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Trait variation in changing environments: Assessing the role of DNA methylation in non-native plant species

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List of papers

This dissertation is a cumulative work that is based on three papers that were published in scientific journals. Two papers share a joint first-authorship (Chapter 2 | Study 1 and Chapter 4 | Study 3) and one paper was published with a single first-authorship (Chapter 3 | Study 2). Manuscripts are provided in ascending order of their appearance as chapters in this thesis:

Chapter 2 | Study 1:

Eckert, S., Herden, J., Stift, M., Joshi, J., van Kleunen, M. (2021). Manipulation of cytosine methylation does not remove latitudinal clines in two invasive goldenrod species in Central Europe. *Molecular Ecology, 30*(1), 222-236. doi: 10.1111/mec.15722

Chapter 3 | Study 2:

Eckert, S., Herden, J., Stift, M., Durka, W., van Kleunen, M., Joshi, J. (2022). Traces of genetic but not epigenetic adaptation in the invasive goldenrod *Solidago canadensis* despite the absence of population structure. *Frontiers in Ecology and Evolution, 10*:856453. doi: 10.3389/fevo.2022.856453

Chapter 4 | Study 3:

Herden, J., **Eckert, S.**, Stift, M., Joshi, J., van Kleunen, M. (2019). No evidence for local adaptation and an epigenetic underpinning in native and non-native ruderal plant species in Germany. *Ecology & Evolution, 9*(17), 9412-9426. doi: 10.1002/ece3.5325

Chapter 1

General Introduction

Chapter 1 | General Introduction

Humans profoundly change ecosystems, often by modifying crucial environmental conditions (Hansen & Clevenger, 2005; Kowarik, 2011; McNeely, 2001). As a result, biogeographical barriers are becoming fuzzy, which is shaping the Anthropocene era (Lewis & Maslin, 2015; Steffen et al., 2011). One of the significant changes associated with the Anthropocene is the spread of non-native plant species: More than 13,000 plant species have now been naturalized into new areas (Pyšek et al., 2017; Seebens et al., 2017; van Kleunen, Dawson, et al., 2015; Weber, 2017). Plant species introduced as ornamentals have outgrown from botanical gardens, such as *Solidago canadensis sensu lato* (L.) (Wulf, 2009). Also, plants were introduced by accident as stowaways of the globalized economy, such as *Alternanthera philoxeroides* (MART.) GRISEB. and *Reynoutria japonica* (HOUTT.) RONSE DECR. (McNeely, 2001). Understanding the mechanisms by which plant species adapt to environmental conditions provided in their new range—especially when they present a severe threat to local biodiversity—is still a major challenge in plant invasion biology (Jeschke et al., 2012; Jeschke & Heger, 2018). Although founder populations are often characterised by impoverished genetic variation limiting their adaptive capacity (Ward et al., 2008), this situation contradicts the successful spread of non-native species and has been deemed the 'invasion paradox' (Fridley et al., 2007; Pérez et al., 2006; Sax & Brown, 2000).

Evidence suggests that adaptive processes in the new range might be facilitated by epigenetic mechanisms, i.e. heritable variation in gene expression states without affecting the underlying DNA sequence (Hawes, 2019; Hawes et al., 2018; Liu et al., 2018; Schrey et al., 2012). Especially variation in cytosine methylation, i.e. the non-random conversion of cytosine nucleotides to 5-methyl-cytosines, was found to affect phenotypic outcome (Zhang et al., 2013). Also, abiotic cues were found to influence cytosine methylation patterns transgenerationally, e.g. transmitting parental 'stress memory' in offspring of the model plant *Arabidopsis thaliana* L. (Hepworth et al., 2018; Iwasaki & Paszkowski, 2014; Kinoshita & Seki, 2014). Moreover, habitat-specific differentiation in cytosine methylation patterns have been demonstrated for the non-native plant species *A. philoxeroides* (Gao et al., 2010) and *R. japonica* (Richards et al., 2012). Results like these form the basis for this thesis, which aims at examining the extent to which this mechanism might shape adaptive processes in non-native plant species in the context of climatic variation in their new environment.

Non-native and invasive plant species

Increased spread of non-native flora coincided with the European colonial and postcolonial history beginning around the fifteenth century (Mack & Lonsdale, 2001; McNeely, 2001). Plants were transferred either accidentally or deliberately, e.g. due to aesthetic reasons, as food resources

and medicines (Essl et al., 2015; Mack & Lonsdale, 2001). Although the rate of introduced plants does not cease today (Seebens et al., 2017), only a minor group of introduced non-native plant species becomes 'invasive' (Falk-Petersen et al., 2006; Turbelin et al., 2017). These plant species form self-sustaining populations that thrive in their new range and may pose a threat to local biodiversity (Powell et al., 2011; Szymura et al., 2015) as well as to human health (Mazza & Tricarico, 2018; Pyšek & Richardson, 2010). For example, the invasive *R. japonica*, that originates from Eastern Asia, forms clonal near-monocultures in invaded areas throughout Europe (Fuchs et al., 2018). Tearing out of a single plant leads to fragmentation of clonal dispersal structures and to the resprouting of several ramets, i.e. clonal offspring (Bailey et al., 2009; Martin et al., 2020; Sásik & Eliáš, 2006). Another classic example of a successful plant invasion across Europe is the health-threatening *Ambrosia artemisiifolia* L. that is native to North America and contains highly allergenic pollen (Chen et al., 2018; Richter et al., 2013). These risks to local biodiversity and human health result into economic losses, e.g. due to increased species management (Keller et al., 2011; Olson, 2006). Local biodiversity has evolved over tens of thousands of years (see e.g. Bischoff et al., 2006; Ehlers & Thompson, 2004; Huntley & Webb III, 2012), hence it remains a challenge to understand how non-native plant species can successfully and rapidly spread in a new area within a few centuries, sometimes even decades (Jeschke et al., 2012; Kowarik, 1995).

Several hypotheses have been proposed in the field of plant invasion biology (reviewed e.g. in Jeschke et al., 2012; Jeschke & Heger, 2018) with the classic ones focusing on shifts in allocated resources from defence to growth (*evolution of increased competitive ability hypothesis*; Blossey & Notzold, 1995) or on reduced herbivore pressure in the new range (*enemy release hypothesis*; Joshi & Vrieling, 2005; Müller-Schärer et al., 2004). More specific hypotheses address that plant defenses may shift away from defense against specialist herbivores in favor of defense against generalist herbivores (*shifting defence hypothesis*; Doorduin & Vrieling, 2011; Joshi & Vrieling, 2005), or emphasize habitat and community invasibility (Lonsdale, 1999; Richardson & Pyšek, 2006). Now and then, hypotheses frameworks have been developed and updated (Catford et al., 2009; Gurevitch et al., 2011; Heger et al., 2019, 2021; Richardson & Pyšek, 2006). Included hypotheses were reviewed whether they are backed up by literature findings (Enders et al., 2020; Jeschke et al., 2012; Jeschke & Heger, 2018) or should be considered as *zombie ideas*, i.e. ideas that do not provide any substantial evidence yet are being investigated (Enders et al., 2020; Fox, 2011).

In general, non-native plant species have been found to undergo multiple stages after introduction, which operate at different temporal and spatial scales (Richardson et al., 2000; Theoharides & Dukes, 2007; Williamson & Fitter, 1996). After introduction, abiotic conditions as

well as resource availability at the local site determine plant colonization success (Lockwood et al., 2009; Theoharides & Dukes, 2007). Here, human-made disturbance regimes have been found to create vacant space susceptible to species invasion (Jauni et al., 2015). Additionally, propagule pressure, i.e. the amount of individuals released at a given site, may increase the probability of successful establishment (Cassey et al., 2018; Theoharides & Dukes, 2007). Up to this point, individuals or small populations of non-native species occur infrequently and isolated at a few locales (Lockwood et al., 2009; Pyšek & Hulme, 2005). This has often been characterised as the *lag*-phase, a period of slow initial spread that might take more than hundred years (Pyšek & Hulme, 2005). Within this stage, non-native species are assumed to develop suitable genotypes capable to spread rapidly (Hobbs & Humphries, 1995). Also, they might already expand via short-distance dispersal still being limited by the lack of suitable habitats (Cousens & Mortimer, 1995) and inclement environmental conditions (Sakai et al., 2001). The increasing number of satellite populations (Cousens & Mortimer, 1995) and the emergence of dispersal corridors (Chapman et al., 2017; Hansen & Clevenger, 2005) finally creates the ground for meta-community dynamics and intraspecific interactions at the regional and interregional scale (Pyšek & Hulme, 2005; Theoharides & Dukes, 2007).

The *lag*-phase has also been suggested a statistical artefact attributed to a bias in changing methodologies over time or in the focus of spatial scales (Pyšek & Hulme, 2005). Nevertheless, an increased rate of expansion with near-monocultures at the final stage has been found for many non-native plant species defining them as ‘pests’ (Williamson & Fitter, 1996) or ‘invasive’ (Richardson et al., 2000). Defining the stages of introduction and the factors that shape them, e.g. using a framework of environmental ‘filters’ through which potential invaders are determined (Colautti & MacIsaac, 2004), is still part of some discussion (Colautti & MacIsaac, 2004; O’Loughlin & Green, 2018; Pearson et al., 2018; Pyšek, 1995; Richardson et al., 2000). In this thesis, plant species will be generally referred to as ‘non-native’ if there is no evidence of increased spread, otherwise they will be indicated as ‘invasive’.

Adaptive processes in non-native plant species

Introduced specimens of non-native species are challenged because they are naïve to the selective pressures in the new range (Pérez et al., 2006). Therefore, non-native species are often characterized to have undergone one or multiple bottleneck events during introduction (Dlugosch & Parker, 2008; Pérez et al., 2006). These events put small founder populations at risk of stochastic extinction or provide them with limited adaptive capacity due to loss of genetic variation (Pérez et al., 2006). Also, formerly rare alleles might become common with increasing population size or growth rate leading to a shift in allele frequency (Nei et al., 1975). Variation in allele frequencies might also occur due to strong genetic drift, i.e. low-density populations at the

edges of the distribution might experience large frequency changes due to stochastic events (Excoffier & Ray, 2008). While most rare alleles are considered deleterious (Dlugosch & Parker, 2008), this shift has been indicated to impact obligatory outcrossing species. For example, for the invasive *Raphanus sativus* L. in the United States in California, Elam et al. (2007) demonstrated that fruit set increased with population size but decreased with genetic relatedness among plants. These effects appear to be contradictory to the observed adaptive variability in non-native plant species, which often develops within a relatively short evolutionary time frame (Dlugosch & Parker, 2008; Pérez et al., 2006).

Self-sustaining populations in the new range might benefit from gene flow due to multiple introductions linking genotypes from different source habitats or via long-distance dispersal between novel environments (Dlugosch & Parker, 2008; Uller & Leimu, 2011; Verhoeven et al., 2011). This admixture in the new range might contribute to standing genetic variation creating novel genotypes and masking deleterious mutations (Verhoeven et al., 2011). Also, Dlugosch et al. (2015) suggested that influential genetic loci might shape quantitative trait variation in founding populations, i.e. large-effect loci that escape bottleneck events in some populations but not others. Despite these potential changes in the new range, comparisons between populations from the native and non-native ranges suggest that pre-adapted traits could also result in increased performance (Elst et al., 2016; Schlaepfer, Glättli, et al., 2010). Using a literature review, Dlugosch & Parker (2008) found that genetic variation frequently decreased in the new range, however, quantitative trait variation was found to rarely decline.

Non-native plant species have been found to be capable to locally adapt to their new environment (Colautti & Lau, 2015; Leimu & Fischer, 2008; Oduor et al., 2016). For example, Colautti & Barrett (2013) demonstrated rapid local adaptation in the invasive *Lythrum salicaria* L. in North America. Latitudinal trait variation, that might represent adaptive differentiation in the new range, has been found for *Mimulus guttatus* DC. (van Kleunen & Fischer, 2008) and *Erigeron canadensis* L. (Abhilasha & Joshi, 2009). Genetic mechanisms, however, require evolutionarily long periods of time and, thus, cannot fully explain recently developed and heritable trait differentiation (Bossdorf et al., 2008; Richards et al., 2010). Yet, short-term plastic responses to environmental cues could likewise be attributed to epigenetic mechanisms (Nicotra et al., 2010) that compensate for limited genetic variation (Schrey et al., 2012).

Cytosine methylation and its impact on adaptive processes

In 1942, Conrad Waddington coined the term ‘epigenetics’ referring to genetic ‘canalization’, i.e. developmental events that lead to a certain phenotype given a certain environmental trigger (Kilvitis et al., 2014; Waddington, 2012). The definition has since been replaced to refer to mechanisms that induce heritable changes in gene expression independent from variation in

nucleotide sequence (Bird, 2007; Holliday, 2006; Kilvitis et al., 2014; Nanney, 1958). Cytosine methylation is one of the best-studied epigenetic mechanisms (Bossdorf et al., 2008) and refers to the transformation of cytosine to 5-methyl-cytosine (Finnegan, Genger, Peacock, et al., 1998). For this, a methyl group (CH_3) is attached to the 5'-position in the pyrimidine ring during DNA replication (Finnegan, Genger, Peacock, et al., 1998; Zhang et al., 2018). In plants, this transformation occurs at CpG, CpNpG and CpNpN sites where p refers to sequences within the DNA strand and N may represent either Cytosine, Guanine, Adenine or Thymine (Finnegan, Genger, Peacock, et al., 1998; Zhang et al., 2018). Cytosine methylation is mediated by specific DNA methyltransferase enzymes that maintain or *de novo* create methylation marks at specific sites (Cao & Jacobsen, 2002). Likewise, cytosine methylation can also be removed during the life cycle of an organism: Demethylation happens either by failure of methylation maintenance, i.e. passive DNA demethylation, or via excision-repair pathways removing methylation marks by DNA demethylase enzymes, i.e. active DNA demethylation (Zhang et al., 2018). Moreover, experimental demethylation can be acquired using cytidine analogues such as zebularine (Baubec et al., 2009) and 5-azacytidine (Griffin et al., 2016), but also non-analogous substances (Causevic et al., 2005; Nowicka et al., 2020). Since methylation patterns were found to be transgenerationally heritable, their variation might add flexibility in the phenotypic outcome of plant species (Bossdorf et al., 2008).

