth, 2017). We further analysed survival throughout the life cycle using mixed effect cox models, which perform proportional hazards regression of time to event data with implementation of random effects. We fitted the Cox models with ecotype and transplant environment and their interaction as predictors and used the same random effect structure as used in the mixed effect models in the package survival 2.44 (Therneau & Grambsch, 2000). All analyses were performed in R v.3.3.2 (R Core Development Team 2016).

Integrated fitness estimate

We formulated age-structured MPMs to obtain an integrated estimate of fitness expressed as population growth rate λ for each ecotype growing in the low and high elevation environment. To obtain survival and reproductive vital rates, we divided the life-cycle of our plants into winter and summer stages (S_i, Figure S2, (Caswell, 2001)). We calculated the survival vital rates as the proportion of individuals transitioning to the next life stage. As an integrated value of the reproductive vital rates (R_i) we used the product of flowering probability, seed count and recruitment. From the seedling experiment, we estimated the establishment rates per ecotype in each transplant environment from generalized linear mixed effect models by implementing the proportion of seedlings established per pot as the response variable and ecotype as the predictors, with binomial error distribution and the same random effects structure as described above for the single vital rate analyses. We recovered similar estimates of establishment rates of the two ecotypes conditional on the growing environment, with overall higher values in the low (i.e., low ecotype: 0.17 ± 0.091; high ecotype: 0.14 ± 0.073; Table S3) compared to the high environment (i.e., low ecotype: 0.02 ± 0.028; high ecotype: 0.06 ± 0.058; Table S3). To assess whether differences in population growth rates between the ecotypes growing in each environment are statistically significant, we performed 20 000 bootstrap replicates of each matrix stratified by population, and constructed bias corrected 95% confidence intervals around estimates. Analyses were performed using packages popbio v. 2.2.4 (Stubben & Milligan, 2007) and boot v. 1.3 (Canty & Ripley, 2021).

To decompose the effects of specific vital rates on population growth rate, we used life-table response experiments (LTRE) (Caswell, 1989). LTREs tests the difference in λ between a matrix of interest and a reference matrix, breaking down the difference into contributions from individual vital rates. We compared the matrix of the foreign ecotype against the matrix of the native ecotype in each environment to quantitatively assess the respective contributions of vital rates to adaptation. LTRE contributions were inferred from 20 000 bootstrap replicates of each matrix stratified by population. Analyses were conducted in the packages popbio v. 2.2.4 and boot v. 1.3.

Fitness trade-offs

To examine the trade-offs involved in plant performance during early life stages, we analyzed one vital rate as a function of another at a preceding stage of the life cycle, the elevational ecotypes, the growing environment, and their interactions. We modelled i) the flowering probability in the summer as a function of plant size at the start of the growing season of the same year, ii) the probability of survival at the end of the summer and winter as a function of plant size at the start of the season and iii) the probability of survival at the end of the summer as a function of flowering probability during the summer. We used generalized linear models to test for trade-offs and differential performance between ecotypes. For each model we implemented a vital rate as response variable in generalized

linear models with binomial error distributions to test for three-way interactions between a vital rate of a preceding life stage, the ecotype and the environment, as well two-way interactions between the vital rate and the ecotype within the separate environments. We determined the significance of the three-way interactions by ANOVA and computed pairwise contrasts between groups as well as confidence intervals of the trends using the R package emmeans (Lenth, 2017).

Life history traits and plasticity

To elucidate the influence of individual life history traits on population growth of the elevational ecotypes growing in the low and high elevation environments, we extracted the elasticities of specific vital rates and stable age distributions from our matrix population models. The elasticities estimate the proportional sensitivities of a change in a specific vital rate to population growth rate, thus their distribution through the life cycle describes the relative importance of vital rates to the population growth rate of each ecotype in a given environment. As an outcome of the trade-offs between survival and reproduction, stable age distributions describe the proportion of the population in each age class at equilibrium. Hence, elasticities and stable age distributions can be considered the expression of individual life history traits under specific growing environments (Caswell, 2001). We examined the difference in estimates of elasticities and stable age distributions for each ecotype when growing in their home or away environment to assess the extent of plasticity expressed by the elevational ecotypes. We compared estimates at different stages of the life cycle by computing 95% bias corrected confidence intervals from 20 0000 bootstrap replicates of the matrix models. To further describe the plastic response of the elasticity values, we correlated the trait shift (i.e, difference in trait value when growing in a home vs. a foreign environment, Ensing & Eckert, 2019) and trait distance (i.e., difference in trait value between the two ecotypes growing in their home environments, Ensing & Eckert, 2019) of the three populations representing each elevational genotype. While trends obtained in these analyses are indicative of co- or counter-gradient patterns of plasticity, we refrained from assessing significance of the correlations because elasticities are non-independent parameters derived from the population growth models.

Results

Evidence for adaptation from separate vital rates

We found significant ecotype x environment interactions for flowering probability in each year, pointing to a consistent differential effect of the environment on the performance of both ecotypes (Figure 2a-c, Table S4). The local ecotype had a significantly higher flowering probability at low elevation the second and third year, whereas at high elevation, the local ecotype performed better only in the third year. The low elevation ecotype also performed significantly better at home than away (high elevation) across the three years.

Reaction norms for seed output recapitulated patterns observed for flowering probability. Significant ecotype x environment interactions in each year (Figure 2d-f, Table S4) were associated with a significant fitness advantage of the local compared to the foreign ecotype at both high and low elevations, although these were largely nonsignificant.

Reaction norms for plant size varied substantially across the different stages of the plant life cycle (Figure 2g-l, Table S5). Significant ecotype x environment interactions were found at the start of each vegetation period (S_1-S_3), and over the second winter (W_2). During the entire duration of the experiment, the low elevation ecotype was consistently larger than the high elevation counterpart, even when growing at high elevation, though not always significantly so.

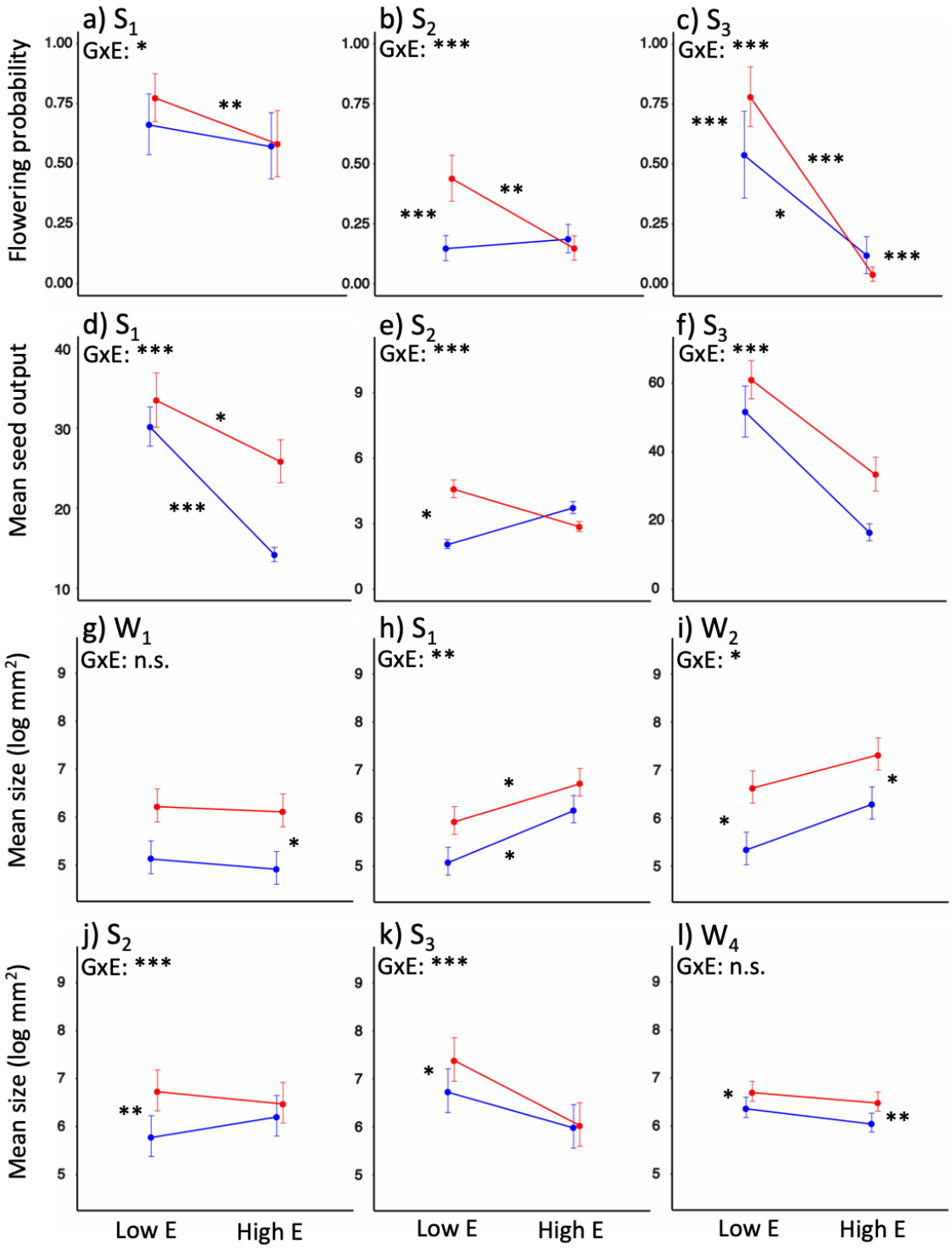

Figure 2. Reaction norms of reproduction and size of elevational ecotypes growing in the transplant experiment at subsequent stages of the life cycle. a-f), Reproduction represented by flowering probability and seed output. g-l), plant size. Symbols indicate mean estimate values inferred from mixed effect models and bars indicate 95% confidence intervals. Mean values are connected by reaction norms depicting the effect of the environment on each elevational ecotype. Red and blue colors denote the low and high elevation ecotype. S_i and W_i denote summers and winters, respectively. Significance of GxE interactions and contrasts consistent to the local vs. foreign and home vs. away criteria are reported (***p<0.001, **p<0.01, *p<0.05).

Cumulative survival was significantly higher in the local ecotype in both environments (Figure 3, Table S6) and the difference between ecotypes was more pronounced at the end of the experiment at low elevation. In contrast to the low elevation ecotype, which had similar cumulative survival at both elevations, the high ecotype performed significantly better in its high elevation home environment, than at low elevation (Table S6). The most pronounced differences between ecotypes in cumulative survival were observed at alternative time points in the two contrasting environments. At low elevation, survival rates of the local low elevation ecotype were significantly higher than of the foreign high ecotype during the first two summer seasons (S_1 and S_2), with a particularly pronounced difference during the first (Figure 3, Table S7). In contrast, at high elevation, survival rates of the local high elevation ecotype were significantly higher than those of the foreign low ecotype during the first and second winter (W_1 and W_2; Figure 3, Table S7).

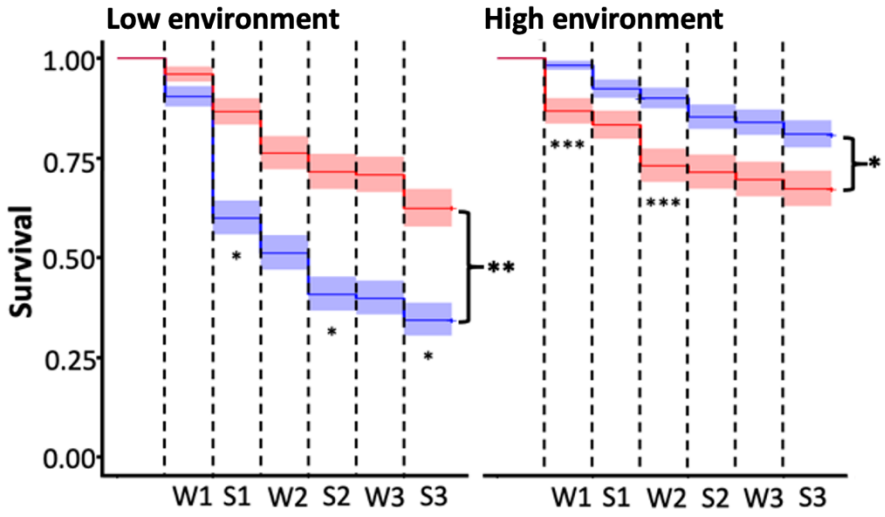

Figure 3. Survival of the elevational ecotypes growing in the transplant experiment. Survival curves represent survival rates of the low (red) and high elevation (blue) ecotypes throughout the experiment at subsequent stages of the life cycle in the low and high elevation environment. Asterisks at the side of each plot indicate significant cumulative differences between the survival curves of the two ecotypes as inferred from cox proportional hazard models and the shaded areas represent 95% confidence intervals. Asterisks underneath the curves indicate significant differences of survival probability between ecotypes at specific life stages, as assessed by generalized linear mixed effect models (***p<0.001, **p<0.01, *p<0.05).

Evidence for adaptation from the integrated fitness estimates

Comparisons of population growth rates inferred from MPMs revealed a significant advantage of the local ecotype in both, the low and high elevation transplant environments, with λ of the local ecotype surpassing that of the foreign ecotype by 35% and 57%, respectively (Figure 4, Table S8). LTRE analyses showed a strong negative impact of the vital rates of the foreign ecotype, predominantly from the reproduction component over survival (Figure 4, Table S8). Notably, strong differences in contribution to population growth occurred primarily in the first year in the low elevation environment, whereas more moderate differences were spread across multiple years in the high elevation environment, with the strongest difference inferred for the third reproductive period (R_3).

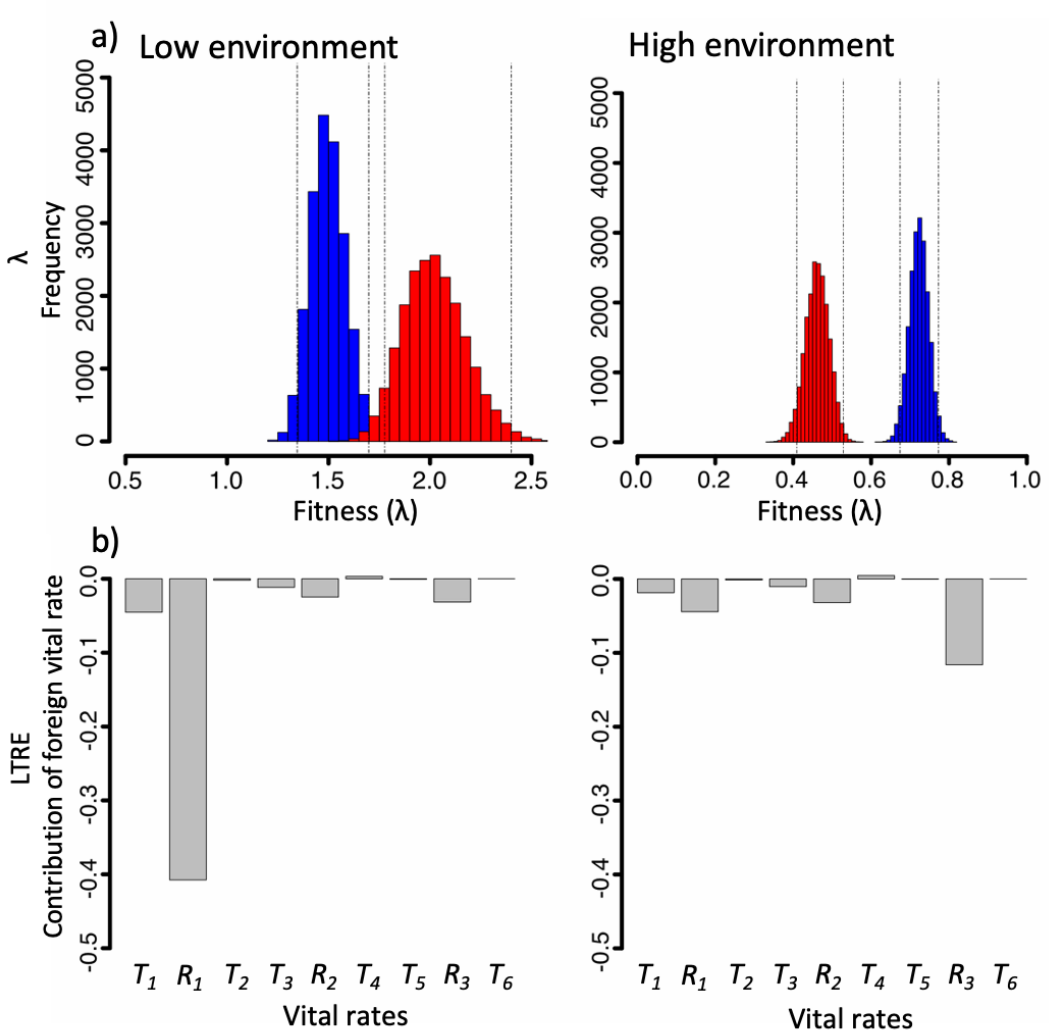

Figure 4. Integrative estimates of ecotype performance expressed as population growth rates (λ) growing in the transplant experiment. a) Histograms representing population growth rate distributions based on 20 000 bootstrap replicates for the low (red) and high (blue) ecotype growing in the in the low and high elevation transplant environments. Dotted lines indicate bias corrected 95% confidence intervals. b) LTRE showing the relative contribution of vital rates expressed as survival and reproduction (T_i and R_i, respectively, with $_i$ indicating the specific vital rates) of the foreign ecotype to population growth at subsequent stages of the life cycle.

Fitness trade-offs

i) Size and flowering probability. The flowering probability varied as function of a significant three-way interaction among plant size, ecotype and environment in the first (Figure 5a, b; Table S9) and second summer (Table S9). Flowering probability consistently increased with increasing plant size, though with a stronger effect in the high compared to the low elevation ecotype in both environments.

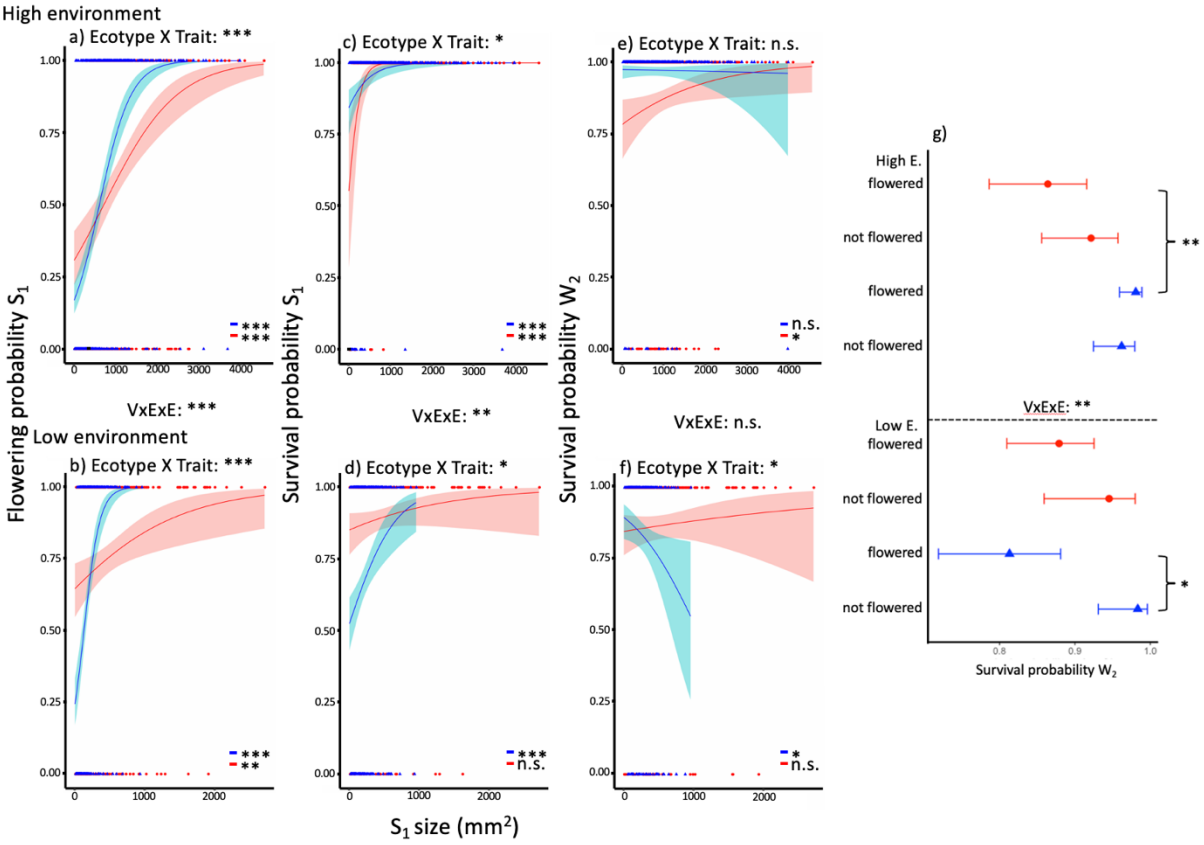

Figure 5. Trade-offs at the early stages of the life-cycle of the elevational ecotypes growing in the reciprocal transplant experiment. a-f) Flowering probability during the first summer (S_1), survival probability during the first summer and the second winter (W_2) as a function of plant size of both ecotypes growing in the high (a, c, e) and low (b, d, f) elevation environments. Red and blue lines indicate predicted relationship from generalized linear model regressions with 95% confidence intervals for the low and high ecotypes, respectively. Corresponding red circles and blue triangles indicate empirical values of plant size and flowering probability. Significant relationships are indicated within each panel as asterisks next to the predicted function. g) Survival probability the second winter (W_2) of flowering and non flowering individuals the first summer (S_1). Symbols indicate mean estimate values inferred from generalized linear models and bars indicate 95% confidence intervals. For each vital rate used as response variable, significance of the three-way interaction between the vital rate used as predictor, the ecotype and the transplant environment (VxGxE), as well as the two-way interaction between the vital rate use as predictor and the ecotype (GxT) within the low and high elevation environment is reported (***p<0.001, **p<0.005, *p<0.05).

ii) Size and survival. Plant size generally showed a positive effect on survival throughout the experiment, with significant effects at multiple stages of the life cycle (Table S10). These effects were particularly relevant following the first year of flowering when similar effects described the survival of the ecotypes as a function of size in the high elevation environment, while these varied in the low elevation environment, with a significant effect only on the high elevation ecotype (Figure 5c, d, Table S10). Notably, however, this was followed by an opposite trend the following winter, when larger high elevation plants showed lower survival (Figure 5f, Table S10).

iii) Flowering probability and survival. Survival varied as a function of a significant three-way interaction among flowering probability, ecotype and environment at the end of the first summer (Table S11) and the following winter (Figure 5g, Table S11). While in the former season flowering had a significant positive effect on survival of each ecotype in each environment, in the latter it negatively affected the survival of the high elevation ecotype in the low elevation environment. Moreover, low elevation plants that flowered in the high elevation environment suffered higher mortality relative to the local flowering plants (Figure 5g, Table S11).

Life history traits and plasticity

Elasticity values showed divergent patterns describing the influence of subsequent vital rates on population growth (Figure 6a, b, Table S12). Survival during the first winter (T_1) and reproduction the first summer (R_1) had the strongest influence at both high and low elevation, but the effect was most pronounced at low elevation. Notably, elasticity values of both ecotypes were similar within environments, with non-significant differences at each stage of the life cycle. Similarly, stable age distributions differed substantially between environments, but much less between ecotypes within environments. At low elevation, populations consisted primarily of young individuals, with a marked shift to adults at high elevation (Figure 6c, d, Table S13). This suggests that shifts in life history traits are primarily driven by environmental differences rather than genetic differences between ecotypes. Correlations between trait shifts and elasticity distance for both ecotypes (Figure 6e, f) support co-gradient plasticity as determinant of the response to the high and low elevation environments.

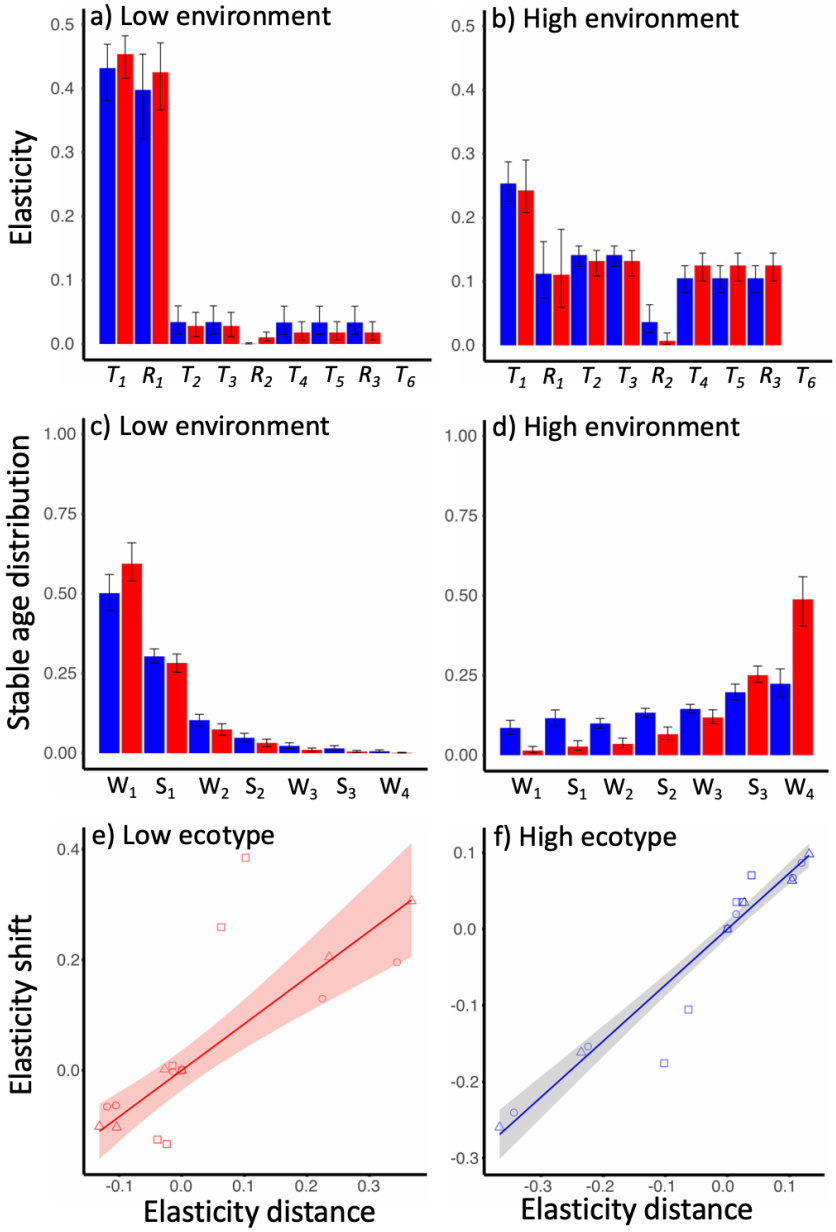

Figure 6. Environmental response of life history traits expressed by the elevational ecotypes growing in the transplant experiment. a and b) Influence of the specific vital rates (i.e., elasticities) survival and reproduction (T_i and R_i, respectively, with $_i$ indicating the specific vital rates) on population growth rates. c and d) Stable age distribution at subsequent summer and winter (S_i and W_i, respectively, with $_i$ representing specific time points) seasons. Elasticity and stable ages distribution values are the mean of 20 0000 bootstrap replicates and error bars indicate bias corrected 95% confidence intervals. In a-d, red and blue bars indicate estimates for the low and high ecotype, respectively. e and f) Plasticity of the life history traits inferred from elasticities is shown as the relationship between trait distance and trait shift for each vital rate expressed by the three focal populations (symbols) representative of each elevational ecotype growing in their respective home and away environment. The shaded area indicates 95% confidence intervals of the trendline.

Discussion

Understanding the processes by which natural selection drive adaptation through alternative fitness components over the course of an organism's life cycle is key to understand the evolution of local adaptation. By using a multi-season reciprocal transplant experiment we obtained compelling evidence of elevational adaptation in *D. carthusianorum*. The adaptive signals were characterized by extensive variation throughout the life cycle, but our recording of performance through multiple life stages allowed us to detect fitness trade-offs between reproduction and survival and provide evidence of the contribution of life history traits to plant fitness. Strikingly, both ecotypes exhibited high levels of plasticity in life history traits. Our study emphasizes the importance of considering temporal variation in performance linked to growth and development, life history traits and environmental heterogeneity to understand the response of plant populations to contrasting environmental conditions.

Reproduction is generally reported as a key determinant of plant performance (Leimu & Fischer, 2008), however, it is not consistently detected as a primary component of adaptation along elevational gradients (Halbritter *et al.*, 2018). On the contrary, survival has been shown to capture the adaptive response in many species. In this study, we found strong evidence for the contribution of both fitness components to elevational adaptation in *D. carthusianorum*. Flowering probability and seed output, our two complementary proxies of reproduction, show strikingly similar patterns that fulfill the criteria of adaptation during the second and third year. Indeed, these estimates are linked to biological processes such as bolting and allocation of resources during seed set that do not have a functional correlation (Angert & Schemske, 2007; Hautier *et al.*, 2009), and thus provide independent evidence of adaptation. Survival of the alternative ecotypes showed specific patterns in line with selective events acting on foreign individuals. The low elevation environment imposed selection during summer, when the seasonal drought that characterizes the continental climate can be a crucial stressor for many plant species (Kim *et al.*, 2013; Bastida *et al.*, 2014; Orsenigo *et al.*, 2014). Contrarily, the high-altitude environment imposed selection during winter, when self-maintenance is vulnerable as a consequence of frost damage and depletion of resources during the extended snow cover. Hence, abiotic factors linked to elevation reduce the survival of foreign ecotypes through selection acting on both survival and reproductive fitness components.

The allocation of resources to reproduction and survival is a fundamental mechanism governs adaptive life history strategies (Stearns, 1992). Our analyses of the correlations between these fitness components and plant size revealed a strong impact of growth on vital rates at subsequent stages of the life cycle. Low elevation plants were consistently larger than high elevation plants regardless of the growing elevation, and similar coefficients of reaction norms across elevation indicate a consistent environmental effect on both ecotypes. In line with overall patterns observed along elevational gradients (Körner, 2003;

Halbritter *et al.*, 2018; Midolo *et al.*, 2020), size in *D. carthusianorum* is thus better interpreted as a divergent phenotypic trait exhibiting spatially plastic variation that feedbacks on key fitness components (Körner, 2003; Laiolo & Obeso, 2017) rather than a fitness proxy *per se*. Larger plants are overall more likely to flower but this effect is influenced by both the ecotype and the environment, pointing to a genetic contribution of the divergent interactions expressed across elevation. Similarly, larger size is overall beneficial for survival the first winter, but leads to a negative impact on high elevation plants growing in the low elevation environment in the following season. This trade-off is triggered by the favorable conditions for reproduction in the low elevation environment on the high altitude plants which exceeded allocation of resources to reproduction and thus compromised their physiological ability to survive the following winter. Reciprocally, low altitude plants achieved smaller size at high elevation compared to their home environment, and flowering results in higher mortality compared to the local ecotype. Overall, our results suggest that genetic variation linked to differential trade-offs between elevational ecotypes underlies adaptation in *D. carthusianorum*.

Our estimates of population growth rates (λ) as integrated fitness estimates confirmed our inference of elevational adaptation. We note that in our experiment, absolute λ values should be interpreted with caution as populations were tested under conditions that are primarily representative of climatic conditions but only partially account for biotic components of selection (Hargreaves *et al.*, 2020), and do not regard local conditions that may differ from the population's native sites. Consistent with different trade-offs governing fitness components in the alternative ecotypes, population growth is driven primarily by early vital rates in the low elevation environment, with stable age distributions consequently skewed towards younger individuals. This is in line with expectations for plant systems inhabiting high-energy environments characterized by abundant resources and high competition. Here, early reproduction and maximized seed output are favored to counterbalance high juvenile mortality (von Arx *et al.*, 2006; Kim & Donohue, 2011; Laiolo & Obeso, 2017). In contrast, as observed in our high elevation sites, a life cycle characterized by reduced annual reproduction and allocation of resources to self-maintenance constitutes a better strategy under a short growing season and limited resources. Hence, the alternative life history strategies expressed by our two ecotypes in their native environments are in line with observed differences in life histories between species occurring along elevation gradients, where short-lived species with high reproductive investment are typical at lower elevation and long-lived species with enhanced offspring quality and bet-hedging strategies predominate at high elevation (Laiolo & Obeso, 2017).

The divergent life history traits expressed by the two ecotypes in their native low and high elevation environments, together with the strong evidence for adaptation to their elevation of origin, suggest that alternative life history strategies are adaptive under different climatic conditions. Interestingly, however, we observed considerable co-gradient plasticity for life

history traits in both ecotypes when grown in the foreign environment. The evolution of plasticity is frequently observed in alpine plants, and is presumably favored by temporal variation of environmental conditions experienced by local populations (Hassel *et al.*, 2005; Ghalambor *et al.*, 2007; Chevin *et al.*, 2013; Botero *et al.*, 2014; Frei *et al.*, 2014; Ensing & Eckert, 2019). While we observed pronounced plasticity in both ecotypes of D. carthusianorum, it was insufficient to allow foreign ecotypes to match the performance of local plants. It would be of interest to test whether the plastic variation observed in the foreign ecotype can buffer the impact of selection and facilitate temporary persistence under adverse foreign conditions. This appears particularly relevant for the high altitude plants that are able to express environmental tolerance to warmer conditions. It has been suggested that rapid shifts in selection imposed by climate change cannot be matched by an evolutionary response in perennial species (Anderson & Song, 2020). However, adaptive plasticity may potentially mitigate the negative effects on population fitness and has been hypothesized to then allow time for an evolutionary response to the novel selection pressures (Ghalambor *et al.*, 2007; Vinton *et al.*, 2022) .

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Trait evolution linked to climatic shifts contributes to adaptive divergence in an alpine carnation (*Dianthus sylvestris*)

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AP, SF and AW designed and set up the transplant experiments. AP collected the field data and performed all analyses. AP wrote the manuscript, which all authors revised.

Abstract

Populations expanding to new habitats may encounter novel selection regimes which can lead to ecotype formation. In the Alps, elevation corresponds to steep ecological gradients, along which ecotype formation has occurred in many species. The majority of alpine plant species are perennial and little is known about how selection acts across different stages of their life-cycles and how fitness trade-offs shape adaptive processes in perennials. We investigated how selection at opposite ends of elevational gradients has driven ecotype formation in *Dianthus sylvestris*, a perennial herb that expanded its ecological niche to low elevation habitats after the Last Glacial Maximum. Through a multi-year reciprocal transplant experiment including parental populations and recombinant crosses we assessed fitness under natural conditions and dissect how adaptation is mediated by different fitness components with inherent trade-offs, and pinpoint the contribution of growth and reproductive traits to this process. We show that the evolution of local adaptation proceeded by selection acting primarily through reproduction and survival at low and high elevation, respectively. At low elevation the primary contribution to adaptation was third year reproduction, concomitant with a left skewed age distribution. At high elevation the contribution to adaptation and the age distribution were more dispersed across the life cycle. We found that large, early flowering plants have a consistent fitness advantage. This was mediated by direct selection favoring large size through reproductive output at low elevation, and early flowering through the probability to produce seeds at high elevation. Our results indicate that the selection regime imposed by the warm low elevation habitat led to the evolution of an ecotype exhibiting a life-history strategy characterized by high investment in rapid growth and early reproduction. In contrast, the high elevation strategy favors high investment in self-maintenance. Our results suggest that weakening of a key fitness trade-off associated with resource allocation contributed to the evolution of distinct ecotypes in this perennial plant species.