Cytosine methylation may shape variation in gene expression at several levels that indirectly affect gene regulation (Henderson & Jacobsen, 2007). For example, cytosine methylation has been found to be involved in reversibly marking the boundaries between euchromatin and heterochromatin in *A. thaliana* (Roudier et al., 2009). As euchromatin is the area of active DNA transcription, cytosine methylation may define genetic accessibility (Zhang et al., 2018). Modelling the impact of 'epimutations', i.e. non-random and non-permanent modifications of methylation states, on adaptive evolution, Kronholm & Collins (2016) showed that the speed of adaptation might depend on the effect size of epimutations. Moreover, Monroe et al. (2022) have shown for *A. thaliana* that the epigenome, i.e. the entirety of an individual's epigenetic states (including both the DNA methylation pattern as well as histone modifications), might be responsible for a 'mutation bias' inducing non-random variation of mutation rates across the plant's genome. These findings highlight that epigenetic mechanisms such as DNA methylation contribute to natural variation and, thus, to evolutionary processes.

Research objectives

Effects of cytosine methylation have especially been analyzed in populations, accessions as well as epigenetic recombinant inbred lines ('epiRILs') of *A. thaliana* (see e.g. Kawakatsu et al., 2016; Quesneville, 2020; Roudier et al., 2009; Schmid et al., 2018). This model species is indispensable

when focusing on the impact of epigenetically controlled genetic regions or epimutations in experiments under laboratory-controlled conditions, e.g. under defined levels of stress exposure. It is, however, much more challenging to place the contribution of cytosine methylation in the evolutionary ecological context of non-model plant species, but can be achieved based on knowledge of epigenetic mechanisms in model organisms and through interdisciplinary approaches (Richards et al., 2017; Thiebaut et al., 2019). Because of their ability to adapt to novel environments in evolutionarily short periods of time, non-native plant species represent a particularly exciting research topic in terms of cytosine methylation (Mounger et al., 2020; Thiebaut et al., 2019). Linking cytosine methylation to plant invasions, however, has been applied predominantly to invasive species that are apomictic or mainly clonally propagating, e.g. *R. japonica* (Douhovnikoff & Dodd, 2015; Richards et al., 2012) and *Ludwigia grandiflora* ssp. *hexapetala* (HOOK. & ARN.) NESOM & KARTESZ (Douhovnikoff & Dodd, 2015; Genitoni et al., 2020). Clonal and apomictic plant species allow for an easier distinction between genetic and epigenetic effects due to environmental variation (Douhovnikoff & Dodd, 2015), but this focus ignores the effects of variation in cytosine methylation patterns under genetic variation. Therefore—growing offspring from seeds—the fundamental aims of this thesis were to explore the putative contribution of cytosine methylation on adaptive mechanisms of mainly sexually reproducing non-native plants.

Cytosine methylation patterns have already been found to differ among wild populations (Husby, 2022; Richards, 2011). Without incorporating offspring, this approach, however, fails to distinguish plastic variation from robustly transmitted epigenetic marks—especially when specimen are genetically non-uniform (Bossdorf et al., 2008; Jablonka & Raz, 2009). Therefore, to explore the transgenerational stability of cytosine methylation patterns, all studies presented in this thesis (Chapter 2–4) utilized artificial demethylation treatments of offspring with the demethylation agent zebularine (Baubec et al., 2009; Griffin et al., 2016), a non-methylatable cytidine analogue. This approach allowed to explore the extent to which cytosine methylation is involved in changing fitness-related plant traits. For example, experimental cytosine demethylation using zebularine has been demonstrated to affect multiple traits in *Taraxacum officinale* (L.) WEBER EX F.H.WIGG., such as plant biomass or root:shoot ratio (Verhoeven & van Gurp, 2012). If cytosine methylation plays a role in adaptive processes of non-native plant species, then expected climatic and spatial patterns should either disappear or at least become weaker in zebularine-treated conspecifics. In particular, a putative demethylation effect would be expected to be more pronounced in non-native plant species due to assumed bottleneck events during introduction compensating for decreased genetic adaptive capacity (Dlugosch & Parker, 2008). Additionally, developmental transitions, that are environmentally induced over long evolutionary time periods, could be epigenetically mediated and stable *sensu* Herman et al.

(2014). Therefore, comparing the phenotypic outcome to non-treated counterparts provides indirect evidence of epigenetic contribution (Bossdorf et al., 2008). To the best of my knowledge, the work outlined in this thesis was the first, in terms of plant traits, that linked environmental variation to cytosine methylation in genetically heterogeneous offspring from non-native plant species by applying experimental demethylation. With the work presented in this thesis, I hope to provide a deeper understanding of plant invasions related to cytosine methylation for future studies in invasion biology, and to help improve the current state of research in this field.

Study design

The experimental design of all three studies presented in this thesis are graphically outlined in Figure 1.1 and relevant parts of this visualization will be provided at the beginning of each of the following three chapters for the readers convenience and visual orientation. All three studies presented utilized experimental demethylation with zebularine, which is applied during the germination phase and incorporated as a cytosine analogue during DNA replication (Champion et al., 2010; Griffin et al., 2016). Contrary to cytosine, zebularine cannot be methylated because it lacks the 4-amino group that seems to be involved in the transfer of the methyl group responsible for cytosine methylation (Champion et al., 2010). Additionally, zebularine covalently binds DNA methyltransferases leading to a depletion of these enzymes that are responsible for creating or maintaining methylation (Champion et al., 2010; Griffin et al., 2016). The formation of these so-called nucleoprotein adducts, however, is considered reversible but competitive, i.e. zebularine displays a high affinity for these targeted enzymes (Champion et al., 2010; van Bemmel et al., 2009). Because no methylation occurs on zebularine (Champion et al., 2010), this mechanism leads to stable but dose-dependent demethylation (Baubec et al., 2009; Cheng et al., 2003). Since cytosine methylation is frequently found in regulatory regions of genes, demethylation may therefore lead to activation of formerly silenced genes (Cheng et al., 2003) and enhance short-term plastic responses (Bossdorf et al., 2008; Nicotra et al., 2010). To study the contribution of cytosine methylation to adaptive processes, two approaches were applied: a common-garden study (Chapters 2–3) and a reciprocal transplant experiment (Chapter 4).

With the common-garden study, I analysed adaptive differentiation in the invasive species *Solidago canadensis sensu lato* (s.l.) L. and *S. gigantea* AITON collected as seeds from source populations along a latitudinal gradient in Central Europe (Chapter 2). Weber & Schmid (1998) had already demonstrated latitudinal differentiation in phenology and life-history traits albeit using rhizomes, i.e. clonal propagules. Both species are native to North America and became invasive in the seventeenth and eighteenth century, respectively (Weber & Schmid, 1998). Using a comparable gradient as in Weber & Schmid (1998), i.e. > 1000 km along 46–59°N, this study

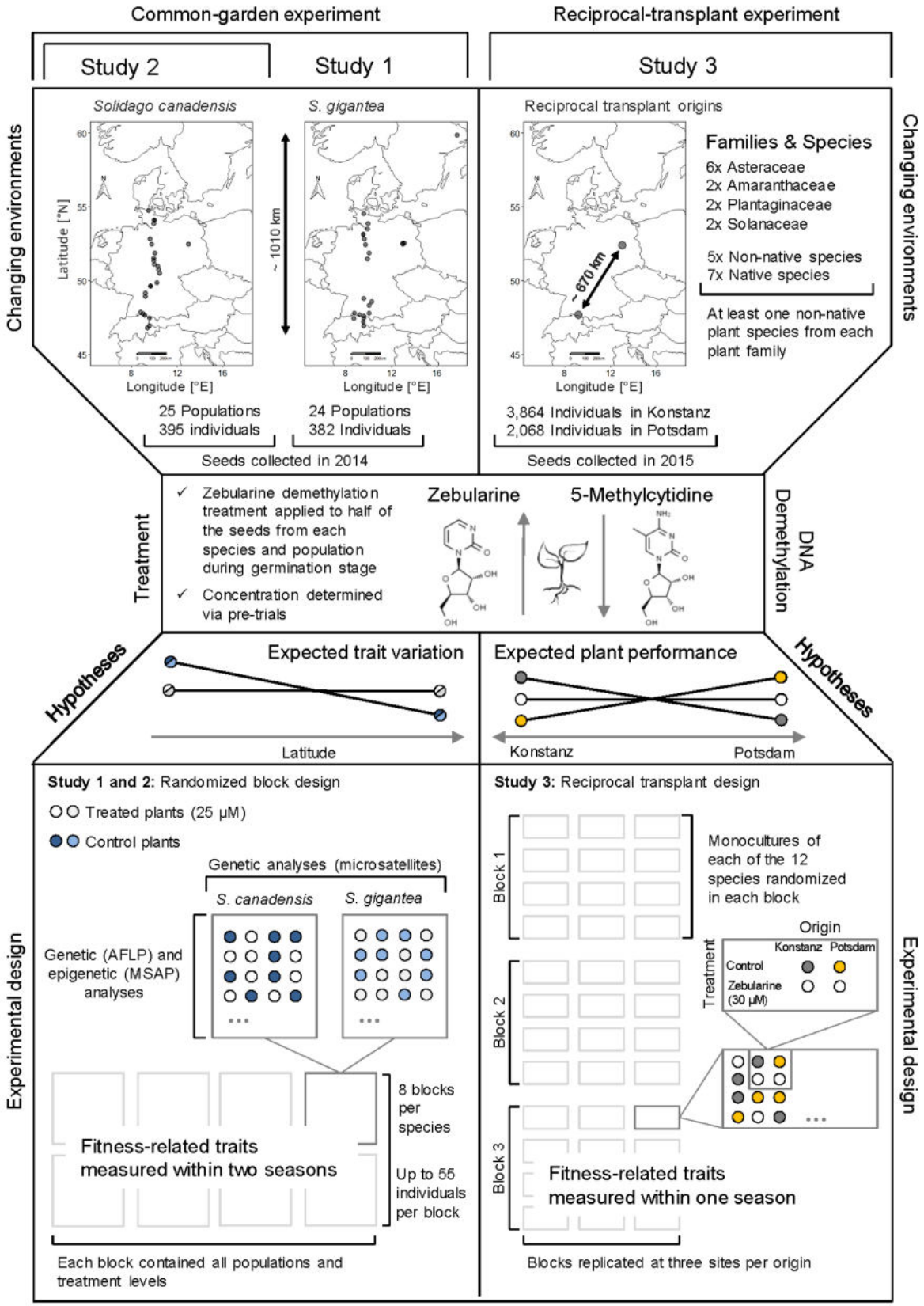


Figure 1.1: Graphical outline summarizing the experimental design of the three studies presented in this thesis. For the readers' convenience and visual orientation, relevant parts are provided at the beginning of each of the following three chapters outlining the study. Chemical structures were obtained from the ChemSpider database (Pence & Williams, 2010).

investigated whether latitudinal trait differentiation persisted in sexually generated offspring and whether these latitudinal clines were partly due to variation in cytosine methylation. Comparing zebularine-treated to non-treated specimen for either species, this study expected that putative latitudinal clines in phenology and life-history traits might be less pronounced in individuals under demethylation treatment. Focusing on the longer established *S. canadensis* and utilizing the same specimen (Chapter 3), I studied whether populations differ in their cytosine methylation pattern and whether this pattern is attributed to climatic and spatial variation along the gradient. For this, I incorporated standard genetic markers as well as methylation-sensitive molecular markers (Reyna-López et al., 1997; Schrey et al., 2013; Vos et al., 1995), and applied genome scans for genetic and epigenetic loci that might show adaptive signatures (Beaumont & Balding, 2004; Bonin, 2008; Holderegger et al., 2008).

To study whether cytosine methylation might be involved in local adaptation of non-native plant species, a reciprocal transplant experiment was conducted using five non-native and seven native plants species from four plant families. These were Amaranthaceae (*Amaranthus retroflexus* L., *Chenopodium album* L.), Asteraceae (*Erigeron canadensis* L., *Erigeron annuus* (L.) PERS., *Lactuca serriola* L., *Senecio vulgaris* L., *Sonchus oleraceus* L., *Tripleurospermum inodorum* (L.) SCH.BIP.), Plantaginaceae (*Plantago major* L., *Veronica persica* POIR.), and Solanaceae (*Datura stramonium* L., *Solanum nigrum* L.). These plant species were selected because they are widespread in Germany and occur in both transplant regions, i.e. Konstanz (47.67°N, 9.16°E) and Potsdam (52.48°N, 13.02°E). In addition, these short-lived species allow interspecific comparisons of multiple life-history traits related to local adaptation and are part of the ruderal vegetation, i.e. disturbed habitats in which non-native species frequently occur (Chytrý, Jarošík, et al., 2008; Chytrý, Maskell, et al., 2008). Both regions, where reciprocal transplants were implemented, differ in their climatic conditions, with Konstanz having temperate-oceanic and Potsdam temperate-continental features (Beck et al., 2018; Peel et al., 2007). Kawecki & Ebert (2004) defined local adaptation using the 'local versus foreign' and 'home versus away' criteria, i.e. local populations should outperform non-local provenances ('local versus foreign') despite the differences in habitat quality ('home versus away'). Additionally, Oduor et al. (2016) found that invasive plant species can be locally adapted in their new range just as native species. Moreover, higher frequencies of self-incompatible invasive plants were found to express local adaptation compared to native species of the same breeding system (Oduor et al., 2016). Based on these findings, this study expected that local populations of both non-native and native plant species should outperform their corresponding non-local provenances. In summary, with the studies presented in this thesis, I investigated three overarching questions:

(i) Is variation in cytosine methylation at least partly responsible for putative latitudinal clines in invasive *Solidago* species? (Chapter 2 | Study 1)

(ii) Does the outcrossing *Solidago canadensis* show signatures of genetic and epigenetic adaptation in its invasive range? (Chapter 3 | Study 2)

(iii) Does variation in cytosine methylation contribute to local adaptation in non-native plant species spanning different plant families? (Chapter 4 | Study 3)

Outline of this thesis

The presented thesis is divided into five chapters, of which the current represents the General Introduction. The three subsequent chapters describe studies already published in scientific journals (Chapter 2–4). The last chapter (Chapter 5) covers the General Discussion.

Study 1 | Common-garden experiment

The first study (Chapter 2) is published in *Molecular Ecology* and represents a follow-up study on Weber & Schmid (1998), who focused on *Solidago canadensis* s.l. (L.) and *S. gigantea* AITON both invasive in Central Europe. Growing clonal propagules in a common-garden environment for one year, Weber & Schmid (1998) found latitudinal clines in phenology and fitness-related traits for both species. This follow-up study extended their approach by growing both species from seeds thereby incorporating genetic variation in the offspring generation. By experimentally demethylating half of the seeds at the germination stage via the demethylation agent zebularine (Baubec et al., 2009), this study investigated genetic population structure using microsatellite markers, and followed the impact of an initial demethylation treatment for two years.

This study highlights that latitudinal clines in almost all fitness-related traits persist in *S. canadensis* offspring grown from seeds despite the lack of population structure. In contrast, *S. gigantea* showed signs of latitudinal population structuring but trait variation was found in only a single trait. Experimental demethylation did not remove latitudinal clines but rather exaggerated them in some traits. Focusing on a large-scale latitudinal gradient in Central Europe, the findings of this study indicate that cytosine methylation might have a negligible impact on adaptive differentiation in both analyzed invasive goldenrod species.

Study 2 | Spatial and genome-scan analyses

The second study (Chapter 3) is published in *Frontiers in Ecology and Evolution* and focuses on the longer established *S. canadensis* from the previous study. Although the analyzed trait variation in the preceding study provided limited evidence for a contribution of cytosine methylation, population structuring in the genetically admixed *S. canadensis* might still be partly attributed to epigenetic variation along the gradient. Epigenetic structuring was, for example, found to exceed

genetic structuring at short-distance spatial scales in *Helleborus foetidus* (Herrera et al., 2016). Therefore, this study utilized the same specimen from the first study focusing on spatial variation of genetic in contrast to epigenetic differentiation. By applying genome scans, this study explored genetic and epigenetic loci putatively susceptible to adaptive processes associated with the latitudinal climatic variation of the corresponding sampled source populations.

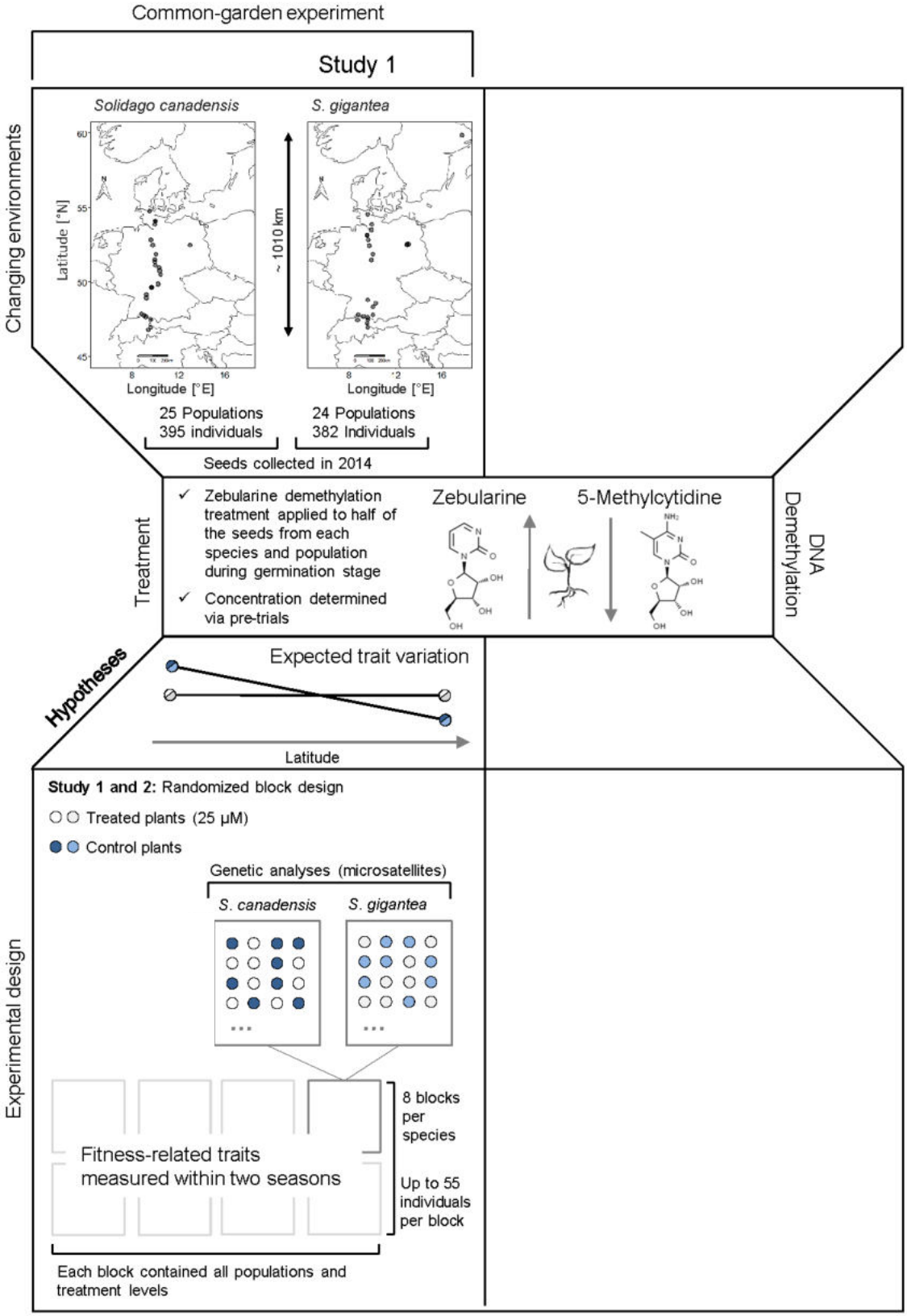
This study highlights that *S. canadensis* contains no epigenetic but spatial genetic variation that shows an autocorrelation structure rather than an isolation-by-distance pattern. Moreover, one genetic and one epigenetic loci revealed to be putatively susceptible to selection associated with the latitudinal gradient. Additionally, the presence of these loci in populations along the latitudinal gradient was partly affected by the demethylation treatment. Besides evidence for spatial genetic variation, population structure was overall surprisingly weak suggesting a negligible contribution of cytosine methylation. The findings of this study strenghten that epigenetic variation does not substantially contribute to spatial variation in this invasive plant species.

Study 3 | Reciprocal-transplant experiment

The third study (Chapter 4) is published in *Ecology & Evolution* and focuses on local adaptation of non-native plant species in Germany. The results from the first (Chapter 2) and second (Chapter 3) study imply that populations of non-native plant species might be subject to selective pressures at the local scale. Indeed, invasive plant species are considered to adapt to local conditions in their new range just like native plants (Colautti & Barrett, 2013; Colautti & Lau, 2015; Oduor et al., 2016). Moreover, cytosine methylation was found to be involved in local adaptation of *A. thaliana* (Dubin et al., 2015) and *Quercus lobata* NÉE (Platt et al., 2015). To disentangle plastic responses from local adaptation, the third study conducted a reciprocal transplant experiment. Considering that local adaptation might have an epigenetic basis (Bossdorf et al., 2008; Dlugosch et al., 2015; Hawes et al., 2018; Suarez & Tsutsui, 2008), this study assessed the contribution of cytosine methylation to local adaptation in six non-native plant species from four plant families in relation to five native congeners utilizing experimental demethylation with zebularine.

This study highlights that there is little evidence for the contribution of cytosine methylation to local adaptation in the analysed non-native but also native species. Contrastingly, some plant species showed maladaptation to their home environment and effects on trait variation between untreated plants and their zebularine-treated counterparts were nondirectional across species and families. Using mainly sexually reproducing ruderal species, the findings of this study point to meta-population dynamics in these disturbed habitats in which they occur.

Chapter 2 | Study 1



Chapter 2 | Study 1: Manipulation of cytosine methylation does not remove latitudinal clines in two invasive goldenrod species in Central Europe

with Jasmin Herden, Marc Stift, Jasmin Joshi and Mark van Kleunen

Abstract

Invasive species frequently differentiate phenotypically in novel environments within a few generations, often even with limited genetic variation. For the invasive plants *Solidago canadensis* and *S. gigantea*, we tested whether such differentiation might have occurred through heritable epigenetic changes in cytosine methylation. In a 2-year common-garden experiment, we grew plants from seeds collected along a latitudinal gradient in their non-native Central European range to test for trait differentiation and whether differentiation disappeared when seeds were treated with the demethylation agent zebularine. Microsatellite markers revealed no population structure along the latitudinal gradient in *S. canadensis*, but three genetic clusters in *S. gigantea*. *Solidago canadensis* showed latitudinal clines in flowering phenology and growth. In *S. gigantea*, the number of clonal offspring decreased with latitude. Although zebularine had a significant effect on early growth, probably through effects on cytosine methylation, latitudinal clines remained (or even got stronger) in plants raised from seeds treated with zebularine. Thus, our experiment provides no evidence that epigenetic mechanisms by selective cytosine methylation contribute to the observed phenotypic differentiation in invasive goldenrods in Central Europe.

Introduction

The collapse of biogeographical barriers during the Anthropocene (Lewis & Maslin, 2015; Steffen et al., 2011) has resulted in >13,000 plant species that have become naturalized outside their native range (Pyšek et al., 2017; van Kleunen, Dawson, et al., 2015). Some of these have become successful invaders (Turbelin et al., 2017). This is remarkable because introduced species should be less adapted to their new environments than native species, and are thought to have limited adaptive potential due to genetic bottleneck events during introduction (Pérez et al., 2006). Nevertheless, several invasive species show signatures of rapid post-introduction evolution (Colautti & Barrett, 2013; Colautti & Lau, 2015; Joshi & Vrieling, 2005; Oduor et al., 2016; Pal et al., 2020; Pérez et al., 2006; Zhang et al., 2018), for example latitudinal clines in flowering phenology (Alexander et al., 2012; Santamaría et al., 2003; Weber & Schmid, 1998). However, little remains known about the mechanisms that allowed alien species to rapidly generate phenotypic differentiation along environmental gradients.

Heritable epigenetic variation has recently been proposed as a putative driver of adaptation to novel environments (Bonduriansky & Day, 2008; Bossdorf et al., 2008; Prentis et al., 2008), indicating a possible role in the rapid adaptation of invasive plants (Banerjee et al.,

2019; Hawes et al., 2018; Prentis et al., 2008; Smithson et al., 2019). Epigenetic variation refers to phenotypic variation that is not caused by changes in nucleotide sequences, but changes in gene expression, for example due to differences in histone packaging (Liu et al., 2010) or cytosine methylation (Bewick & Schmitz, 2017; Niederhuth & Schmitz, 2017). The latter has to date been the most thoroughly studied epigenetic mechanism inducing variation in gene expression at several levels that may indirectly affect gene regulation (Henderson & Jacobsen, 2007). Examples of such methylation-based regulatory mechanisms include reversible marking of boundaries between euchromatin and heterochromatin at ^{m}CHH (H = A, C, T) sites mediated by small interfering RNA (siRNA) (Khraiwesh et al., 2012; Li et al., 2015; Roudier et al., 2009), changes in the expression state of epialleles mediated by repeat sequences near the affected gene (Weigel & Colot, 2012), or methyltransferase enzymes that transfer or *de novo* create methylation marks at CpG, CpHpH or CpNpG (N = A, C, G, T) sites (Cao & Jacobsen, 2002). These mechanisms play a role in phenotypic plasticity (Nicotra et al., 2010) and stress-related adaptation in plants (Boyko & Kovalchuk, 2008) and can be transmitted to the next generation (Henderson & Jacobsen, 2007; Jablonka & Raz, 2009; Thamban et al., 2019; Verhoeven & van Gurp, 2012). The involvement of epigenetic regulation in ecological and evolutionary responses could resolve the paradox of rapid local phenotypic differentiation in invasive plant species with limited genetic variation and a short time since introduction (Richards et al., 2012; Schrieber & Lachmuth, 2017). Although several papers support this idea (Baker et al., 2018; Cubas et al., 1999; Herman & Sultan, 2016; Kalisz & Purugganan, 2004; Richards et al., 2012; Vergeer et al., 2012), experimental studies that disentangle epigenetic and genetic contributions to phenotypic variation are still relatively rare.