Keywords

Natural selection, elevational adaptation, matrix population model, aster model, trade-off, life history traits, population growth rate, reciprocal transplant experiment

Introduction

Populations facing novel environments may experience selection regimes that favor traits and trait combinations that differ from those favored in the original habitats. For a large number of species in the northern hemisphere, current distributions result from demographic responses to climatic changes following the Last Glacial Maximum (Davis & Shaw, 2001; Petit *et al.*, 2003). As environments suitable to species persistence opened up beyond glacial refugial ranges upon the retreat of the glaciers, populations expanding into deglaciated areas either tracked their ecological niche or expanded their niche into warmer habitats. The response to novel selection regimes has likely driven the formation of warm-adapted ecotypes (Hargreaves *et al.*, 2014). In temperate mountain ranges, recolonization of elevational gradients exposed populations to strong clinal variation in key abiotic factors that frequently assert strong selection pressures (Körner, 2003; Keller *et al.*, 2013; Halbritter *et al.*, 2018). This has resulted in local adaptation in a large number of species, accompanied by changes in both phenotypic and life history traits (Halbritter *et al.*, 2018). Dissecting the underlying processes offers an excellent opportunity to gain insights into the evolution of contemporary ecotypes in response to climate-driven selection.

Elevational gradients form steep ecological gradients that are primarily shaped by changes in physical parameters. The shift to warmer temperatures at the onset of the present interglacial period and the retreat of the glaciers have facilitated the formation and colonization of novel, warmer habitats. Today, rapid anthropogenic climate change is predicted to bear major impacts on species' ecological niches and distribution ranges (Tito *et al.*, 2020; Pörtner *et al.*, 2022). In most mountain systems, temperature constitutes a primary determinant of the abiotic environment, with cascading effects on multiple selective agents (Körner, 2003). Typically, increased elevation corresponds to a decrease in temperature, and lower temperatures at high elevation imply a longer period of snow cover, with consequently shorter summer seasons (Körner, 2003). This can directly slow down organisms' metabolic processes and affect their physiology (Körner, 2006; Poorter *et al.*, 2011). Temperature further impacts biotic interactions, such as e.g., between plants and pollinators or hosts and their parasites, which are fundamental for species persistence (López-Goldar & Agrawal, 2021).

In plants, elevational ecotypes commonly exhibit phenotypic divergence with a strong genetic basis, and shared trends in fitness-related traits are observed across a diversity of taxa. In a meta-analysis on ecological evidence of elevation adaptation, Halbritter *et al.,* (2018) found that elevational ecotypes display pronounced divergence in flowering time and size, expressed both as plant height and biomass. Both plant height and flowering time are essential for successful reproduction in many plant species, thus bearing direct effects on individual fitness (Gervasi & Schiestl, 2017; Gaudinier & Blackman, 2019). While selection on these traits is typically mediated by interactions with other biotic agents such as pollinators (Zu & Schiestl, 2017), climate directly impacts the fine-tuning of this crucial life stage by

governing the plants' ability to achieve physiological thresholds (Amasino, 2010; Cho *et al.*, 2017; Ehrlén *et al.*, 2020). Most plants must reach a minimum size before they can reproduce (Weiner *et al.*, 2009; Younginger *et al.*, 2017), and individuals with a higher ability to accumulate biomass have more resources to invest in reproductive structures (Bonser & Aarssen, 2009; Cheplick, 2020; Proulx, 2021). Because size shares a positive allometric relationship with temperature, this physical parameter affects the evolution of reproductive traits and of life-history strategies that optimize the antagonistic resource requirements for self-maintenance and reproduction (Stearns, 1992). This is reflected in vegetation at the warmer end of elevational gradients that often consists of plants exhibiting a life-history strategy characterized by large, fast-growing plants with high investment in reproduction, whereas at the opposite end, populations typically display a more conservative strategy, characterized by slower growth and a longer lifespan (von Arx *et al.*, 2006; Hautier *et al.*, 2009; Kim & Donohue, 2011; Kim & Donohue, 2013; Laiolo & Obeso, 2017; Rosbakh & Poschlod, 2018). While the phenotypic variation in plant size, height and phenology and the consequential divergence in life-history traits along elevational gradients is well documented, the mechanisms by which these traits interact in their contribution to adaptation remain largely unknown.

Unravelling the evolution of phenotypic traits in different environments requires to assess how variation in trait values expressed by different individuals correlates with relative fitness. Heritable trait variation that is consistently associated with fitness may drive trait divergence between environments, thus resulting in phenotypic divergence between locally adapted ecotypes. Fitness itself results from the combined effects of separate components, such as survival and reproduction (Orr, 2009; Acerenza, 2016), which may act coherently in favoring optimal trait values, but also drive contrasting trajectories (Wadgymar *et al.*, 2017). In *Mimulus guttatus*, for example, selection favors larger flowers through reproduction while simultaneously favoring smaller flowers through survivorship, whose stronger effect during the entire life cycle eventually results in smaller flowers being advantageous (Mojica & Kelly, 2010). These complex trait-fitness interactions complicate the study of adaptation, and call for the identification of fitness components with a major role in ensuring population persistence. Moreover, as the relative contribution of separate fitness components may vary depending on the environment, selection may act through alternative fitness components in different ecotypes (Goebl *et al.*, 2022). Hence, understanding the role of phenotypic traits in adaptation requires experiments to dissect the process of phenotypic selection though both individual and combined analyses of multiple fitness components.

Studies of local adaptation are ideally performed using reciprocal transplant experiments where genotype by environment interactions can be tested (Johnson *et al.*, 2021). In such experiments, populations are reciprocally transplanted between habitats and local adaptation is inferred through two criteria (Kawecki & Ebert, 2004). The native populations display higher fitness in their native habitat relative to the foreign population (local vs.

foreign criterion) and they have higher fitness in their native habitat than when growing in the foreign habitat (home vs. away criterion). The first of these criteria is generally regarded as a stronger indicator of local adaptation. While ecotypes constitute natural units to test for local adaptation, they are of limited utility to dissect the role of individual traits in this process. In locally adapted populations, selection has driven the evolution of different phenotypes that maximize fitness, so that individuals express correlated trait values, with limited variation centered around optimum values (Lexer *et al.*, 2003; Ferris & Willis, 2018). Phenotypic selection analyses, however, require substantial trait variation to assess its effects on fitness. This can be achieved by exposing genotypes from experimental crosses to natural selection, where reshuffling of the genomes through recombination can generate a wider distribution of phenotypic values than those expressed in local ecotypes.

Dianthus sylvestris (Caryophyllaceae) is a widespread perennial herb that occupies elevational gradients from the colline to the alpine belt of the European Alps (Collin & Shykoff, 2003; Info Flora 2016). Previous work has shown that during the Last Glacial Maximum, *D. sylvestris* survived in south-eastern refugia characterized by narrow climatic conditions similar to those of present day high elevation habitats (Luqman *et al.*, 2022). Post-glacial warming has then facilitated recolonization of the Alpine Arch concomitant with the expansion of the species' climatic niche through adaptive evolution in the warm habitats at low elevation. Consequently, contemporary elevational ecotypes show substantial divergence in fitness-related traits consistent with the physiological response to climate-driven selection of many alpine species, in particular in plant size, plant height and flowering time. The derived condition of these traits in *D. sylvestris* offers an excellent opportunity to study the evolutionary trajectories underlying the species' response to recent climate-driven selection.

In this study, we use ecologically diverged elevational populations of *D. sylvestris* from the central Alps to dissect how selection acting through alternate fitness components has driven ecotype formation and assess the contribution of phenotypic divergence in plant size, plant height and flowering time to this process. We use a transplant experiment of wild populations from the extremes of the elevational gradient occupied by the species to find evidence of adaptation and complement our field trials with recombinant populations to perform phenotypic selection analyses and dissect the adaptive role of fitness-related traits. Specifically, we ask; 1) Does the phenotypic divergence of plant size, plant height and flowering time between populations inhabiting the high elevation and low elevation habitats have a genetic basis? 2) Has the colonization of the low elevation habitat driven the evolution of elevational adaptation? 3) If so, is this concomitant with variation in life history traits favoring alternative strategies of resource allocation to self-maintenance vs. reproduction? 4) How does the phenotypic divergence in plant size, plant height and flowering time between the elevational ecotypes contribute to the recent adaptation to a warmer environment?

Methods

Experimental set up

Reciprocal transplantation of wild populations

We sampled seeds from 13 to 41 individuals in wild populations of *D. sylvestris* at three low- (i.e., <1000 m.a.s.l.) and three high- (i.e., > 2000 m.a.s.l.) elevation sites in the central Alps (Valais, Switzerland; Figure S1, Table S1). During summer 2015, seeds were germinated in a greenhouse (Lindau-Eschikon, Switzerland) in peat moss based soil (Klasmann Deilmann Gmbh) under a 12-hour day/night cycle at 20/18°C and relative humidity of 50-60%. In fall 2015, seedlings were transferred to our transplant sites in geographic proximity to the six source populations (chapter 1: Figure 1). Climatic conditions at our two high and two low elevation sites resemble those of our six natural populations, in particular in respect to temperature and precipitation (chapter 1: Figure 1). We transplanted ~300 seedlings into each of four transplant sites, providing even representation of populations and seed families across the four sites. Seeds were arranged randomly in 25 cm grids in 6 blocks of ~72 individuals each. Blocks further included 3-22 F1 individuals that were not used in this study and were randomly placed within each site, along with 8 blocks of *Dianthus carthusianorum* that were also not used here. We fenced transplant sites to exclude mammalian herbivores and regularly trimmed the vegetation surrounding our plants to prevent our plants from being overgrown. Hence, our experiments primarily test responses to abiotic factors, although biotic components such as below-ground competition or plant-pollinator interactions are integral part of the observed natural processes. To ensure successful establishment, we watered the plants twice a week during the first winter of 2015/2016. Plants that died during this period were attributed to transplant shock and excluded from further analyses. At the start of 2016, 690 and 645 plants were alive in the low and high elevation sites, respectively, and form the basis of our analyses (Table S2).

Seedling recruitment experiment

In fall 2020, we sowed seeds produced in 2018 in each of our transplant sites to obtain an *in-situ* estimate of seedling recruitment. Whenever possible, we pooled 100 seeds per population equally representing five maternal families and sowed five seeds per family in each of 20 biodegradable pots (approximately: 10x8x6 cm, 0.4 litres) containing peat moss soil (Klasmann Deilmann Gmbh) (Table S3). Due to variation in seed production among populations and sites, in some cases the number of seeds sown per population and seed family varied (Table S3). We embedded the pots in positions within the blocks of the original experiment. Seedlings alive at the end of 2021 were considered successful recruitments.

Transplantation of recombinant populations

We generated recombinant (F2) plants derived from two F1 families that were derived from controlled crosses between one low- and one high-elevation individual (Table S4). During the flowering season 2017, we placed 15-28 F1 individuals in each of five cages at the research station Lindau-Eschikon and weekly supplemented butterflies (*Pieris brassicae*) to

act as pollinators. With this approach, three types of recombinant groups are potentially produced in each cage: descendants of each of the two F1 lines, and hybrids between these. Seeds were then germinated as reported above and ~1000 seedlings representative of the three types of crosses were transplanted into 14 new blocks each added to one low- and one high-elevation site with the same grid design as described above. In spring 2018, 621 and 555 plants survived in the low- and high-elevation site, respectively (Table S5).

Data collection

We collected data on flowering time (i.e., date of anthesis of the first flower), plant height, plant size, and fitness over five (2016 - 2020) and three (2018 - 2020) growing seasons for the wild and the F2 populations, respectively. The growing season typically spans March to November in the low sites and May to October in the high sites (for details see, chapter 1). At the start and end of each growing season, we took high resolution images (Nikon D810; 7360x4912 pixels) of all individual plants, scored survival and estimated plant size as the mean of two orthogonal diameters of the rosettes measured using image J v.2.0 (Schindelin *et al.*, 2012). During the growing seasons, we visited each site twice a week in 2016 and once a week in the following years. For the recombinant populations, no flowering occurred in 2018, and we visited sites twice a week during the 2019 and 2020 growing seasons. After wilting of the flowers, we bagged inflorescences in organza bags and harvested all stalks when the seeds had ripened. Plants typically produce stalks of similar length, and we estimated plant height as the mean length of three representative stalks. In the laboratory we extracted seeds from capsules and estimated individual seed output for the wild populations as the average number of seeds from two independent runs of an elmor C3 High Sensitive Seed Counter (elmor Ltd, Schwyz, Switzerland). For F2 individuals, we weighed all seeds produced per plant using a Mettler Toledo Ae240 Balance (precise to the nearest 0.0001 gram). For analyses requiring count data, cumulative seed weight was transformed to seed number using correlations inferred from a subset of ~80 individuals from the low- and high-elevation site (Figure S2).

Statistical analyses

In all analyses of the wild populations, we considered the low and high elevation populations as representative of the low and high elevation ecotypes, and transplant sites at the same elevation as replicates of the low and high elevation environments. All analyses were performed in R v. 3.3.2 (R Core Development Team 2016).

Phenotypic divergence between elevational ecotypes

We tested for phenotypic divergence between elevational ecotypes in flowering time, plant height and plant size, both in mean treat expression and at subsequent time points across the life cycle. We standardized the traits within each year and elevational environment to a mean of 0 and standard deviation of 1. Traits were set as response variable in linear mixed effect models (LMMs) with Gaussian error distributions and ecotype, transplant environment and their ecotype by environment interaction set as predictor variables. Trait values were log transformed when this improved distribution of the residuals (see results). To uncover genetic correlations between plant size, plant height and flowering time, we fitted the latter two as response variables and plant size, ecotype, and transplant environment and their interaction as predictor variables in LMMs. In all analyses, we nested seed families within population and block nested within site as random effects. These were excluded in a few cases when low variation caused singular fits of the models (see results).

Elevational adaptation

To test for elevational adaption of the two ecotypes, we formulated age-structured matrix population models (MPM) yielding an integrated estimate of fitness expressed as population growth rate (λ), as described in chapter 1. Briefly, we divided the life cycle into winter (W_i) and summer (S_i) stages and calculated the survival vital rates (T_i) as the proportion of individuals transitioning across stages (Figure S3) (Caswell, 2001). Reproductive vital rates (R_i) were estimated as the product of flowering probability, seed count and recruitment. Our empirical recruitment estimates recovered overall higher estimates for both ecotypes in the low (i.e., low ecotype: 0.19 ± 0.19; high ecotype 0.16 ± 0.20) compared to high environment (i.e., low ecotype: 0.06 ± 0.06; high ecotype: 0.13 ± 0.16). To assess whether differences in population growth rates of ecotypes growing in each environment are statistically significant, we performed 20 000 bootstrap replicates of each matrix stratified by population, and estimated bias corrected 95% confidence intervals around all means. These analyses were performed in the packages popbio v. 2.2.4 (Stubben & Milligan, 2007) and boot v. 1.3 (Canty & Ripley, 2021). To further investigate the contribution of individual fitness components to adaptation in the transplant environments, we decomposed the contributions of specific vital rates to variation in λ by using life-table response experiments (LTRE) (Caswell, 1989). These tested the matrix of the foreign ecotype against the matrix of the native ecotype in each environment. LTRE contributions were inferred from 20 000 bootstrap replicates of each matrix stratified by population.

Contributions of single fitness components to adaptation

We dissected the contributions of three separate fitness components to adaptation: flowering probability, survival probability and seed count. For each fitness component, we tested ecotype x environment interactions, and for differential performance of the alternative ecotypes growing within each environment (local vs. foreign criterion) and for the effect of the environment on each ecotype (home vs. away criterion; Kawecki & Ebert, 2004). We used generalized linear mixed effect models (GLMMs) implementing the fitness component as response variable and ecotype, transplant environment, and their interaction as predictor variables, using a binomial error distribution for the categorical variables (i.e., flowering probability and survival probability) and zero-inflated Poisson distributions for seed count. We further analysed survival throughout the life cycle using mixed effect Cox models, which perform proportional hazards regression of time to event data with implementation of random effects. We fitted the Cox models with ecotype, transplant environment and their interaction as predictor variables using the package survival 2.44 (Therneau & Grambsch, 2000).

Life-history variation

To elucidate whether adaptation is accompanied by life-history differences we estimated elasticities of individuals vital rates and compared stable age distributions of each ecotype growing under contrasting environments. Elasticities estimate the proportional sensitivities of a change in a specific vital rate to population growth rate and their distribution through the life cycle describes the relative influence of vital rates on the population growth rate of each ecotype in a given environment. Stable age distributions describe the proportions of individuals in in each age class. Values and confidence intervals were inferred from the same matrix population models and bootstrapping procedures as described above.

Impact of plant size on fitness

To investigate the impact of plant size on fitness, we implemented the fitness components at each life stage as a function of plant size at the previous stage, together with ecotype, transplant environment and their interactions as predictor variables in GLMMs. To gain an overall estimate of the effect of plant size across the life cycle, we standardized plant size and seed count within life stage and site to a mean of 0 and standard deviation of 1. We then used standardized plant size as predictor variable while considering each repeated measure of the same individual plant as a unique sample and included life stage as a covariate. For the modelling of the effect at subsequent life stages we used the non-standardized data. We used binomial error distribution for the modelling of survival and flowering probability and a zero-inflated Poisson distribution for seed count. For the modelling of seed count we assumed that the probability of a structural zero varies with plant size (Brooks *et al.*, 2017). For the GLMMs and the Cox models, we included seed families nested within population and block nested within site as random effects, except in a few cases when low variation caused singular fits of the models (see results). We applied

mixed effect models using the package lme4 v1.1 (Bates *et al.*, 2015) and zero-inflated models using glmmTMB v. 1.3 (Brooks *et al.*, 2017). We assessed significance levels of the interactions using likelihood ratio tests with package lmerTest v.3.3 (Kuznetsova *et al.*, 2017) and obtained estimates and significance levels of fixed effects with package emmeans v.1.5 (Lenth, 2017).

Recombinant populations

A principal component analysis (PCA) based on the ddRAD data identified population structure in the F2 plants that were grouped them into three genetic clusters (see chapter 3). In all statistical models of the F2 data described below, we accounted for variation between these clusters by including them as a covariate in the models and tested their interaction with other predictors, as well as their effect on the response variables. The genetic clusters were included in the final, most parsimonious, models if either the interaction with other predictors or the effect on the response variables were statistically significant as determined with likelihood ratio-tests (LRTs). Further, we accounted for the effect of transplant blocks as a random effect when this exerted a significant effect on the response variable. To improve model fit, for all studied traits, as well as the fitness proxies cumulative seed weight and seed count, values exceeding two standard deviations from the mean within each genetic cluster and site were excluded from the analyses (i.e., low environment cluster 1: 3, cluster 2: 7, cluster 3: 6; high environment, cluster 1: 6, cluster 2: 7, cluster 3: 2).

We aimed to compare selection estimates of plants at the same stage of the life cycle and therefore used data from the first year of flowering, i.e., 2019 and 2020, for plants growing in the low and high site, respectively. We investigated whether the phenotypic variation expressed in our F2 populations encompassed the phenotypic variation of the wild populations and if the dependence of flowering time and plant high on plant size was recapitulated in the F2 populations. We produced density plots of the trait distributions of the low and high elevation ecotypes and the F2s, growing at both elevations. For this visualization we used the mean flowering time, plant height and start of the season plant size across the experiment for the wild populations. We examined the genetic correlations between the traits by fitting plant height and flowering time as response variables and plant size, transplant site and their interaction as predictor variables in LMMs, using a Gaussian error distribution.

Cumulative fitness estimates for phenotypic selection analyses

To obtain a cumulative estimate of relative fitness for the phenotypic selection analyses, we employed the aster modelling framework, which accounts for the hierarchal structure of fitness by incorporating multiple co-dependent components. Following Geyer *et al.,* (2007), we modelled separate fitness components into hierarchal life-history stages following a graph structure (Figure S4). We used survival probability, flowering probability and seed

production as the three layers of our models. Each layer contained two nodes representing performance in the individual fitness components during 2019 and 2020, where the performance in each of the nodes depends on the predecessor node. The binary variables survival and flowering probability were modelled with a Bernoulli error distribution and the seed count data with a Poisson error distribution. We scaled flowering time to start at 1 in both sites to account for the different start of the growing season in Julian days between the sites. We globally standardized flowering time, plant height and plant size to a mean of zero and standard deviation of one. We then identified the most parsimonious model within each site using a step-wise model selection approach by testing full models including all relevant two-way interactions against reduced models using LRTs. The full models included as predictor variables flowering time, plant height, plant size, genetic cluster, as well as two-way interactions between flowering time and plant height, and between each trait and genetic cluster. We subsequently used the output of the most parsimonious models to predict the expected fitness for each individual plant in each site separately. Aster models were fitted using the package Aster v1.1-1 (Geyer *et al.*, 2007). We relativized the cumulative fitness obtained from the aster models to the global mean across sites in line with recommendation for selection analyses that can be assumed to be non-frequency dependent (De Lisle and Svensson (2017).

Phenotypic selection analyses

We estimated selection differentials, i.e., total selection acting on each trait, through cumulative fitness from the aster models by implementing it as response variable in LMMs. We modelled fitness functions using each trait separately as predictor variable, and included site and the interaction with site as covariate, using a Gaussian error distribution. Significance and effect sizes of the differentials were estimated from parametric bootstrap (n=5000) models and included either a linear or a quadratic predictor. The quadratic regression coefficients were subsequently doubled to obtain the quadratic selection differentials (Stinchcombe *et al.*, 2008).

To identify the targets of selection and dissect how selection acts through different fitness components, we estimated selection gradients using multivariable LMMs for cumulative fitness and total seed weight and GLMM for the probability to produce seeds. We relativized total seed weight to the global mean across sites. These models included all traits simultaneously, as well as the interaction between flowering time and plant height as predictor variables. For cumulative fitness and relative total seed weight we used Gaussian error distribution, and for the probability to produce seeds, binomial error distribution. We modelled the data of the two sites together and used a parametric bootstrap (n=5000) to obtain the selection coefficients within each site simultaneously. Statistical significance was determined according to 95% bias-corrected confidence intervals (i.e., non-overlapping zero) estimated on the same parametric bootstrap replicates that were used to obtain the selection coefficients.

Results

Phenotypic divergence

The elevational ecotypes displayed phenotypic divergence in plant height, flowering time and plant size throughout the life cycle, although the effect depended on the growing environment. Plants originating from low elevation produced significantly taller stalks, and flowered later than the high ecotype in both environments, albeit flowering time divergence was only statistically significant in the high environment (Figure 1a and b, Table S6, Table S7). Plants of the low elevation ecotype grew larger the high ecotype when growing in their home environment, but this difference was not expressed at high elevation (Figure 1a Table S8). The divergence in all traits was accompanied by yearly variation, particularly in the statistical significance of the contrasts, whereas general patters remained consisted across the life cycle (Figure S5, Figure S6, Table S6, Table S7, Table S8).

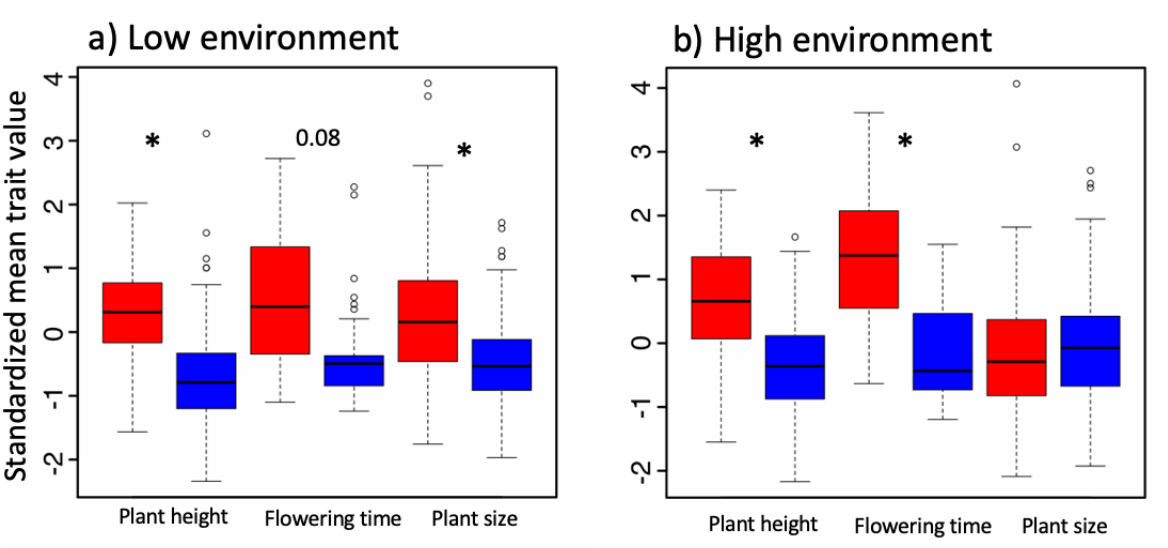

Figure 1. Phenotypic divergence in plant height, flowering time and plant size of the elevational ecotypes growing in the a) low and b) high environment. Boxes represent trait values standardized by life-stage and environment and statistical significance is inferred from linear mixed effect models. Red and blue denote the low and high elevation ecotypes, respectively. Significance of contrasts consistent to differential performance within each transplant environment are reported (**p<0.01, *p<0.05).

Genetic correlations between traits

For both ecotypes, genetic correlations underly a positive effect of plant size on plant height (Figure 2a - b, Table S9). Furthermore, larger plants of the low elevation ecotype flowered earlier in both environments (Figure 2c – d, Table S9). The traits dependence on plant size displayed strong yearly variation. Although not always statistically significant, the direction of the trends was consistent with the patterns observed in the mean values in all years except year five (Figure S7, Table S9).

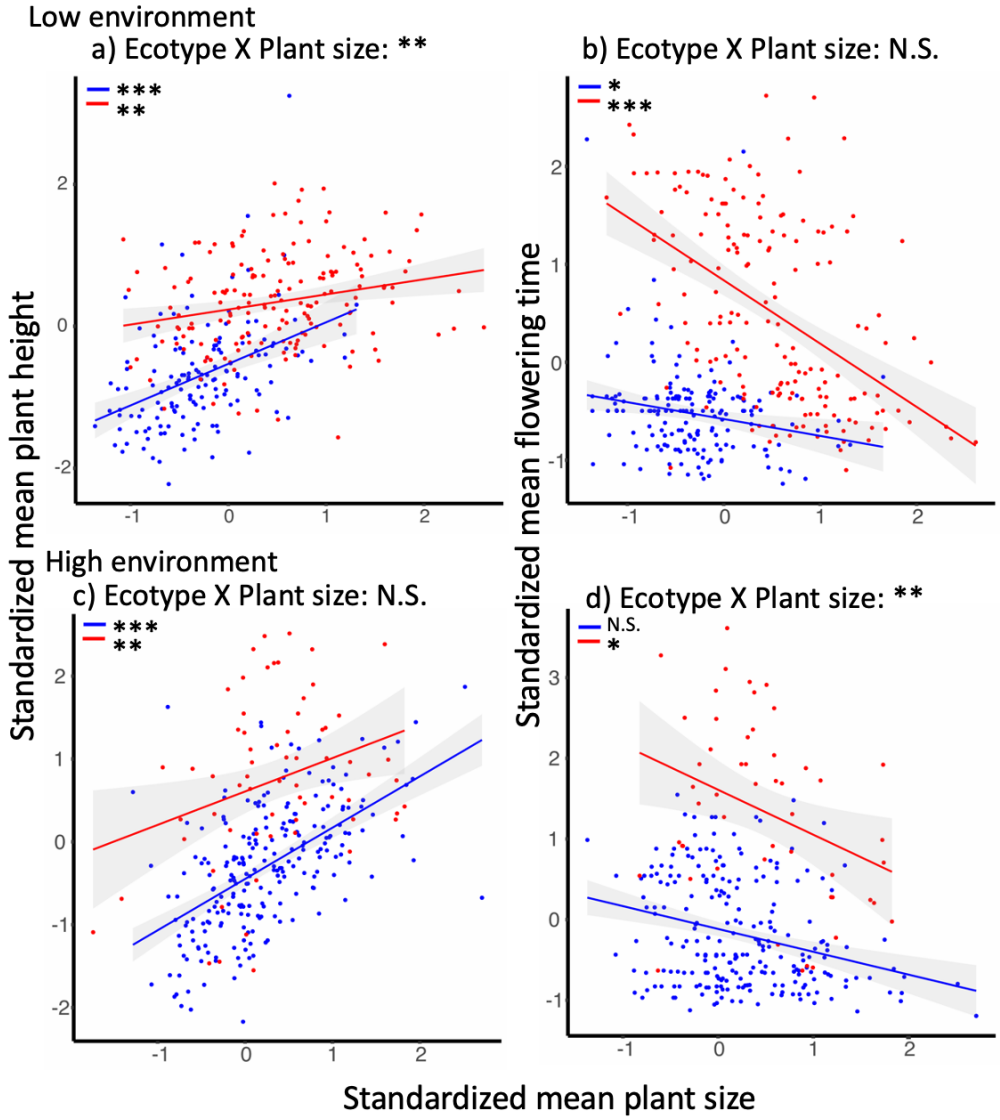

Figure 2. Relationship between plant height, flowering time and plant size of elevational ecotypes growing in the low and high environments. Left panels (a, c), relationship between plant height and plant size. Right panels (b, d), relationship between flowering time and plant size. Trait values are standardized by life-stage and environment and statistical significance is inferred from linear mixed effect models. Red and blue denote the low and high elevation ecotypes, respectively. Significance of the two-way interaction between ecotype and plant size and relationships between traits within each ecotype and transplant environment are reported. Short red and blue lines denote statistical significance of trait correlations for the low and high elevation ecotypes, respectively (***p<0.001, **p<0.01, *p<0.05).

Elevational adaptation

Population growth rates estimated from MPMs revealed a significant advantage of the local over the foreign ecotype in both environments. The local ecotype always showed positive population growth rate whereas the foreign displayed negative population growth rate (low environment: low ecotype: 1.5 (CI: 1.437, 1.575), high ecotype: 1.03 (CI: 0.968, 1.113); high environment: low ecotype: 0.815 (CI: 0.764, 0.870), high ecotype: 1.08 (CI: 1.042, 1.112)) (Figure 3a and b). The LTRE analyses revealed that the strongest negative impact of the vital rates of the foreign ecotype in low environment was reproduction the third year and fifth year whereas in the high environment, early survival and fourth and fifth year's reproduction had a stronger relative impact (Figure 4d and e, Table S10).

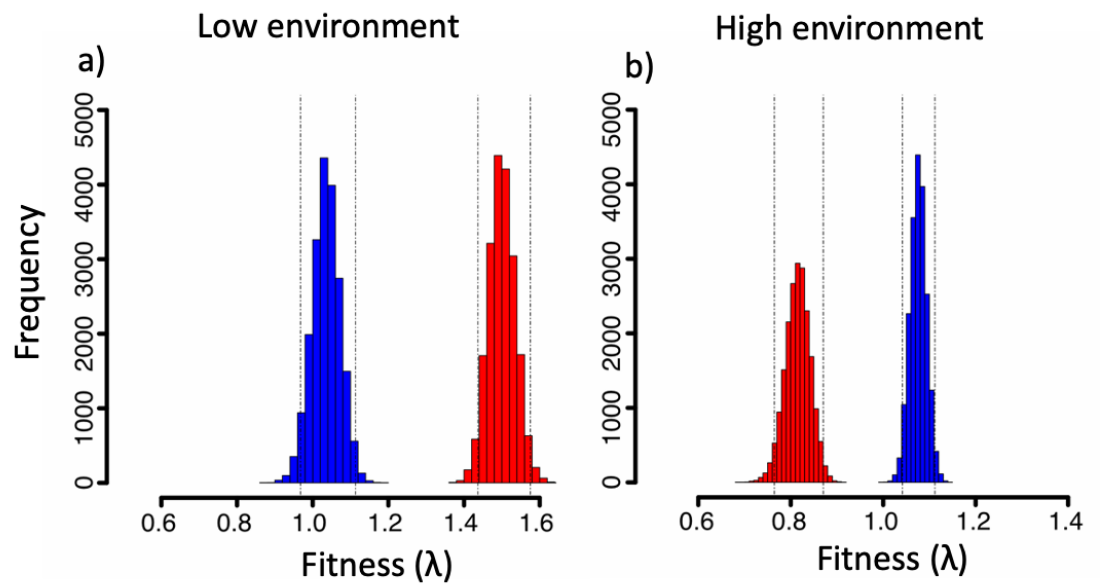

Figure 3. Integrative estimates of ecotype performance expressed as population growth rates (λ) growing in the transplant environments. a and b) Histograms representing population growth rate distributions based on 20 000 bootstrap replicates for the low (red) and high (blue) elevation ecotypes growing in the in the low and high environments. Dotted lines indicate bias corrected 95% confidence intervals.

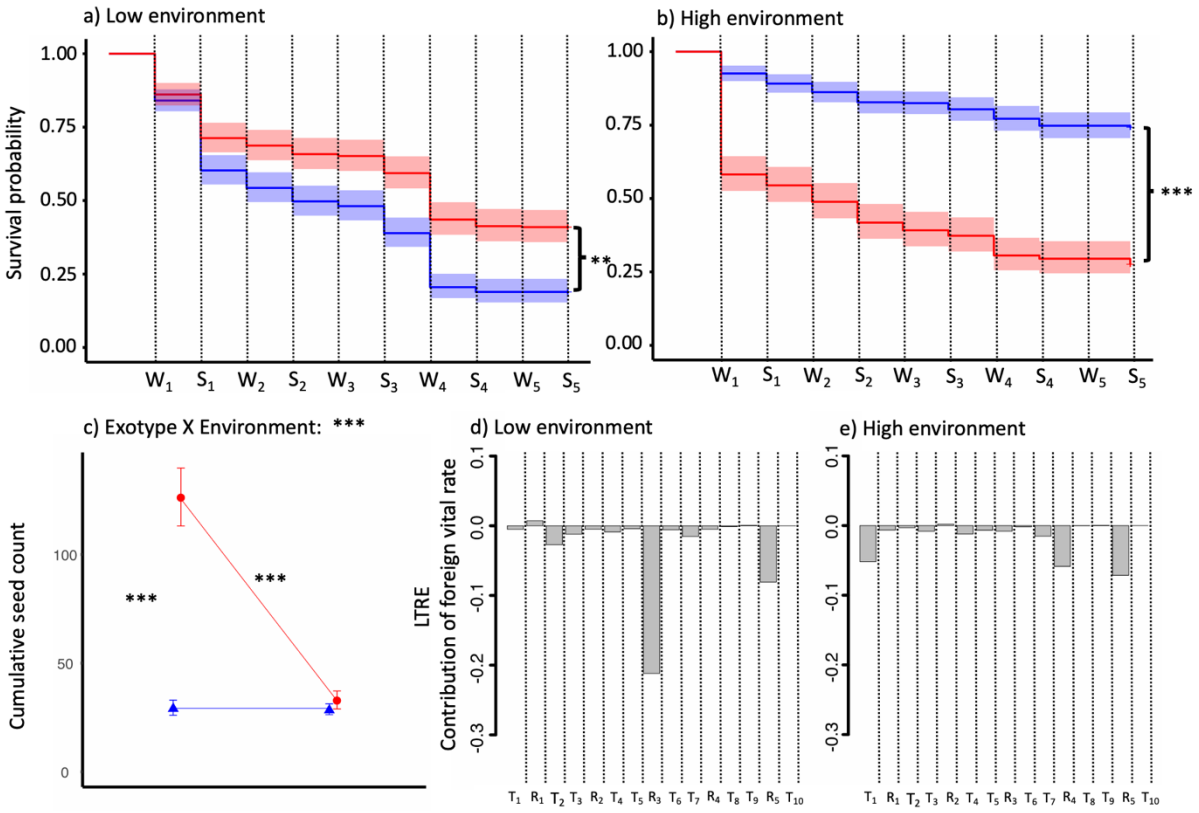

Figure 4. Contribution of individual fitness components to adaptation in the elevational environments. A and B) survival curves representing survival rates of the low (red) and high (blue) elevation ecotypes throughout the experiment at subsequent stages of the life cycle in the low a) and high b) environment. W_i and S_i denote the life stages (W_i winter survival and S_i summer survival). Asterisks at the side of each plot indicate significant cumulative differences between the survival curves of the two ecotypes as inferred from cox proportional hazard models and the shaded areas represent 95% confidence intervals. c) Performance in cumulative seed count of elevational ecotypes growing in the low and high environments. Symbols indicate mean estimate values inferred from generalized linear mixed effect models and bars indicate standard errors. Mean values are connected by reaction norms depicting the effect of the environment on each elevational ecotype. Red and blue colors denote the low and high ecotypes. Significance of ecotype by environment interactions and contrasts consistent to the local vs. foreign and home vs. away criteria are reported. d and e) LTRE showing the relative contribution of the vital rates expressed as survival (T_i) and reproduction (R_i) of the foreign ecotype to population growth rate at subsequent stages of the life cycle in the low d) and high e) environments. $_i$ Indicate specific vital rates and life stages. (***p<0.001, **p<0.01).