Here, we tested for two invasive goldenrod species, *Solidago canadensis* sensu lato (s.l.) L. and *S. gigantea* Aiton (Asteraceae), whether they show heritable phenotypic latitudinal clines in fitness-related traits in their invasive range in Europe, and whether this variation is affected by cytosine demethylation. We also used microsatellite markers to analyse population structure in both species. *Solidago canadensis* and *S. gigantea* are native to North America, but were introduced to Europe in the 17th and 18th century, respectively (Aiton, 1813; Weber, 2017). Although their introduction dates back at least 250 years, both species show reduced genetic variation in their invaded European range, indicating a bottleneck or founder effect (Alexander, Poll, et al., 2009; Schlaepfer et al., 2008). Weber and Schmid (1998) previously conducted a common-garden experiment with plants from invasive populations of *S. canadensis* s.l. (named *S. altissima* in their publication, but which is now considered the morphologically similar diploid *S. canadensis* s.l.; hexaploid plants of *S. altissima* have so far been found only in Belgium; (Verloove et al., 2017)) and *S. gigantea* raised from field-collected rhizome cuttings, and showed that with increasing latitude of origin, plants flowered earlier and at a smaller size. These clines in phenology and size could reflect vegetative carry-over effects (Dong et al., 2018). To test whether

such clines are transmitted sexually, we collected seeds of 25 *S. canadensis* and 24 *S. gigantea* populations along a latitudinal gradient from Switzerland to southern Sweden, and grew the offspring in a common garden in Konstanz, Germany. To test for the role of cytosine methylation on phenotypic expression, we treated half of the seeds per mother plant with the demethylation agent zebularine (Baubec et al., 2009).

Our study aimed to answer three main questions: (i) Do *S. canadensis* and *S. gigantea* show latitudinal clines in phenology and fitness-related traits when plants are grown from seeds (and not from rhizomes as in Weber & Schmid, 1998)? (ii) Does treatment with a demethylation agent affect phenology and fitness-related traits, and putative latitudinal clines therein? Specifically, do clines become less pronounced after treatment of seeds with zebularine? (iii) Do *S. canadensis* and *S. gigantea* show neutral molecular genetic variation, and if so, is this variation structured along the analysed latitudinal gradient?

Material and Methods

Study species and seed collection

Solidago canadensis and *S. gigantea* are native to North America, but were introduced to Europe in17th and 18th century, respectively (Aiton, 1813; Weber, 2017). Both species are perennial herbaceous plants that can grow over 2 m in height, spread vegetatively via rhizomes (Klimešová et al., 2017), and usually flower in late summer and early autumn with branched inflorescences that can produce >10,000 seeds (Weber, 2000). The above-ground parts die back in winter, and plants resprout, usually with multiple ramets, from their rhizomes (Egli & Schmid, 2000; Weber & Jakobs, 2005). Both species have become highly invasive in ruderal and disturbed areas (*S. canadensis*; van Kleunen & Schmid, 2003), and in mesic habitats such as wetlands (*S. gigantea*; Weber & Jakobs, 2005). Whereas in their native range both species have multiple ploidy levels (Semple & Cook, 2005), in Europe *S. canadensis* s.l. is diploid (van Kleunen & Schmid, 2003; but see Verloove et al., 2017, for a recent single record of hexaploid *S. altissima* in Belgium) and *S. gigantea* is tetraploid (Schlaepfer, Edwards, et al., 2010; Schlaepfer et al., 2008). We collected ripe seeds from 25 populations of *S. canadensis* and 24 populations of *S. gigantea* along a latitudinal gradient in Central Europe between October 2014 and March 2015 (Figure 2.1). For each population and species, we collected seeds from at least five mother plants that were at least 1 m apart to minimize sampling from the same clones. Information on sampling location, elevation (m a.s.l.), estimated numbers of shoots per population and the number of maternal lines are given in Tables A2.1 and A2.2.

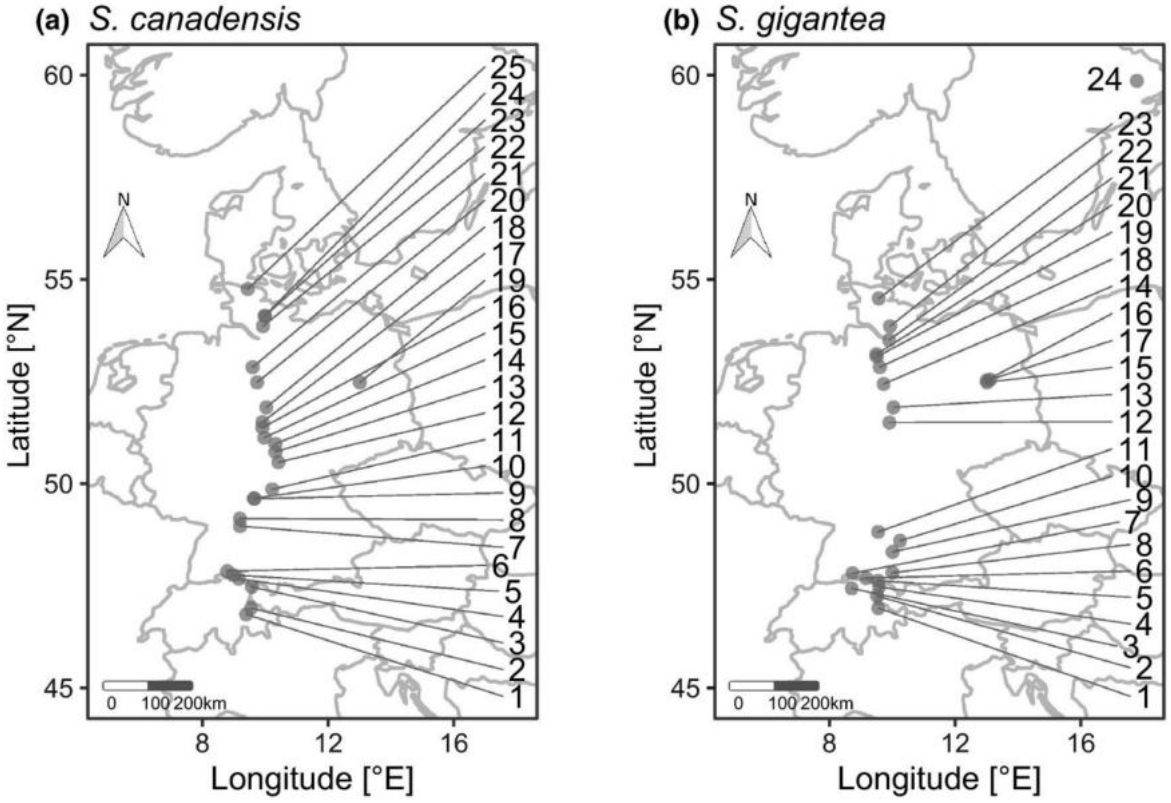


Figure 2.1: Sampling locations along the analysed latitudinal gradient in Central Europe. Populations of (a) *Solidago canadensis* and (b) *S. gigantea* are numbered according to their positions along the gradient (see Tables A2.1 and A2.2 for additional information).

Zebularine demethylation treatment

Zebularine is a cytidine analogue ($C_9H_{12}N_2O_5$) that is commonly used as a demethylation agent (Alonso et al., 2017; Baubec et al., 2009; Herman & Sultan, 2016; Verhoeven & van Gurp, 2012). By inhibiting DNA methyltransferases, zebularine changes cellular methylation patterns on cytosine locations and causes hypomethylation during mitosis (Baubec et al., 2009; Griffin et al., 2016). For example, treatment of *Arabidopsis thaliana* seeds with 80 µm zebularine during germination decreased methylation levels of plants from 81.4% to 58.8% and, in *Medicago sativa* seedlings, treatment with 40 µm zebularine decreased methylation levels from 20.6% to 17.6% on average (Baubec et al., 2009). Additionally, it reduced methylation levels at CpG sites from 17.8% to 15.9% in *A. thaliana* seedlings treated with 25 µm zebularine (Griffin et al., 2016). Although the effects are likely to be transient and restoring mechanisms are triggered already at the seedling stage (Liu et al., 2015), zebularine-derived hypomethylation has been reported to modify stress responses in different plant species (Baker et al., 2018; Verhoeven & van Gurp, 2012). However, treatment with zebularine was also reported to induce growth retardation and increased mortality of seedlings at concentrations >50 µm for *Taraxacum officinale* (Verhoeven & van Gurp, 2012) and *Polygonum persicaria* (Herman & Sultan, 2016), and at concentrations >40 µm for *A. thaliana* (Baubec et al., 2009). Based on a pilot experiment using different

concentrations of the demethylation agent zebularine (0, 12.5, 25, 50, 100 and 200 µm; see Method A2.1), we determined that goldenrod seedlings showed impaired growth at concentrations exceeding 25 µm when germinating on filter paper moistened with zebularine solution. We therefore chose to use the concentration at which growth disruptions were minimal (25 µm), but which was still likely to result in demethylation (see also Herman & Sultan, 2016).

For the main experiment, in mid-April 2015, seeds were surface-sterilized for 3 min in a 5% sodium hypochlorite (NaOCl) solution, rinsed in double-distilled water (ddH_2O) and dried with paper tissues. Two batches per maternal line, each containing 10–20 seeds, were placed on filter paper (Ø 2.7 cm, VWR) in separate Petri dishes (Ø 3.5 cm). Filter paper was moistened with either 200 µl ddH_2O (hereafter called control) or an aqueous solution of 25 µm zebularine (hereafter called zebularine-treated). Petri dishes were sealed (Parafilm, Bemis) and placed in randomized order in a growth chamber at the University of Konstanz (14-hr photoperiod, night-day cycle with temperature range 17.5–22.5°C and light intensity 110–135 µmol m^{-2} s^{-1}) and covered with a single layer of regular white paper to minimize condensation on the lids. Because of the potential instability of zebularine (Cho et al., 2011; Marquez, Kelley, et al., 2005), every second day, we transferred all zebularine-treated and control seeds to new Petri dishes with freshly prepared solutions until at least three seeds per dish had germinated (i.e., clearly visible cotyledons). This process was repeated until the beginning of May 2015 (*S. canadensis*) and end of April 2015 (*S. gigantea*).

Plant cultivation

Two 3–5-day-old seedlings of each maternal line and treatment were transplanted into 7 × 7 × 6.5-cm pots (substrate: Pikiererde Classic, Einheitserdewerke Werkverband e.V.) and kept in a glasshouse until the end of May (*S. canadensis*) or mid-May (*S. gigantea*), when they were moved outside for acclimatization. One week later, plants were transplanted into circular 7-L pots (substrate as before with addition of 5 g/L Osmocote slow-release fertilizer; release: 12–14 months; Everris International B.V). In total, we transplanted 400 *S. canadensis* plants (206 controls and 194 zebularine-treated plants, representing 25 populations and 224 maternal lines) and 336 *S. gigantea* plants (184 controls and 152 zebularine-treated plants, representing 24 populations and 189 maternal lines) to the common garden. Sample sizes per population and treatment are given in Tables A2.1 and A2.2.

Experimental set-up of 2-year common-garden experiments

For each of the two species separately, we used a (incomplete) randomized block design, with eight blocks per species. We aimed to have each population represented in each block with one control and one zebularine-treated offspring of the same maternal line. For cases where control

and zebularine-treated plants of the same maternal line were not available, we paired control and treated plants of different maternal lines from the same population. Blocks were arranged in two rows of four and each block consisted of 55 individuals in an 11 × 5 grid for *S. canadensis* and up to 46 individuals in an 11 × 4+2 grid for *S. gigantea*. Gaps due to an unequal number of surviving seedlings were filled with left-over seedlings of the same species as buffer plants. In addition, blocks were surrounded at each side by one row of buffer plants to reduce edge effects. Blocks were spaced at least 75 cm apart. The experimental area was exposed to full sunlight throughout the day and watered *ad libitum* to ensure moist substrate. A molluscicide (Schneckenkorn, Spiess-Urania Chemicals GmbH) was applied around pots and scattered on top of the soil surface inside pots at the beginning and middle of June in 2015 and at the end of April in 2016. In spring 2016, an additional 10 g of Osmocote slow-release fertilizer (release: 2–3 months; Everris International B.V.) was scattered on top of the soil surface inside pots.

Plant measurements

At the beginning of the common-garden experiment in May 2015, we counted the number of true leaves (i.e., excluding cotyledons) and measured the length and width of the longest leaf per plant. We multiplied those values as an estimate of the initial leaf area per seedling (cm^2; hereafter initial size). The onset of flowering of each plant was monitored every other day, and defined as the time point of the first unfolded ray floret on any of the capitula. Additionally, stretched height (cm; hereafter called plant height) at flowering (when height growth ceases) was measured for each plant in both years. To test whether zebularine treatment during the seedling stage caused a sustained treatment effect reflected in plant growth at the beginning of the experiment, we analysed plant height measured on four occasions after transfer to the common garden (Method A2.2, Figures A2.1 and A2.2).

As a proxy for allocation to sexual reproduction, we harvested inflorescences from the tip down to the lowest side shoot once ripe seeds were observed (i.e., plants were harvested multiple times if they formed new flowers after harvesting; Figure A2.3). This was done to avoid the release of mature seeds and subsequent spread of the species in the garden and its surroundings. At the end of October 2015, when flowering had ceased, we harvested the remaining (vegetative) above-ground biomass to assess complete above-ground biomass as a fitness-related parameter and proxy for growth. Harvested biomass was dried at 70°C for >72 hr before weighing. After drying, we separated stem tissue from the inflorescence to get the reproductive biomass (g dry weight). Stems were then added to the vegetative biomass (i.e., any above-ground tissue not part of the reproductive biomass). The pots with roots and rhizomes were kept outside over winter. At the beginning of the second vegetation period (April 2016), we counted the number of ramets as a measure of vegetative reproduction.

Molecular genetic variation

To analyse population genetic structuring, we genotyped one representative of each maternal line in the control treatment using 11 microsatellite markers (Table A2.3) that had been developed for *Solidago* spp. (Beck et al., 2014; Wieczorek & Geber, 2002). Two monomorphic markers (Sg1, Sg12) were excluded for both species and two further markers (Sg6, SS19D) for *S. canadensis* due to poor quality.

Two fully expanded, healthy leaves per plant were collected in August 2015 and silica-dried for DNA extraction (Chase & Hills, 1991) with a modified CTAB protocol (Doyle & Doyle, 1990) using 20 mg of dry leaf material per plant. DNA was amplified with the microsatellite markers and 5-FAM dye (Thermo Fisher Scientific) during polymerase chain reaction (PCR; 94°C for 4 min; three cycles of 94°C for 30 s; 63°C for 60 s; 72°C for 45 s; 35 cycles 94°C for 30 s; 61°C for 60 s; 72°C for 45 s; 72°C for 15 min) and samples were sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Peaks were scored manually using GENEMAPPER software v5.0 (Applied Biosystems). This succeeded for 203 *S. canadensis* (25 populations) and 174 *S. gigantea* (24 populations) plants. We analysed microsatellite data in allelic format (see Tables A2.4 and A2.5 for summary statistics) and conducted a discriminant analysis of principal components (DAPC; Jombart et al., 2010) to assess population stratification of genotyped individuals per species using the package ADEGENET v2.1.3.

DAPC does not assume any underlying population model or linkage disequilibrium and is a two-step procedure starting with dimension reduction of (scaled and centred) raw microsatellite data via principal components analysis (PCA). We retained the principal components (PCs) that accounted for 95% of the cumulative variance in the data for use in linear discriminant analysis that computes the contribution of each allele to each predefined cluster from the PCA step. We used the *k*-means clustering algorithm (Jain, 2010) with $k = 2–20$ clusters to derive the optimal k based on differences between successive values of the Bayesian information criterion (BIC) as a goodness-of-fit measure (Figure A2.4). The resulting DAPC is then followed by a cross-validation step via successively repeating DAPCs with an increasing number of retained PCs while keeping all other parameters constant. In this step, the DAPCs are repeated using 90% of the data as training data to assess the proportion of successful prediction outcome of the remaining 10% to calculate the root mean square error (RMSE), an indicator of successful re-assignment to each cluster. The number of PCs with the lowest RMSE was used for the final DAPC (Figure A2.4). Missing alleles in the tetraploid *S. gigantea* were coded as zeros and excluded from analysis using the *recode_polyploids* function from the package POPPR v2.8.3. For marker Sg6, a 236-bp fragment occurred in all samples and was therefore excluded from analysis. To infer population stratification with adjacent *k*-clusters and to assess the robustness of DAPC results to variation in

the number of retained PCs, DAPC was repeated with $k = 2–5$ (Figure A2.5) and with a varying number of retained PCs (Figure A2.6).

To test for isolation-by-distance, we correlated the log-transformed pairwise geographical distance (km) between populations with the corresponding pairwise genetic distance (Wright's F_{ST}, standardized as $F_{ST}/(1–F_{ST})$ according to Rousset, 1997; see also Slatkin, 1993; Wright, 1946), using the Mantel test. For the self-incompatible (Voser-Huber, 1983; Weber & Jakobs, 2005) *S. gigantea*, allele frequencies were estimated using the method of De Silva et al. (2005) developed for autopolyploid organisms under polysomic inheritance and implemented in the *deSilvaFreq* function from the package POLYSAT v1.7-4. This function is suitable if all individuals have even-numbered ploidies and the selfing rate is known. Additionally, it is robust against underestimating common allele frequencies and overestimating rare frequencies (De Silva et al., 2005).

Statistical analyses

As five *S. canadensis* and 10 *S. gigantea* plants died during the experiment or were replaced by buffer plants, our final 2-year data set comprised 395 plants for *S. canadensis* and 326 plants for *S. gigantea*. As some traits could not be measured on all plants (e.g., some plants did not flower), the number of plants per analysis varied (Table 2.1; Table A2.6). For all analyses of flowering phenology, we excluded plants without mature inflorescences.

We analysed flowering phenology, quantified as the number of days to flowering after June 1 with generalized linear mixed-effects models (GLMMs) suitable for count data. GLMMs were fitted with either a Poisson distribution or a negative-binomial distribution both with a log-link function. Models were analysed using zero-truncated distributions as there are no zero days-to-flowering. Phenology data from 2015 for *S. canadensis* showed signs of underdispersion in preliminary models using either untransformed values, scaling or rank transformation (dispersion parameter $\sigma^2 < 0.6$). Data were then transformed using ordered quantile normalization (Peterson & Cavanaugh, 2019) and analysed with a linear mixed-effects model (LMM). Initial size, total above-ground biomass measured in the first year and plant height measured in both years were analysed with LMMs. The ratio of reproductive-to-total above-ground biomass, a measure of allocation to sexual reproduction, was analysed with a GLMM using a logit-link beta distribution. For both species, initial size was transformed using Yeo–Johnson transformation (YJ; Yeo & Johnson, 2000). For *S. canadensis*, plant height in 2016 and the number of ramets were transformed using ordered quantile normalization. For *S. gigantea*, plant height in 2015 and in 2016 was transformed using Box–Cox transformation (BC; Box & Cox, 1964) and YJ transformation, respectively. The type of data transformation was assessed via cross-validation (Pearson's ρ-statistic divided by its degrees of freedom) using the *bestNormalize* function in the

package BESTNORMALIZE v1.5.0. Dispersion of GLMMs (except those with a beta distribution) was assessed using the function *sigma* from the packages SPAMM v3.0.0 and GLMMTMB v0.2.3 and *dispersion_glmer* from the package BLMECO v1.3.

All models were fitted separately for each of the two species and included initial size (except when used as the response variable) and latitude of the source population as covariates, and zebularine treatment as a fixed factor. The covariates initial size and latitude were scaled and centred to a mean of zero and a standard deviation of one to facilitate interpretation of model coefficients. As latitude was strongly correlated with climatic variables (Method A2.3, Table A2.7 and Figure A2.7), we also ran models in which we replaced latitude with climatic variables (Method A2.4). However, we only present results of models that included latitude in the Results section (for the effect of climatic variables see Tables A2.8 and A2.9). To test whether zebularine treatment affected latitudinal clines, we included the interaction of latitude and zebularine treatment. For *S. gigantea*, we also repeated the analysis after omitting the northernmost population to evaluate the influence of this outlying population on the expression of latitudinal clines (Tables A2.10 and A2.11). Furthermore, to test whether the geographical gap in the latitudinal transect for *S. gigantea* (48.82–51.50°N; Figure 2.1) is reflected in phenotypic variation, we ran an additional analysis where we included the southern group (populations 1–11) versus the northern group (populations 12–24) as an additional fixed factor (Tables A2.12 and A2.13). Finally, to test whether phenotypic variation is associated with the DAPC cluster that is most dominant in each individual, we ran a separate analysis in which we added cluster membership as a fixed factor (Tables A2.14 and A2.15). Furthermore, models included block, source population and maternal line nested within population as random-intercept factors to account for nonindependence of plants in the same block and for nonindependence of plants from the same maternal lines nested within populations. All random effects were kept in the models (Barr et al., 2013) despite boundary (singular) fit as they were part of the experimental design and only removed if models did not converge. Models were fitted using the functions *fitme* from the package SPAMM v3.2.0, *glmmTMB* from the package GLMMTMB v1.0.1, and *(g)lmer* from the package LME4 v1.1-23.

The significance of fixed model terms was tested via likelihood-ratio tests (Lewis et al., 2011; Pinheiro & Bates, 2009; Zuur et al., 2009). While we are aware that multiple testing increases the risk of type I errors, the methods available to adjust *p*-values vary considerably (García, 2004; Verhoeven et al., 2005) and have been criticized for being overly conservative (Nakagawa, 2004; Perneger, 1998). Therefore, we based our interpretation on the unadjusted *p*-values, but we also provide *p*-values adjusted by the Benjamini–Hochberg correction (Benjamini & Hochberg, 1995; Verhoeven et al., 2005). Marginal and conditional R^2 (not available for

Conway–Maxwell–Poisson, Beta and zero-truncated distributions) were calculated using the function *r.squaredGLMM* from the package MUMIN v1.43.17. Model assumptions were checked using model diagnostics (Bolker et al., 2009; Zuur et al., 2009; Zuur et al., 2010). Results were visualized using the R packages GGPLOT2 v3.3.0, SJPLOT v2.8.3, COWPLOT v1.0.0, GGPUBR v0.3.0, GGPLOTIFY v0.0.5, GGTHEMES v4.2.0 and DOTWHISKER v0.5.0. All analyses were performed in R v3.6.3 (R Core Team, 2020) and all data underlying the analyses are available in the Dryad repository (Eckert et al., 2020).

Results

Phenotypic traits of populations from different latitudes

In the *Solidago canadensis* common-garden experiment, we found that with increasing latitude of origin plants started to flower earlier and at a smaller height, both in 2015 and in 2016 (Table 2.1; Figure 2.2b,c,e,f; Figure A2.8). Moreover, with increasing latitude of origin, plants of *S. canadensis* produced less above-ground biomass and invested a larger proportion of it into sexual reproduction (Table 2.1; Figure 2.2g,h; Figure A2.8). In contrast, the number of ramets produced by *S. canadensis* was not significantly affected by latitude of origin (Table 2.1; Figure 2.2d; Figure A2.8).

In *S. gigantea*, latitude of origin had no significant effect on flowering phenology, plant height, total above-ground biomass or the relative investment of biomass into sexual reproduction (Table 2.1; Figure 2.3b,c,e–h; Figure A2.8). However, in contrast to *S. canadensis*, the number of ramets produced by *S. gigantea* declined with increasing latitude of origin (Table 2.1; Figure 2.3d). This effect, however, became nonsignificant after removing the northernmost population (Tables A2.10 and A2.11), and also when we replaced latitude with a population-grouping factor (south versus north of the distributional gap of the species in Germany; Tables A2.12 and A2.13). These alternative analyses, on the other hand, indicated that the southern populations flowered significantly earlier than the northern ones (Tables A2.10–A2.13).

Effect of zebularine treatment on latitudinal clines

In *S. canadensis*, the zebularine treatment reduced initial size (Figure 2.2a) and delayed early-stage plant height-growth (Figures A2.1 and A2.2). Furthermore, it led to a small but significant delay in the onset of flowering (Figure 2.2b; Figure A2.8). Zebularine treatment also affected the expression of latitudinal clines for plant height and total biomass in the first year of the experiment (Table 2.1; Figure A2.8). However, contrary to our expectations, zebularine-treated

Table 2.1: Summary of likelihood-ratio tests for mixed-effects models. Analysed response variables included initial (seedling) size (number of first true leaves × length × width of the longest true leaf; cm^2), phenology (days to flowering), plant height (cm), reproductive-to-total above-ground biomass ratio, total above-ground biomass (g), and the number of ramets in the second year for *S. canadensis* and *S giagntea*. Fixed effects included initial size (not included if response variable), latitude of source populations, zebularine treatment and the interaction of the latter two. Significant *p*-values in likelihood-ratio tests ($p < 0.05$) are given in bold. All *p*-values were adjusted ($p_{adjusted}$) for multiple comparisons (Benjamini & Hochberg, 1995). Detailed model parameters are given in Table A2.6.