Analyses of individual fitness component showed that survival has a strong impact on adaptation, as significant ecotype by environment interactions result in the local ecotype performing significantly better than the foreign ecotype in both environments (Figure 4a and b, Table S11). This difference was particularly pronounced in the high elevation environment, where the proportion of surviving local ecotypes was approximately double by the end of the experiment. Separate comparisons at subsequent stages of the life cycle identified significant ecotype by environment interactions, thus supporting these findings (Figure S8, Table S12).

Comparisons of cumulative seed count revealed a significant ecotype by environment interaction and strong signal for adaptation in the low elevation environment (Figure 4c, Table S13). In contrast, the two ecotypes yielded similarly low seed count at high elevation. The low ecotype produced significantly more seeds when growing in its home environment. We further detected strong inter seasonal variation in both seed count and flowering probability, with overall greater seed production in the third and fifth year (Figure S9, Figure S10, Table S13 and Table S14).

Elasticities and stable age distributions depend on ecotype and environment

The elasticity values extracted from the MPM showed overall strong influence of survival (i.e. T_i) throughout the life cycle for both ecotypes in both environments (Figure 5a and b, Table S15). Elasticities of the reproductive vital rates were low for most years, with larger effects only in the third and fifth year in the low and high elevation environment, respectively. Estimates across ecotypes growing in the same environment were similar, with no statistically significant difference for any vital rate.

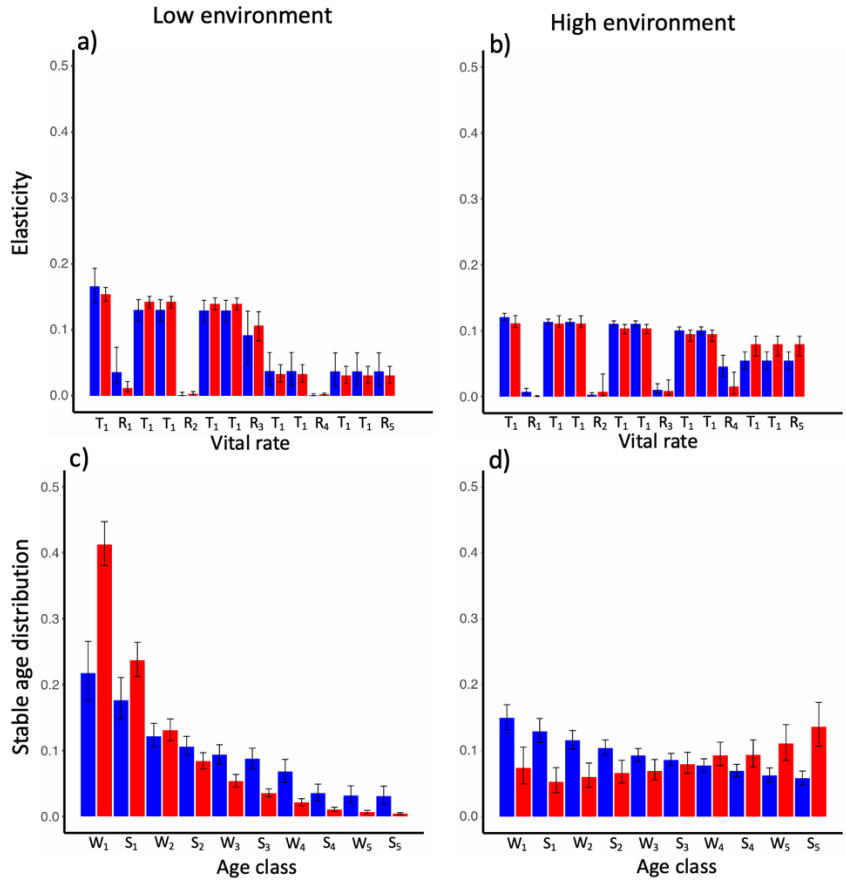

Figure 5. Environmental response of life-history traits expressed by the elevational ecotypes growing in the transplant experiment. a and b) Influence of the specific vital rates (i.e., elasticities) of survival (T_i) and reproduction (R_i) on population growth rates. c and d) Stable age distribution at subsequent summer (Si) and winter (W_i) seasons. Elasticity and stable age distribution values are the mean of 20 0000 bootstrap replicates and error bars indicate bias corrected 95% confidence intervals. $_i$ Indicate specific vital rates and life stages and red and blue bars indicate estimates for the low and high ecotype, respectively.

The stable age distributions revealed that in the low elevation environment, populations of both ecotypes, but in particular of the low ecotype, will consist primarily of young individuals (Figure 5c and d, Table S16). At high elevation we detected a divergent pattern between the ecotypes. The distributions of the low ecotype shifted towards an increased proportion of older individuals whereas the age distribution of the high ecotype remained skewed towards younger individuals.

Plant size affects performance in single fitness components

Plant size tended to have a positive effect on individual fitness components. In both environments, regardless of ecotype, larger plants were both more likely to flower and to survive (Figure 6a – d, Table S17, Table S18). Larger plants also produced more seeds, but in each environment, this effect was significant only in the local ecotype (Figure 6e and f, Table S19). Separate analyses at the subsequent life-stages revealed that impact of plant size on survival probability was statistically significant only for the time points when plants experienced strong mortality events (Figure S11, Figure S8). In these cases, the effect was consistently stronger for the foreign ecotype, except in the high environment the second winter (W2; (Table S18). Plant size overall positively affected the probability of flowering during the first four years of the experiment, but not during the fifth year, and was consistently associated with increased seed output (Figure S12, Figure S13).

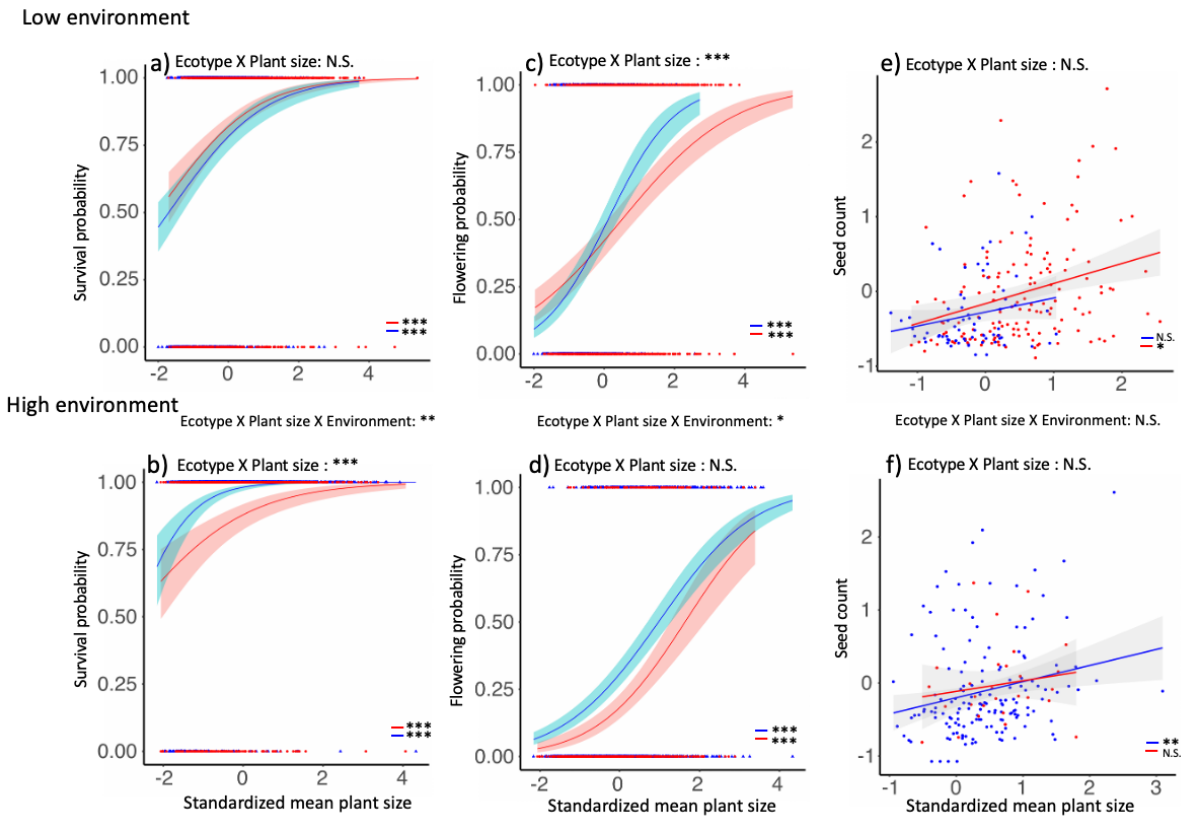

Figure 6. The effect of plant size on survival probability, flowering probability and seed count of elevational ecotypes growing in the low (a, c, e) and high (b, d, f) environment. Plant size and seed count are standardized by life-stage and environment. Red and blue lines indicate predicted relationships from generalized and linear mixed effect model regressions with 95% confidence intervals for the low and high elevation ecotypes, respectively. Corresponding red and blue triangles indicate values of plant size. Significance of the three-way interaction between ecotype, plant and the environment and of the ecotype X plant interactions and of the relationship between plant size and the separate fitness components are reported. Short red and blue lines denote statistical significance for the low and high elevation ecotypes, respectively (***p<0.001, **p<0.01, *p<0.05).

Recombinant populations

The phenotypic distribution present in the wild populations was partly covered in the F2 populations (Figure S15). The dependence of plant height and flowering time on plant size identified in the wild populations was recapitulated in the F2 populations (Figure S14, Table S20).

We detected significant directional selection differentials acting towards earlier flowering and larger plants in both elevational sites, and taller stalks in the high site (Table 1). For flowering time, we further identified significant negative and positive nonlinear selection in the low and high site, respectively, and significant positive nonlinear selection for plant height in the high site. Selection for larger size was stronger in the low than in the high site (Table 1).

Table 1. Estimates of linear (*S*) and nonlinear (*C*) selection differentials for plant size, flowering time and plant height at the low and high elevation transplant sites. Selection differentials are estimated based on parametric bootstrap (n=5000) using univariable regression models and include 95% bias corrected confidence intervals. Significant results (i.e., estimates whose 95% confidence intervals do not overlap zero) are in bold.

	Directional selection		Nonlinear selection	
Traits	*S* cumulative fitness		*C* cumulative fitness	
	Low site	High site	Low site	High site
Plant size	**1.29 (0.86, 1.70)**	**0.28 (0.18, 0.383)**	-1.78 (-4.97, 0.75)	0.01 (-0.21, 0.27)
Plant height	0.11 (-0.01, 0.23)	**0.3 (0.23, 0.37)**	-0.22 (-0.40, 0.002)	**0.15 (0.06, 0.25)**
Flowering time	**-0.35 (-0.44, -0.26)**	**-0.27 (-0.34, -0.21)**	**-0.23 (-0.39, -0.08)**	**0.2 (0.12, 0.3)**

The selection gradients based on cumulative fitness revealed strong directional selection for larger size in the low site and selection for earlier flowering in the high site (Table 2). We found significant selection gradients for earlier flowering and taller plants through the probability to produce seeds at the high elevation site. In the low elevation site, we did not find evidence for selection acting through this fitness proxy. Based on relative total seed weight we inferred significant selection for larger plant size at both elevations.

Table 2. Estimates of linear (β) selection gradients for plant size, flowering time and plant height at the low and high elevation transplant sites. Selection gradients are estimated based on parametric bootstrap (n=5000) using multivariable regression models and include 95% bias corrected confidence intervals. Results for cumulative fitness, relative total seed weight and probability to produce seeds, are reported. Significant results (i.e., estimates whose 95% confidence intervals do not overlap zero) are in bold.

Traits	β cumulative fitness	
	Low site	High site
Plant size	**1.00 (0.49, 1.47)**	0.02 (-0.06, 0.1)
Plant height	0.13 (-0.07, 0.33)	0.11 (-0.04, 0.25)
Flowering time	0.02 (-0.17, 0.21)	**-0.24 (-0.36, -0.14)**
	β relative total seed weight	
Plant size	**1.26 (0.03,2.54)**	**0.61 (0.14,1.1)**
Plant height	0.01 (-0.35,0.36)	0.50 (-0.04,1.02)
Flowering time	-0.07 (-0.37,0.22)	-0.11 (-0.76,0.56)
	β probability to produce seeds	
Plant size	0.50 (-1.68,2.73)	-0.42 (-1.38,0.39)
Plant height	-0.27 (-0.85,0.29)	**1.48 (0.91,2.13)**
Flowering time	-0.47 (-0.98, 0.03)	**-0.8 (-1.34,-0.34)**

Discussion

Understanding how ecotype formation proceeds following range expansion into new habitats requires uncovering how adaptive traits contribute to the evolutionary response to novel selection regimes. We combined transplant experiments of wild populations and recombinant crosses to uncover the evolution of key traits in *D. sylvestris* following the recolonization of low elevation habitats in the central Alps after the Last Glacial Maximum. We show that populations inhabiting the extremes of elevational gradients evolved phenotypic divergence with a strong genetic basis, accompanied by adaptation to the contrasting selection regimes at high and low elevations. This process was driven by selection acting through alternate fitness components in the two environments, and was tightly linked to variation in life history traits. As a response to the warmer climate, the low elevation ecotype evolved a shorter life cycle characterized by high reproduction, in contrast to ancestral strategies apt to ensure self-maintenance under colder climates that constrains plant physiological processes. This process was mediated by the evolution of a plastic response to the higher energy environment at the warm end of the elevational gradient, thus enabling plants to achieve larger size in response to strong direct selection. Combined with selection for early flowering at the cold end of the gradient, these genetic responses underly the phenotypic divergence observed between the elevational ecotypes.

Strong evidence of elevational adaptation in *D. sylvestris* emerged from population growth models, where the local ecotype consistently outperformed the foreign ecotype in both the high and low elevation environment. While these models provide compelling evidence of local adaptation as inferred from integrative fitness estimates for these perennial plants, they also allow dissecting the contributions of separate life stages to fitness and help formulate hypotheses about how selection acts in different environments (Caswell, 1989; Caswell, 2001; Peterson *et al.*, 2016; Goebl *et al.*, 2022). In *D. sylvestris*, adaptation in the low elevation habitat was primarily driven by reproduction in the third year, whereas at high elevation, contributions of different stages of the life cycle were more uniformly distributed. Dissecting ecotype by environment interactions of complementary fitness components revealed that reproduction confers a strong advantage of the local ecotype at low elevation, concomitant with a skewed age distribution towards young individuals with high reproductive output. Contrarily, contrasts of reproductive output at high elevation yield similar estimates for both ecotypes. Here, plant performance was characterized by a strong adaptive signal in survival resulting in age classes similarly represented in the population, which indicates that population growth at high elevation relies on a conservative strategy. These inferences are in line with strategies of ensuring self-maintenance which commonly prevails under colder alpine conditions (von Arx *et al.*, 2006; Šťastná *et al.*, 2012; Laiolo & Obeso, 2017; Rosbakh & Poschlod, 2018). On the other hand, the low elevation habitat selected for a life history strategy that favors investment in early reproductive fitness at the cost of shorter life cycles. This response is consistent with patterns commonly recovered along elevational gradients (Hautier et al., 2009; Kim & Donohue, 2011; Kim & Donohue,

2013; Laiolo & Obeso, 2017; Pérez *et al.,* 2020). A recent inference similar to ours was recovered by Peterson *et al.,* (2020) who used a common garden experiment to expose annual and perennial *Mimulus guttatus* populations to experimental drought conditions, resembling our low elevation environment, and revealed that selection favored allocation to reproduction under warmer and drier conditions. Warmer habitats typically allow for longer growing seasons, resulting in an overall higher energy input and resource availability that allow for increased reproductive output and competitive ability (Stearns, 1992; Laiolo & Obeso, 2017).

The shift to a faster life cycle at low elevation is associated with an increase in plant size expressed by the local compared to the foreign ecotype across all stages of the life cycle. In plants, size is a common indicator of resource accumulation driven by metabolic rates as defined by resource availability and physical parameters (Poorter *et al.*, 2011; Younginger *et al.*, 2017). Among these, temperature and length of the growing season play a key role (Körner, 2003; Davies, 2006; Körner, 2006; Körner, 2015). In *D. sylvestris,* we found that plant size was positively correlated with plant height and with earlier flowering. Plant size was also positively associated with survival and reproduction. Thus, size acts as a key determinant of both reproductive traits and fitness. This is a frequently reported physiological relationship (Bonser & Aarssen, 2009; Weiner *et al.*, 2009; Younginger *et al.*, 2017; Cheplick, 2020; Fournier *et al.*, 2020; Proulx, 2021), and that similar correlations between plant size and fitness components were recovered across ecotypes of *D. sylvestris* suggests that altered genetic correlations do not justify the observed shift in life-history traits. The ability of the low elevation ecotype to increase its size was only expressed when growing in its home environment while remaining latent under the contrasting environmental conditions at high elevation. This environmental dependence indicates that the faster life cycle achieved by the low ecotype constitutes an inducible strategy set off by the climate at low elevation, where higher resource acquisition allows faster growth. We deduce that selection in the low environment acted on the genetic basis of the plastic response to the warmer climate, fueling a shorter cycle with resource acquisition devoted to intense reproduction.

At both elevations, total selection indicated that plants that flowered earlier, with tall stalks and large size had higher fitness, with particularly high coefficients for selection on size at low elevation. Multivariable analyses corroborate that this signal in plant size is the result of direct selection, while genetic correlations underlie the selection differentials estimated for the other traits. Notably, dissecting the impact of selection on separate fitness components revealed that direct selection for large size was driven by reproductive output, while the coefficient estimated for the probability to reproduce was close to zero. This is in line with the adaptive role of size at low elevation as a key driver of a fast life cycle where reproduction can be achieved by most individuals, but differential fitness is primarily driven by the investment in large reproductive output.

In the high elevation habitat, a contrasting scenario was recovered, as direct selection favored early flowering, while total selection estimated for plant size results from the strong genetic correlation between these two traits. Moreover, significant gradients were driven by the effect of selection on the probability to produce seeds rather than on reproductive output. Flowering time is a crucial transition that, for entomophilous plants like *D. sylvestris*, must coincide with the availability of pollinators and is necessarily linked to abiotic conditions allowing the reproductive stage of the life cycle (Elzinga *et al.*, 2007; Amasino, 2010; Ehrlén, 2015; Gaudinier & Blackman, 2019; Ehrlén *et al.*, 2020). Early flowering ensures successful reproduction in alpine environments where the short summer season constrains fruit maturity but can come at the cost of diminished resources that can be allocated to reproductive output (Obeso, 2002; Stinson, 2004; Gimenez-Benavides *et al.*, 2007; Anderson *et al.*, 2012; Hamann *et al.*, 2021). This trade-off can be exacerbated in low resource environments, or alternatively be reduced following the release from limitations imposed by the abiotic environment (Tuomi *et al.*, 1983; Obeso, 2002; Shefferson *et al.*, 2003; Sletvold & Ågren, 2015; Willi & Van Buskirk, 2022).

Our analyses of total selection acting on flowering time and plant size indicated that adaptation to low elevation evolved from relaxed trade-offs between the probability to produce seeds and reproductive output as mediated by the warmer climate. Importantly, selection gradients for large size at low elevation and early flowering at high elevation are in line with genetic divergence in phenotypic traits between ecotypes, which thus results from alternate evolutionary response to selection regimes at the extreme of the elevational gradient. On the other hand, despite our expectations informed by general patterns across plant systems (Halbritter *et al.*, 2018), we did not find evidence for direct selection acting on plant height. Selection for tall stalks is typically related to competition for light and pollinators, and is frequently observed in highly competitive environments (Gervasi & Schiestl, 2017; Zu & Schiestl, 2017; Halbritter *et al.*, 2018). However, *D. sylvestris* naturally inhabits rocky outcrops which are largely void of density dependent competition from direct neighbors, and long stalks are typically arcuate, thus favoring visits through lateral approach by pollinators. Thus, we infer that in contrast to plant size and flowering time, ecotypic divergence in plant height in *D. sylvestris* is not mediated by spatially divergent natural selection but is instead a consequence of genetic correlations with these adaptive traits.

The adaptive shift towards a shorter life cycle under warmer conditions in *D. sylvestris* recapitulates common trends observed across many plant species along temperature gradients. Our results shed light on processes that governed the recent response to climate-driven selection in this species, and while mechanisms remain specific to each system, similar principles may underlie evolutionary trajectories shared across species. Our results show that the recent response of *D. sylvestris* to postglacial warming proceeded through selection acting on adaptive variants that underlie the plastic response of physiological

processes associated with increased plant growth. These variants were likely present as standing genetic variation in refugial populations during the LGM and can be expected to be maintained in the gene pool of current high elevation populations descendant from recolonizing lineages. As anthropogenic climate change imposes novel selection regimes, this adaptive potential can form the basis of a continued evolutionary response through the realization of past evolutionary trajectories such as those described in this study. On the other hand, whether low elevation populations harbor the genetic potential for further evolution, and which trajectories this may imply, remains unknown. Our study is a first step to unravel past evolutionary responses to climate warming and further experiments are needed to uncover the existence of latent phenotypes that may be expressed and become adaptive under future climatic conditions.

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The fitness effects of allelic variation underlying adaptive traits in an alpine plant mediate divergence between elevational ecotypes

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AP, SF and AW designed and set up the transplant experiments. AP collected the field data and performed all analyses. AP wrote the manuscript, which all authors revised.

Abstract

Alpine plant populations have recolonized formerly glaciated areas after the Last Glacial Maximum and evolved adaptations to novel habitats along elevational gradients. Dissecting the genetic basis of adaptation to shifting selection regimes is key for understanding how climate has driven local adaptation and ecotype formation in alpine plants. Using recombinant crosses between elevational ecotypes transplanted into high and low elevation sites, we studied the fitness effects of alleles underlying adaptive traits in the alpine carnation *Dianthus sylvestris*. We combined uni- and multi-variate GWAS of plant size, height and flowering time and tested fitness effects at candidate loci through both integrative estimates and analyses of separate components under the contrasting elevational environments. We found a polygenic trait architecture with the effects of individual loci consistent with either antagonistic pleiotropy or conditional neutrality, and in the latter cases, neutral effects were primarily found in the derived, low elevation habitat. We further inferred that adaptation to this environment proceeded through a shift from a negative to a positive correlation between the probability to reproduce successfully and fecundity. Our findings suggest that selection has driven the evolution of elevational ecotypes through pleiotropic effects on alternative components of fitness, which in turn mediate the divergence of life history traits between ecotypes.

Keywords

Adaptation, antagonistic pleiotropy, aster models, conditional neutrality, ddRAD, ecotype divergence, GWAS, fitness trade-off, transplant experiment

Introduction

Natural selection acts on phenotypic traits when they have a consistent relationship with fitness. Under contrasting environmental conditions, spatially divergent selection can lead to the evolution of phenotypically divergent populations that have a fitness advantage in their respective habitats over foreign populations, resulting in local adaptation (Clausen *et al.*, 1940; Kawecki & Ebert, 2004). The evolution of such locally adapted ecotypes has long captured the interest of evolutionary biologists and a wealth of ecological literature has revealed that local adaptation is common, yet not ubiquitous, in nature (Leimu & Fischer, 2008; Hereford, 2009). Because selection pressures can change when climatic conditions change over time, local adaptation can be a transient phenomenon (Hargreaves *et al.*, 2014; Anderson & Song, 2020). The evolutionary response to altered selection regimes may lead to a shift or an expansion of the ecological niche occupied by the species (Hargreaves *et al.*, 2014). As the adaptive traits characterizing ecotypes are an expression of their underlying genetic architecture, such a response is ultimately a genetic phenomenon (Savolainen *et al.*, 2013; Bomblies & Peichel, 2022). Understanding how adaptation proceeds following exposure to novel environmental conditions requires identifying the genetic architecture underlying adaptive traits and dissecting the fitness effects of the uncovered allelic variants.

The genetic architecture underlying adaptation has been investigated in a diversity of study systems (see e.g., Bamba *et al.*, 2019 and Bomblies & Peichel, 2022, and references therein). Estimates of the fitness effects of adaptive variants under natural conditions, however, are rare and largely limited to major-effect loci in a few well known study systems. Primary examples in animals include adaptation to freshwater in threespined sticklebacks (*Gasterosteus aculeatus*) (Barret *et al.*, 2008) and wild Soay sheep (*Ovis aries*) (Johnston *et al.,* 2013). Examples in plants include adaptation to dune habitats in *Mimulus guttatus* (Hall *et al.*, 2010); phenology in *Boechera stricta* and *Arabidopsis* (Anderson *et al.,* 2013; Leinonen *et al.,* 2013) and freezing tolerance in *Arabidopsis* (Oakley *et al*., 2014). Studies using these systems show that well designed experiments have the capacity to capture the fitness effects of allelic variants driving the evolution of adaptive traits. Studies incorporating the polygenic architecture of adaptive quantitative traits or fitness components are even rarer but provide unique examples of the cumulative contributions of multiple loci to fitness (Troth *et al.*, 2018; Gramlich *et al.*, 2022). Except for few cases, in the majority of study systems our knowledge about the genetic variants mediating evolutionary change relies on functional annotations of candidate loci and presumed genotype by environment interactions for fitness. Hence, we know surprisingly little about the relationship between genetic variation and fitness as mediated by phenotypic expression. Filling this gap is relatively rarely done because it requires large field experiments to dissect genotype-fitness associations while accounting for genetic correlations among phenotypic traits (Chandler *et al.*, 2013; Savolainen *et al.*, 2013).

The genetic basis of fitness variation can be assessed using field experiments under the environmental conditions that two or more ecotypes experience in their natural habitats. Because of past selection acting on natural populations, fitness related traits are expected to display limited phenotypic variation and to be correlated across individuals within each ecotype, resulting in strong linkage disequilibrium at underlying loci. Thus, while wild populations are ideal units for reciprocal transplant experiments to test for local adaptation, they are of limited use to dissect the fitness effects of the genetic architecture underlying this process. This is better achieved in admixed populations (Lexer *et al.,* 2003) by creating recombinant populations derived from artificial crosses between ecotypes, where the decoupling of loci across the genome is expected to break up genetic correlations and allow to assess locus-specific effects (Lexer *et al.*, 2003; Chandler *et al.*, 2013). Such recombinant populations between divergent genotypes are also expected to express broad phenotypic variation, which facilitates phenotypic selection analyses. Studies that combine genetic mapping with transplant experiments therefore have the potential to uncover the genetic architecture of adaptive traits expressed under natural environmental conditions, and assess the fitness effects of genetic variation under the contrasting selection regimes that have led to the formation of distinct ecotypes.

The fitness effects of alleles underlying adaptive traits in different environments can broadly express two forms (Savolainen *et al.*, 2013). Antagonistic pleiotropy is inferred when alternate alleles at a specific locus are favored in two different environments, whereas conditional neutrality refers to the situation in which an allele confers a fitness advantage in one environment but is neutral in the other. Antagonistic pleiotropy can be a strong driver of ecotype formation even in the face of recurrent gene flow, as divergent selection acting across environments can maintain polymorphisms at adaptive loci (Anderson *et al.*, 2011; Savolainen *et al.*, 2013; Luqman *et al.,* 2022). Conditional neutrality, on the other hand, can underlie population divergence when adaptation is governed by multiple loci with significant fitness effects in alternate environments, or else gene flow will cause the genotype adaptive in one environment to spread as a generalist. Conditional neutrality is the more prevalent pattern reported in field studies (e.g.,Wadgymar *et al.*, 2017 and references therein; Gramlich *et al*., 2022). On the other hand, opposite fitness effects compatible with antagonistic pleiotropy are often detected in controlled experiments (Bono *et al.*, 2017). They are further expected to be associated with strong genomic signatures of selection at candidate loci with major phenotypic effects (Anderson *et al.*, 2011; Savolainen *et al.*, 2013), but are rarely reported in field studies (but see e.g., Ågren *et al.*, 2013; Troth *et al.*, 2018; Expósito-Alonso *et al.*, 2019). This discrepancy is often attributed to the greater statistical power needed to find significant fitness effects in both environments, compared to the single statistically significant comparison required to corroborate conditional neutrality, or to the effects of temporal variation in environmental factors (Wadgymar *et al.*, 2017).

Species inhabiting steep climatic gradients provide excellent study systems to investigate the genetic architecture of recent adaptation emerged from plant responses to climate-driven selection. In continental mountain ranges, phenotypic variation and patterns of local adaptation along elevational gradients result from selection regimes that set in after the Last Glacial Maximum (Davis & Shaw, 2001; Petit *et al.*, 2003). With increasing temperatures, plant species recolonized habitats freed from the retreating glaciers, facing highly heterogeneous environmental conditions. The steep shift in abiotic conditions along elevational gradients contributes to a geographical compression of vegetation zones, and populations experiencing contrasting conditions can occur in close proximity (Körner, 2003; Tito *et al.*, 2020). Elevational gradients have long been used as natural laboratories for the study of the organismal responses to varying abiotic conditions (Clausen *et al.*, 1940; Tito *et al.*, 2020). A particularly rich literature exists for plants, due to their amenability to transplant experiments (Leimu & Fischer, 2008; Hereford, 2009). Divergent selection acting on key traits, such as plant size and flowering time, is common across multiple species and mountain ranges (Halbritter *et al.*, 2018), and alternative life-history strategies are linked to selection driven by resource availability (Laiolo & Obeso, 2017). Slow growth and self-maintenance strategies characterize plants in high elevation habitats, whereas abundant reproduction characterizes plants with faster life cycles in the more resource-rich low elevation habitats.

Dianthus sylvestris is a widespread perennial carnation commonly occurring on rocky outcrops along elevational gradients throughout the European Alps (Collin & Shykoff, 2003; Info Flora 2022). During the Last Glacial Maximum, *D. sylvestris* survived in glacial refugia in the South-Eastern Prealps characterized by climatic conditions similar to nowadays' high elevation habitats, and subsequently recolonized the Alps (Luqman *et al.*, 2022). In this range expansion, adaptation to warmer climate at low elevation led to the formation of a divergent ecotype characterized by marked differences in phenotypic and life-history traits. In previous work, we conducted a reciprocal transplant experiment of high and low elevation ecotypes of the Central Alps along with a recombinant population to uncover the role of natural selection in driving the evolution of divergent traits and their contribution to adaptation. We found that the low elevation ecotype displays a delayed flowering time, taller stalks and larger plant size relative to its high elevation counterpart. These traits are strongly genetically correlated, as size governs the plants' ability to produce taller stalks and achieve flowering (chapter 2). Moreover, size is tightly linked to plant fecundity, and selection on this trait results in life history traits that maximize reproduction in the low elevation habitat, in contrast to self-maintenance strategies favored in the more resource-limited high elevation habitat. While the low elevation ecotype is characterized by fast growth and investment in fecundity at the expense of a shorter life cycle, the high elevation ecotype displays early flowering and lower seed output to maximize the probability of seed ripening before the end of the short alpine vegetation period. Studies on *D. sylvestris* populations growing at the upper and lower elevation limits in the Alps therefore offer the

opportunity to unravel the evolution of elevational ecotypes as a result of divergent selection in response to climate-driven selection.

In this study, we leverage on this experiment to uncover the fitness effects of alleles underlying the traits that drove the adaptive divergence between low and high elevation populations of *D. sylvestris* in the central Alps. We conduct univariate GWA analyses on plant size and multivariate GWA analyses of plant size, height and flowering time to identify loci underlying their phenotypic variation. We further associate allelic variation to fitness to assess how selection has driven ecotype formation in response to climate variation along the elevational gradient. Specifically, we ask 1) has evolution of the low-elevation ecotype proceeded through selection on polygenic trait architecture with opposite fitness effects across environments, or are alleles conditionally neutral? 2) Does the allelic effect on fitness act through alternative fitness components as reflected in life-history traits linked to plants' ability to allocate resources under differently limiting environments?

Methods

Experimental procedures

For this study, we relied on recombinant populations transplanted to one low and one high elevation experimental site (hereafter low- and high-environment) in the region of Valais (Switzerland) as described in chapter 2. Briefly, F1 families were first obtained from multiple crosses between low- and high-elevation individuals (Table S1). Different combinations of F1 individuals were subsequently placed in separate cages supplemented with pollinators to maximize seed production. In summer 2017, seeds were germinated in a greenhouse and in fall seedlings were transplanted to each of the two environments. Each environment received 1000 seedlings arranged in 14 randomized blocks, with 72 plants in each block. The transplant environments were fenced to exclude mammalian herbivores and we continuously trimmed the vegetation surrounding our plants to minimize above ground biotic interactions. In spring 2018, 621 and 554 plants survived in the low and high environment, respectively, and were used as experimental populations employed in the analyses (Table S2). A detailed description of the experimental design is provided in chapter 2.

Phenotypic traits and fitness

We collected data on plant size, plant height, flowering time and fitness over three growing seasons (2018 – 2020), following the protocol described in chapter 2. Briefly, the growing seasons typically lasts from March to November in the low environment and from May to October in the high environment (for details see, chapter 1). At the start of each growing season, we estimated survival (yes/no), and plant size as two orthogonal diameters of the basal rosette measured on photos of each individual plant. In 2018, plants displayed only vegetative growth and the transplant sites were not visited regularly. During the growing

seasons of 2019 and 2020 we visited each site twice a week and recorded flowering time (i.e., date of anthesis of the first flower) for each plant. At the end of each growing season, we harvested all stalks and determined plant height as the mean length of three representative stalks. We extracted the seeds from all capsules and weighed all seeds per plant using a Mettler Toledo Ae240 Balance (precise to the nearest 0.0001 gram). We counted the seeds of a subset of plants (84 and 82 from the low- and high-environment, respectively) and estimated separate correlation coefficients between seed weight and seed number (Figure S1). We used the linear relationship across sites to estimate the number of seeds produced by each plant for use in aster models (see below).

Genetic data

At the start of the 2018 growing season, we sampled leaf tissues for DNA extraction from each individual plant in both environments and dried them in silica gel. DNA extraction and subsequent ddRAD genotyping were performed using a customized version of the original protocol of Peterson *et al.,* (2012) as described in Westergaard *et al.,* (2019), including three replicates of three random samples per library. The raw data were demultiplexed using the internal barcode, using Stacks v.2.41 (Rivera-Colón & Catchen, 2021) with default settings. The raw data were mapped to the reference genome of *D. sylvestris* (Fior *et al*., unpublished results) using the default settings of BWA mem v0.7.17 (Li & Durpin, 2009). Reads with low mapping quality (Q20) were subsequently removed. SNPs were called in the four grandparents using FreeBayes v1.3.1 (Garrison & Marth, 2012) and the resulting VCF was filtered using vcftools (Danecek et al., 2011) and vcflib (Garrison *et al*., 2022) for a minimum quality score of 20 and a minimal mean depth of 3 and a mean depth of 10, a minor allele count of 3 and a minor allele frequency of 0.05. After removal of complex SNPs, 235356 sites were genotyped. Freebayes was then run to call these SNPs on 2318 individuals from the F1 and F2 generations and the resulting vcf was filtered for the same parameters described above, and a maximum number of missing sites across individuals of 5%. Samples with more than 50% missing sites were also removed. To avoid paralogues, loci with excessive heterozygosity were removed using a p-value threshold of 0.00001, resulting in 42827 high-quality SNPs in 2318 samples, of which 21419 SNPs are located in scaffolds anchored in the linkage map of 15 chromosomes of *D. sylvestris* (Fior *et al*., unpublished results). We explored genetic groups in a principal component analysis (PCA) performed in PLINK 1.9 (Purcell *et al.*, 2007) on a data set including the grandparents, F1 and F2 plants.