			Initial size			**Latitude (L)**			**Zebularine (Z)**			**L × Z**		
	Response variable	***n***	χ^2	***p***	$p_{adjusted}$	χ^2	***p***	$p_{adjusted}$	χ^2	***p***	$p_{adjusted}$	χ^2	***p***	$p_{adjusted}$
S. canadensis	Initial size	395	—	—	—	3.02	0.082	0.123	**20.85**	**<0.001**	**<0.001**	0.00	0.995	0.995
	Phenology 2015	388	**58.84**	**<0.001**	**<0.001**	**17.36**	**<0.001**	**<0.001**	**5.81**	**0.016**	**0.021**	0.38	0.540	0.540
	Phenology 2016	381	**11.84**	**<0.001**	**0.001**	**11.89**	**<0.001**	**0.001**	0.95	0.331	0.441	0.07	0.797	0.797
	Height 2015	384	0.42	0.517	0.517	**6.16**	**0.013**	0.052	1.56	0.212	0.282	**4.42**	**0.035**	0.071
	Height 2016	381	0.71	0.400	0.533	**7.61**	**0.006**	**0.023**	3.11	0.078	0.156	0.39	0.533	0.533
	Total biomass 2015	390	**67.00**	**<0.001**	**<0.001**	**5.80**	**0.016**	**0.032**	1.69	0.193	0.193	**4.16**	**0.041**	0.055
	Biomass ratio 2015	384	0.18	0.670	0.760	**8.27**	**0.004**	**0.016**	1.33	0.248	0.496	0.09	0.760	0.760
	Ramets 2016	395	1.59	0.207	0.415	2.24	0.134	0.415	0.09	0.768	0.768	0.31	0.581	0.768
S. gigantea	Initial size	326	—	—	—	0.04	0.835	0.835	**32.10**	**<0.001**	**<0.001**	0.21	0.650	0.835
	Phenology 2015	288	**26.81**	**<0.001**	**<0.001**	0.55	0.456	0.608	0.88	0.884	0.608	0.22	0.636	0.636
	Phenology 2016	314	**6.93**	**0.008**	**0.034**	0.04	0.842	0.850	0.04	0.850	0.850	0.71	0.399	0.799
	Height 2015	286	0.57	0.450	0.636	1.23	0.267	0.636	0.51	0.477	0.636	0.05	0.819	0.819
	Height 2016	313	1.95	0.163	0.651	0.29	0.589	0.671	0.18	0.671	0.671	0.37	0.543	0.671
	Total biomass 2015	322	**57.34**	**<0.001**	**<0.001**	2.94	0.087	0.146	2.56	0.109	0.146	0.03	0.54	0.854
	Biomass ratio 2015	284	0.05	0.831	0.969	0.84	0.359	0.717	2.11	0.146	0.586	0.00	0.969	0.969
	Ramets 2016	326	0.00	0.982	0.999	**4.68**	**0.031**	0.122	0.00	0.999	0.999	0.14	0.711	0.999

plants showed more instead of less pronounced latitudinal clines compared to control plants (Figure 2.2e,g).

In *S. gigantea*, the zebularine treatment also reduced initial size (Figure 2.3a) and delayed early-stage height growth (Figures A2.1 and A2.2), but did not affect any of the other measured traits. Moreover, the zebularine treatment did not significantly affect latitudinal clines in *S. gigantea* (Table 2.1; Figure 2.3; Figure A2.8).

Molecular genetic structure of populations from different latitudes

In *S. canadensis*, DAPC analysis gave $k = 7$ genetic clusters based on six discriminant functions and retained 15 PCs (Figure 2.4a). However, almost all individuals belonged to multiple genetic clusters, and the clusters did not reveal any association with latitude. In line with this, genetic distance in terms of F_{ST} did not increase with geographical distance between populations (Mantel test: $r_M = 0.013$; $p = 0.451$; $n = 1{,}000$; Figure 2.4b).

In *S. gigantea*, DAPC identified $k = 3$ clusters based on two discriminant functions and retained 25 PCs (Figure 2.4c). Overall, the populations could be grouped into a southern, a central and a northern genetic cluster, whereby the cluster that was dominant at intermediate latitude was also frequently found in southern and northern populations. In line with this, genetic distance in terms of F_{ST} increased with geographical distance between populations (Mantel test: $r_M = 0.288$; $p < 0.001$; $n = 1{,}000$; Figure 2.4d). DAPC cluster membership was not significantly associated with trait variation (Tables A2.14 and A2.15).

Discussion

In our 2-year common-garden experiments with two highly invasive goldenrod species, *Solidago canadensis* and *S. gigantea*, we tested whether plants grown from seeds showed latitudinal phenotypic clines and whether there is a potential epigenetic contribution mediated by cytosine methylation. Treatment of seeds with the demethylation agent zebularine had an overall negative effect on initial size (Figures 2.2a and 2.3a) and plant height (Table A2.16, Figures A2.1 and A2.2), and, for *S. canadensis*, delayed the onset of flowering in the first year (Figure 2.2b). By growing our plants from seeds, we showed that the latitudinal clines in phenological and performance traits previously reported for both species grown from rhizomes (Weber & Schmid, 1998) are also inherited to the sexually produced offspring generation. The clines persisted or even became slightly stronger in plants grown from seeds treated with zebularine, arguing against an epigenetic underpinning through cytosine methylation. Therefore, the observed heritable phenotypic differentiation along latitudinal gradients in the analysed traits of the two invasive goldenrod species probably has a genetic basis.

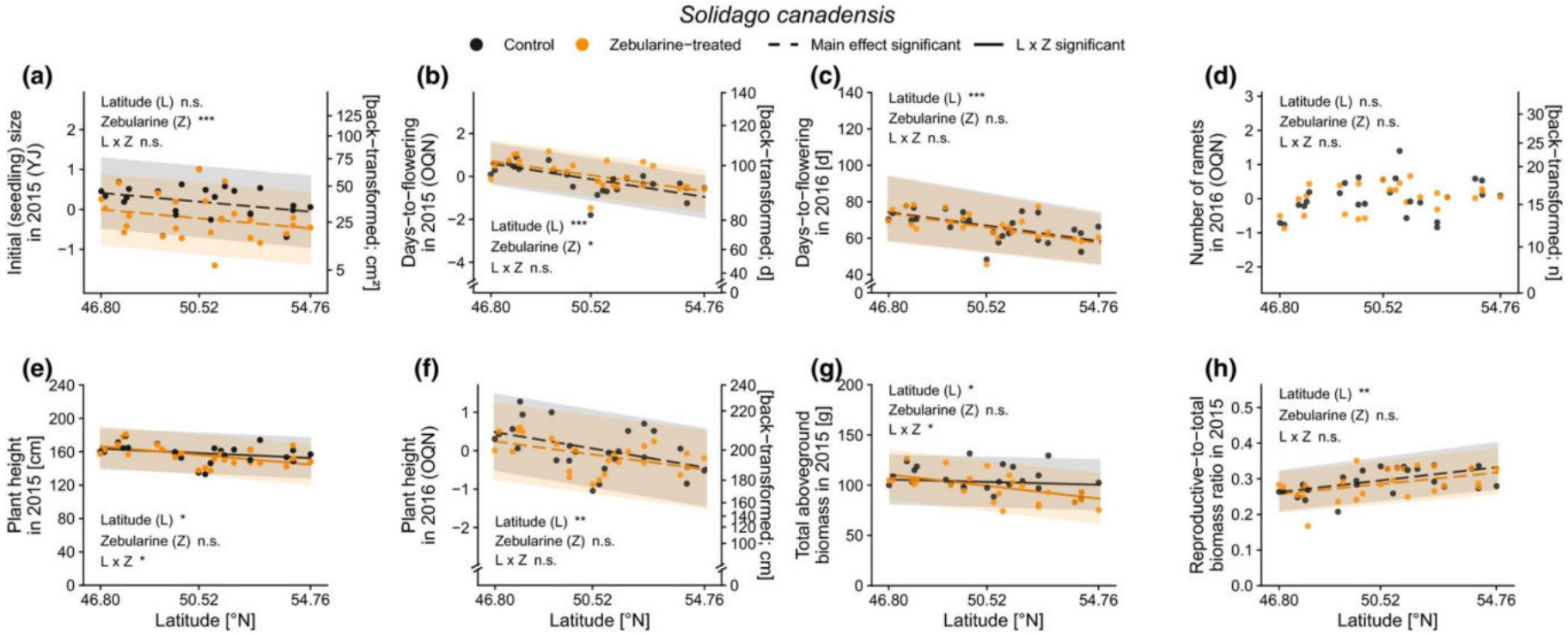


Figure 2.2: Plant traits along the latitudinal gradient for *Solidago canadensis*. Population means for control plants (black) and zebularine-treated plants (orange) related to latitude. Lines indicate the predicted marginal-effect values and their 95% confidence intervals (dashed = effect of main factors is significant; solid = effect of the interaction between latitude and zebularine treatment is significant). The effect of latitude, seed treatment and their interaction was assessed using (generalized) linear mixed-effects models and subsequent likelihood-ratio tests (Table 2.1; significance levels: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s., not significant). In cases where data have been transformed, the second y-axis displays the back-transformed data. (a) Initial (seedling) size in 2015 (data transformed using Yeo–Johnson transformation; YJ); (b) days to flowering in 2015 (data transformed using ordered quantile normalization; OQN); (c) days to flowering in 2016; (d) the number of ramets in spring 2016 (data transformed using OQN); (e) plant height (cm) at flowering stage in 2015; (f) plant height (cm) in 2016 (data transformed using OQN); (g) total above-ground biomass (g) harvested in 2015; (h) the ratio of reproductive-to-total above-ground biomass in 2015.

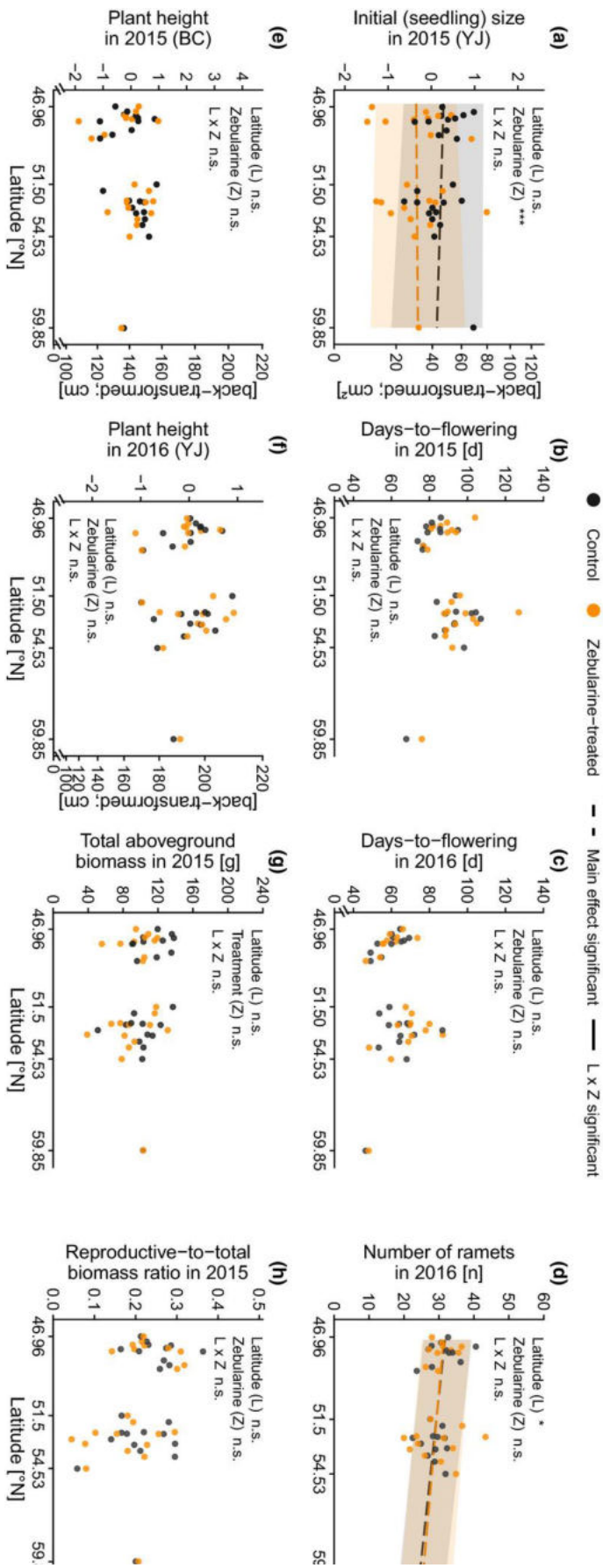


Figure 2.3: Plant traits along the latitudinal gradient for Solidago gigantea. Population means for control plants (black) and zebularine-treated plants (orange) related to latitude. Lines indicate the predicted marginal-effect values and their 95% confidence intervals (dashed = effect of main factors is significant). The effect of latitude, seed treatment and their interaction was assessed using (generalized) linear mixed-effects models and subsequent likelihood-ratio tests (Table 1; significance levels: ***p < .001; **p < .01; *p < .05; n.s, not significant). In cases where data have been transformed, the second y-axis displays the back-transformed data. (a) Initial (seedling) size in 2015 (data transformed using Yeo–Johnson transformation; YJ); (b) days to flowering in 2015; (c) days to flowering in 2016; (d) the number of ramets in spring 2016; (e) plant height (cm) at flowering stage in 2015 (data transformed using Box–Cox transformation; BC); (f) plant height (cm) (data transformed using YJ); (g) total above-ground biomass (g) harvested in 2015; (h) the ratio of reproductive-to-total above-ground biomass in 2015.

Phenotypic latitudinal clines

In a previous common-garden experiment with plants grown from field-collected rhizomes, Weber & Schmid (1998) found that plants from northern European populations of both species flowered earlier and at a smaller size than those from southern populations. In our study, we found a similar phenotypic latitudinal cline for *S. canadensis* (Figure 2.2), but not for *S. gigantea* (Figure 2.3), when plants were grown from seeds instead of rhizomes. This suggests that at least for *S. canadensis* the phenotypic latitudinal clines found by Weber & Schmid (1998) are not simply parental–environmental effects carried over through rhizomes. Interestingly, while Weber & Schmid (1998) found that the size of the inflorescences decreased with latitude for both species, we found that the relative allocation of biomass to sexual reproduction actually increased for *S. canadensis* (Figure 2.2h). This might be because in our study the total above-ground biomass of *S. canadensis* decreased with latitude (Figure 2.2g), resulting in a larger relative allocation to reproduction. Thus, the results of our study are in line with those of Weber & Schmid (1998) for *S. canadensis*, but not for *S. gigantea*.