Genome-wide association analyses

To find loci associated with plant size, plant height and flowering time we employed univariate and multivariate mixed effect models as implemented in GEMMA 0.98.1 (Zhou & Stephens, 2012). We used univariate models to associate SNPs with phenotypic variation in plant size, and three multivariate models for plant size and plant height, plant size and flowering time and plant size, plant height and flowering time to best describe the genetic architecture of these traits, considering their genetic correlations and identify potentially

pleiotropic loci (Zhou & Stephens, 2014; Chhetri *et al.*, 2019). Because the genetic architecture of traits may vary across the life cycle (e.g., Bac-Molenaar *et al.,* 2015) and the life history variation between *D. sylvestris* growing low and high elevation environments (chapter 2), we investigated plants at comparable life stages by using data from the first year of flowering in each environment. We combined data from 132 individuals in the low environment in 2019 and 115 individuals in the high environment in 2020 to performed association analyses accounting for the transplant environment as a covariate. Our sample size limited statistical power to also test for GxE interactions in the GWA analyses. Flowering time was assessed relative to the date of the first flowering plant in each environment, which was set to day one. Values for all traits exceeding two standard deviations of the mean within each genetic cluster and environment were excluded from the analyses. Population structure may bias GWA analyses by increasing the number of false positives (Tibbs Cortes *et al.*, 2020). Therefore, in both the univariate and multivariate analyses, a relatedness matrix estimated in GEMMA was included as a covariate. Statistical significance of identified associations was determined using the Wald test (Wald, 1943). Significant associations were classified according to two criteria: a p-value cutoff based on the Bonferroni correction (alpha < 0.05) and FDR-correction (5%) for genome-wide significance (Benjamini & Hochberg, 1995). Due to the very stringent nature of the Bonferroni correction, all downstream analyses were performed for SNPs passing the FDR threshold, but both significance levels are reported in Table S3. To account for linkage between SNPs, we estimated correlations between pairwise combinations of significantly associated SNPs in vcftools (Danecek *et al.*, 2011) and retained the SNP with the highest p-value for pairs with Pearson's r2 >0.60. As multiple associated SNPs were in strong linkage within separate scaffolds, this pruning resulted in retaining one significant SNP per scaffold.

Analysis of phenotypic and fitness data

Relationship between plant size, flowering probability and growth environment

To test differences between plant size achieved by our experimental population in the low and high environment, we tested the effect of environment as predictor variable on plant size in a linear mixed effect model (LMM). We further tested the effect of plant size on flowering probability in each environment by modelling flowering probability as a function of plant size, environment and their interaction as predictor variables in generalized linear mixed effect models (GLMMs). The models were fitted with Gaussian error distribution for plant size (a continuous variable) and binomial error distribution for flowering probability (a categorical variable). Detailed model structure is reported below. Mixed effect models were employed using the package lme4 v1.1 (Bates *et al.*, 2015)

Fitness effects of identified alleles

We used integrative fitness estimates obtained through the aster modelling framework (Geyer *et al.*, 2007) to investigate the fitness effects of alternate alleles at loci identified in the GWA analyses. We constructed a hierarchical graph model incorporating the separate

co-dependent fitness components of yearly survival, flowering, and seed production, into a cumulative fitness estimate as reported in chapter 2. Briefly, the hierarchical structure uses survival probability, flowering probability and seed production as the three layers of the model (Figure S2). Each layer contains two nodes corresponding to performance in the fitness components during 2019 and 2020, where the performance in each node depends on the predecessor node. To facilitate interpretation of the allelic effects on fitness, we polarized the alleles at each locus according to the allele that conferred a positive effect on plant size. The sample sizes of the homozygotes of the minor alleles were very low, therefore we excluded these homozygotes and estimated the allelic effects as the effect size of the contrast between homozygous and heterozygous individuals. A similar approach was employed by Troth *et al.,* (2018). We investigated antagonistic pleiotropy and conditional neutrality separately for each locus identified in the GWA by implementing the locus as predictor variable and testing for genotype by environment interactions and for differences between genotypes within each environment using likelihood ratio tests (LRTs). Detailed model structure is reported below. We predicted the mean expected cumulative fitness per genotype using the output of the most parsimonious model for each locus separately. We relativized cumulative fitness obtained from the aster models to the global mean across environments, according to recommendations for analyses of selection that can be assumed to be non-frequency dependent (De Lisle and Svensson, 2017). We then calculated the allelic effect as the contrast between the homo- and heterozygotes mean difference in cumulative fitness for each locus in the two environments separately. We controlled for multiple testing using FDR correction. Aster models were employed using the package Aster v1.1-1 (Geyer *et al.*, 2007).

Trade-offs between fitness components

We estimated the correlation between the allelic effects on probability of producing seeds and total seed weight. To achieve this, we calculated the cumulative probability to produce seeds (hereafter probability to produce seeds) and the cumulative total seed weight by combining data from 2019 and 2020. Cumulative seed weight was calculated only for plants that produced seeds. We relativized the cumulative total seed weight (hereafter relative seed weight) to the global mean across environments (De Lisle & Svensson, 2017). We modelled the two separate fitness components as response variables using locus as predictor variable in GLMMs and LMMs for probability to produce seeds and relative seed weight, respectively. We implemented a binomial error distribution for the probability to produce seeds (a categorical variable), and for relative seed weight (a continuous variable) a Gaussian error distribution. Detailed model structure is reported below. Relative seed weight was log transformed to improve the distribution of the residuals. To subsequently achieve an estimate for the allelic effects through these separate fitness components and to investigate potential trade-offs, we estimated the contrast between the homozygotes and heterozygotes at each locus from the separate GLMMs and LMMs. Hence, for the probability to produce seeds, we report the odds ratio of producing seeds between homo-

and heterozygous individuals. We then performed correlation tests between the allelic effects on relative seed weight and probability to produce seeds, separately for each environment using Pearson's correlation test. Since the goal was to provide an overall estimate of the direction of the trends, we included all loci in these analyses, not only those that had a significant effect on cumulative fitness.

Covariates and model selection

In all statistical models described above we accounted for the effect of the genetic clusters, and the elevational origin of the genetic background (estimated as the relatedness to the alternate (low/high) origin grandparent individuals), by including them as covariates in the models and used LRTs to test their interaction with the other predictors and their effect on the response variables. In case either the interaction with other predictors or the effect on the response variables were statistically significant we included them in the final, most parsimonious models. Additionally, in the mixed effect models, to account for the effect of transplant blocks, we implemented them as a random effects when they had a significant effect on the response variable. In addition to outliers removed from the GWAS analyses, values exceeding two standard deviations of the mean within each genetic cluster and environment of seed count and weight were excluded from the analyses of the phenotypic and fitness data. All analyses were performed in R v. 3.3.2 (R Core Development Team 2016).

Results

GWA analyses

The F2 plants fall into three genetic clusters according to the possible crosses derived from the low- and high-elevation grandparents (Figure S3). Two clusters thus represent either offspring of F1 derived from the alternate grandparents (Table S1) and the third represents the outcrossed offspring between different F1 crosses. We performed uni- and multi-variate GWA analyses to uncover loci underlying phenotypic variation in plant size, plant height and flowering time. After pruning, we retained 45 SNPs that were significantly associated with plant size in the univariate analyses (Figure 1A; Figure S4A; Table S3). Multivariate analyses revealed 9 SNPs associated with plant size and plant height and 27 associated with plant size and flowering time (Figure 1B-1C; Figure S4B-S4C; Table S3). The multivariate analyses using all three traits simultaneously identified 8 SNPs (Figure 1D; Figure S4D; Table S3). Four SNPs, significant in all analyses, passed the Bonferroni threshold whereas all other SNPs reached significance level according to the FDR correction. Numerous SNPs overlap between the different analyses (Table S3). All SNPs associated with plant size and plant height were overlapping with associations with flowering time. Therefore, the SNPs can be grouped into three sets: 10 SNPs associated with all traits, 17 SNPs associated with plant size and flowering time, and 21 SNPs associated only with plant size. Across analyses, we recovered QQ plots expected for analyses of polygenic traits and λ values ranging from 0.98 to 1.08

supporting that our analyses accounted for population structure related to the three genetic clusters emerging from the crossing design (Figure S5). The effect sizes of the alleles in set three (associated only with plant size) ranged between -1.44 and 1.34 cm^2 (Table S3). Effect sizes from multivariate analyses show that alleles that have positive effects on plant size (ranging from 0.55 to 1.73 cm^2) have positive effects on plant height (ranging from 0.371 to 2.846 cm) and negative effects on flowering time (ranging from -3.33 to -0.92 days) (Table S3). In other words, plants carrying alleles conferring larger sizes are taller and initiate flowering earlier.

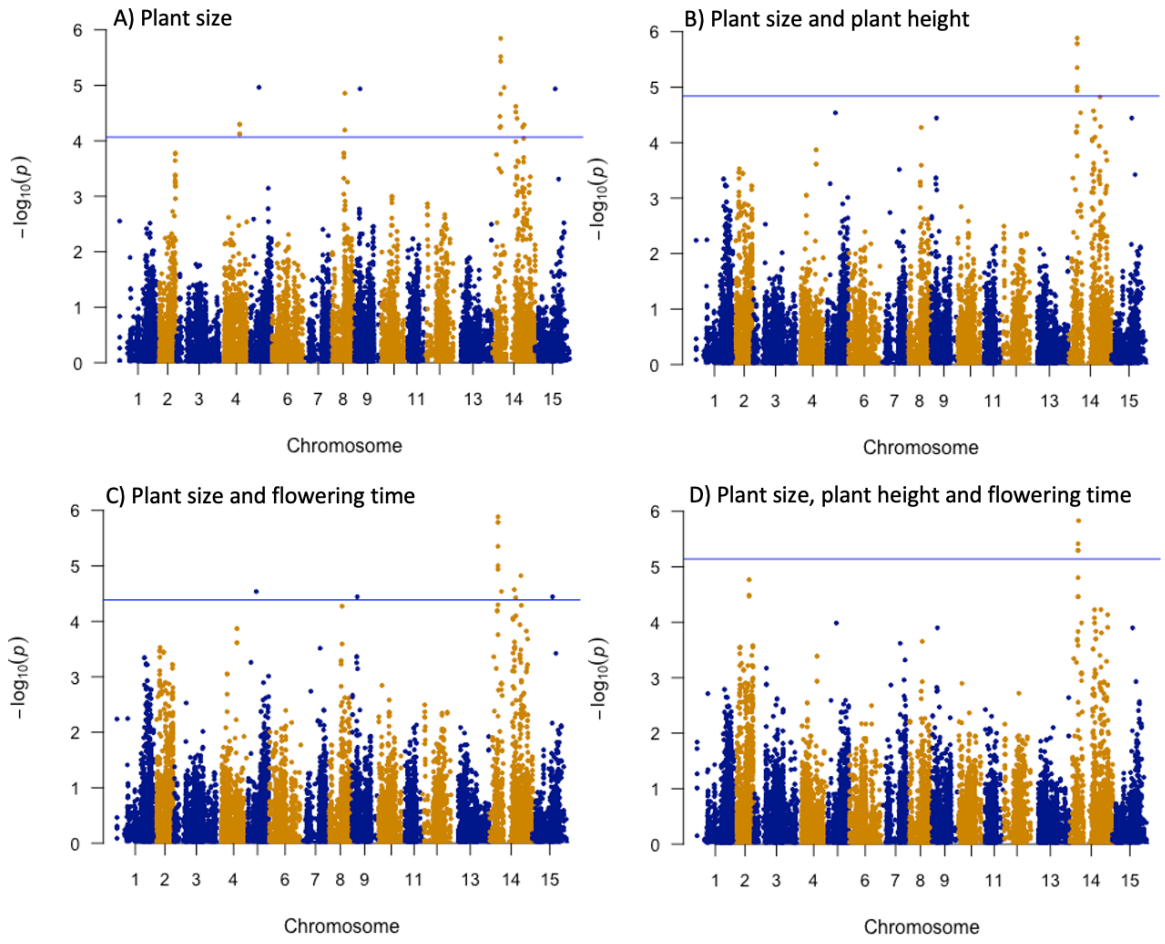

Figure 1. Results of the univariate and multivariate GWA analyses presented as and Manhattan plots of associations with A) plant size, B) plant size and plant height, C) plant size and flowering time and D) plant size, plant height and flowering time. Blue lines indicate genome-wide significance threshold based on the FDR corrected p-values. The SNPs before LD pruning are reported and only SNPs on scaffolds anchored to the 15 chromosomes are plotted.

Relationships between plant size, flowering probability and the growing environment

Plants growing in the low environment attained significantly larger sizes than those in the high environment in 2019. In 2020, this pattern was reversed, as plants growing in the high environment were significantly larger as a result of continued vegetative growth in the previous year, in contrast to a large proportion of plants that flowered in 2019 in the low environment (Figure 2A and 2B; Table S4). The probability to flower increased with plant size (Figure 2C and 2D; Table S4), and there was a significant plant size by environment interaction in 2020. Plants were significantly more likely to flower in the low environment than in the high environment in both 2019 and 2020 (Figure 2E and 2F; Table S4).

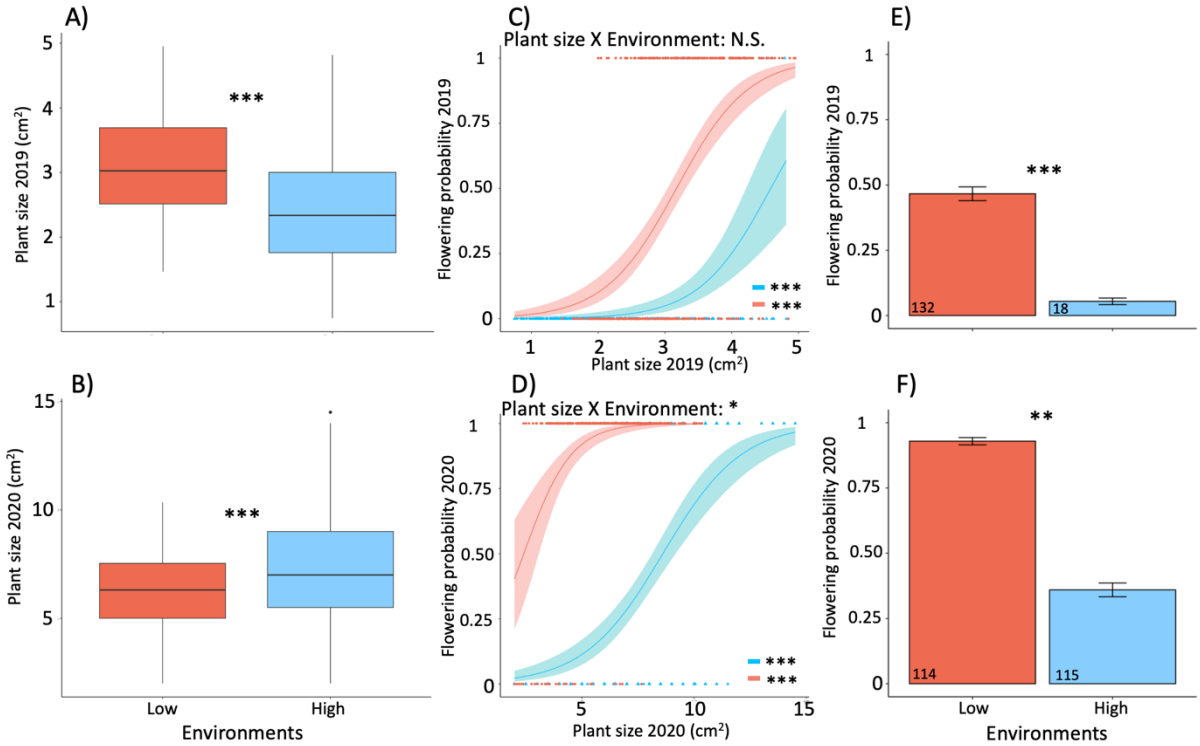

Figure 2. Plant size, flowering probability, and their interaction in the two elevational environments. A - B) Plant size (cm^2) in 2019 and 2020 in the low and high environments. C - D) Flowering probability in 2019 and 2020 in the low and high environments modelled as a function of plant size in 2019 and 2020, respectively. Significance of plant size by environment (Plant size X Environment) interactions are reported. E-F) Flowering probability in 2019 and 2020 in the low and high environment, number of flowering plants in each transplant environment noted in the bars. (***p<0.001, **p<0.01, *p<0.05).

Fitness effects of the identified alleles

Loci showing a significant genotype by environment interaction associated with negative effects of alternate alleles across environments were considered indicative of antagonistic pleiotropy. Loci with a significant genotype by environment interaction and a significant beneficial effect of one allele in only one environment were considered indicative of conditional neutrality. Two loci associated with plant size and three associated with plant size and flowering time displayed antagonistic pleiotropy (Table 1). Conditional neutrality was inferred for two loci associated with plant size, two with plant size and flowering time and two associated with all traits (Table 1). All except one of these loci displayed the neutral effect in the low elevation environment. Nine, two, and two loci associated with plant size, plant size and flowering time and all traits, respectively, displayed significant fitness effects in one environment but a nonsignificant genotype by environment interaction (Table S5). Two loci (SNP ID 18 and ID 13), associated with all traits, showed a significant positive effect on fitness in both environments and 23 loci did not show any statistically significant effects on cumulative fitness.

Table 1. Output of LRTs of the aster models testing for genotype by environment interaction and differential performance in cumulative fitness of genotypes within environments, loci with significant genotype by environment interaction shown. Fitness effect and trait(s) associated with the SNP, SNP id (corresponding to SNP ids in table S3), FDR corrected p-values of genotype by environment interaction, contrast between alternate genotypes and FDR corrected p-values in the high and low environment and environment with significant fitness effect contrast reported.

SNP id	Genotype by environment interaction, p-value	High environment : contrast; p-value	Low environment : contrast; p-value	Environment with significant fitness effect
Antagonistic pleiotropy				
plant size				
10	**0.0004**	**0.17; 0.01**	**-0.45; 0.001**	both
44	**0.0004**	**0.22; 0.0004**	**-0.46; 0.01**	both
plant size + flowering time				
8	**0.0002**	**0.22; 0.005**	**-0.22; 0.0002**	both
14	**0.0002**	**0.29; 0.0004**	**-0.14; 0.0002**	both
15	**0.0002**	**0.34; 0.0004**	**-0.11; 0.0002**	both
Conditional neutrality				
plant size				
42	**0.05**	**0.17; 0.02**	-0.1; 0.08	high
35	**0.0004**	**0.19; 0.0004**	-0.13; 0.26	high
plant size + flowering time				
27	**0.03**	-0.01; 0.524	**-0.44; 0.0002**	low
21	**0.02**	**0.18; 0.03**	-0.23; 0.18	high
plant size + height + flowering time				
20	**0.03**	**0.19; 0.03**	0.14; 0.86	high
24	**0.01**	**0.2; 0.01**	-0.24; 0.13	high

To evaluate whether there is a trade-off between performance in the different fitness components, we conducted correlation analyses between the allelic effects on the probability to produce seeds and relative seed weight in the separate environments. We detected a significant negative correlation between the effect on probability to produce seeds and the effect on relative seed weight in the high environment (Figure 3A). In the low environment, this pattern was reversed and instead showed a positive correlation between the allelic effects on these two fitness components (Figure 3B).

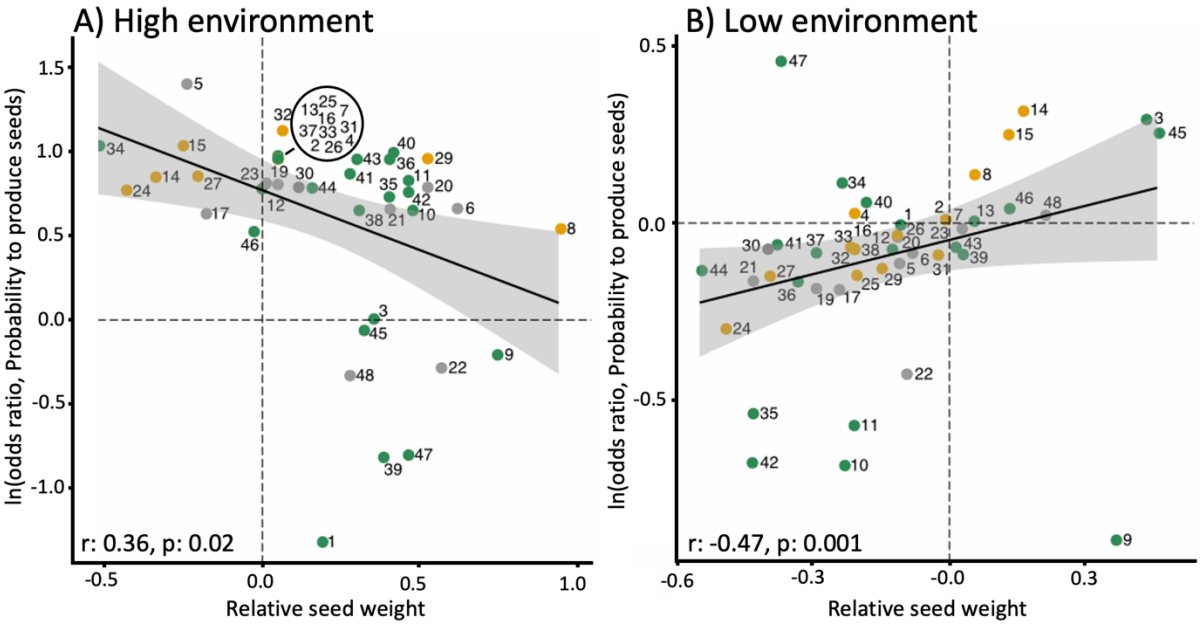

Figure 3. Correlation plots of allelic effect on single fitness components of SNPs significantly associated with plant size (green), plant size and flowering time (orange) and plant size, flowering time and plant height (grey). A) the high- and B) the low- environment. Numbers indicate SNP ids in table S3 lines indicate predicted relationships from linear model regressions with 95% confidence intervals and correlation coefficients and p-values are reported.

Discussion

Uncovering how fitness acts on the genetic variants underlying adaptive trait variation is pertinent to develop a better understanding of the evolution of local adaptation. In this study, we used recombinant F2 crosses transplanted into high and low elevation environments to unravel how natural selection acts on alleles underlying adaptive traits that distinguish ecotypes in *D. sylvestris* that evolved along elevational gradients after the LGM. We found that both antagonistic pleiotropy and conditional neutrality act simultaneously in our recombinant F2 crosses. The majority of the conditionally neutral loci showed a neutral effect at low elevation. By dissecting the impact of selection through different fitness components we revealed an environment-dependent trade-off between the allelic effects on probability of successful reproduction and reproductive output. The environment

dependence reversed a negative relationship expressed at high elevation to a positive at low elevation. Overall, our findings reveal that the studied adaptive traits have a polygenetic basis and that the alternate selection regimes affect the relationship between individual fitness components.

In a previous study, we showed that elevational divergence in plant size, plant height and flowering time has a genetic basis and that the recent adaptive divergence of low and high elevation populations of *D. sylvestris* was driven by natural selection acting on plant size and flowering time (chapter 2). We also found that plant size is a strong indicator of resource allocation to reproductive traits in *D. sylvestris.* Here, we identified multiple loci associated with either plant size using alone or with plant size and all possible combinations of the studied traits. These complementary approaches can provide a more comprehensive description of the genetic architecture underlying genetically correlated traits than single trait approaches alone. Multivariate models can both identify potentially pleiotropic loci and increase statistical power by exploiting the covariance between the traits, even when a locus is associated with only one of the traits included in the model (Zhou & Stephens, 2014; Chhetri *et al.*, 2019). Hence, simultaneous identification of a SNP using these and univariate models enhances the confidence in the accuracy of the associations. This strategy has been successfully applied to dissect the genetic architecture of blood pressure in humans (Liang *et al*., 2017), yield in beans (Oladzad *et al*., 2019), meat production in cattle (Niu *et al*., 2021), and of anatomical traits in giant sequoia (De La Torre *et al*., 2022). It is therefore particularly suited for polygentic traits controlled by loci with potentially pleiotropic effects, as we hypothesised for the genetic architecture of size and reproductive traits in *D. sylvestris* on the basis of their genetic correlations. In line with these expectations, multiple both overlapping and non-overlapping loci were identified between the univariate and multivariate models.

We associated alleles at significant loci with cumulative fitness and uncovered that while many of them did not have a detectable effect on fitness, a few showed evidence consistent with antagonistic pleiotropy or conditional neutrality. The evidence for antagonistic pleiotropy is notable, given the relative scarcity of examples reported in the literature. This scarcity of evidence for genetic trade-offs is not due to a sparsity of these mechanisms acting in nature but more plausibly due to the fact that the majority of adaptive traits are often polygenic, implying low effect sizes of individual loci which makes it difficult to detect antagonistic effects (Le corre & Kremer, 2012; Barghi *et al.*, 2020; Hayward & Sella, 2022). Consequently, the inference of conditional neutrality across study systems may be inflated (Mee & Yeaman, 2019; Wadgymar *et al.*, 2017). However, conditional neutrality might be promoted in cases in which natural selection acts on different targets under contrasting environmental conditions, as is the case in our study system (chapter 2). Our evidence of both types of fitness effects simultaneously is in agreement with expectations that adaptive divergence driven by natural selection acting on traits with a polygenic genetic architecture,

as in our study system, the genotype by environment interactions for fitness could be governed by both conditional neutrality and antagonistic pleiotropy (Anderson *et al.*, 2013; Hämälä & Savolainen, 2019; Kawecki & Ebert, 2004; Savolainen *et al.*, 2013). A recent study conducted by Wright *et al.,* (2021) using F2 populations showed that the genetic basis of local adaptation in white clover (*Trifolium repens*) was governed by both conditional neutrality and antagonistic pleiotropy, through divergence in life history strategies between locally adapted ecotypes. A notable pattern that emerged in our results was that the loci showing conditional neutrality primarily displayed a neutral effect at low elevation. This directionality likely facilitated the recent range expansion to low elevation habitats of *D. sylvestris* in the central Alps, which facilitated the evolutionary response to the novel climate driven selection regime at low elevation. If alleles at loci underlying adaptive traits primarily exhibited detrimental fitness effects at low elevation, this would have hampered the rapid evolutionary response that accompanied the niche expansion into low elevation environments. Furthermore, we hypothesise that the loci showing neutral effects in the low environment, yet with a non-significant GxE interaction, would be identified as conditionally neutral with a larger sample size. These results suggest that adaptation to the novel low elevation environment was facilitated by the recruitment of alleles with neutral effects

A key aspect of the effect of natural selection acting on *D. sylvestris* at the opposite ends of the elevational gradient is that the adaptive traits contribute to cumulative fitness through alternate fitness components (chapter 2). At low elevation, selection acts through reproductive output, whereas at high elevation, where investment in reproduction is constrained, selection instead acts through the probability of successful reproduction. Consistent with this finding, we recovered that the fitness effects of adaptive alleles at high elevation seem to be governed by a trade-off between the probability to produce seeds and reproductive output, while the positive correlations between these two components at low elevation indicates that plants that achieve successful reproduction are also likely to have higher seed production. Hence, the environment dependent shift in resource allocation that we identified through analyses of trait-fitness associations (chapter 2) is recapitulated in the fitness effects of alleles underlying these same traits. This corroborates the role of climate driven selection as a strong driver of the evolution of adapted ecotypes that maximise resource acquisition through shifts in life history traits. High elevation habitats characterized by low resource availability appear to select for strategies that preserve self-maintenance at the cost of low investment in reproduction. Because of the short growing season and low temperature that constraints physiological processes, selection for early flowering, as seen in *D. sylvestris* (chapter 2), enables plants to achieve fruit maturity, but on the other hand, shortens resource acquisition in the preceding vegetative stage and thus entails lower capacity for reproductive output. In contrast, in environments without strong resource limitation, such a trade-off would not necessarily be expressed and continuous resource uptake could allow the reversal of the relationship between the fitness components (Hamann *et al.*, 2021).

The shift in the direction of the relationship between the allelic effects on the separate fitness components likely affects the observed pattern of allelic effects on cumulative fitness. Adaptive alleles at high elevation that appear to act through the probability of producing seeds would likely not exhibit a detrimental effect at low elevation where the majority of plants that flower also reproduce successfully. On the other hand, the adaptive alleles at low elevation seem to act through reproductive output, which could entail a cost under high elevation environmental conditions, where allocation to seeds is constrained. Consequently, we propose that the trade-off linked to resource allocation induces a cascading effect on cumulative fitness and therefore at least partly explains the mechanisms underlying the patterns of antagonistic pleiotropy and conditional neutrality. This implies that niche expansion into the warmer low elevation habitat by *D. sylvestris* populations was facilitated by a release from the constraining environmental conditions at high elevation. Exposure to the warmer climate at lower elevation shifted the direction of the interaction between fitness components which subsequently reduced selection against the invading populations and hence facilitated an evolutionary response to the low elevation environmental conditions.

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Discussion

Synthesis

This thesis contributes to our understanding of the evolution of local adaptation in response to climate-driven selection in perennial plant species. By reciprocally transplanting elevational populations belonging to two perennial carnation species, we drew evidence of local adaptation from estimates of population growth rates, in which for both species, the local populations performed better than the foreign, and thus correspond to elevational ecotypes. Moreover, as plant performance has a functional dependence on individual fitness components, we dissected the impact of selection on fitness proxies for reproduction and survival throughout the life cycle. This allowed us to show that in both species, fitness components linked to reproduction appear to be key in driving adaptation to low elevation. In line with evolutionary theory on life history traits, we interpret this as evidence that investment in reproductive fitness components is advantageous under environmental conditions characterized by high-energy input and high resource availability (Stearns, 1992; Kim & Donohue, 2011; Wenk & Falster, 2015; Salguero-Gomez *et al.*, 2016). In contrast, at high elevation, the main agent of selection was survival, highlighting the relevance of investment in self maintenance under environmentally constrained conditions (Laiolo & Obeso, 2017; Rosbakh & Poschlod, 2018; Friedman, 2020). The impact of alternate selection regimes at the extreme ends of the elevational gradient resulted in the evolution of ecotypes characterised by divergent life history traits.

An important finding revealed by this thesis is that plant size acts as a major determinant of both, phenotypic traits and fitness components. In both our focal species we found a strong genetic basis for ecotypic divergence in this trait. In *D. sylvestris*, we documented that natural selection has driven the evolution of the low elevation ecotype as mediated by the higher reproduction achieved by larger plants. This evolutionary response is reflected in the fitness effects of alleles that underly the genetic architecture of plant size and of genetically correlated traits. This thesis therefore provides strong evidence that ecotype formation in two species of carnation is tightly associated with alternative strategies of resource allocation to self-maintenance and reproduction in response to environmental constraints. As these processes encompass trade-offs between linked components of plant performance expressed at subsequent stages of the life cycle, our work emphasizes the need for long-term experiments and integrative fitness estimates to gain a comprehensive understanding of processes underlying local adaptation in perennial plants.

Limitation and perspectives

In this thesis, we aimed to dissect adaptive processes in two perennial species and we achieved this by using our long term reciprocal transplant experiments where we combined data collected for three and five years for *D. carthusianorum* and *D. sylvestris*, respectively.

Yet, our experiments still only cover a portion of the perennial life cycle. While obtaining life-time fitness estimates would be desirable, given the differential influence of early vs. late life stages to the adaptive response of the alternative ecotypes, we expect that continuing the experiments for a longer period of time would likely only increase the contrasts in fitness. We therefore argue that our experiments are likely to recapitulate the fitness consequences of the climate driven selection regimes. On the other hand, the limited temporal survey of the recombinant populations of *D. sylvestris* represents a likely more relevant limitation for the interpretation of the phenotypic selection analyses. Here, we only used one time point of trait measuring, i.e., the first year of reproduction, but we are aware that the impact of selection might be life stage dependent (Ehrlén, 2015; Goebl *et al.*, 2022). Indeed, it has been argued that particularly selection acting on phenology should be considered in a life cycle context because the timing of reproductive events is fundamentally a property of the life cycle and selection acting pre- and post-reproduction and on non-reproducing individuals might affect inferences on selection acting on phenological traits (Ehrlén, 2015). We therefore propose that in the future, selection analyses should be performed over multiple seasons including both mean trait values, averaged across life stages, and multiple time specific measurements to allow dissection of life stage specific variation in the direction and strength of selection.

Our work focused on abiotic factors linked to the climate related selection regime at the opposing ends of the elevational gradient. As this was our primary interest, we did not consider biotic factors that are nonetheless likely to act as relevant agents of selection (e.g., Hargreaves *et al.*, 2020 and references therein; Benning & Moeller, 2021; Wadgymar *et al.*, 2022). Particularly, competition is expected to significantly affect plant fitness along elevational gradients (Alexander *et al.*, 2015). We hypothesize that this is primarily relevant for *D. carthusianorum,* which at low elevation inhabits dense meadows with many potential competitors. On the other hand, while competition is a likely determinant of the ecological niche of *D. sylvestris*, both high and low elevation populations grow on rocky outcrops where few other species manage to establish. Differences in competitors, therefore, may not bear strong effects on the evolution of divergent ecotypes in this latter species.

Because natural selection simultaneously acts on multiple traits, our focal traits covary with elevation in the wild populations, which limits the power of identifying loci underlying our focal traits. We overcame this issue by producing recombinant F2 crosses, yet this approach comes at the cost of important aspects. F2 crosses have undergone limited recombination and hence contain long chromosome tracts in linkage disequilibrium. For this reason, we relied on a reduced representation sequencing approach (RADseq) for genotyping. While this strategy is cost-effective, it is unlikely to genotype causal variants, but instead will yield associations at SNPs that are linked to the causal variants. Because our crosses originate from four parental genotypes, different alleles at RAD markers could be associated with causal variants, which inherently limits our ability to detect association signals. An additional

caveat stems from the very rationale that warrants the use of crosses to assess allele-specific fitness effects. In fact, while crosses decouple traits and the underlying loci from one another, they also break up gene complexes and the effects of epistatic interactions that may be key to expression of the phenotypes we aimed to study (Carter *et al.*, 2005; Csillery *et al.*, 2018). We suggest that future work could aim to achieve a larger sample size and potentially combine GWAS on recombinant crosses with association analyses performed on populations originating from an intermediate elevation to firmly link fitness effect of the allelic variation underlying the adaptive traits to the action of natural selection in the wild populations.

Despite the limitations, our work provides insights into the evolutionary and plastic responses to climate driven selection at opposite ends of an elevational gradient in perennial carnations and lays the foundation for future, more targeted studies. Such studies could involve detailed experiments exploring the roles of individual selective agents to provide a refined understanding of population responses. For example, the prolonged snow cover at high elevation provides insulation against frost damage, determines the start of the growing season and, we hypothesize, also exerts strong selection against the low elevation ecotype of both species. Contrarily, at low elevation, summer drought appears to impose strong selection, potentially causing the maladaptive response of the high elevation ecotype. Thus, dissecting the role of these potential selective agents could be achieved using field experiments including manipulations, such as snow removal (e.g., Anderson *et al.*, 2019) or drought treatments (e.g., Bushey *et al.*, 2023).

Relevance

By taking advantage of transplant experiments of seedlings of both wild populations and complementary germination experiments, We built projections on ecotype-specific population growth representative of the plant life cycle, as well as attained comprehensive integrative fitness estimates. Using this framework combined with experimental crosses, We unraveled how ecotype formation in two perennial carnations has proceeded in response to climate driven selection through divergence of life history and phenotypic traits. This thesis provides new insights into ecotype formation in response to climate driven selection at opposite ends of a steep elevational gradient and shows how the evolution of local adaptation in two closely related perennial plants proceeds through species-specific life history responses and trade-offs.

Commentary on the relevance in the context of anthropogenic climate change

Understanding the evolution of local adaptation in response to climate driven selection is crucial for predicting population persistence under future climates. Local adaptation is a transient phenomenon and populations that are locally adapted today may be maladapted under future selection regimes (Anderson & Song, 2020; Bontrager *et al.*, 2020). In *D.*

carthusianorum, We speculate that the substantial phenotypic plasticity of the life history traits might temporarily facilitate population persistence by mitigating the negative impact of changing future climatic conditions. This is a phenomenon that has been hypothesised to allow time for an evolutionary response to the novel selection regimes (Ghalambor *et al.*, 2007; Fox *et al.*, 2019; Radersma *et al.*, 2020; West-Eberhard, 2003). In *D. sylvestris* We gained insights into a recent evolutionary response to the warmer low elevation habitats. Our results suggest that the contemporary high elevation populations harbor the necessary genetic variation to exert an evolutionary response to warming conditions. However, whether this response will be fast enough to cope with the rapidly changing climate will constitute the main determinant of the species' ability to persist under future conditions. Future experiments to address this timely question could build on the knowledge acquired in this thesis and further exploit the space-for-time experimental framework provided by elevational gradients to assess the populations' ability to respond to climate change along a more continuous suite of climatic conditions within the extremes explored in the current work.

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Chapter I. Supplementary materials

Supplementary figures

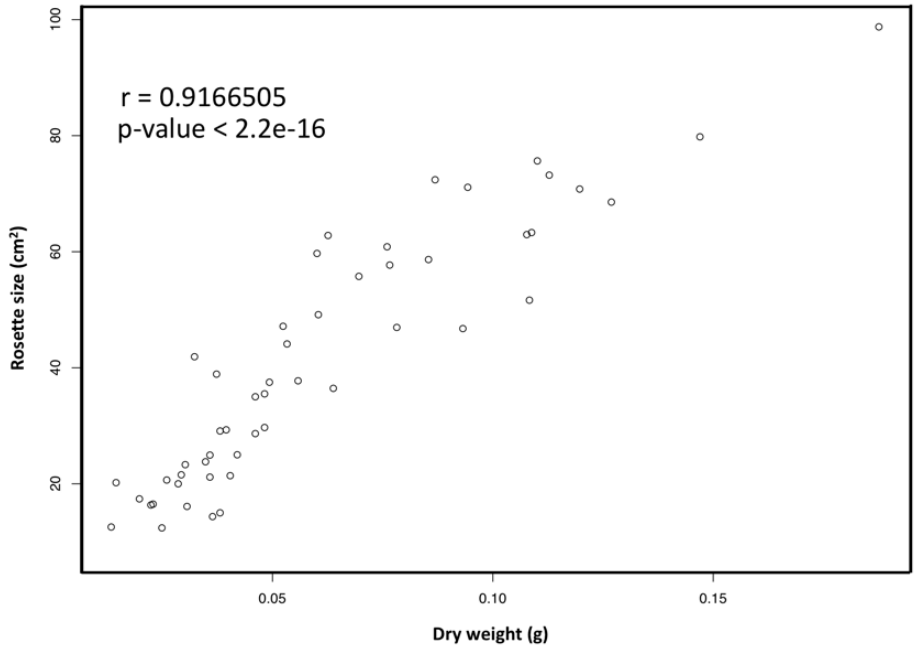

Figure S1. Correlation between rosette size (cm^2) of and dry weight (g) of above ground tissue, based on data from 53 plants growing in a greenhouse facility (Lindau-Eschikon, Switzerland).

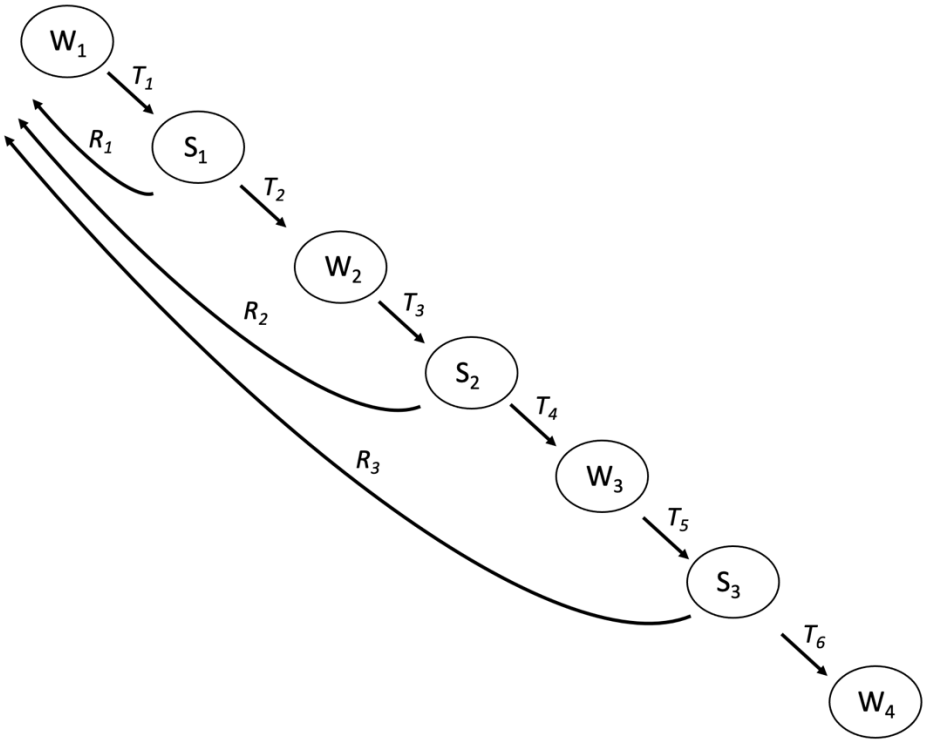

Figure S2. Age-classified life-cycle graph for *D. carthusianorum* used for the matrix population models. The life cycle is divided in subsequent summer (S_i) and winter (W_i) stages, represented by indexes in circles. The vital rates are inferred as transitions between the stages (i.e., survival T_i and reproduction R_i).

Supplementary tables

Table S1. Low and high elevation populations used in the transplant experiment. Population, elevation, meters above sea level, coordinates, number of maternal families and mean nr. of seedling per family ± SD reported.

Population	Elevation	Meters above sea level	Coordinates: latitude, longitude	Number of maternal families	Mean nr. of seedlings per family ± SD
Unterstalden	Low	754	46.26, 7.88	39	16.74 ± 12.93
Niedergampel	Low	688	46.31, 7.71	30	13.17 ± 14.77
Grengiols	Low	898	46.38, 8.11	23	16.48 ± 10.62
Simplonpass	High	2002	46.25, 8.03	20	18.10 ± 12.68
Gibidumsee	High	2211	46.26, 7.94	32	13.91 ± 10.58
Faldumalp	High	2029	46.38, 7.74	29	9.97 ± 7.05

Table S2. Transplant sites of the reciprocal transplant experiment. Elevation, meters above sea level, coordinates, number of maternal families per site, and mean nr. of seedlings per family ± SD reported.

Transplant Site	Altitude	Meters above sea level	Coordinates: latitude, longitude	Number of maternal families	Mean nr. of seedlings per family ± SD
Leuk	Low	890	46.27, 7.88	133	4.67 ± 3.48
Zeneggen	Low	930	46.31, 7.66	135	5.26 ± 3.63
Findeln	High	2120	46.01, 7.76	127	4.63 ± 2.88
Oberu	High	2150	46.35, 7.67	135	4.48 ± 2.98

Table S3. Results of the seed establishment experiment. We modelled establishment as the response variable in generalized linear mixed effect models and a binomial error distribution. We tested the difference in establishment rates between ecotypes in the contrasting environments and obtained significance levels using likelihood ratio tests. We report mean establishment rates and its SD per ecotype extracted from the models, Estimate, SE and p-values.

Model	**Data**	**Mean establishment; SD**	**Estimate; SE; P-value**
Establishment ~ Ecotype	Low Environment	Low: 0.17; 0.091 High: 0.14; 0.073	0.2598; 2548; 0.311
	High Environment	Low: 0.02; 0.028 High: 0.06; 0.06	-0.6352; 0.3636; 0.096

Table S4. Results of flowering probability and seed output analyses. We tested for adaptation in the reproductive vital rates, flowering probability and seed output, by modelling them as the response in generalized linear mixed effect models with binomial and poisson error distribution for flowering probability and seed output, respectively. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. S_i indicate the summers. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold.

Test	**S_1: χ^2; P-value**	**S_2: χ^2; P-value**	**S_3: χ^2; P-value**
Flowering probability			
Ecotype X Environment	**5.046; 0.025**	**38.165; <0.001**	**46.306; <0.001**
Local vs. foreign	**S_1: Estimate; SE; P-value**	**S_2: Estimate; SE; P-value**	**S_3: Estimate; SE; P-value**
Low Environment	0.575; 0.431; 0.461	**0.223; 0.065; <.0001**	**0.329; 0.107; <0.001**
High Environment	0.964; 0.721; 0.961	1.320; 0.360; 0.310	**3.221; 1.135; <0.001**
Home vs. away			
Low	**0.407; 0.136; 0.007**	**0.223; 0.117; 0.004**	**0.012; 0.012; <0.001**
High	0.684; 0.220; 0.237	1.322; 0.701; 0.598	**0.117; 0.117; 0.032**
Seed output	**S_1: χ^2; P-value**	**S_2: χ^2; P-value**	**S_3: χ^2; P-value**
Ecotype X Environment	**87.829; <0.001**	**69.125; <0.001**	**78.41; <0.001**
Local vs. foreign	**S_1: Estimate; SE; P-value**	**S_2: Estimate; SE; P-value**	**S_3: Estimate; SE; P-value**
Low Environment	0.835; 0.233; 0.518	**0.481; 0.165; 0.033**	-0.70;2 0.509; 0.168
High Environment	0.609; 0.171; 0.077	1.407; 0.482; 0.319	-0.99; 0.510; 0.052
Home vs. away			
Low	**0.689 ; 0.126; 0.042**	0.628; 0.269; 0.277	-3.24; 2.179; 0.137
High	**0.502; 0.092; <.0001**	0.839; 0.790; 0.156	-3.53; 2.179; 0.105

Table S5. Results of the size analyses. We tested for adaptation in size by modelling it as the response in linear mixed effect models with a gaussian error distributions. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold. W_i and S_i indicate winter and summer, respectively.

Test	**χ^2; P-value**					
Size	W_1	S_1	W_2	S_2	S_3	W_4
Ecotype X Environment	1.872; 0.171	**10.716; 0.001**	**4.456; 0.035**	**48.66; <0.001**	**45.93; <0.001**	0.700; 0.403
Local vs. foreign	**Estimate; SE; P-value**					
Low E	-1.079; 0.427; 0.064	-0.848; 0.361; 0.076	**1.282; 0.363; 0.021**	**-0.952; 0.197; 0.005**	**-0.650; 0.169; 0.012**	**-0.334; 0.137; 0.028**
High E	**-1.197; 0.427; 0.048**	-0.559; 0.360; 0.193	**-1.023; 0.358; 0.044**	-0.270; 0.192; 0.227	-0.038; 0.164; 0.829	**-0.436; 0.104; 0.009**
Home vs. away						
Low	0.129; 0.172; 0.352	**0.796; 0.202; 0.048**	0.687; 0.322; 0.157	-0.259; 0.572; 0.694	-1.356; 0.622; 0.160	-0.214; 0.275; 0.511
High	0.221; 0.284; 0.364	**1.085; 0.200; 0.028**	0.947; 0.322; 0.090	0.422; 0.572; 0.537	-0.744; 0.622; 0.353	-0.316; 0.278; 0.361

Table S6. Results of the cumulative survival analyses. We modelled survival throughout the experiment using cox proportional hazard models and tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation.We report coefficients, hazard ratios and p-values, significant results are in bold.

Test	**Coefficients**	**Hazard ratio**	**P-values**
Ecotype X Environment	-1.726	0.178	**<0.001**
Local vs. foreign			
Low Environment	-1.044	0.352	**0.022**
High E Environment	0.762	2.143	**0.003**
Home vs. away			
Low	0.015	1.016	0.960
High	1.797	6.030	**<0.001**

Table S7. Results of single time point survival analyses. We tested for adaptation in survival at specific stages of the life cycles by modelling them the response in generalized linear mixed effect models with binomial error distribution. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold. W_i and S_i indicate winter and summer, respectively.

Test				**χ^2; P-value**			
Survival	W_1	S_1	W_2	S_2	W_3	S_3	W_4
Ecotype X Environment	**40.695; <0.001**	**11.353; <0.001**	**24.089; <0.001**	1.385; 0.239	**3.917; 0.048**	0.037; 0.848	3.045; 0.081
Local vs. foreign				**Estimate; SE; P-value**			
Low Environment	0.549; 0.251; 0.190	**0.168; 0.083; <0.001**	0.797; 0.212; 0.394	**0.195; 0.083; <0.001**	0.236; 0.219; 0.119	0.857; 0.237; 0.577	**0.424; 0.179; 0.042**
High Environment	**10.757; 5.30 <0.001**	0.676; 0.373; 0.478	**5.214; 1.808; <0.001**	0.381; 0.203; 0.070	1.645; 1.042; 0.4323	0.944; 0.389; 0.8889	1.153; 0.405; 0.686
Home vs. away							
Low	**0.140; 0.091; 0.002**	**2.705; 1.107; 0.015**	1.005; 0.354; 0.988	3.363; 2.8256; 0.149	0.316; 0.368; 0.322	3.615; 2.589; 0.073	1.004; 1.249; 0.999
High	2.740; 1.800; 0.142	**10.874; 3.824; <0.001**	**6.574; 2.754; <0.001**	**6.552; 4.949; 0.013**	2.200; 2.314; 0.453	**3.981; 2.778; 0.048**	2.732; 3.358; 0.414

Table S8. Population growth rate and LTRE at the two transplant environments. Population growth rate of the elevational ecotypes growing in the low and high elevation transplant environments, mean values based on 20 000 bootstrap replicates and 95% bias corrected confidence intervals are reported. Results of the LTRE showing the contribution of the vital rates of the foreign ecotype to population growth rate in the two transplant environments. Survival and reproductive vital rates throughout the life cycle are indicated by T_i and R_i, respectively. Mean estimates based on 20 000 bootstrap replicated are reported.

Environment	**Lambda, high ecotype**	**Lambda, low ecotype**	**T_1**	**R_1**	**T_2**	**T_3**	**R_2**	**T_4**	**T_5**	**R_3**	**T_6**
High	0.72 (0.67, 0.77)	0.46 (0.41, 0.53)	-0.019	-0.044	-0.001	-0.010	-0.032	0.005	<-0.001	-0.116	0
Low	1.50 (1.35, 1.70)	2.03 (1.77, 2.40)	-0.045	-0.408	-0.002	-0.012	-0.025	0.003	<-0.001	-0.031	0

Table S9. Results of trade-off analyses for size and flowering probability. We tested for trade-offs between size and flowering throughout the experiment by modelling flowering probability as a function of size using generalized linear mixed effect models. We tested for three way interactions between ecotypes, size and the transplant environment and two-way interactions between ecotype and size within the low and high elevation transplant environments, respectively as well as trends within experimental groups. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using the emmeans R package; Estimate, SE, z.ratio and p-values are reported. S_i denote the summer. Significant results in bold.

Model	**S_1: χ^2; P-value**	**S_2: χ^2; P-value**	**S_3: χ^2; P-value**
Flowering ~ Size X Ecotype X Environment	**28.1; <0.001**	**5.4; 0.02**	0.2; 0.69
Flowering ~ Size X Ecotype	**Low E: -50.106; <0.001, High E: -19.529; <0.001**		

Time point	**Environment**	**Ecotype**	**Trend, size**	**SE**	**df**	**z.ratio**	**p-value**
S_1	**Low**	**Low**	**1.08E-03**	**3.88E-04**	**Inf**	**2.797**	**0.005**
	Low	**High**	**8.81E-03**	**1.19E-03**	**Inf**	**7.426**	**<.001**
	High	**Low**	**1.14E-03**	**2.10E-04**	**Inf**	**5.562**	**<.001**
	High	**High**	**2.63E-03**	**2.73E-04**	**Inf**	**9.615**	**<.001**
S_2	**Low**	**Low**	1.31E-04	1.42E-04	Inf	1.597	0.110
	Low	**High**	**1.18E-03**	**1.57E-04**	**Inf**	**3.162**	**0.002**
	High	**Low**	**6.73E-04**	**1.55E-04**	**Inf**	**4.337**	**<.0001**
	High	**High**	**9.46E-04**	**1.42E-04**	**Inf**	**6.648**	**<.0001**
S_3	**Low**	**Low**	**3.90E-04**	**1.25E-04**	**Inf**	**3.117**	**0.002**
	Low	**High**	**1.13E-03**	**2.49E-04**	**Inf**	**4.545**	**<.0001**
	High	Low	**1.20E-03**	**3.81E-04**	**Inf**	**3.148**	**0.002**
	High	**High**	**2.16E-03**	**2.88E-04**	**Inf**	**7.518**	**<.0001**

Table S10. Results of trade-off analyses for size and survival probability. We tested for trade-offs between size and survival throughout the experiment by modelling survival probability as a function of size using generalized linear mixed effect models. We tested for three way interactions between ecotypes, size and the transplant environment and two-way interactions between ecotype and size within the low and high elevation transplant environments, respectively as well as trends within experimental groups. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using the emmeans R package; Estimate, SE, z.ratio and p-values are reported. W_i and S_i indicate winter and summer, respectively. Significant results in bold.

Model	W_1: χ^2; P-value	S_1: χ^2; P-value	W_2: χ^2; P-value	S_2: χ^2; P-value	S_3: χ^2; P-value	W_4: χ^2; P-value
Survival~ Size X Ecotype X Environment	0.375; 0.540	**10.693; 0.001**	2.049; 0.152	**5.818; 0.016**	0.298; 0.585	0.659; 0.417
Survival ~ Size X Ecotype		**Low E: 4.736; 0.03**, **High E: 6.203; 0.013**	**Low E: 5.314; 0.021** , High E: 2.130; 0.144			

Time point	Environment	Ecotype	Trend, size	SE	df	z.ratio	p-value
Survival W_1	**Low**	**Low**	5.32E-04	4.82E-04	Inf	1.103	0.27
	Low	**High**	**3.51E-03**	**1.07E-03**	**Inf**	**3.28**	**0.0010**
	High	**Low**	**1.71E-3**	**4.82E-04**	**Inf**	**4.504**	**<0.0001**
	High	High	6.96E-03	3.76E-03	Inf	1.849	0.0645
Survival S_1	Low	Low	8.44E-04	5.36E-04	**Inf**	1.576	0.115
	Low	**High**	**2.29E-03**	**7.81E-04**	**Inf**	**3.735**	**0.0002**
	High	**Low**	**6.71E-03**	**1.76E-03**	**Inf**	**3.805**	**0.0001**
	High	**High**	**2.53E-03**	**7.24E-03**	**Inf**	**3.496**	**0.0005**
Survival W_2	Low	Low	3.18E-04	4.20E-04	**Inf**	0.758	0.449
	Low	**High**	**-2.04E-3**	**9.25E-04**	**Inf**	**-2.06**	**0.027**
	High	**Low**	**6.41E-04**	**2.87E-04**	**Inf**	**2.235**	**0.025**
	High	High	-1.08E-04	3.93E-04	Inf	-0.274	0.784

Table S10. Continued.

Time point	Environment	Ecotype	Trend, size	SE	df	z.ratio	p-value
Survival S_2	Low	Low	2.97E-04	2.99E-04	Inf	0.992	0.321
	Low	**High**	1.25E-03	8.46E-04	Inf	1.481	0.139
	High	**Low**	**1.68E-02**	**7.08E-03**	**Inf**	**2.377**	**0.0175**
	High	**High**	**4.81E-03**	**1.35E-03**	**Inf**	**3.554**	**0.0004**
Survival S_3	Low	Low	2.35E-04	1.42E-04	Inf	1.661	0.097
	Low	High	3.41E-04	2.84E-04	Inf	1.200	0.230
	High	Low	1.67E-03	1.21E-03	Inf	1.383	0.167
	High	High	1.0E-03	7.06E-04	Inf	1.427	0.154
Survival W_4	Low	Low	1.33E-04	1.88E-04	Inf	0.703	0.482
	Low	High	1.84E-04	3.56E-04	Inf	0.515	0. 606
	High	**Low**	**4.05E-03**	**1.30E-03**	**Inf**	**3.119**	**0.002**
	High	**High**	**2.74E-03**	**1.00E-03**	**Inf**	**2.726**	**0.006**

Table S11. Results of trade-off analyses for survival and flowering probability. We tested for trade-offs between survival and flowering throughout the experiment by modelling flowering probability as a function of survival using generalized linear mixed effect models. We tested for three way interactions between ecotypes, flowering and the transplant environment and two-way interactions between ecotype and survival within the low and high elevation transplant environments, respectively as well as trends within experimental groups. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using the emmeans R package; Estimate, SE, z.ratio and p-values are reported. W_i and S_i indicate winter and summer, respectively. Significant results in bold.

Model	S_1: χ^2; P-value	W_2: χ^2; P-value	S_2: χ^2; P-value	W_3: χ^2; P-value	S_3: χ^2; P-value	W_4: χ^2; P-value
Survival ~ Flowering X Ecotype X Environment	**8.42; 0.004**	**8.81; 0.003**	0.08; 0.77	0.0; 0.99	0.01;0.94	0.98; 0.32
Survival ~ Flowering X Ecotype	**Low E: 5.18; 0.02** , **High E: 4.21; 0.04**	Low E: 8.14; 0.004**, High E: 2.42; 0.12**				

Time point	Environment	Ecotype	Contrast, flowering	Estimate	SE	df	z.ratio	p-value
Survival S_1	**Low**	**Low**	**yes - no**	**3.272**	**0.431**	**Inf**	**7.596**	**<0.001**
	Low	**High**	**yes - no**	**1.619**	**0.237**	**Inf**	**6.835**	**<0.001**
	High	**Low**	**yes - no**	**3.447**	**0.531**	**Inf**	**6.487**	**<0.001**
	High	**High**	**yes - no**	**3.321**	**0.729**	**Inf**	**4.556**	**<0.001**
Survival W_2	Low	Low	yes - no	-0.833	0.51	Inf	-1.632	0.6
	Low	**High**	**yes - no**	**-2.571**	**0.728**	**Inf**	**-3.533**	**0.005**
	High	Low	yes - no	-0.645	0.359	Inf	-1.796	0.479
	High	High	yes - no	0.894	0.576	Inf	1.553	0.657

Table S11. Continued.

Time point	Environment	Ecotype	Contrast, flowering	Estimate	SE	df	z.ratio	p-value
Survival W_2	Low	yes	low-high	0.509	0.27	Inf	1.882	0.419
	Low	no	low-high	-1.23	0.849	Inf	-1.448	0.73
	High	**yes**	**low-high**	**-2.413**	**0.497**	**Inf**	**-4.86**	**<0.001**
	High	no	low-high	-0.874	0.469	Inf	-1.865	0.43
Survival S_2	Low	Low	yes - no	18.593	3563.284	Inf	0.005	1
	Low	High	yes - no	19.56	6070.393	Inf	0.003	1
	High	Low	yes - no	17.494	6098.544	Inf	0.003	1
	High	High	yes - no	18.13	4738.979	Inf	0.004	1
Survival W_3	Low	Low	yes - no	-0.879	1.243	Inf	-0.707	0.991
	Low	High	yes - no	-0.16	1.173	Inf	-0.136	0.999
	High	Low	yes - no	0.03	1.117	Inf	0.027	1
	High	High	yes - no	17.024	63.634	Inf	0.268	0.999
Survival S_3	Low	Low	yes - no	0.608	0.384	Inf	1.582	0.638
	Low	High	yes - no	0.454	0.38	Inf	1.196	0.877
	High	Low	yes - no	-0.487	1.046	Inf	-0.465	0.999
	High	High	yes - no	0.465	0.751	Inf	0.62	0.997
Survival W_4	Low	Low	yes - no	-0.874	0.65	Inf	-1.344	0.797
	Low	High	yes - no	0.226	0.53	Inf	0.428	0.999
	High	Low	yes - no	-1.19	1.04	Inf	-1.145	0.899
	High	High	yes - no	0.116	0.988	Inf	0.117	0.999

Table S12. Influence of specific vital rates on population growth rate at the two transplant environments. Influence of specific vital rates on population growth rates extracted from the matrix population models of the elevational ecotypes growing in the low and high elevation transplant environments expressed as elasticities. Survival and reproductive vital rates throughout the life cycle are indicated by T_i and R_i, respectively, Mean values are based on 20 000 bootstrap replicates and 95% bias corrected confidence intervals are reported

Environment	Ecotype	T_1	R_1	T_2	T_3	R_2	T_4	T_5	R_3	T_6
High	High	0.254 (0.226, 0.287)	0.112 (0.074, 0.162)	0.141 (0.124, 0.156)	0.141 (0.124, 0.156)	0.036 (0.02, 0.064)	0.105 (0.082, 0.125)	0.105 (0.082, 0.125)	0.105 (0.082, 0.125)	0
High	Low	0.243 (0.208, 0.29)	0.111 (0.06, 0.182)	0.132 (0.109, 0.148)	0.132 (0.109, 0.148)	0.007 (0.002, 0.019)	0.125 (0.101, 0.144)	0.125 (0.101, 0.144)	0.125 (0.101, 0.144)	0
Low	High	0.432 (0.381, 0.469)	0.398 (0.321, 0.454)	0.034 (0.016, 0.06)	0.034 (0.016, 0.06)	0.0005 (0, 0.002)	0.034 (0.015, 0.059)	0.034 (0.015, 0.059)	0.034 (0.015, 0.059)	0
Low	Low	0.454 (0.416, 0.483)	0.426 (0.366, 0.471)	0.028 (0.011, 0.05)	0.028 (0.011, 0.05)	0.01 (0.005, 0.019)	0.018 (0.006, 0.035)	0.018 (0.006, 0.035)	0.018 (0.006, 0.035)	0

Table S13. Stable age distribution. Stable age distribution of the elevational ecotypes growing in the low and high elevation transplant environments. Winter and summer stages are represented by W_i and S_i, respectively. Values are based on 20 000 bootstrap replicates and 95% bias corrected confidence intervals are reported.

Environment	Ecotype	W_1	S_1	W_2	S_2	W_3	S_3	W_4
High	High	0.085 (0.065, 0.109)	0.116 (0.093, 0.142)	0.099 (0.085, 0.115)	0.133 (0.121, 0.147)	0.145 (0.133, 0.159)	0.197 (0.173, 0.223)	0.224 (0.182, 0.27)
High	Low	0.015 (0.007, 0.027)	0.027 (0.016, 0.045)	0.035 (0.023, 0.053)	0.066 (0.049, 0.088)	0.118 (0.099, 0.142)	0.251 (0.228, 0.279)	0.488 (0.405, 0.559)
Low	High	0.501 (0.448, 0.56)	0.303 (0.283, 0.327)	0.104 (0.085, 0.122)	0.048 (0.035, 0.062)	0.023 (0.014, 0.032)	0.015 (0.008, 0.023)	0.006 (0.003, 0.01)
Low	Low	0.594 (0.54, 0.659)	0.283 (0.253, 0.311)	0.074 (0.056, 0.092)	0.032 (0.02, 0.044)	0.01 (0.005, 0.016)	0.005 (0.002, 0.009)	0.001 (0.001, 0.003)

Chapter II. Supplementary materials

Supplementary figures

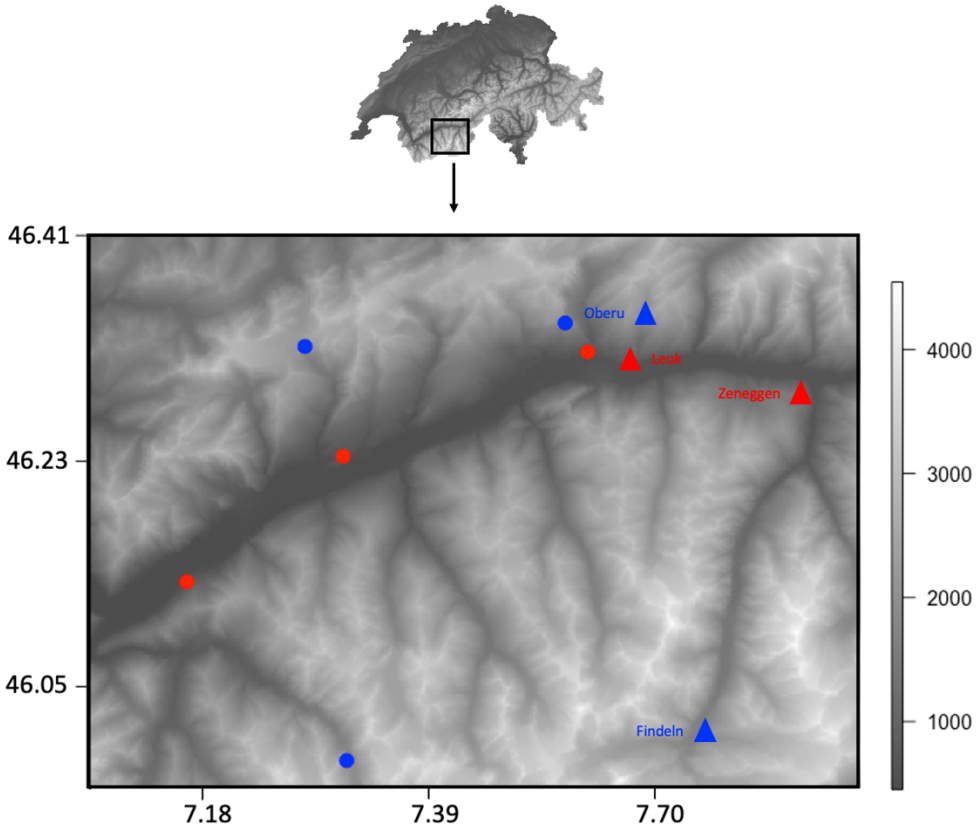

Figure S1. Map of the study area in the central Swiss Alps (Upper Rhône Valley). Locations of the three high (blue) and low (red) elevation populations of *D. sylvestris* (circles) and four transplant sites (triangles).

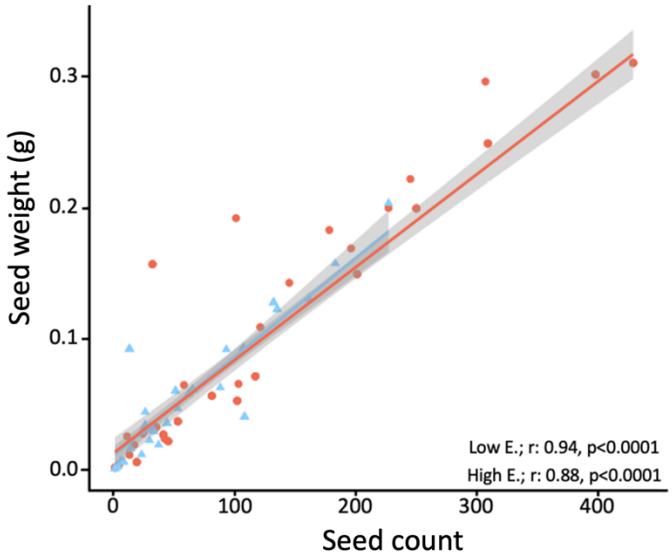

Figure S2. Correlation between seed weight (g) and seed count for seeds from 84 and 82 F2 individuals growing in the low (red) and the high (blue) elevation transplant site, respectively. Blue and red lines with 95% confidence intervals indicate the predicted relationship based on linear model regressions. Correlation coefficients (r) and p-values are reported.

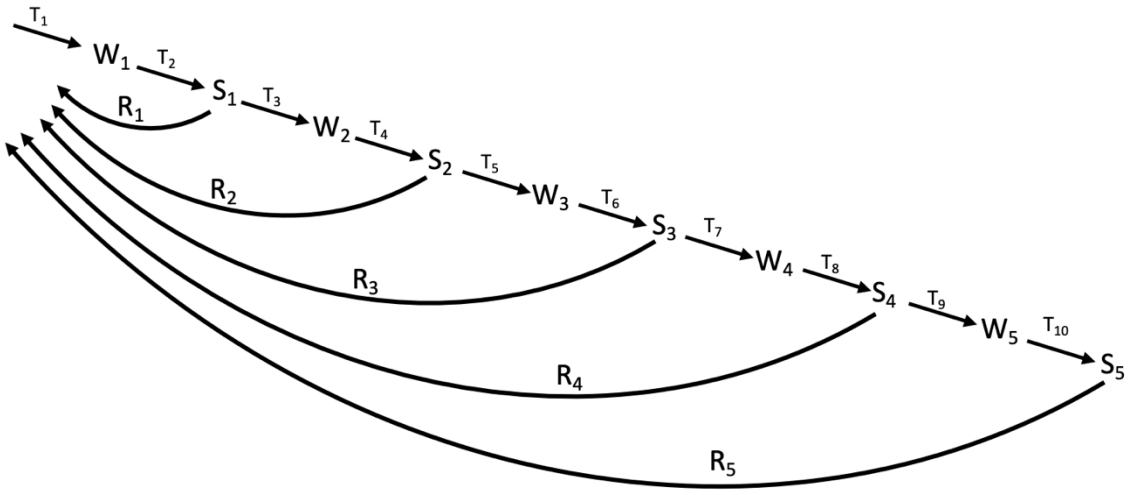

Figure S3. Age-classified life-cycle graph for *D. sylvestris* used for the matrix population models. The life cycle is divided into subsequent summer (S_i) and winter (W_i) stages, represented by indexes. The vital rates (i.e., survival T_i and reproduction R_i) are inferred as transitions between the stages.

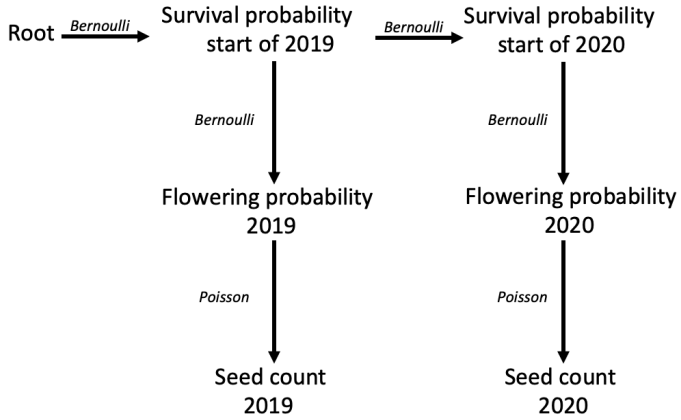

Figure S4. Life-history graph depicting the structure of the aster models. The graph consists of three layers representing, survival probability, flowering probability and seed count. Each node in the graph represents performance in the separate fitness components for the different seasons. The binary variables of survival and flowering probability were modelled using Bernoulli error distribution and the seed count data using Poisson error distribution.

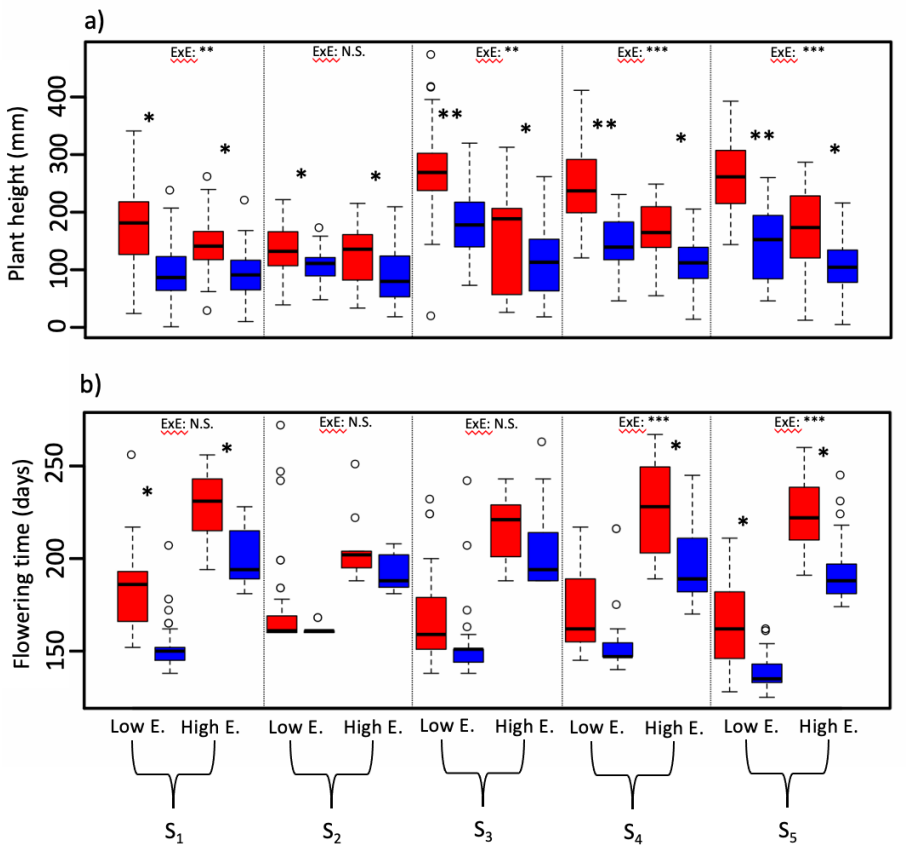

Figure S5. Phenotypic divergence in a) plant height (mm) and b) flowering time (days) of elevational ecotypes growing in the low and high environment at subsequent growing seasons. Boxes represent the raw values and statistical significance is inferred from linear mixed effect models. S_i denote the growing seasons. Low env. and High env. denote low and high environments, respectively. Red and blue denote the low and high elevation ecotypes, respectively. Significance of ecotype by environment interactions (ExE) and contrasts consistent to differential performance within each transplant environment are reported (***p<0.001, **p<0.01, *p<0.05).