While *S. gigantea* did not show latitudinal clines with regard to flowering time, height, above-ground biomass production and relative allocation to sexual reproduction, it showed, in contrast to *S. canadensis*, a significant decrease in the production of ramets with latitude (Figure 2.3d). *Solidago gigantea* thus forms an exception to the pattern of increased clonality in colder environments at higher elevations and latitudes (Klimeš et al., 1997). Our unexpected finding, however, could simply reflect that the climate in our southern common garden was less suitable for plants from the north. This would also explain why plants from northern populations of *S. canadensis* grew less tall and produced less biomass than plants from southern populations in our experiment, but not when measured in their original sites (Weber & Schmid, 1998). Moreover, the previously significant decrease in the production of ramets disappeared, when the northernmost *S. gigantea* population was excluded from analysis or when genetic clusters were taken into account. However, without further experiments, we cannot exclude alternative explanations, including the possibility that the northern populations were founded from different source populations (which happened to have lower clonality) than the southern populations.

Our latitudinal transect covered a range from 46°N to 59°N and spanned about 1,800 km, similar to the previous study by (Weber & Schmid, 1998) on both *Solidago* species (44–61°N), and other studies on latitudinal adaptation in invasive plants (Colautti & Lau, 2015; Kollmann & Bañuelos, 2004). Both our study and that of Weber & Schmid (1998) contained a gap in the transect for *S. gigantea*, which is in line with the overall low number of records of this species in Central Germany (BfN, 2020). Latitude correlated significantly with the principal component axis (PC1) that mainly represented variation in wind speed, solar radiation and mean temperature of

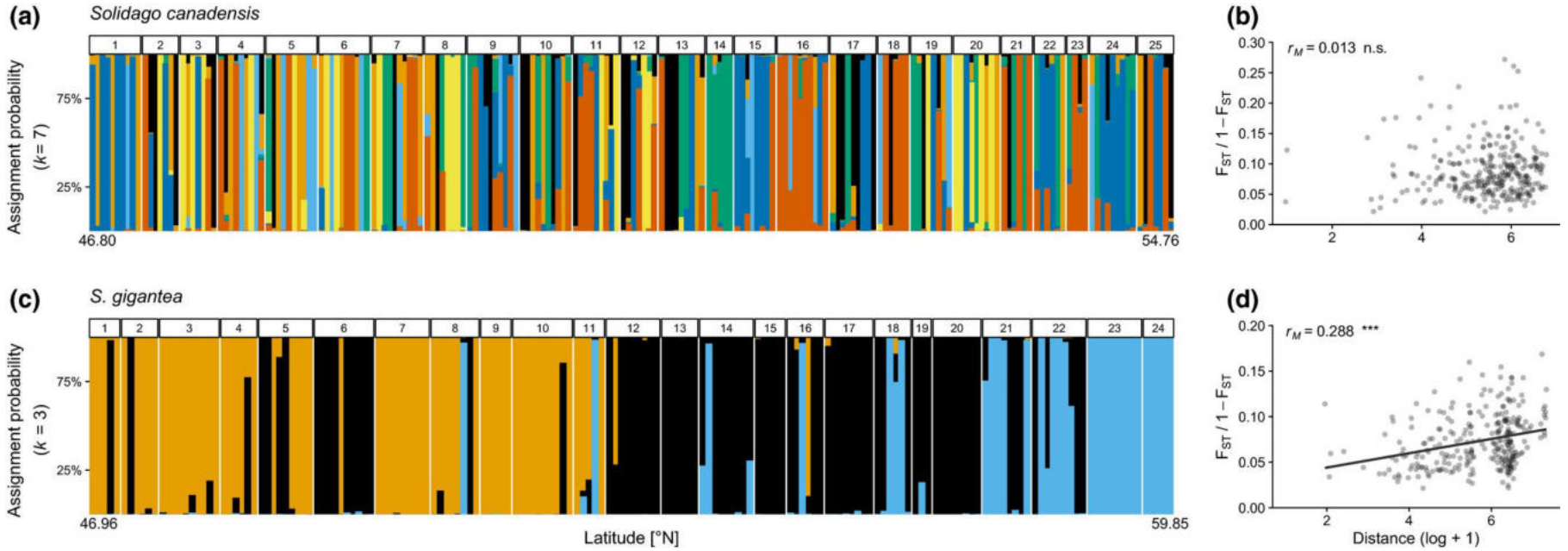


Figure 2.4: Genetic structuring in 25 *Solidago canadensis* and 24 *S. gigantea* populations. Control plants were analysed with discriminant analysis of principal components (DAPC; Jombart et al., 2010) and isolation-by-distance (Rousset, 1997) on a population-level. Isolation-by-distance was obtained by correlating the population-based pairwise fixation index F_{ST} with the logarithm of geographical distances between source populations using the Mantel test with 1,000 repetitions (r_M, Mantel's coefficient of correlation; significance levels: *** $p < 0.001$; n.s., not significant). Stacked bars display the probability (%) of membership (a) *S. canadensis* control plants to $k = 7$ genetic clusters based on the Bayesian information criterion (BIC) and (c) *S. gigantea* control plants to $k = 3$ genetic clusters. Scatter plots display genetic variation correlated against geographical distance for (b) *S. canadensis* ($r_M = 0.013$; n.s.) and (d) *S. gigantea* ($r_M = 0.288$; $p < 0.001$).

the driest annual quarter (Figure A2.7). When we replaced latitude with principal component scores of the multivariate climatic data, PC1 had strong effects on the onset of flowering, height, above-ground biomass and relative allocation to sexual reproduction in *S. canadensis* (Table A2.8). Moreover, for *S. gigantea*, PC1 had a significant effect on the onset of flowering and plant height in 2015, as well as the relative allocation to sexual reproduction. Surprisingly, these traits were not significantly affected by latitude alone, perhaps because for *S. gigantea* the correlation between latitude and PC1 was not as strong ($R^2 = 0.62$) as for *S. canadensis* ($R^2 = 0.84$; Figure A2.7). Overall, these findings suggest that climate is a strong driver of phenotypic differentiation in both *Solidago* species.

Gradual latitudinal changes in climate and in day length (Figure A2.9) affect flowering phenology, plant life cycles and growth (Woodward & Williams, 1987). Generally, plants at low latitudes are likely to flower earlier and grow more vigorously compared to plants at high latitudes. However, because plants at high latitudes have shorter growing seasons, there may be counter-gradient selection (Conover & Schultz, 1995) for plants that flower early at a smaller size. This could explain why, when grown in a common garden, the *S. canadensis* plants from northern populations flowered earlier and at a smaller size than the plants from southern populations. While such climatic–ecotypic differentiation has since long been shown to be adaptive in other species, such as in *Achillea* spp. (Hiesey et al., 1942) and *Bouteloua curtipendula* (Olmsted, 1944), this remains to be tested explicitly for our *Solidago* species.

Effects of the demethylation agent zebularine

Our study is the first common-garden study that used a demethylation agent in an attempt to experimentally disentangle epigenetic and genetic contributions to phenotypic variation in offspring of invasive plants sampled along a latitudinal gradient. Demethylation agents have been used by previous studies on the role of epigenetics in an ecological and evolutionary context (Bossdorf et al., 2010; Herden et al., 2019; Verhoeven & van Gurp, 2012).

Zebularine reduces cytosine methylation in a dose-dependent manner and may cause side-effects, although it has been found to have a longer half-life and fewer side-effects than, for example, 5-azacytidine (Baubec et al., 2009; Cheng et al., 2003). We applied a zebularine concentration of 25 µm, which was the highest concentration at which *S. gigantea* seedlings were not visibly affected in a pilot experiment testing a range of concentrations (0, 12.5, 25, 50, 100 and 200 µm; see Method A2.1 and Figure A2.10). In the common-garden experiment, we found that zebularine-treated plants of both species had smaller initial sizes (Figures 2.2a and 2.3a) and a delayed height growth compared to the control plants (Figures A2.1 and A2.2). *Arabidopsis thaliana* and *Medicago sativa* showed similar delays in plant growth at zebularine concentrations of more than 20 µm (Baubec et al., 2009). On the other hand, Wilschut et al. (2016) found no

negative growth effects treating seeds of asexual lines of *Taraxacum officinale* at comparatively low concentrations (1 and 10 µm, respectively) of agar-dissolved zebularine. Although we have not performed a comparative assessment of methylation levels between treated and untreated plants, we assume that the initial reduction in seedling size and plant growth indicates that our treatment with 25 µm zebularine was probably effective. However, we strongly recommend that future studies on experimental demethylation quantify the effectiveness of the used chemical by measuring the reduction in global methylation levels.

If phenotypic differentiation in the two goldenrod species was entirely or partly caused by changes in cytosine methylation patterns, and under the assumption that the zebularine treatment was effective in reducing genome-wide methylation levels, one would expect the latitudinal clines in flowering phenology and fitness-related traits to disappear or become weaker in plants treated with the demethylation agent zebularine. We found that zebularine-treated *S. canadensis* plants flowered slightly, but significantly, later than untreated plants in the first year. However, the zebularine treatment did not remove any phenotypic latitudinal clines (Figures 2.2 and 2.3). On the contrary, it even resulted in slightly steeper clines for *S. canadensis* in plant height and total above-ground biomass (Figure 2.2e,g). This suggests that, although our demethylation treatment did not remove latitudinal clines in our experiment, it may still have contributed to unmasking cryptic genetic variation that otherwise would have been silenced. For example, variation in DNA methylation levels has been shown to be involved in tagging the boundary between euchromatin and heterochromatin in *Zea mays* (Li et al., 2015; Niederhuth & Schmitz, 2017) and *A. thaliana* (Roudier et al., 2009). Furthermore, (Dubin et al., 2015) found that so-called gene-body methylations (GbMs) in *A. thaliana* were significantly correlated with latitude; that is, accessions from colder environments contained a higher number of GbMs, and that these may contribute to local adaptation. GbMs are cytosine-related gene methylations (i.e., they occur at CG sites) that are characterized by depletion of methylation levels at the gene's regulatory sites and are mostly associated with housekeeper genes (Bewick & Schmitz, 2017). Although it remains to be tested whether and how such methylation-based variations in gene-regulatory mechanisms might affect the analysed traits of our study species, the limited effects of our demethylation treatment on trait variation fits the idea that DNA methylation contributes much less to variation in gene expression than genetic mechanisms such as single nucleotide polymorphisms (e.g., Meng et al., 2016).

It is challenging to quantify epigenetic versus genetic contributions to phenotypic variation (Herman & Sultan, 2016), particularly because there are multiple epigenetic mechanisms, such as DNA methylation (Bewick & Schmitz, 2017; Finnegan, Genger, Peacock, et al., 1998; Herman & Sultan, 2016), histone modification (Bastow et al., 2004; Zhang et al., 2007) and small regulatory RNAs (Castel & Martienssen, 2013; Matzke & Mosher, 2014; Morgado et

al., 2017). Furthermore, many epigenetic mechanisms have been shown to be dependent on genetic loci associated with adaptive mechanisms (Banerjee et al., 2019; Cortijo et al., 2014; Dubin et al., 2015; Nicotra et al., 2010). We focused on cytosine methylation, because this is the most thoroughly studied epigenetic mechanism to date, and has been shown to have transgenerational heritability and to play a role in adaptation to environmental stress (Hawes et al., 2018; Herrera et al., 2014; Herrera & Bazaga, 2010). Studies of epigenetic effects in nonmodel organisms have mostly focused on populations in contrasting natural environments (Gao et al., 2010; Herrera & Bazaga, 2016; Lira-Medeiros et al., 2010), and on apomictic or asexual plant species, such as *Taraxacum officinale* (Verhoeven & van Gurp, 2012; Wilschut et al., 2016) and *Festuca rubra* (Münzbergová et al., 2019). There is, to the best of our knowledge, only one other study that has addressed epigenetic latitudinal variation. Preite et al. (2015) assessed methylation states in offspring of the apomictic species *T. officinale* collected along a latitudinal gradient in Europe, and found that the investigated regions differed in epigenetic variation based on methylation-sensitive amplification length polymorphism (MS-AFLP) markers, but also due to genetic AFLP variation. Note that all studies mentioned above did not analyse entire genomes, and can therefore not exclude that some of the apparent epigenetic effects actually have a genetic cause. It thus remains challenging to separate epigenetic from genetic contributions to latitudinal clines.

Molecular genetic differentiation

In *S. canadensis*, our clustering analysis of microsatellite variation revealed seven genetic clusters, but these did not correspond to different populations or groups thereof, and most of the genetic clusters were represented along the entire latitudinal range (Figure 2.4a). In other words, we found considerable genetic variation but did not find any molecular genetic structure for *S. canadensis* along the sampled latitudinal gradient. Letters document that gardeners in Europe, and particularly in England, repeatedly received *Solidago* seeds (along with soil to promote their acclimatization to the new area) from early settlers in North America over a long period of time (Wulf, 2009); this could explain the relatively high degree of genetic variation. Nevertheless, genetic variation of *S. canadensis* is still lower in invaded areas than in its native range (Alexander, Poll, et al., 2009).