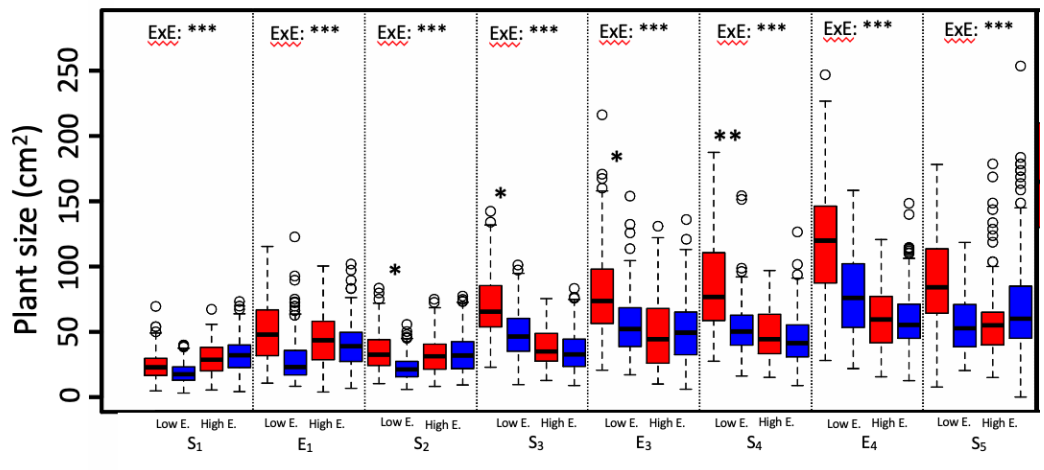

Figure S6. Phenotypic divergence in plant size (cm^2) of elevational ecotypes growing in the low and high environment at subsequent stages of the life cycle. Boxes represent the raw values and statistical significance is inferred from linear mixed effect models. Low env. and High env. denote low and high environment, respectively. Red and blue denote the low and high elevation ecotypes, respectively. Significance of ecotype by environment interactions (ExE) and contrasts consistent to differential performance within each transplant environment are reported (***p<0.001, **p<0.01, *p<0.05).

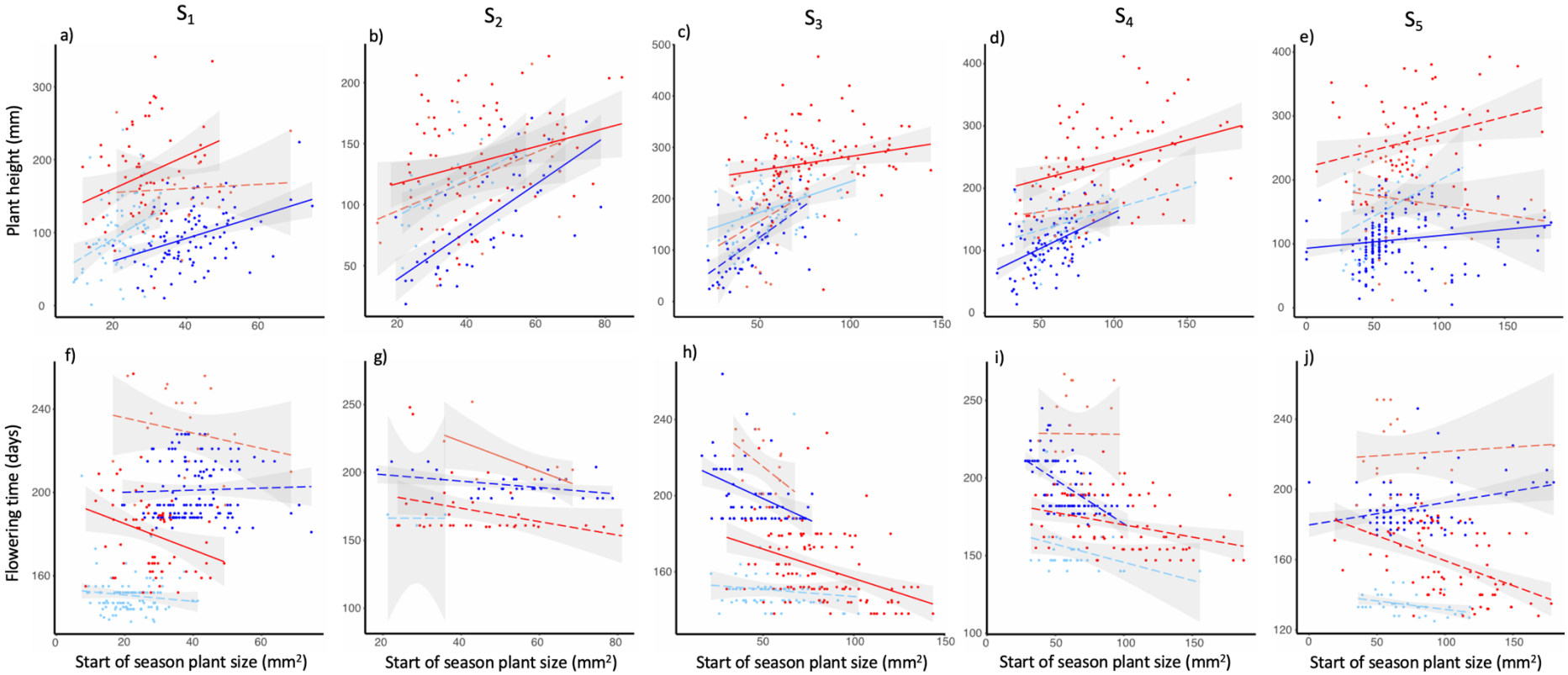

Figure S7. Relationship between plant height (mm), flowering time (days) and plant size (mm^2) of elevational ecotypes growing in the low and high environments at subsequent growing seasons. Top panels (a-e), relationship between plant height and plant size. Bottom panels (f-j), relationship between flowering time and plant size. S_i denote the growing seasons. Dark red and blue indicate low and high elevational ecotypes growing in their home low and high environment, respectively. Light red and blue indicate low and high and high elevational ecotypes growing in their respective away environments. Filled and dashed lines indicate statistically significant (0.05>p) and nonsignificant relationships, respectively.

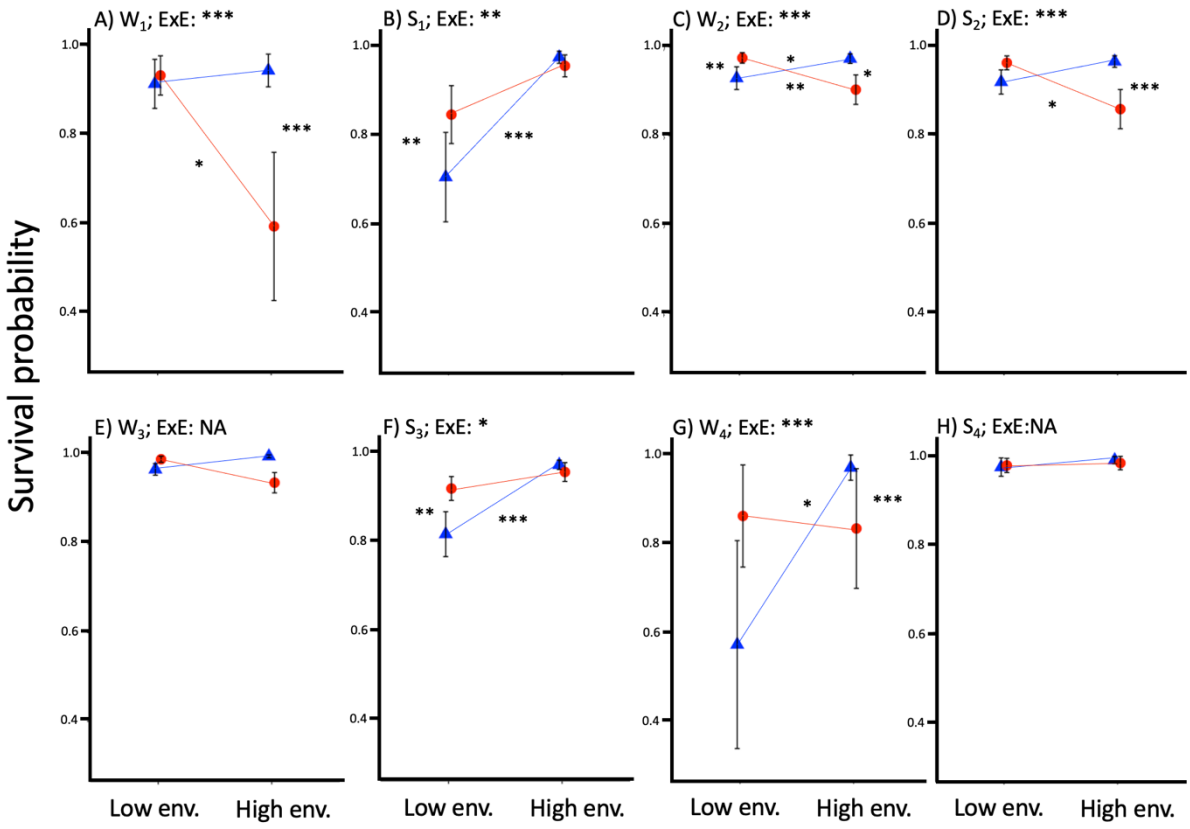

Figure S8. Performance in survival probability of elevational ecotypes growing in the low and high environment at subsequent growing seasons. Symbols indicate mean estimate values inferred from generalized linear mixed effect models and bars indicate standard errors. Mean values are connected by reaction norms depicting the effect of the environment on each elevational ecotype. Red and blue colors denote the low and high ecotype, respectively and Low and High env. indicate the low and high environments and. W_i and S_i denote the life stages (W winter survival and S summer survival). Significance of ecotype by environment interactions (ExE) and contrasts consistent to the local vs. foreign and home vs. away criteria are reported (***p<0.001, **p<0.01, *p<0.05).

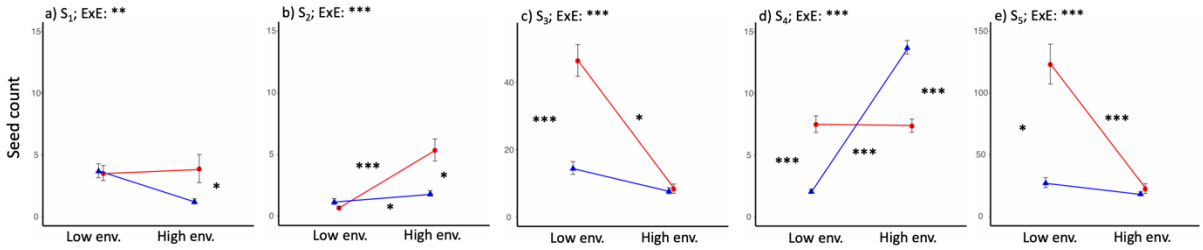

Figure S9. Performance in seed count of elevational ecotypes growing in the low and high environment at subsequent growing seasons. Symbols indicate mean estimate values inferred from generalized linear mixed effect models and bars indicate standard errors. Mean values are connected by reaction norms depicting the effect of the environment on each elevational ecotype. Red and blue colors denote the low and high ecotype, respectively and Low and High env. indicate the low and high environments and S_i denote the growing seasons. Significance of ecotype by environment interactions (ExE) and contrasts consistent with the local vs. foreign and home vs. away criteria are reported (***p<0.001, **p<0.01, *p<0.05).

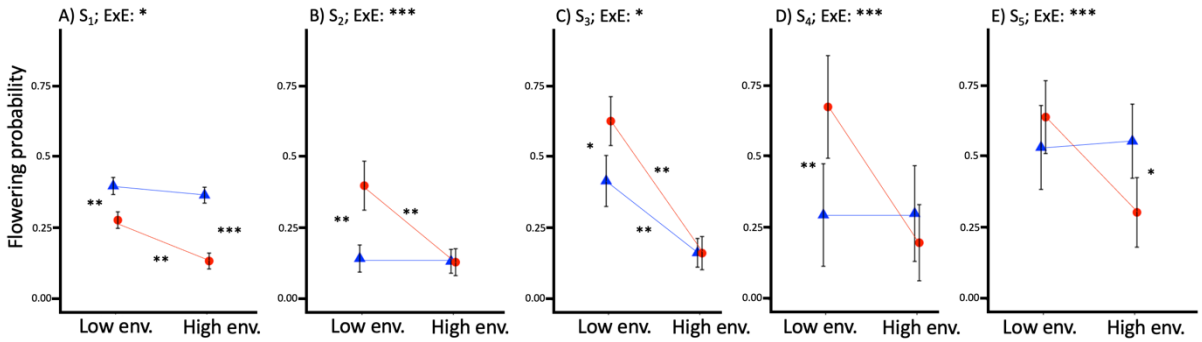

Figure S10. Performance in flowering probability of elevational ecotypes growing in the low and high environment at subsequent growing seasons. Symbols indicate mean estimate values inferred from generalized linear mixed effect models and bars indicate standard errors. Mean values are connected by reaction norms depicting the effect of the environment on each elevational ecotype. Red and blue colors denote the low and high ecotype, respectively, and low and high env. indicate the low and high environments and S_i denote the growing seasons. Significance of ecotype by environment interactions (ExE) and contrasts consistent with the local vs. foreign and home vs. away criteria are reported (***p<0.001, **p<0.01, *p<0.05).

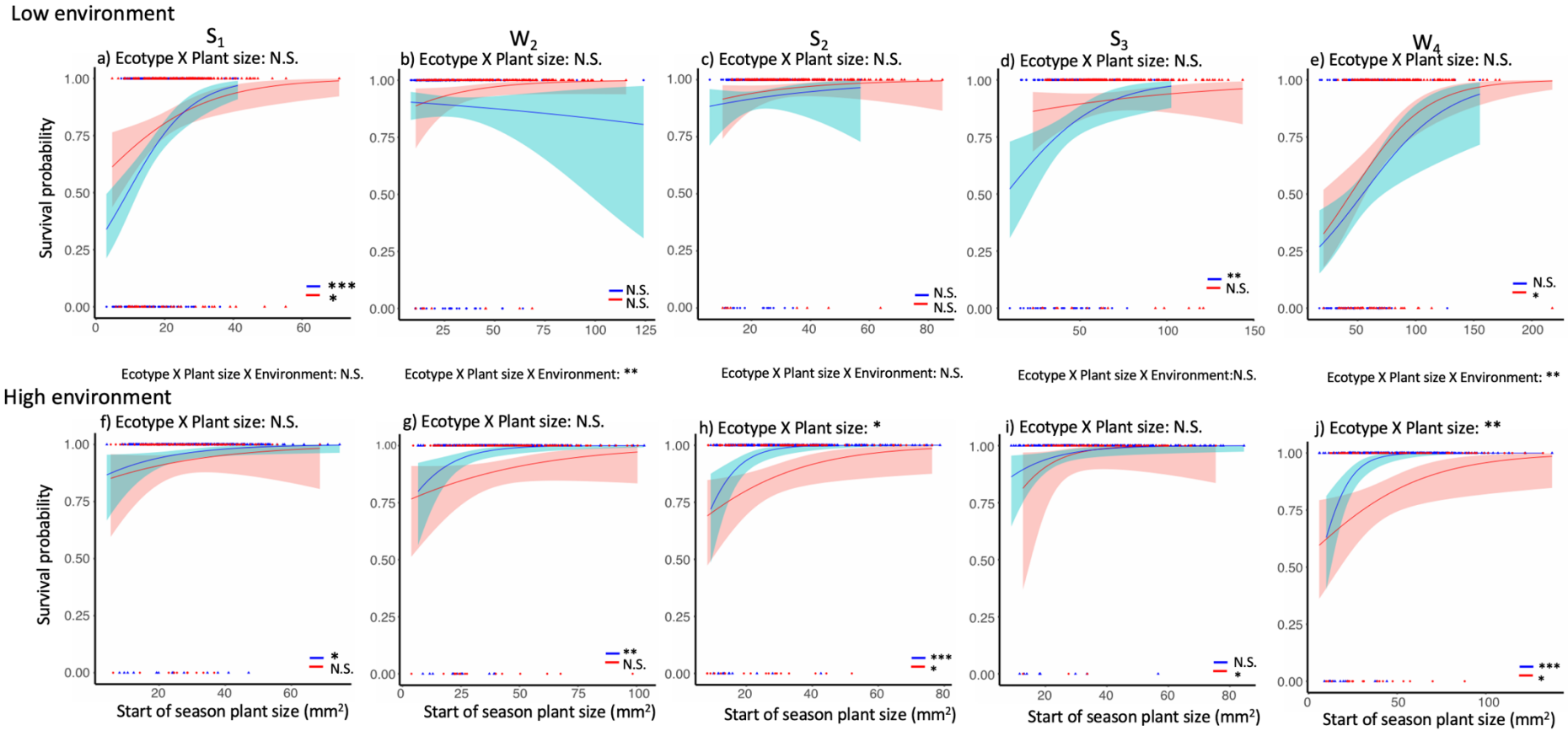

Figure S11. Effect of plant size on survival probability of elevational ecotypes growing in the low (a-e) and high (f-j) environments. W_i denotes the winter seasons. Red and blue lines indicate predicted relationships from generalized linear model regressions with 95% confidence intervals for the low and high elevation ecotypes, respectively. Corresponding red and blue triangles indicate empirical values of plant size at the start of each growing season. Significance of the three-way interaction between ecotype, environment and trait and ecotype by trait interactions within each environment and of the relationships between plant size at the start of the previous growing season and survival probability are reported (***p<0.001, **p<0.01, *p<0.05).

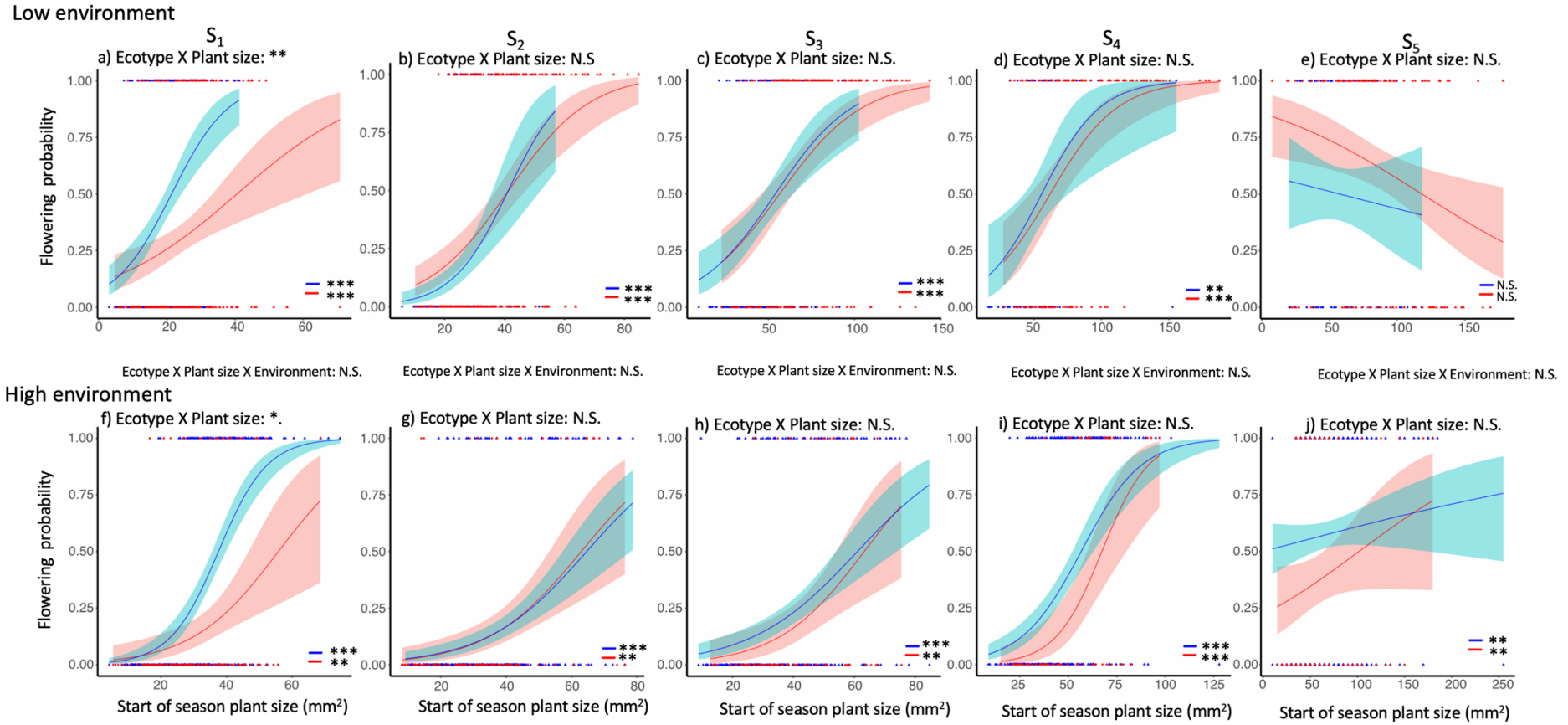

Figure S12. Effect of plant size on flowering probability of elevational ecotypes growing in the low (a-e) and high (f-j) environment. S_i denote the growing seasons. Red and blue lines indicate predicted relationships from generalized linear model regressions with 95% confidence intervals for the low and high elevation ecotypes, respectively. Corresponding red and blue triangles indicate empirical values of plant size at the start of each growing season. Significance of the three-way interaction between ecotype, environment and trait and ecotype by trait interactions within each environment and of the relationships between plant size at the start of each growing season and flowering probability are reported (***p<0.001, **p<0.01).

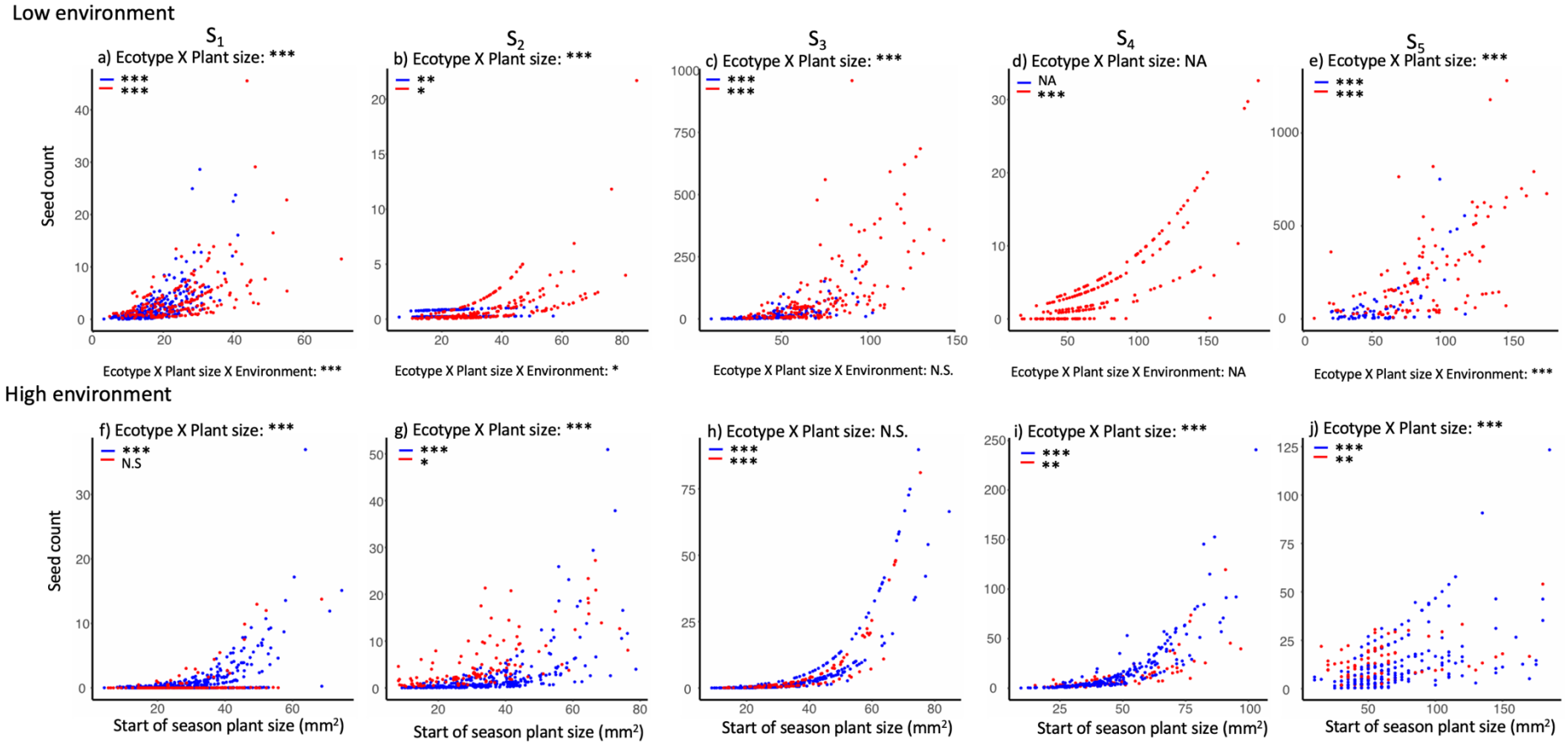

Figure S13. Effect of plant size on seed count of elevational ecotypes growing in the low (a-e) and high (f-j) environment. W_i denotes the winter seasons. Red and blue lines are added linear regression lines (with 95% confidence intervals), for the low and high ecotypes, respectively, on top of the relationship between plant size and seed count, estimated using zero-inflated poisson models. Significance of the three-way interaction between ecotype, environment and trait and ecotype by trait interactions within each environment and of the relationships between plant size at the start of the previous growing season and seed count are reported (***p<0.001, **p<0.01, *p<0.05).

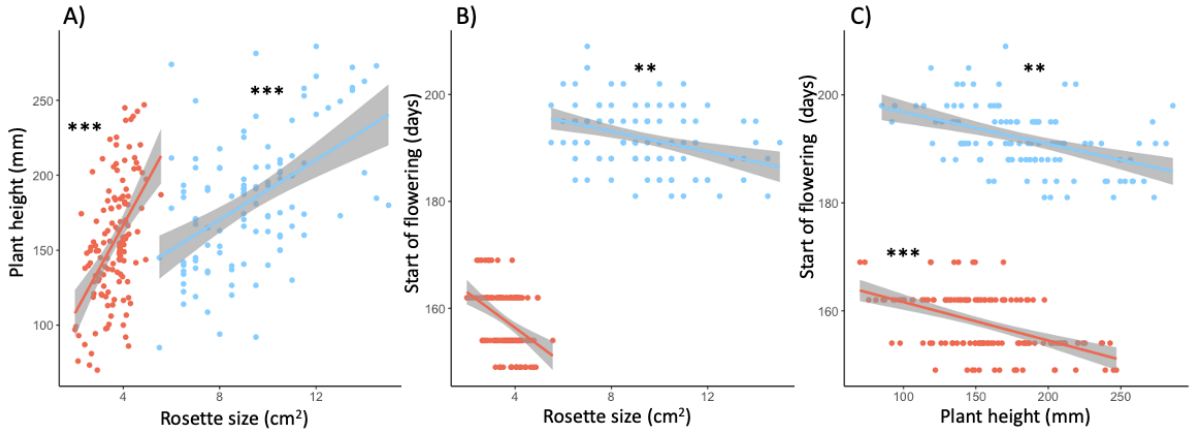

Figure S14. Relationship between plant size, plant height and flowering time at the low and high elevation transplant sites in 2019 and 2020, respectively. A) Plant height (mm) and plant size (cm^2), B) flowering time (days) and plant size (cm^2), C) flowering time (days) and plant height (cm^2), Red and blue denote data from the low site in 2019 and the high site in 2020, respectively. Significance of the effect of the predictor trait (X axes) on the response trait (Y axes) as extracted from linear mixed effect models are reported (***p<0.001, **p<0.01)

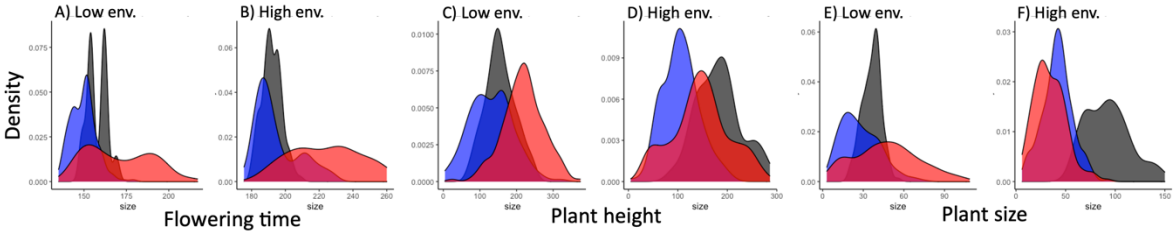

Figure S15. Density plots over trait distributions of wild and F2 populations growing in low and high environments and transplant sites (Low env. and High env.). Red, blue and black denote data of the low ecotype, high ecotype and the F2 populations, respectively.

Supplementary tables

Table S1. Locations of the wild *D. sylvestris* populations included in the study. Population name, elevation class (low versus high), elevation (meters above sea level), coordinate (latitude, longitude) and year sampled are reported.

Population	Elevation class	Elevation (m.a.s.l.)	Coordinates	Year sampled
Varen	Low	768	46.32, 7.62	2012
Saxon	Low	570	46.13, 7.16	2012
Saviese	Low	746	46.24, 7.34	2014
Chäller	High	2062	46.34, 7.60	2012
Tsanfleuron	High	2110	46.32, 7.30	2014
Val de Bagne	High	2281	45.99, 7.34	2014

Table S2. Overview of the reciprocal transplant experiment of wild *D. sylvestris* populations. Transplant site name, elevational environment denominator (i.e., low or high), elevation (meters above sea level), coordinates (latitude, longitude), nr. of plants per population and transplant site and nr. of maternal families and mean nr. of plants per family (± SD) are reported. Note that all the plant nrs. refer to plants alive after transplant shock and hence form the basis of our analyses.

Site	Elevational environment	Meters above sea level	Coordinates: latitude, longitude	Population	Number of plants alive after transplant shock	Number of maternal families	Mean nr. of plants per family ± SD
Leuk	Low	890	46.27, 7.88	Varen (low origin)	48	10	4.8±3.260
				Saxon (low origin)	42	8	5.25±2.252
				Saviese (low origin)	69	14	4.929±2.870
				Chäller (high origin)	48	17	2.824±1.667
				Tsansfleuron (high origin)	59	20	2.95±2.328
				Val de Bagne (high origin)	82	25	3.28±2.492
Zeneggen	Low	930	46.31, 7.66	Varen (low origin)	48	11	4.364±3.641
				Saxon (low origin)	38	7	5.429±0.976
				Saviese (low origin)	70	16	4.375±2.446
				Chäller (high origin)	54	16	3.375±1.5
				Tsansfleuron (high origin)	58	18	3.222±2.045
				Val de Bagne (high origin)	74	25	2.96±2.189
Findeln	High	2120	46.01, 7.76	Varen (low origin)	50	8	6.25±2.915
				Saxon (low origin)	31	8	3.875±1.727
				Saviese (low origin)	64	13	4.923+2.753
				Chäller (high origin)	52	16	3.25±2.082
				Tsansfleuron (high origin)	53	17	3.118±2.027
				Val de Bagne (high origin)	88	26	3.385±2.192
Oberu	High	2150	46.35, 7.67	Varen (low origin)	43	10	4.3±2.163
				Saxon (low origin)	32	8	4±1.604
				Saviese (low origin)	48	14	3.429±2.277
				Chäller (high origin)	66	19	3.474±2.366
				Tsansfleuron (high origin)	49	17	2.882±1.996
				Val de Bagne (high origin)	69	24	2.875±2.133

Table S3. Overview of the recruitment experiment of wild *D. sylvestris* populations. Transplant site name, elevational environment denominator (i.e., low or high), nr. of seeds sown per population and transplant site and nr. of maternal families and mean nr. of seeds per family (± SD) reported.

Transplant site	Transplant environment	Population	Nr. of seeds sown	Nr. of maternal families represented	Mean nr. of seeds per maternal family, ± SD
Leuk	Low	Varen (low origin)	100	5	20±0
		Saxon (low origin)	100	5	20±0
		Saviese (low origin)	100	4	25±4.082
		Châller (high origin)	100	5	20±0
		Tsansfleuron (high origin)	100	4	25±0
		Val de Bagne (high origin)	100	5	20±0
Zeneggen	Low	Varen (low origin)	100	5	20±0
		Saxon (low origin)	100	5	20±0
		Saviese (low origin)	100	5	20±0
		Châller (high origin)	100	5	20±0
		Tsansfleuron (high origin)	100	3	33.333±23.094
		Val de Bagne (high origin)	100	2	50±14.142
Findeln	High	Varen (low origin)	100	2	50±49.497
		Saxon (low origin)	100	4	25±4.082
		Saviese (low origin)	0	0	0
		Châller (high origin)	100	3	33.333±2.887
		Tsansfleuron (high origin)	100	4	25±4.082
		Val de Bagne (high origin)	100	4	25±4.082
Oberu	High	Varen (low origin)	100	2	50±49.497
		Saxon (low origin)	100	4	25±4.082
		Saviese (low origin)	0	0	0
		Châller (high origin)	100	3	33.333±2.887
		Tsansfleuron (high origin)	100	4	25±4.082
		Val de Bagne (high origin)	100	4	25±4.082

Table S4. Creation of F1 and F2 populations. Top: Sampling location of plants used to generate F1 crosses, including population name, elevational origin denominator (i.e., low or high) and nr. of grandparent plants used for F1 crosses reported. Bottom: production of F2 crosses, cage, F1 cross and nr. of plants per cross and cage reported. Note that the number after F1s refers to the different grandparent plants.

F0 plants used in production of F1 the generation		
Population	**Elevational origin**	**Nr. grandparent plants**
Saxon	Low	1
Varen	Low	1
Tsanfleuron	High	2
F2 crosses		
Cage	**F1**	**Nr. of plants**
1	Saxon_X_Tsanfleuron2	28
2	Saxon_X_Tsanfleuron2	15
2	Tsanfleuron_X_Varens1	15
3	Tsanfleuron_X_Varens1	13
3	Saxon_X_Tsanfleuron2	13
4	Saxon_X_Tsanfleuron2	10
4	Tsanfleuron_X_Saxon2	10
4	Tsanfleuron_X_Varens1	10
5	Tsansfleuron_X_Varens1	15

Table S5. Overview of the transplant experiment of F2 crosses. Transplant site, elevational environment denominator (i.e., low or high), cage number, number of plants per transplant size and cage and number of plants per genetic cluster as identified by PCA analysis (nrs. 1 to 3 arbitrarily chosen, Pålsson 2023, chapter 3) reported. Note that all the plant nrs. refer to plants alive at the start of 2018 and hence form the basis of our analyses.

Site	**Elevational environment**	**Cage**	**Number of plants alive after transplant shock**	**Number of plants per genetic cluster, 1; 2; 3**
Zeneggen	Low	1	122	122; 0; 0
		2	134	43; 67; 24
		3	132	52; 56; 24
		4	119	57;42;20
		5	114	144; 0; 0
Findeln	High	1	101	100; 1; 0
		2	122	32; 60; 30
		3	103	47; 47; 9
		4	124	62; 48; 14
		5	104	104; 0; 0

Table S6. Results of the plant height analyses. We tested for divergence in plant height by modelling it as the response variable in linear mixed effect models with a Gaussian error distribution. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold. S_i denote the growing seasons, Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**	**Standardized mean**
Model	plant height s1~ecotype*environment+(1|site) + (1|population/maternal family)	plant height s2~ecotype*environment+(1|site) + (1|population)	plant height s3~ecotype*environment+(1|site) + (1|population/maternal family)	plant height s4~ecotype*environment+(1|site) + (1|population/maternal family)	plant height s5~ecotype*environment+(1|site) + (1|population/maternal family)	mean plant height~ecotype*environment+(1|site/block) + (1|population/maternal family)
			χ^2; P-value			
GxE	**7.616; 0.005**	0.051; 0.821	**7.898; 0.005**	**15.921; <.0001**	**22.359; <.0001**	0.406; 0.524
Local vs. foreign			**Estimate; SE; p-value**			
Low env.	**-84.33; 18.5; 0.008**	**-26.2; 9.79; 0.022**	**-88.2; 15.7; 0.003**	**-114.81; 16.4; 0.0003**	**- 123.1; 19.4; 0.001**	**-1.010; 0.232; 0.010**
High env.	**-54.20; 19.3; 0.035**	**-29.1; 11.40; 0.02**	**-43.1; 19.4; 0.05**	**-52.04; 17.6; 0.017**	**- 60.0; 19.2; 0.023**	**-0.935; 0.238; 0.012**
Home vs. away						
Low eco.	-36.41; 25.0; 0.26	-11.7; 30.23; 0.732	-139.7; 61.9; 0.145	-66.65; 35.2; 0.182	-109.3; 33.6; 0.071	0.179; 0.376; 0.678
High eco.	-6.28; 24.1; 0.817	-14.6; 30.12; 0.672	-94.7; 61.3; 0.26	-3.88; 34.9; 0.921	-46.2; 33.3; 0.291	0.254; 0.371; 0.562

Table S7. Results of the flowering time analyses. We tested for divergence in flowering time by modelling it as the response variable in linear mixed effect models with a Gaussian error distribution. We tested for environment by environment interactions and differential performance of the environments according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold. S_i denote the growing seasons, Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**	**Standardized mean**
Model	flowering time s1~ecotype*environment +(1|site/site_plot) + (1|population/maternal family)	flowering time s2~ecotype*environment+(1|site) + (1|population/maternal family)	flowering time s3~ecotype*environment+(1|site) + (1|population/maternal family)	log(flowering time s4)~ecotype*environment+(1|site) + (1|population/maternal family)	flowering time s5~ecotype*environment+(1|site) + (1|population/maternal family)	mean flowering time~ecotype*environment+(1|site/block) + (1|population/maternal family)
			χ^2; P-value			
GxE	0.006; 0.940	0.277; 0.599	0.108; 0.743	**11.457; <.0001**	**6.062; 0.014**	**22.842; <.0001**
Local vs. foreign			**Estimate; SE; p-value**			
Low env.	**-26.1; 8.34; 0.034**	-12.4; 8.30; 0.202	-13.8; 7.98; 0.156	0.901; 0.048; 0.117	**-23.3; 9.47; 0.068**	-1.029; 0.445; 0.081
High env.	**-26.0; 8.54; 0.033**	-14.9; 8.80; 0.143	-15.1; 8.45; 0.131	**0.835; 0.045; 0.023**	**-29.4; 9.51; 0.034**	**-1.480; 0.449; 0.028**
Home vs. away						
Low eco.	**53.3; 12.81; 0.048**	**35.9; 7.66; 0.018**	**57.2; 11.66; 0.032**	**1.414; 0.102; 0.034**	**59.6; 9.94; 0.024**	0.982; 0.571; 0.224
High eco.	53.5; 12.64; 0.051	33.4; 7.25; 0.031	**55.9; 11.46; 0.037**	1.309; 0.094; 0.059	**53.4; 9.88; 0.031**	0.531; 0.567; 0.448

Table S8. Results of the plant size analyses. We tested for divergence in plant size by modelling it as the response variable in linear mixed effect models with a Gaussian error distribution. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Estimate, SE and p-values are reported, significant results are in bold. Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**S_1**	**E_1**	**S_2**	**S_3**	**E_3**	**S_4**	**E_4**	**S_5**	**Standardized mean**
Model	plant size start of s1 ~ecotype*environment+(1|site/block)+(1|population/maternal family)	plant size e1~ecotype*environment+(1|site/block)+(1|population/maternal family)	plant size s2~ecotype*environment+(1|site/block)+(1|population/maternal family)	plant size s3~ecotype*environment+(1|site/block) + (1|population/ maternal family)	plant size e3~ecotype*environment+(1|site/block) + (1|population/maternal family)	plant size s4~ecotype* environment +(1|site/block) + (1|population/maternal family)	plant size e4~ecotype*environment+(1|site/block) + (1|population /maternal family)	plant size s5~ecotype*environment+(1|site) + (1|population/maternal family)	mean plant size~ecotype*environment+(1|site/block) + (1|population/maternal family)
				χ^2; P-value					
GxE	**40.603; <.0001**	**51.014; <.0001**	**66.5; <.0001**	**49.221 ; <.0001**	**51.263; <.0001**	**40.968 ; <.0001**	**66.129; <.0001**	**41.765; <.0001**	**86.851; <.0001**
Local vs. foreign				**Estimate; SE; P-value**					
Low env.	-5.52 ; 2.60; 0.093	**-22.00; 6.19; 0.021**	**-13.00; 3.5; 0.016**	**-22.01; 6.19; 0.021**	**-26.39 ; 8.31; 0.030**	**-32.41; 7.92; 0.009**	**-43.70; 10.6; 0.010**	**-35.56; 8.46; 0.005**	**-0.736; 0.242; 0.035**
High env.	2.93; 2.64; 0.319	-3.97; 6.21; 0.554	1.73; 3.52; 0.646	-3.29; 6.24; 0.623	-1.24; 8,32; 0.888	-5.03; 7.70; 0.545	-2.94; 10.4; 0.789	5.51; 8.03; 0.522	0.156; 0.244; 0.555

Table S8. Continued.

Test	S_1	E_1	S_2	S_3	E_3	S_4	E_4	S_5	Standardized mean
				Estimate; SE; P-value					
Home vs. away									
Low eco.	5.45; 3.45; 0.241	-6.37; 7.58; 0.483	-3.8; 7.55; 0.663	-31.95; 9.38; 0.075	-32.56 ; 19.28; 0.230	-35.59; 12.95; 0.103	-56.09; 15.3; 0.061	-21.77; 20.25; 0.389	-0.473; 0.318; 0.268
High eco.	13.91; 3.38; 0.051	11.65; 7.51; 0.256	10.93; 7.50; 0.280	-13.23 ; 9.30; 0.288	-7.40; 19.23; 0.737	-8.20; 12.96; 0.588	-15.33; 15.3; 0.146	19.29; 20.21; 0.436	0.419; 0.315; 0.312

Table S9. Linear mixed effect models, and linear models for the effect of plant size on plant height and flowering time. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using pairwise contrasts in the emmeans R package; Response, models, estimates, SE, degrees of freedom (df), t value, and p-values are reported. S_i denote the growing seasons. Significant results are in bold.

Respone and fixed effects	**plant size * ecotype: 2-way interaction; χ^2; P-value**	**plant size * environment: 2-way interaction; χ^2; P-value**	**plant size * ecotype * environment: 3-way interaction; χ^2; P-value**	**Model**
plant height s1 ~ plant size start of s1*ecotype* environment	0.081; 0.776	0.271; 0.603	1.280;0.258	plant height s1 ~ plant size start of s1 *ecotype*environment +(1|site) + (1|population/maternal family)
plant height s2 ~ plant size start of s2*ecotype* environment	1.605; 0.205	0.000; 0.999	-0.0062; 0.804	plant height s2 ~ plant size start of s2*ecotype* environment+(1|site/block) + (1|population)
plant height s3 ~ plant size start of s3*ecotype* environment	0.427; 0.514	5.600; 0.014	-0.247; 0.619	plant height s3 ~ plant size start of s3*ecotype* environment+(1|site/block) + (1|population/maternal family)
plant height s4 ~ plant size start of s4*ecotype* environment	0.306; 0.580	0.623; 0.30	0.015; 0.902	plant height s4 ~ plant size start of s4*ecotype* environment+(1|site) + (1|population/maternal family)
plant height s5 ~ plant size start of s5*ecotype* environment	1.534; 0.215	0.381; 0.537	0.487; 0.485	plant height s5 ~ plant size start of s5*ecotype*environment+(1|site/block) + (1|population/maternal family)

Table S9. Continued.

Respone and fixed effects	plant size * ecotype: 2-way interaction; χ^2; P-value	plant size * environment: 2-way interaction; χ^2; P-value	plant size * ecotype * environment: 3-way interaction; χ^2; P-value	Model
flowering time s1 ~ plant size start of s1*ecotype* environment	0.373; 0.541	**4.361; 0.037**	0.251; 0.616	flowering time s1 ~ plant size start of s1*ecotype*environment+(1|site) + (1|population/maternal family)
flowering time s2 ~ plant size start of s2*ecotype* environment	**4.293; 0.038**	2.505; 0.113	0.191; 0.662	flowering time s2 ~ plant size start of s2*ecotype*environment+(1|site) + (1|population/maternal family)
flowering time s3 ~ plant size start of s3*ecotype* environment	0.711; 0.400	0.132; 717	0.004; 0.950	flowering time s3 ~ plant size start of s3*ecotype*environment+(1|site) + (1|population/maternal family)
flowering time s4 ~ plant size start of s4*ecotype* environment	**4.86; 0.026**	0.216; 0.642	0.815; 0.367	flowering time s4 ~ plant size start of s4*ecotype*environment+(1|site) + (1|population/maternal family)
flowering time s5 ~ plant size start of s5*ecotype* environment	3.210; 0.073	1.543; 0.214	0.234; 0.625	flowering time s5 ~ plant size start of s5*ecotype*environment+(1|site) + (1|population/maternal family)

Table S9. Continued.

	Within environments							
Response	**Environment**	**Ecotype**	**Trend; plant size start of season**	**SE**	**df**	**t.ratio**	**p-value**	**Model**
plant height s1	High environment	high	**1.324**	**0.323**	**104.2**	**4.097**	**0.0001**	plant height s1 ~ plant size start of s1*ecotype+(1|site) + (1|population)
		low	0.027	0.842	91.4	0.032	0.975	
	Low environment	high	1.34	1.217	125	1.101	0.273	plant height s1 ~ plant size start of s1 *ecotype+(1|site) + (1|population/maternal family)
		low	**1.84**	**0.858**	**123**	**2.145**	**0.034**	
plant height s2	High environment	high	**1.138**	**0.389**	**57.6**	**2.928**	**0.005**	plant height s2 ~ plant size start of s2*ecotype+(1|site) + (1|population/maternal family)
		low	0.924	0.475	54.8	1.943	0.057	
	Low environment	high	1.495	0.832	100	1.798	0.075	plant height s2 ~ plant size start of s2 *ecotype)
		low	**0.755**	**0.292**	**100**	**2.583**	**0.011**	
plant height s3	High environment	high	0.692	0.487	63.9	1.421	0.160	plant height s3 ~ plant size start of s3 *ecotype+(1|site) + (1|population/maternal family)
		low	1.671	1.135	51.4	1.472	0.147	
	Low environment	high	**1.195**	**0.347**	**171**	**3.449**	**0.001**	plant height s3 ~ plant size start of s3 *ecotype+(1|site) + (1|population/maternal family)
		low	**0.788**	**0.221**	**154**	**3.570**	**0.001**	
plant height s4	High environment	high	**1.132**	**0.365**	**8.28**	**3.104**	**0.014**	plant height s4 ~ plant size start of s4*ecotype+(1|site)
		low	0.325	0.612	111.05	0.531	0.597	
	Low environment	high	0.753	0.411	98.9	1.831	0.070	plant height s4 ~ plant size start of s4*ecotype+(1|site) + (1|population/maternal family)
		low	**0.678**	**0.174**	**67.2**	**3.901**	**0.0002**	

Table S9. Continued.

Within environments

Response	Environment	Ecotype	Trend; plant size start of season	SE	df	t.ratio	p-value	Model
plant height s5	High environment	high	**0.561**	**0.103**	**188**	**5.434**	**<.0001**	plant height s5 ~ plant size start of s5 *ecotype+(1|site)
		low	0.196	0.211	188	0.928	0.354	
	Low environment	high	0.287	0.458	102	0.627	0.32	plant height s5 ~ plant size start of s5*ecotype+(1|site) + (1|population/maternal family)
		low	0.390	0.222	102	1.759	0.082	
flowering time s1	High environment	high	-0.005	0.138	142	-0.038	0.969	flowering time s1 ~ plant size start of s1*ecotype + (1|population)
		low	-0.329	0.294	113	-1.116	0.267	
	Low environment	high	-0.292	0.168	164	-1.741	0.0834	flowering time s1 ~ plant size start of s1*ecotype + (1|population/maternal family)
		low	**-0.330**	**0.155**	**160**	**-2.126**	**0.035**	
flowering time s2	High environment	high	-0.195	0.097	35.8	-2.012	0.052	flowering time s2 ~ plant size start of s2*ecotype + (1|population)
		low	**-0.917**	**0.231**	**35.6**	**-3.969**	**0.0003**	
	Low environment	high	-0.004	1.637	31	-0.002	0.998	flowering time s2 ~ plant size start of s2*ecotype + (1|population)
		low	-0.284	0.247	32.7	-1.152	0.258	
flowering time s3	High environment	high	**-0.522**	**0.112**	**71.2**	**-4.650**	**<.0001**	flowering time s3 ~ plant size start of s3*ecotype + (1|population)
		low	-0.457	0.412	61.1	-1.111	0.271	
	Low environment	high	-0.072	0.99	172	-0.726	0.469	flowering time s3 ~ plant size start of s3*ecotype + (1|population)
		low	**-0.165**	**0.063**	**174**	**-2.631**	**0.009**	

Table S9. Continued.

Within environments								
Response	**Environment**	**Ecotype**	**Trend; plant size start of season**	**SE**	**df**	**t.ratio**	**p-value**	**Model**
flowering time s4	High environment	high	-0.209	0.095	107	-2.207	0.029	flowering time s4 ~ plant size start of s4*ecotype +(1|site/block) + (1|population)
		low	0.222	0.202	112	1.100	0.274	
	Low environment	high	-0.189	0.111	110	-1.697	0.092	flowering time s4 ~ plant size start of s4*ecotype + (1|population)
		low	-0.052	0.047	110	-1.095	0.276	
flowering time s5	High environment	high	0.001	0.028	216	3.539	0.001	flowering time s5 ~ plant size start of s5*ecotype +
		low	-0.077	0.056	216	-1.393	0.165	
	Low environment	high	-0.209	0.076	165	-2.738	0.007	flowering time s5 ~ plant size start of s5*ecotype+(1|population)
		low	**-0.220**	**0.038**	**165**	**-5.751**	**<.0001**	
Response	**Environment**	**Ecotype**	**ExT (trait) interaction, χ^2; P-value**	**Trend; plant size start of season**	**SE**	**df**	**z value**	**p-value**
mean flowering time	High environment	high	**8.629; 0.003**	-0.069	0.059	279	-1.161	0.247
		low		**-0.459**	**0.120**	**279**	**-3.829**	**0.0002**
	Low environment	high	1.058, 0.304	**-0.217**	**0.088**	**341**	**-2.473**	**0.014**
		low		**-0.330**	**0.067**	**342**	**-4.928**	**<.0001**
mean height	High environment	high	2.744; 0.098	**0.494**	**0.070**	**291**	**7.084**	**<.0001**
		low		**0.277**	**0.116**	**291**	**2.390**	**0.0097**
	Low environment	high	**3.930; 0.003**	**0.586**	**0.105**	**312**	**5.600**	**<.0001**
		low		**0.210**	**0.070**	**312**	**2.990**	**0.003**

Table S9. Continued.

Respone and fixed effects	plant size * ecotype: 2-way interaction; χ^2; P-value	plant size *environment: 2-way interaction; χ^2; P-value	plant size * ecotype * environment: 3-way interaction; χ^2; P-value	Model
mean flowering time ~ mean size * ecotype *environment	0.626; 0.429	**4.281; 0.039**	0.112; 0.738	mean flowering time ~ mean plant size*ecotype*environment+(1|site/block) + (1|population/maternal family)
mean height ~ mean size * ecotype *environment	1.491; 0.222	0.029; 0.864.	0.012; 0.911	mean height ~ mean plant size*ecotype*environment+(1|site/block) + (1|population/maternal family)

Table S10. Results of the LTRE showing the contribution of the vital rates of the foreign ecotype to population growth rate in the two transplant environments. Survival and reproductive vital rates throughout the life cycle are indicated by T and R, respectively. Mean estimates based on 20 000 bootstrap replicated are reported.

Environment	T_1	R_1	T_2	T_3	R_2	T_4	T_5	R_3	T_6	T_7	R_4	T_8	T_9	R_5	T_{10}
High	-0.052	-0.006	-0.003	-0.008	0.002	--0.012	-0.007	-0.008	-0.002	-0.015	-0.059	-2.43E-04	1.89E-04	-0.071	0
Low	-0.005	0.007	-0.027	-0.012	-0.005	-0.009	-0.004	-0.212	-0.006	-0.015	-0.005	-0.001	0.001	-0.081	0

Table S11. Results of the cumulative survival analyses. We modelled survival throughout the experiment using cox proportional hazard models and tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We report coefficients, hazard ratios and p-values, significant results are in bold. Low env. and High env. denote low and high environment, respectively.

Test	Coefficients	Hazard ratio	p-values
GxE	**-2.457**	**0.086**	**<.0001**
Local vs. foreign			
Low env.	**-0.734**	**0.48**	**0.002**
High env.	**1.627**	**5.088**	**<.0001**
Home vs. away			
Low ecotype	-0.587	0.556	0.066
High ecotype	**1.96**	**7.099**	**0.016**

Table S12. Results of survival probability analyses. We tested for adaptation in survival probability by modelling it as the response variable in generalized linear mixed effect models with binomial error distribution. We tested for eccotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Models, estimate, SE and p-values are reported, significant results are in bold. W_i and S_i denote the season. Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**W_1**	**S_1**	**W_2**	**S_2**	**W_3**	**S_3**	**W_4**	**S_4**	**W_5**
Model	survival probability w1 (y/n) ~ecotype*environment+(1|site/block)	survival probability s1 (y/n)~ecotype*environment+(1|site/block) + (1|population/maternal family)	survival probability w2 (y/n)~ecotype*environment+(1|site/block)	survival probability s2 (y/n)~ecotype*environment+(1|site/block)	NA	survival probability s3 (y/n)~ecotype*environment+(1|site/block)	survival probability w4 (y/n)~ecotype*environment+(1|site/block)+ (1|population/maternal family)	NA	NA
				χ^2; P-value					
GxE	**62.668; <.0001**	**7.610; 0.006**	**17.01; <.0001**	**16.795; <.0001**	NA	**4.674; 0.031**	**42.559; <.0001**	NA	NA
								NA	NA
Local vs. foreign				**Estimate; SE; P-value**				NA	NA
Low env.	0.76; 0.191; 0.2743	**0.436; 0.132; 0.0062**	**3.878; 1.650; 0.0015**	0.444; 0.188; 0.0547	NA	**0.380; 0.121; 0.003**	0.804; 1.089; 0.872	NA	NA
High env.	**11.814; 3.017; <.0001**	1.805; 0.839; 0.2034	**0.339; 0.148; 0.0134**	**4.613; 1.779; 0.0001**	NA	1.72; 0.997; 0.353	**6.492; 2.832; <.0001**	NA	NA
Home vs. away								NA	NA
Low eco.	**0.103; 0.103; 0.023**	3.928; 2.897; 0.0636	**0.242; 0.146; 0.019**	**0.236; 0.133; 0.010**	NA	2.01; 1.329; 0.291	0.804; 1.089; 0.872	NA	NA
High eco.	1.604; 1.612; 0.6384	**16.275; 11.609; 0.0001**	2.760; 1.513; 0.0640	2.450; 1.304; 0.092	NA	**9.07; 5.068; 0.0001**	**24.266; 33.034; 0.019**	NA	NA

Table S13. Results of seed count analyses. We tested for adaptation in seed count by modelling it as the response variable in generalized linear mixed effect models with zero inflated poisson error distribution. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Models, estimate, SE and p-values are reported, significant results are in bold. S_i denote the growing season. Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**	**Cumulative seed count**
Model	seed count s1~ecotype*environment+(1|site/block) + (1|population/maternal family)	seed count s2~ecotype*environment+(1|site/block) + (1|population)	seed count s3~ecotype*environment+(1|site/block) + (1|population)	seed count s4~ecotype*environment+(1|site/block) + (1|population)	seed count s5~ecotype*environment+(1|site/block) + (1|population)	cumulative seed count~ecotype+environment+(1|site/block) + (1|population/maternal family)
			χ^2; P-value			
GxE	**10.349; 0.001**	**103.218; <.0001**	**319.57; <.0001**	**92.661; <.0001**	**704.72; <.0001**	**3262.9; <.0001**
Local vs. foreign			**Estimate; SE; P-value**			
Low env.	1.105; 0.356; 0.757	1.85; 1.0; 0.256	**0.311; 0.101; <0.001**	**0.31; 0.084; <.0001**	**0.279; 0.138; 0.010**	**0.214; 0.061; <.0001**
High env.	0.350; 0.159; 0.021	**0.33; 0.17; 0.028**	0.832; 0.274; 0.577	**1.94; 0.295; <.0001**	0.892; 0.441; 0.817	0.982; 0.281; 0.951
Home vs. away						
Low eco.	0.391; 0.564; 0.515	**11.27; 3.56; <.0001**	0.094; 0.103; 0.031	0.90; 0.227; 0.663	**0.169; 0.050; <.0001**	**0.250; 0.067; <.0001**
High eco.	0.124; 0.175; 0.139	2.03; 0.63; 0.023	0.252; 0.275; 0.207	**5.69; 1.927; <.0001**	**0.541; 0.160; 0.038**	1.146; 0.305; 0.608

Table S14. Results of flowering probability analyses. We tested for adaptation in flowering probability by modelling it as the response in generalized linear mixed effect models with binomial error distribution. We tested for ecotype by environment interactions and differential performance of the ecotypes according to the local vs. foreign and home vs. away criteria of local adaptation. We tested the significance of the interaction between elevation and ecotype using likelihood ratio tests and report χ^2 and p-values. Local vs. foreign and home vs. away contrasts were estimated using pairwise contrasts in the emmeans R package; Models, estimate, SE and p-values are reported, significant results are in bold. S_i denote the growing season. Low env. and Low eco. and High env. and High eco. the low and high environments and ecotypes, respectively.

Test	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
Model	flowering probability s1 (y/n) ~ecotype*environment +(1|site)	flowering probability s2 (y/n) ~ecotype* environment+(1|site/ block) + (1|population/maternal family)	flowering probability s3 (y/n) ~ecotype*environment+(1|site/block) + (1|population/maternal family)	flowering probability s4 (y/n)~ecotype*environment+(1|site/block) + (1|population/maternal family)	flowering probability s5 (y/n)~ecotype*environment+(1|site/block) + (1|population/maternal family)
			χ^2; P-value		
GxE	**6.298; 0.012**	**13.264; <.0001**	**5.444; 0.012**	**17.242; <.0001**	**11.145; <.0001**
Local vs. foreign			**Estimate; SE; P-value**		
Low env.	**1.716; 0.308; 0.003**	**0.253;0.1063; 0.0011**	**0.425; 0.163; 0.026**	**0.202; 0.105; 0.002**	0.643; 0.297; 0.339
High env.	**3.722;0.968; <.0001**	1.027; 0.460; 0.953	1.009; 0.433; 0.983	1.734; 0.726; 0.188	**2.839; 1.208; 0.014**
Home vs. away					
Low eco.	**0.402; 0.113; 0.001**	**0.227; 0.097; 0.001**	**0.116; 0.054; <.0001**	0.119; 0.136; 0.062	0.247; 0.183; 0.059
High eco.	0.872; 0.152; 0.432	0.922; 0.369 ; 0.839	**0.275; 0.115; 0.002**	1.734; 0.726; 0.188	1.093; 0.797; 0.9032

Table S15. Influence of specific vital rates on population growth rate at the two transplant environments. Influence of specific vital rates on population growth rates extracted from the matrix population models of the elevational ecotypes growing in the low and high environments expressed as elasticities. Survival and reproductive vital rates throughout the life cycle are indicated by T and R, respectively, Mean values are based on 20 000 bootstrap replicates and 95% bias corrected confidence intervals are reported. Env. and Eco. denote the environments and the ecotypes, respectively.

Env.	**Eco.**	**T_1**	**R_1**	**T_2**	**T_3**	**R_2**	**T_4**	**T_5**	**R_3**	**T_6**	**T_7**	**R_4**	**T_8**	**T_9**	**R_5**	**T_{10}**
High	High	0.121 (0.115, 0.126)	0.007 (0.004, 0.012)	0.113 (0.109, 0.118)	0.113 (0.109, 0.118)	0.003 (0.001, 0.006)	0.111 (0.107, 0.115)	0.111 (0.107, 0.115)	0.01 (0.005, 0.019)	0.1 (0.094, 0.106)	0.1 (0.094, 0.106)	0.046 (0.03, 0.063)	0.055 (0.041, 0.068)	0.055 (0.041, 0.068)	0.055 (0.041, 0.068)	0 (0.041, 0.068)
High	Low	0.111 (0.105, 0.123)	0 (0, 0.001)	0.111 (0.105, 0.123)	0.111 (0.105, 0.123)	0.007 (0.002, 0.034)	0.103 (0.095, 0.109)	0.103 (0.095, 0.109)	0.009 (0.002, 0.025)	0.095 (0.084, 0.101)	0.095 (0.084, 0.101)	0.015 (0.004, 0.037)	0.08 (0.062, 0.092)	0.08 (0.062, 0.092)	0.08 (0.062, 0.092)	0 (0.062, 0.092)
Low	High	0.166 (0.141, 0.193)	0.036 (0.019, 0.074)	0.13 (0.113, 0.146)	0.13 (0.113, 0.146)	0.001 (0, 0.005)	0.129 (0.112, 0.145)	0.129 (0.112, 0.145)	0.092 (0.048, 0.128)	0.037 (0.016, 0.065)	0.037 (0.016, 0.065)	0.001 (0, 0.003)	0.037 (0.015, 0.065)	0.037 (0.015, 0.065)	0.037 (0.015, 0.065)	0 (0.015, 0.065)
Low	Low	0.154 (0.144, 0.164)	0.012 (0.006, 0.022)	0.143 (0.133, 0.151)	0.143 (0.133, 0.151)	0.003 (0.001, 0.006)	0.14 (0.13, 0.148)	0.14 (0.13, 0.148)	0.107 (0.083, 0.127)	0.033 (0.021, 0.047)	0.033 (0.021, 0.047)	0.002 (0.001, 0.004)	0.031 (0.019, 0.045)	0.031 (0.019, 0.045)	0.031 (0.019, 0.045)	0 (0.019, 0.045)

Table S16. Stable age distribution. Stable age distribution of the elevational ecotypes growing in the low and high environments. Winter and summer stages are represented by W and S, respectively. Values are based on 20 000 bootstrap replicates and 95% bias corrected confidence intervals are reported. Env. and Eco. denote the environments and the ecotypes, respectively.

Env.	Eco.	W_1	S_1	W_2	S_2	W_3	S_3	W_4	S_4	W_5	S_5
High	High	0.15 (0.132, 0.17)	0.129 (0.113, 0.149)	0.116 (0.102, 0.131)	0.104 (0.094, 0.116)	0.093 (0.084, 0.103)	0.086 (0.077, 0.096)	0.078 (0.068, 0.088)	0.069 (0.06, 0.079)	0.063 (0.052, 0.074)	0.058 (0.049, 0.069)
High	Low	0.074 (0.05, 0.105)	0.053 (0.036, 0.074)	0.06 (0.045, 0.081)	0.066 (0.051, 0.085)	0.069 (0.056, 0.087)	0.08 (0.066, 0.098)	0.093 (0.077, 0.113)	0.094 (0.075, 0.116)	0.111 (0.086, 0.14)	0.136 (0.107, 0.173)
Low	High	0.217 (0.176, 0.266)	0.176 (0.147, 0.211)	0.122 (0.106, 0.141)	0.106 (0.094, 0.122)	0.094 (0.08, 0.109)	0.054 (0.045, 0.064)	0.068 (0.051, 0.087)	0.036 (0.024, 0.049)	0.032 (0.02, 0.047)	0.031 (0.018, 0.046)
Low	Low	0.413 (0.381, 0.447)	0.237 (0.212, 0.264)	0.131 (0.115, 0.148)	0.084 (0.072, 0.097)	0.054 (0.045, 0.064)	0.054 (0.045, 0.064)	0.022 (0.016, 0.027)	0.011 (0.008, 0.014)	0.007 (0.005, 0.009)	0.004 (0.003, 0.006)

Table S17. Generalized linear mixed effect models, and generalized linear models for the effect of plant size on flowering probability. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using pairwise contrasts in the emmeans R package; Response, models, estimates, SE, degrees of freedom (df), z value, and p-values are reported. S_i denote the growing seasons. Significant results are in bold.

Respone and fixed effects	**plant size*ecotype: 2-way interaction; χ^2; P-value**	**plant size*environment: 2-way interaction; χ^2; P-value**	**plant size * ecotype * environment: 3-way interaction; χ^2; P-value**	**Model**
flowering probability s1 ~ plant size start of s1 *ecotype* environment	**13.12; 0.0003**	0.00; 0.99	0.15; 0.697	flowering probability s1 (y/n) ~ plant size start of s1 ecotype*environment+(1|site) + (1|population/maternal family)
flowering probability s2 ~ plant size start of s2*ecotype* environment	1.13; 0.288	0.01; 0.936	0.63; 0.427	flowering probability s2 (y/n) ~ plant size start of s2*ecotype*environment)
flowering probability s3 ~ plant size start of s3*ecotype* environment	0.0; 0.937	2.1; 0.149	0.4; 0.536	flowering probability s3 (y/n) ~ plant size start of s3*ecotype*environment
flowering probability s4 ~ plant size start of s4*ecotype* environment	0.1; 0.736	**4.4; 0.036**	0.8; 0.375	flowering probability s4 (y/n) ~ plant size start of s4*ecotype*environment

Table S17. Continued.

Respone and fixed effects	plant size*ecotype: 2-way interaction; χ^2; P-value	plant size*environment: 2-way interaction; χ^2; P-value	plant size * ecotype * environment: 3-way interaction; χ^2; P-value	Model
flowering probability s5 ~ plant size start of s5*ecotype* environment	0.99; 0.319	-8.34; 0.004	2.52; 0.112	flowering probability s5 (y/n) ~ plant size start of s5*ecotype*environment+(1|site/block) + (1|population)
flowering probability ~ plant size *ecotype* environment + year	3.12; 0.077	0.01; 0.942	**6.54; 0.011**	flowering probability ~ plant size*ecotype*environment+year+(1|site/block)+(1|population)

Within environments

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size start of season	SE	df	z value	p-value	Model
flowering probability s1	High environment	high	**4.4; 0.036**	**0.136**	**0.017**	**Inf**	**8.200**	**<.0001**	flowering probability s1 (y/n) ~ plant size start of s1*ecotype + (1|population)
		low		**0.076**	**0.023**	**Inf**	**3.340**	**0.0008**	
	Low environment	high		**0.176**	**0.027**	**Inf**	**6.510**	**<.0001**	flowering probability s1 (y/n) ~ plant size start of s1*ecotype+(1|site/block) + (1|population/maternal family)
		low	**11.6; 0.001**	**0.078**	**0.018**	**Inf**	**4.390**	**<.0001**	
flowering probability s2	High environment	high	0.03; 0.85	**0.065**	**0.012**	**Inf**	**5.390**	**<.0001**	flowering probability s2 (y/n) ~ plant size start of s2*ecotype + (1|population/maternal family)
		low		**0.069**	**0.018**	**Inf**	**3.810**	**0.0001**	
	Low environment	high		**0.124**	**0.028**	**Inf**	**4.5**	**<.0001**	flowering probability s2 (y/n) ~ plant size start of s2*ecotype+(1|site/block) + (1|population/maternal family)
		low	1.75; 0.19	**0.085**	**0.016**	**Inf**	**5.330**	**<.0001**	

Table S17. Continued.

Within environments

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size start of season	SE	df	z value	p-value	Model
flowering probability s3	High environment	high		**0.063**	**0.011**	**Inf**	**5.690**	**<.0001**	flowering probability s3 (y/n) ~ plant size start of s3*ecotype + (1|population)
		low	0.53; 0.47	**0.081**	**0.024**	**Inf**	**3.400**	**0.0007**	
	Low environment	high		**0.0460**	**0.011**	**Inf**	**4.310**	**<.0001**	flowering probability s3 (y/n) ~ plant size start of s3*ecotype + (1|population)
		low	0.22; 0.64	**0.040**	**0.009**	**Inf**	**4.650**	**<.0001**	
Flowering probability s4	High environment	high		**0.064**	**0.009**	**Inf**	**6.930**	**<.0001**	flowering probability s4 (y/n) ~ plant size start of s4*ecotype
		low	0.8; 0.373	**0.083**	**0.020**	**Inf**	**4.110**	**<.0001**	
	Low environment	high		**0.046**	**0.016**	**Inf**	**2.810**	**0.0050**	flowering probability s4 (y/n) ~ plant size start of s4*ecotype
		low	0.1; 0.74	**0.040**	**0.009**	**Inf**	**4.440**	**<.0001**	
Flowering probability s5	High environment	high		**0.013**	**0.004**	**Inf**	**3.000**	**0.003**	flowering probability s5 (y/n) ~ plant size start of s5*ecotype+(1|site) + (1|population)
		low	3.09; 0.079	**0.029**	**0.008**	**Inf**	**3.460**	**0.0005**	
	Low environment	high		0.002	0.011	Inf	0.2	0.841	flowering probability s5 (y/n) ~ plant size start of s5*ecotype+(1|site) + (1|population)
		low	0.88; 0.35	-0.009	0.007	Inf	-1.22	0.223	
flowering probability	High environment	high		**0.908**	**0.073**	**Inf**	**12.370**	**<.0001**	flowering probability ~ plant size*ecotype+year+(1|site/block) + (1|population/maternal family)
		low	0.95; 0.33	**1.048**	**0.126**	**Inf**	**8.320**	**<.0001**	
	Low environment	high		**1.256**	**0.135**	**Inf**	**9.300**	**<.0001**	flowering probability ~ plant size*ecotype+year+(1|site/block) + (1|population/maternal family)
		low	**15.3; <.0001**	**0.663**	**0.082**	**Inf**	**8.090**	**<.0001**	

Table S18. Generalized linear mixed effect models, and generalized linear models for the effect of plant size on survival probability. S_i and W_i indicate summer and winter stages, respectively, s_i and e_i denote start and end of growing seasons, respectively. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using pairwise contrasts in the emmeans R package; Response, models, estimates, SE, degrees of freedom (df), z value, and p-values are reported. Significant results are in bold.

Respone and fixed effects	**plant size*ecotype: 2-way interaction; χ^2; P-value**	**plant size*environment: 2-way interaction; χ^2; P-value**	**plant size size * ecotype * environment: 3-way interaction; χ^2; P-value**	**Model**
survival probability S1 ~ plant size s1 *ecotype* environment	2.50; 0.114	1.15; 0.285	0.21; 0.643	survival S1 (y/n) ~ plant size s1*ecotype*environment+ (1|site)
survival probability W2 ~ plant size e1 *ecotype* environment	0.17; 0.677	2.43; 0.119	**6.77; 0.009**	survival W2 (y/n) ~ plant size e1*ecotype*environment+ (1|site)
survival probability S2 ~ plant size s2 *ecotype* environment	2.13; 0.145	2.72; 0.099	3.34; 0.068	survival S2 (y/n) ~ plant size s2*ecotype*environment
survival probability W3 ~ plant size s2 *ecotype* environment	NA	NA	NA	NA
survival probability S3 ~ plant size s3 *ecotype* environment	2.0; 0.161	3.6; 0.057	0.6; 0.457	survival S3 (y/n) ~ plant size s3*ecotype*environment
survival probability W4 ~ plant size e3 *ecotype* environment	0.9; 0.351	3.5; 0.062	**8.3; 0.004**	survival W4 (y/n) ~ plant size e3*ecotype*environment
survival probability S4 ~ plant size s4 *ecotype* environment	NA	NA	NA	NA
survival probability W5 ~ plant size e4 *ecotype* environment	NA	NA	NA	NA
survival probability S5 ~ plant size s5 *ecotype* environment	NA	NA	NA	NA
survival probability ~ plant size * ecotype * environment	**4.83; 0.028**	**3.95; 0.047**	**8.54; 0.004**	survival probability ~ plant size*ecotype*environment+year+(1|site)+(1|population)

Table S18. Continued.

Within environments

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size start of season	SE	df	z value	p-value	Model
survival probability S1	High environment	high		**0.067**	**0.027**	**Inf**	**2.436**	**0.015**	survival S1 (y/n) ~ plant size
		low	0.36; 0.55	0.041	0.032	Inf	1.271	0.204	s1*ecotype + (1|site)
	Low environment	high		**0.094**	**0.0237**	**Inf**	**3.950**	**0.0001**	
			2.42; 0.12						survival S1 (y/n) ~ plant size
		low		**0.045**	**0.022**	**Inf**	**2.070**	**0.0389**	s1*ecotype+(1|site/block)
survival probability W2	High environment	high		**0.0837**	**0.0273**	**Inf**	**3.062**	**0.0022**	survival W2 (y/n) ~ plant size
		low	3.67; 0.055	0.0268	0.0157	Inf	1.711	0.0872	e1*ecotype + (1|site/block)
	Low environment	high		-0.014	0.014	Inf	-1.015	0.310	
									survival W2 (y/n) ~ plant size
		low	3.76; 0.052	0.029	0.018	Inf	1.587	0.113	e1*ecotype+(1|site/block)
survival probability S2	High environment	high		0.157	0.046	Inf	1.344	0.0006	survival S2 (y/n) ~ plant size
		low	5.38; 0.02	**0.050**	**0.022**	**Inf**	**2.270**	**0.0233**	s2*ecotype
	Low environment	high		0.025	0.033	Inf	0.778	0.437	
									survival S2 (y/n)~ plant size
		low	0.084; 0.77	0.038	0.030	Inf	1.266	0.206	s2*ecotype
survival probability W3	High environment	high		NA	NA	NA	NA	NA	
		low	NA	NA	NA	NA	NA	NA	NA
	Low environment	high		NA	NA	NA	NA	NA	
		low	NA	NA	NA	NA	NA	NA	NA
survival probability S3	High environment	high		0.079	0.035	Inf	2.251	0.024	survival S3 (y/n) ~ plant size
		low	0.16; 0.692	0.108	0.066	Inf	1.635	0.102	s3*ecotype
	Low environment	high		**0.036**	**0.0131**	**Inf**	**2.739**	**0.006**	survival S3 (y/n) ~ plant size
		low	2.25; 0.13	0.010	0.0110	Inf	0.936	0.349	s3*ecotype+(1|site)

Table S18. Continued.

Within environments

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size start of season	SE	df	z value	p-value	Model
survival probability W4	High environment	high	**9.04; 0.003**	**0.012**	**0.031**	**Inf**	**3.890**	**0.0001**	survival W4 (y/n) ~ plant size e3*ecotype
		low		**0.034**	**0.014**	**Inf**	**2.430**	**0.015**	
	Low environment	high	0.01; 0.91	0.020	0.012	Inf	1.715	0.086	survival W4 (y/n) ~ plant size e3*ecotype+(1|site/block)+(1|population)
		low		**0.022**	**0.010**	**Inf**	**2.223**	**0.026**	
survival probability S4	High environment	high	NA	NA	NA	NA	NA	NA	
		low		NA	NA	NA	NA	NA	
	Low environment	high	NA	NA	NA	NA	NA	NA	
		low		NA	NA	NA	NA	NA	
survival probability	High environment	high	**13.7; 0.0002**	**1.525**	**0.190**	**Inf**	**8.030**	**<.0001**	survival probability ~ plant size*ecotype+year+(1|site) + (1|population)
		low		**0.739**	**0.129**	**Inf**	**5.750**	**<.0001**	
	Low environment	high	0.02; 0.89	**0.783**	**0.119**	**Inf**	**6.569**	**<.0001**	survival probability ~ plant size*ecotype+year+(1|site/block) + (1|population)
		low		**0.806**	**0.109**	**Inf**	**7.372**	**<.0001**	

Table S19. Generalized linear mixed effect models, and generalized linear models for the effect of plant size on seed count. S_i indicate stage, and s_i the start of the growing season. We tested the significance of the interactions using likelihood ratio tests and report χ^2 and p-values. Trends were estimated using pairwise contrasts in the emmeans R package; Response, models, estimates, SE, degrees of freedom (df), z value, and p-values are reported. Significant results are in bold.

Respone and fixed effects	**plant size size*ecotype: 2-way interaction; χ^2; P-value**	**plant size size*environment: 2-way interaction; χ^2; P-value**	**plant size size*ecotype * environment: 3-way interaction; χ^2; P-value**	**Model**
seed count S1 ~ plant size s1 *ecotype* environment	**110.04; <.0001**	**42.20; <.0001**	**11.51; <.0001**	seed count S1 ~ plant size s1*altitude*environment+(1|site/block)
seed count S2 ~ plant size s2 *ecotype* environment	**41.53; <.0001**	**58.78; <0.0001**	**5.42; 0.02**	seed count S2 ~ plant size s2*altitude* environment +(1|site/block) + (1|population)
seed count S3 ~ plant size s3 *ecotype* environment	**16.49; <.0001**	2.61; 0.106	0.12; 0.725	seed count S3 ~ plant size s3*altitude* environment+(1|site/block)+(1|population/maternal family)
seed count S4 ~ plant size s4 *ecotype* environment	NA	NA	NA	NA
seed count S5 ~ plant size s5 *ecotype* environment	**25.90; <.0001**	**3.94; 0.047**	**25.06; <.0001**	seed count S5 ~ plant size s5*altitude* environment+(1|site/block)+(1|population)
seed count ~ plant size size * ecotype * Environment	0.00; 0.94	0.01; 0.90	0.51; 0.47	mean seed ~ mean size*altitude* environment+(1|site/block) + (1|population)

Table S19. Continued.

Within environments

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size size start of season	SE	df	t. ratio	p-value	Model
seed count S1	High environment	high		**0.035**	**0.006**	**470**	**5.520**	**<.0001**	seed count S1 ~ plant size
		low	**27.3; <.0001**	0.011	0.028	**470**	0.410	0.683	s1*altitude+(1|site/block) + (1|population)
	Low environment	high	**146; <.0001**	**0.110**	**0.007**	**485**	**16.520**	**<.0001**	seed count S1 ~ plant size
		low		**0.024**	**0.005**	**485**	**4.920**	**<.0001**	s1*altitude+(1|site/block)
seed count S2	High environment	high		**0.019**	**0.004**	**428**	**4.570**	**<.0001**	seed count S2 ~ plant size
		low	**23.6; <.0001**	-0.01	**0.005**	**428**	**-1.820**	**0.07**	s2*altitude+(1|site/block) + (1|population)
	Low environment	high		-0.055	**0.020**	**385**	**-2.7191**	**0.006**	seed count S2 ~ plant size
		low	**33.9; <.0001**	**0.014**	**0.006**	**385**	**2.495**	**0.0130**	s2*altitude+(1|site)+(1|population)
seed count S3	High environment	high		**0.034**	**0.002**	**370**	**14.360**	**<.0001**	seed count S3 ~ plant size s3*altitude+(1|site)
		low	2.17; 0.14	**0.042**	**0.008**	**370**	**5.620**	**<.0001**	+(1|population)
	Low environment	high		**0.028**	**0.002**	**340**	**12.930**	**<.0001**	seed count S3 ~ plant size
		low	**53.9; <.0001**	**0.012**	**0.0004**	**340**	**28.600**	**<.0001**	s3*altitude+(1|site/block)+(1|population/maternal family)

Table S19. Continued.

Response	Environment	Ecotype	ExT (trait) interaction, χ^2; P-value	Trend; plant size size start of season	SE	df	t. ratio	p-value	Model
seed count S4	High environment	high	**20.3; <.0001**	**0.039**	**0.001**	**314**	**34.500**	**<.0001**	seed count S4 ~ plant size s4*altitude+(1|site/block)+(1|population)
		low		**0.018**	**0.005**	**314**	**3.800**	**0.0002**	
	Low environment	plant size s4	NA	**0.005**	**0.001**	**185**	**4.98**	**<.0001**	seed count S4 ~ plant size s4 +(1|site)+(1|population)
seed count S5	High environment	high	**18; <.0001**	**0.012**	**0.001**	**308**	**15.820**	**<.0001**	seed count S5 ~ plant size s5*altitude+(1|site/block)+(1|population)
		low		**0.006**	**0.002**	**308**	**3.680**	**0.0003**	
	Low environment	high	**179; <.0001**	**0.020**	**0.001**	**181**	**15.530**	**<.0001**	seed count S5 ~ plant size s5*altitude +(1|site/block)+(1|population)
		low		**0.004**	**0.0002**	**181**	**15.290**	**<.0001**	
seed count	High environment	high	0.15; 0.69	**0.247**	**0.085**	**160**	**2.907**	**0.004**	mean seed ~ mean size*altitude+(1|site/block) + (1|population)
		low		0.139	0.202	176	0.687	0.493	
	Low environment	high	0.1; 0.75	0.145	0.150	212	0.998	0.319	mean seed ~ mean size*altitude+(1|site/block) + (1|population)
		low		**0.183**	**0.076**	**189**	**2.422**	**0.016**	

Table S20. Linear mixed effect models for the relationship between plant size, plant height and flowering time in the F2 populations in the low site in 2019 and high site in 2020. Significance of the trends, SE df, z values and p-values within environments for each trait. Significant results are in bold.

Model	Site	Trend; plant size/plant height	SE	df	z value	p-value
plant height 2019 ~ plant size 2019	**Low site**	**2.496**	**0.465**	**128**	**5.362**	**<.0001**
flowering time 2019 ~ plant size 2019	Low site	-0.214	0.111	126	-1.932	0.056
flowering time 2019 ~ plant height 2019	**Low site**	**-0.057**	**0.011**	**128**	**-5.366**	**<.0001**
plant height 2020 ~ plant size 2020	**High site**	**6.76**	**1.46**	**396**	**4.636**	**<.0001**
flowering time 2020 ~ plant size 2020	**High site**	**-0.622**	**0.23**	**399**	**-2.703**	**0.007**
flowering time 2020 ~ plant height 2020	**High site**	**-0.041**	**0.012**	**401**	**-3.397**	**0.001**

Chapter III. Supplementary materials

Supplementary figures

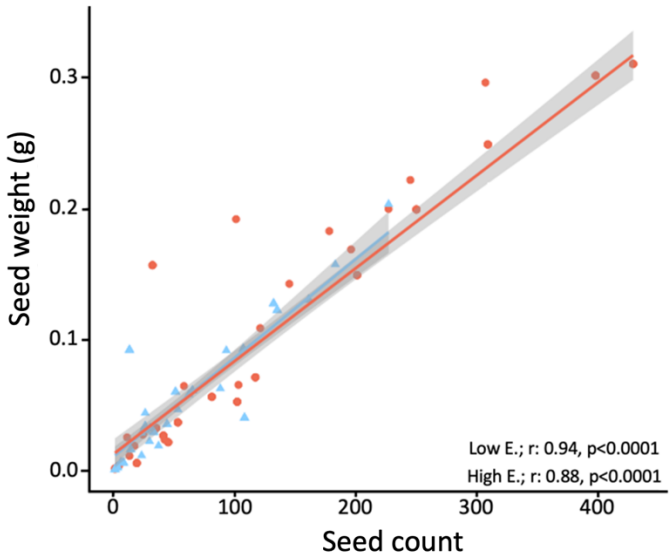

Figure S1. Correlation between seed weight (g) and seed count, based on data of a subset of F2 plants, 84 growing in the low site (red) and 82 in the high site (blue). Lines indicate predicted relationship from linear model regressions with 95% confidence intervals and correlation coefficients and p-values are reported. This figure is also reported in chapter 2.

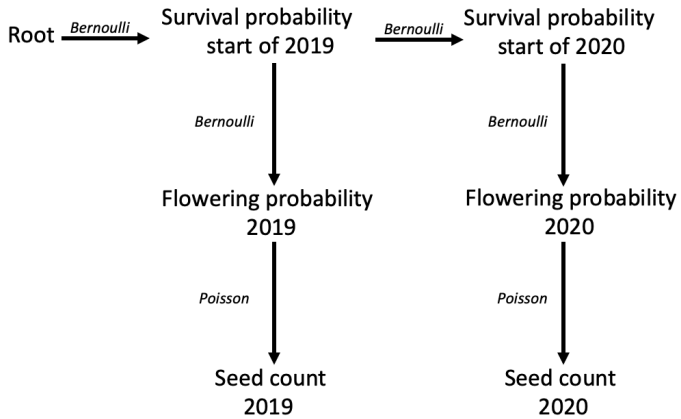

Figure S2. Life-history graph depicting the structure of the aster models. The graph consists of three layers representing, survival probability, flowering probability and seed count. Each node in the graph represent performance in the separate fitness components for the different seasons. The binary variables of survival and flowering probability were modelled using Bernoulli error distribution and the seed count data using Poisson error distribution. This figure is also reported in chapter 2.

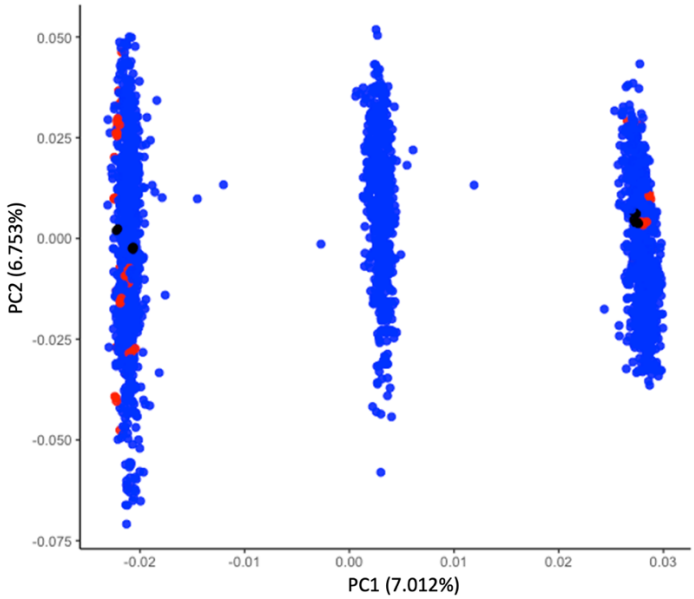

Figure S3. PCA plot showing the three genetic clusters. Black, red, and blue data points indicate grandparents, F1 and F2 plants, respectively.

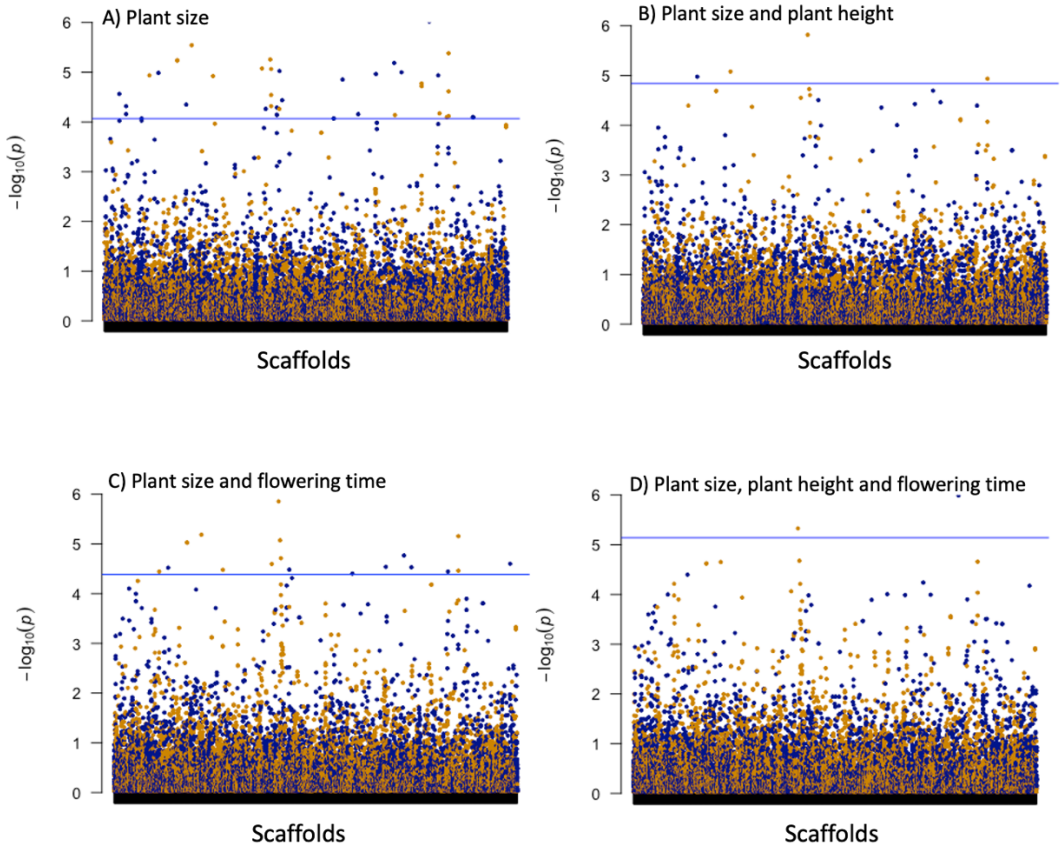

Figure S4. Results of the univariate and multivariate GWA analyses presented as and Manhattan plots of associations with A) plant size, B) plant size and plant height, C) plant size and flowering time and D) plant size, plant height and flowering time. Blue lines indicate genome-wide significance threshold based on the FDR corrected p-values. The SNPs before collapsing high LD pairs are reported and only unanchored SNPs are plotted.

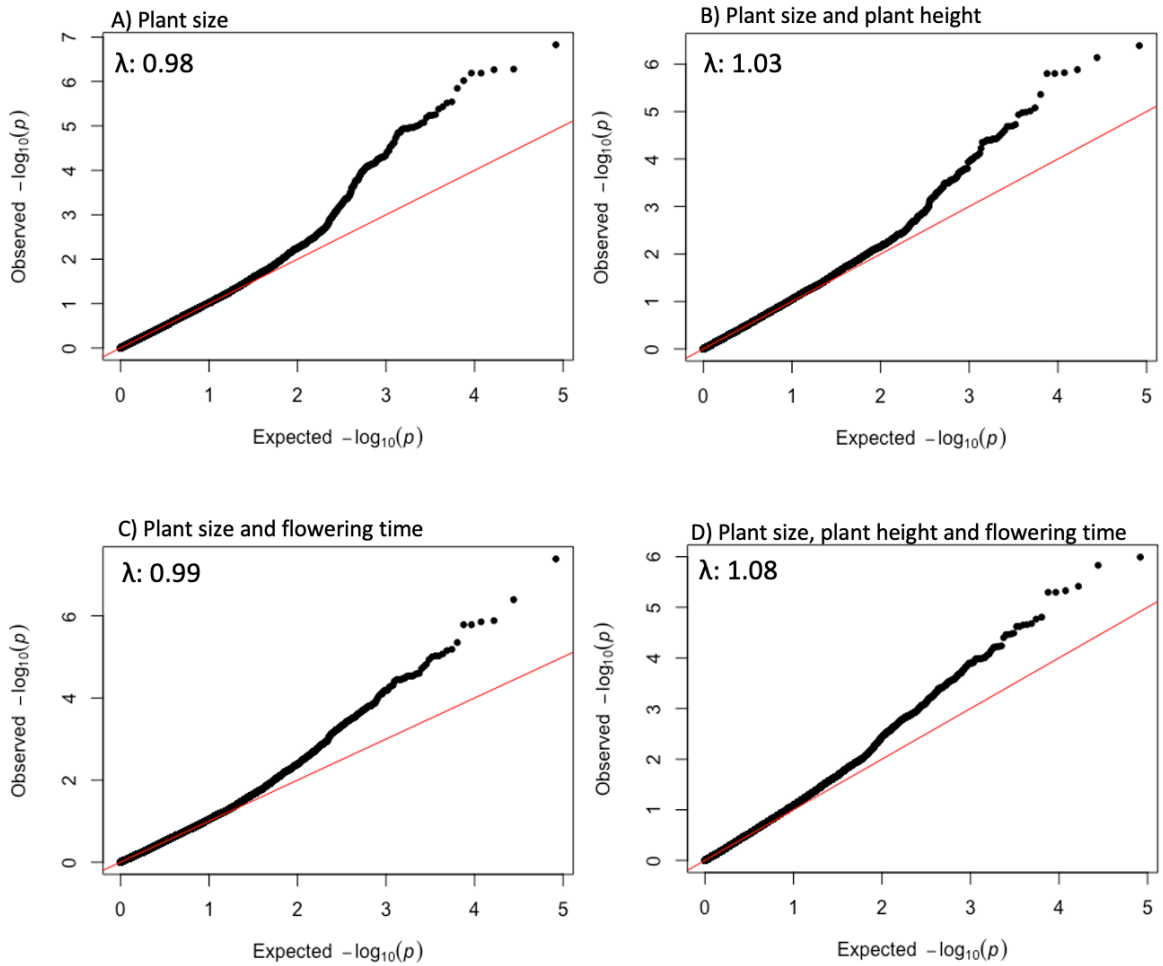

Figure S5. QQ plots from the univariate and multivariate GWA analyses test for associations with A) plant size, B) plant size and plant height, C) plant size and flowering time and D) plant size, plant height and flowering time. Genomic inflation factors (λ) are reported. Both unanchored and anchored SNPs included.

Supplementary tables

Table S1. Production of F1 and F2 populations. Top part, Population origin of plants used to generate F1 crosses, Population name (as referenced in chapter 2, this thesis), elevational origin denominator (i.e., low or high) and nr. of grandparent plants used for F1 crosses reported. Bottom part, production of F2 crosses, cage, F1 cross and nr. of plants per cross and cage reported. Note that the number after F1s descendants refers to the different grandparent plants. This table is also reported in chapter 2.

F0 plants used in production of F1 the generation

Population	Elevational origin	Nr. grandparent plants
Saxon	Low	1
Varen	Low	1
Tsanfleuron	High	2

F2 crosses

Cage	F1	Nr. of plants
1	Saxon_X_Tsanfleuron2	28
2	Saxon_X_Tsanfleuron2	15
2	Tsanfleuron_X_Varens1	15
3	Tsanfleuron_X_Varens1	13
3	Saxon_X_Tsanfleuron2	13
4	Saxon_X_Tsanfleuron2	10
4	Tsanfleuron_X_Saxon2	10
4	Tsanfleuron_X_Varens1	10
5	Tsansfleuron_X_Varens1	15

Table S2. Overview of the transplant experiment of the F2 crosses. Transplant site, elevational, denominator (i.e., low or high), cage number, number of plants per transplant site and cage and number of plants per genetic cluster as identified by PCA analysis (nrs. 1 to 3 arbitrarily chosen, see Figure S3) reported. Note that all the plant nrs. refer to plants alive at the start of 2018 and hence form the basis of our analyses. This table is also reported in chapter 2 of this thesis.

Site	Elevation	Cage	Number of plants alive after transplant shock	Number of plants for genetic cluster 1; cluster 2 and cluster 3
Zeneggen	Low	1	122	122; 0; 0
		2	134	43; 67; 24
		3	132	52; 56; 24
		4	119	57;42;20
		5	114	144; 0; 0
Findeln	High	1	101	100; 1; 0
		2	122	32; 60; 30
		3	103	47; 47; 9
		4	124	62; 48; 14
		5	104	104; 0; 0

Table S3. SNPs identified and retained after collapsing high LD pairs in the GWA analyses. Chromosome (CHR), scaffold, SNP number, SNP id (a unique arbitrary number to increase interpretability across results from different analyses), major/minor allele, minor allele frequency (MAF), type of analysis identified in (S denotes SNPs identified in the univariate analyses for plant size, S+H, S+F, and S+H+F in the multivariate analyses for plant size + plant height, plant size + flowering time and plant size + plant height + flowering time, respectively), effect sizes (β) (i.e. the mean effect on the trait of the alternative allele replacing the reference, note that the β values are reported on the scale of the traits), for plant size (cm^2), flowering time (days) and plant height (cm), standard error (SE) (note that SE is only available from the univariate GWA analyses, hence in the case where a SNP was identified in both uni- and multi-variate analyses the SE is extracted from the univariate results and is unavailable for the SNPs only identified in the multivariate analyses), SNP category, p-value and significance level are reported.

CHR	scaffold	SNP	SNP_id	Major/Minor allele	MAF	S	S+H	S+F	S+H+F	β S (cm^2)	SE S (cm^2)	β F (days)	β H (cm)	SNP category	p-value	signficance level
4	scaffold790_size106621	30763	1	C/A	0.079	x				1.172	0.284			S	5.05E-05	fdr
5	scaffold3776_size35700	8823	2	T/G	0.085	x		x		1.208	0.269	-2.146		S+F	1.09E-05	fdr
8	scaffold190_size186885_131261_186885	41960	3	T/C	0.091	x				1.074	0.264			S	6.40E-05	fdr
9	scaffold317_size154501	153516	4	A/C	0.081	x		x		1.224	0.273	-1.976		S+F	1.16E-05	fdr
14	scaffold1984_size63601	48539	5	G/A	0.08	x	x	x	x	1.472	0.275	-2.101	1.518	S+H+F	1.49E-07	bonferroni/fdr
14	scaffold623_size118343_1_108361	50991	6	C/T	0.087	x	x	x	x	1.249	0.242	-0.928	1.4803	S+H+F	5.41E-07	bonferroni/fdr
14	scaffold1506_size75553	60666	7	G/C	0.085	x		x		1.207	0.269	-2.146		S+F	1.09E-05	fdr
14	scaffold85_size245077	73776	8	G/C	0.073	x		x		1.175	0.28	-3.102		S+F	3.94E-05	fdr
14	scaffold10_size403606	53381	9	G/T	0.32	x				0.671	0.164			S	5.65E-05	fdr
14	scaffold1764_size68837	20572	10	A/C	0.069	x				1.22	0.29			S	3.64E-05	fdr
14	scaffold3049_size45039	1149	11	C/A	0.075	x				1.156	0.282			S	5.77E-05	fdr
14	scaffold4049_size32949	32418	12	T/C	0.092	x				0.955	0.232			S	5.12E-05	fdr
14	scaffold897_size99964_14872_99964	47804	13	C/A	0.091	x				1.102	0.259			S	3.03E-05	fdr
14	scaffold42_size311157	22215	14	G/C	0.095			x		0.905		-3.104		S+F	3.75E-05	fdr
14	scaffold985_size94949	90489	15	C/T	0.087			x		1.021		-3.328		S+F	1.50E-05	fdr
15	scaffold5691_size21091	1665	16	A/G	0.081	x		x		1.224	0.273	-1.976		S+F	1.16E-05	fdr

Table S3. Continued.

CHR	scaffold	SNP	SNP_id	Major/Minor allele	MAF	S	S+H	S+F	S+H+F	β S (cm^2)	SE S (cm^2)	β F (days)	β H (cm)	SNP category	p-value	signficance level
NA	scaffold3066_size44812	28555	17	T/C	0.051	x	x	x	x	1.732	0.337	-2.615	1.698	S+H+F	5.27E-07	bonferroni/fdr
NA	scaffold64_size265011	162000	18	A/C	0.496	x	x	x	x	0.68	0.145	0.315	0.371	S+H+F	9.55E-07	bonferroni/fdr
NA	scaffold1864_size66233	18007	19	T/C	0.079	x	x	x	x	1.242	0.27	-2.681	1.176	S+H+F	5.80E-06	fdr
NA	scaffold2047_size62230_17008_62230	39391	20	A/G	0.081	x	x	x	x	1.324	0.276	-2.412	1.83	S+H+F	2.87E-06	fdr
NA	scaffold703_size112596	29529	21	A/T	0.078	x	x	x	x	1.242	0.262	-2.417	1.485	S+H+F	4.16E-06	fdr
NA	scaffold1649_size71619	11417	22	C/T	0.059	x	x	x		-1.437	0.316	1.872	-2.846	S+H+F	1.03E-05	fdr
NA	scaffold547_size152864_28368_152864	77659	23	A/G	0.085	x	x	x		1.233	0.268	-2.105	1.578	S+H+F	6.50E-06	fdr
NA	scaffold309_size155518	54308	24	A/T	0.084	x		x	x	1.207	0.26	-2.587	1.155	S+H+F	5.55E-06	fdr
NA	scaffold156_size201049_1_36169	29800	25	C/G	0.081	x		x		1.224	0.273	-1.976		S+F	1.16E-05	fdr
NA	scaffold2336_size55757	8210	26	C/T	0.083	x		x		1.212	0.271	-2.096		S+F	1.20E-05	fdr
NA	scaffold2974_size45843	5074	27	A/T	0.092	x		x		1.104	0.243	-1.801		S+F	8.40E-06	fdr
NA	scaffold3114_size44160	29496	28	A/G	0.055	x		x		1.496	0.317	-2.737		S+F	2.86E-05	fdr
NA	scaffold3220_size71569_32000_71569	15866	29	C/A	0.085	x		x		1.199	0.265	-1.717		S+F	9.46E-06	fdr
NA	scaffold4325_size30621	19799	30	A/G	0.083	x		x		1.197	0.27	-2.072		S+F	1.40E-05	fdr
NA	scaffold5034_size25308	9058	31	A/G	0.085	x		x		1.208	0.269	-2.146		S+F	1.09E-05	fdr
NA	scaffold5645_size21377	19857	32	T/G	0.083	x		x		1.221	0.27	-2.049		S+F	1.00E-05	fdr
NA	scaffold6730_size16006	8959	33	T/C	0.081	x		x		1.224	0.273	-1.976		S+F	1.16E-05	fdr
NA	scaffold1188_size89006	46579	34	T/C	0.073	x				1.273	0.298			S	2.73E-05	fdr
NA	scaffold1276_size82621	51365	35	C/T	0.076	x				1.147	0.283			S	6.96E-05	fdr
NA	scaffold146_size205089	17539	36	G/A	0.081	x				1.099	0.275			S	8.48E-05	fdr

Table S3. Continued.

CHR	scaffold	SNP	SNP_id	Major/Minor allele	MAF	S	S+H	S+F	S+H+F	β S(cm²)	SE S (cm²)	β F (days)	β H (cm)	SNP category	p-value	signficance level
NA	scaffold1963_size64119	49178	37	C/A	0.077	x				1.143	0.275			S	4.48E-05	fdr
NA	scaffold3023_size45286	18319	38	A/C	0.083	x				1.085	0.264			S	5.45E-05	fdr
NA	scaffold3201_size42846	28491	39	T/C	0.084	x				1.069	0.259			S	5.12E-05	fdr
NA	scaffold3220_size71569_1_28967	5874	40	T/A	0.08	x				1.089	0.265			S	5.48E-05	fdr
NA	scaffold326_size153415	137100	41	G/A	0.103	x				0.905	0.215			S	3.66E-05	fdr
NA	scaffold4169_size31942	6692	42	G/A	0.078	x				1.117	0.279			S	8.52E-05	fdr
NA	scaffold4619_size28270	24097	43	C/T	0.082	x				1.096	0.271			S	7.00E-05	fdr
NA	scaffold5485_size22438	606	44	T/C	0.055	x				1.348	0.334			S	7.28E-05	fdr
NA	scaffold617_size118679	72595	45	T/G	0.088	x				1.174	0.267			S	1.68E-05	fdr
NA	scaffold6753_size15907	15171	46	T/A	0.07	x				1.246	0.307			S	6.73E-05	fdr
NA	scaffold7964_size11211	1962	47	A/T	0.075	x				1.136	0.283			S	8.01E-05	fdr
NA	scaffold935_size97769	61243	48	T/C	0.389			x		-0.553	0.161			S+F	2.51E-05	fdr

Table S4. Generalized and linear mixed effect models for the effect of the growing environment on plant size and for the effect of the growing environment and plant size on flowering probability. Response and fixed effects of the final models, estimate (E), SE, z value and p-value for the interaction are reported as well as trends within environments. Significant results are in bold.

Model	**Environment: E; SE; z; P-value**					
plant size 2019 ~ environment	**-0.663; 0.063 ;-10.558; <.0001**					
plant size 2020 ~ environment * cluster	**0.962; 0.172; 5.583; <.0001**					
flowering probability 2019 ~ environment * cluster	**-1.954; 0.392; -4.989; <.0001**					
flowering probability 2020 ~ environment	**1.545; 0.551; 2.806; 0.005**					
Model	**E; SE; z; P-value**					
flowring probability 2019 ~ plant size 2019 * environment	-0.009; 0.404; -0.021; 0.983					
Response	**Environment**	**Trend; plant size 2019**	**SE**	**df**	**z value**	**p-value**
flowering probability 2019	**high environment**	**1.87**	**0.346**	**Inf**	**5.40E+00**	**<.0001**
	low environment	**1.86**	**0.209**	**Inf**	**8.885**	**<.0001**
Model	**E; SE; z; P-value**					
flowering probability 2020 ~ plant size 2020 * environment	**0.373; 0.189; 1.973; 0.048**					
Response	**Environment**	**Trend; plant size 2020**	**SE**	**df**	**z value**	**p-value**
flowering probability 2020	**High environment**	**0.57**	**0.07**	**Inf**	**8.16**	**<.0001**
	Low environment	**0.943**	**0.175**	**Inf**	**5.377**	**<.0001**

Table S5. Output of LRTs of the aster models testing for genotype by environment interaction and differential performance in cumulative fitness of genotypes within environments, for loci without significant genotype by environment interaction shown. Fitness effect and trait(s) associated with the SNP, SNP id (corresponding to SNP ids in table S3), FDR corrected p-values of genotype by environment interaction, contrast between alternate genotypes and FDR corrected p-values in the high and low environment and environment with significant fitness effect contrast reported.

SNP id	Genotype by environment interaction, p-value	High environment : contrast; p-value	Low environment : contrast; p-value	Environment with significant fitness effect
		plant size		
9	0.35	0.08; 0.19	**0.32; 0.08**	low
46	0.69	0.09; 0.16	**0.26; 0.08**	low
11	0.07	**0.14; 0.02**	-0.13; 0.44	high
28	0.35	**0.27; 0.0004**	0.29; 0.30	high
29	0.36	**0.17; 0.02**	0.11; 0.61	high
36	0.12	**0.19; 0.01**	0.003; 0.48	high
37	0.35	**0.19; 0.02**	0.02; 0.74	high
40	0.69	**0.19; 0.01**	0.13; 0.44	high
47	0.12	**0.16; 0.02**	-0.05; 0.34	high
		plant size + flowering time		
29	0.37	**0.17; 0.04**	0.11; 0.61	high
32	0.37	**0.18; 0.03**	0.1; 0.61	high
		plant size + height + flowering time		
23	0.24	0.13; 0.12	**0.32; 0.13**	low
19	0.19	**0.16; 0.05**	0.54; 0.86	high

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