The frequent representation of multiple genetic clusters within single individuals indicates that there has been frequent admixture. This might have happened prior to the establishment of wild populations (see Dlugosch & Parker, 2008). However, as the seeds of *Solidago* species are light and have a pappus, and thus can be dispersed over long distances by wind (Melville & Morton, 1982), another likely scenario is that admixture followed after major gene flow among populations along the sampled gradient in Central Europe. Admixture may facilitate the spread of

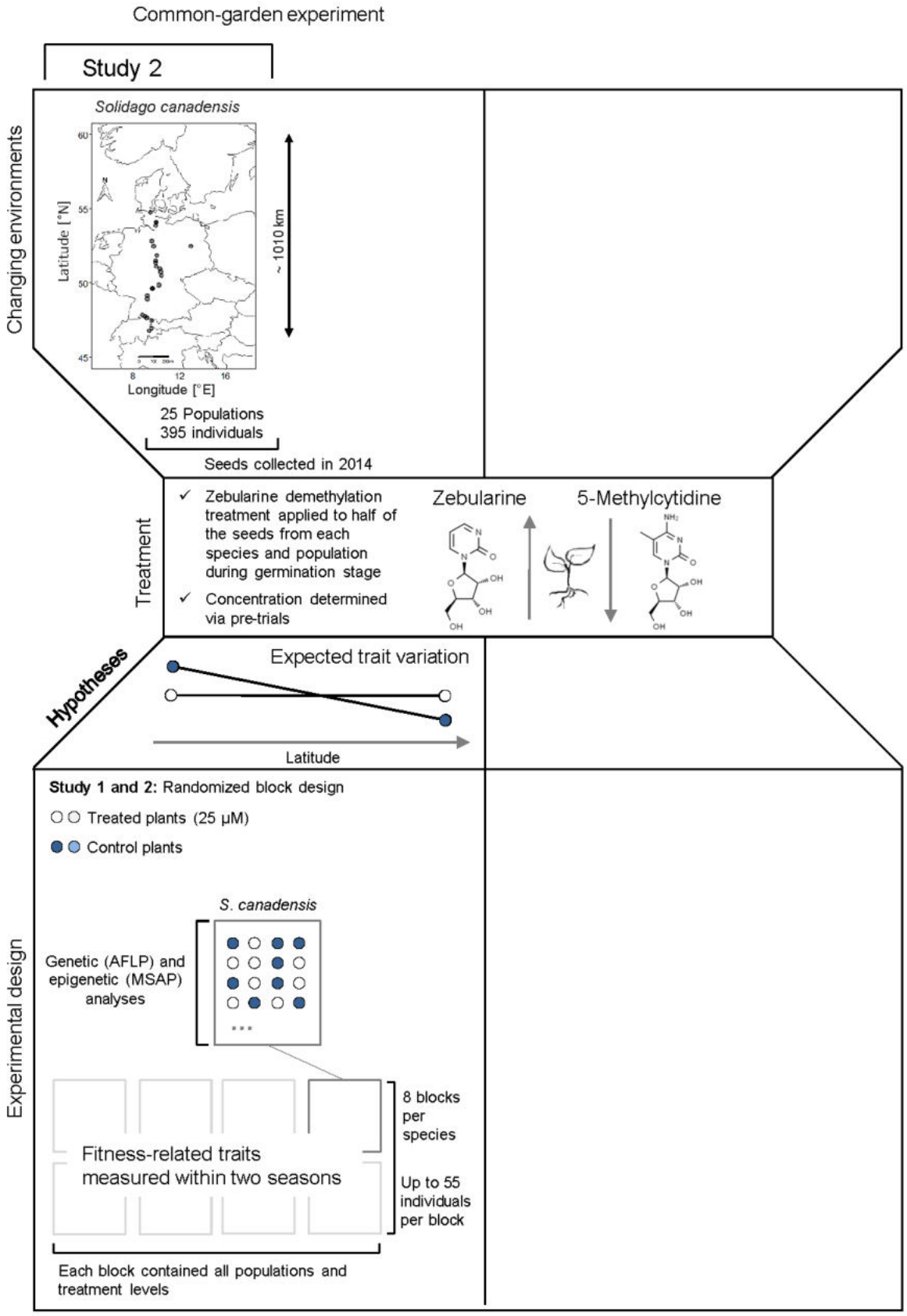
alien species by increasing the overall genetic variability and fitness (Rius & Darling, 2014; van Kleunen, Röckle, et al., 2015). A high degree of admixture has also been shown for invasive *S. canadensis* populations in China (Zhao et al., 2015) and Japan (Sakata et al., 2015). So, while there is considerable variation in presumably neutral microsatellite loci in *S. canadensis* in Central Europe, it does not show any latitudinal population structure, probably as a consequence of extensive admixture. Combined with the fact that latitudinal clines in flowering phenology and growth were not removed by demethylation, these results might suggest that selection resulted in latitudinal genetic variation at loci of adaptive significance.

In contrast to *S. canadensis*, *S. gigantea* showed a clear genetic structure along the sampled latitudinal transect. We detected three genetic clusters, and the populations could roughly be grouped into a southern, a central and a northern genetic cluster (Figure 2.4c). In line with this, we also found that genetic differentiation between populations correlated positively with geographical distance (Figure 2.4d). Interestingly, although Schlaepfer et al. (2008) found isolation-by-distance for chloroplast-DNA variation in the native North American range of *S. gigantea*, they did not find it in the European range. This discrepancy probably reflects the scale-difference between this broad Europe-wide study and our more fine-scale latitudinal transect. *Solidago canadensis* and *Solidago gigantea* both produce large amounts of lightweight, wind-dispersed seeds and are outcrossing (Alexander, Naylor, et al., 2009; Weber & Jakobs, 2005), but, in contrast to *S. canadensis*, *S. gigantea* is restricted to more moist and wet habitats, such as fen meadows (Weber & Jakobs, 2005)—ecosystems that have declined in area and frequency in Central Europe. Apparently, this limits gene flow at the scale of our sampled transect, and may also explain the gap in the distribution of *S. gigantea* in Central Germany (Figure 2.1; also see Weber & Schmid (1998).

Conclusions

Although epigenetic variation was shown to contribute to adaptation in *A. thaliana*, its broader significance remains unclear (Schmid et al., 2018). Recent literature has shown that DNA methylation appears to have only little influence on variation in gene expression (Meng et al., 2016; Moorsel et al., 2019), and might largely reflect genetic differences (Moorsel et al., 2019). In line with this, we found no evidence that latitudinal clines in two invasive species, *S. canadensis* and *S. gigantea*, can be explained by changes in cytosine methylation. We cannot exclude other potential epigenetic mechanisms, for example through the interplay with cryptic genetic variation (Gibson & Dworkin, 2004; Kalisz & Kramer, 2008; Zabinsky et al., 2019) or through shifts in the boundaries between euchromatin and heterochromatin (Li et al., 2015; Roudier et al., 2009), but, based on our findings, it seems most likely that genetic mechanisms underlie the latitudinal clines.

Chapter 3 | Study 2



Chapter 3 | Study 2: Traces of genetic but not epigenetic adaptation in the invasive goldenrod *Solidago canadensis* despite the absence of population structure

with Jasmin Herden, Marc Stift, Walter Durka, Mark van Kleunen and Jasmin Joshi

Abstract

Biological invasions may result from multiple introductions, which might compensate for reduced gene pools caused by bottleneck events, but could also dilute adaptive processes. A previous common-garden experiment showed heritable latitudinal clines in fitness-related traits in the invasive goldenrod *Solidago canadensis* in Central Europe. These latitudinal clines remained stable even in plants chemically treated with zebularine to reduce epigenetic variation. However, despite the heritability of traits investigated, genetic isolation-by-distance was non-significant. Utilizing the same specimens, we applied a molecular analysis of (epi)genetic differentiation with standard and methylation-sensitive (MSAP) AFLPs. We tested whether this variation was spatially structured among populations and whether zebularine had altered epigenetic variation. Additionally, we used genome scans to mine for putative outlier loci susceptible to selection processes in the invaded range. Despite the absence of isolation-by-distance, we found spatial genetic neighborhoods among populations and two AFLP clusters differentiating northern and southern *Solidago* populations. Genetic and epigenetic diversity were significantly correlated, but not linked to phenotypic variation. Hence, no spatial epigenetic patterns were detected along the latitudinal gradient sampled. Applying genome-scan approaches (BAYESCAN, BAYESCENV, RDA, and LFMM), we found 51 genetic and epigenetic loci putatively responding to selection. One of these genetic loci was significantly more frequent in populations at the northern range. Also, one epigenetic locus was more frequent in populations in the southern range, but this pattern was lost under zebularine treatment. Our results point to some genetic, but not epigenetic adaptation processes along a large-scale latitudinal gradient of *S. canadensis* in its invasive range.

Introduction

Heritable variation within a plant species is essential to adapt to new environments in response to natural selection (Darwin, 1859). Invasive plant species often have lower genetic variation in their new habitat due to founder effects or bottleneck events (Dlugosch & Parker, 2008), which makes it a challenge to explain their successful spread (Dlugosch et al., 2015; Estoup et al., 2016). Some clonal plants have become dominant in multiple habitats across a broad geographical range with only a single or a few genotypes, e.g., *Reynoutria japonica* (Hollingsworth & Bailey, 2000). It is still unclear how such species managed to adapt to a wide range of environmental conditions without genetic diversity to generate heritable variation for selection to act on.

Epigenetic mechanisms in plants are increasingly discussed as a potential explanation because these mechanisms may provide an additional source of phenotypic variation in genetically impoverished invasive species (Banerjee et al., 2019). Cytosine methylation is an adaptive epigenetic mechanism that may cause phenotypic plasticity and transgenerational evolutionary change (Bewick & Schmitz, 2015; Münzbergová et al., 2019). Methylated cytosines frequently occur in regulatory regions of genes, such as CpG islands or as part of gene-body methylations (Platt et al., 2015). Although it has been suggested that cytosine methylation is associated with local adaptation and could counteract possible bottleneck events during introduction processes (Banerjee et al., 2019), the results of the few studies analyzing this have been inconsistent (Herden et al., 2019; Sork, 2018). Therefore, further studies are needed to understand whether and how cytosine methylation is linked to transgenerational and persistent adaptation of plants in new environments.

Several studies have shown that variation in cytosine methylation might be associated with differentiation between local habitat conditions or along climatic gradients: for example in the clonal invasive species *Reynoutria japonica* (Richards et al., 2012) and the poleward range-expanding apomictic plant species *Taraxacum officinale* (Preite et al., 2015). Since variation in cytosine methylation was suggested as a response to changing environments (Münzbergová et al., 2019), it could also be involved in the formation of altitudinal (Alexander, Naylor, et al., 2009; Moran et al., 2017) and latitudinal clines across the invasive range (Eckert et al., 2021; Kollmann & Bañuelos, 2004), or related to invasion dynamics due to landscape heterogeneity (Deutschewitz et al., 2003; Eschtruth & Battles, 2009). Invasive plant species are exposed to novel environmental conditions often combined with high disturbance levels in their new range (Theoharides & Dukes, 2007). Therefore, to locally adapt, rapid, and heritable changes are crucial to counter impoverished genetic variation during introduction (Theoharides & Dukes, 2007). Heritable epigenetic mechanisms like changes in cytosine methylation patterns may expand phenotypic variability or the expression of cryptic genotypes in response to changing environmental cues (Marin et al., 2020; Mounger et al., 2021).

Several studies have shown the evolutionary significance of cytosine methylation in inbreeding and clonal non-native plant species (Dong et al., 2019; Douhovnikoff & Dodd, 2015), but studies on non-native outcrossing plants are still underrepresented. As rare examples for studies on native outcrossers using genetically diverse seed material, epigenetic variation was detected, e.g., for *Viola cazorlensis* (Herrera & Bazaga, 2010), and *Plantago lanceolata* (Gáspár et al., 2019). Moreover, variation in DNA methylation of genomic regions containing transposable elements has been suggested to mediate phenotypic variation in response to environmental change thus triggering rapid evolutionary processes (Baduel & Colot, 2021). Schmid et al. (2018) even

suggest that epigenetic variation may be more involved in adaptive processes among genetically diverse species compared to asexually reproducing ones. Thus, studying invasive outcrossing plant species in the context of cytosine methylation related to climatic variation is necessary to infer the adaptive potential of cytosine methylation, and its contribution to invasion success given the increasing number of non-native plants worldwide (Seebens et al., 2017). To the best of our knowledge, our study is the first to analyze the spatial structuring of non-clonal offspring of an invasive plant species at the epigenetic level quantifying its contribution based on experimental cytosine demethylation at the seedling stage.

For this purpose, we focused on *Solidago canadensis* s.l., a perennial Asteraceae, which is invasive in Central Europe, as well as in many other parts of the world, and which has formed latitudinal clines that persist even when plants are grown in common-garden environments (Eckert et al., 2021; Li et al., 2016; Weber & Schmid, 1998). Eckert et al. (2021) found that in Central Europe these clines persisted in offspring that have been treated with the cytosine demethylation agent zebularine. Although this suggests that variation in cytosine methylation does not play a role in generating adaptive variation along geographic clines, it might still reflect fine-scale spatial separation between populations as has been found, for example, for *Helleborus foetidus* (Herrera et al., 2016) and *Fragaria vesca* (De Kort et al., 2020). Additionally, De Kort et al. (2020) suggested that population history rather than short-term environmental stress might shape epigenetic signatures. For *S. canadensis*, tracing established populations back to their donor population in the native range is hardly feasible, but this species has been known in Central Europe since the seventeenth century (Aiton, 1813). Therefore, a reasonable period of time might have passed for *S. canadensis* populations in Central Europe to develop epigenetic and genetic signatures at varying spatial scales that are reflected in phenotypic variation. Because the effect of clinal variation in *S. canadensis* related to climatic and latitudinal gradients has been analyzed before (Eckert et al., 2021; Weber & Schmid, 1998), we focused on possible associations between (epi)genetic variation and phenotypic variation, and to what extent these associations might have been affected by the zebularine treatment. For this purpose, we incorporated the effect of spatial structure in the invasive range. Genetic structuring may vary at different spatial scales due to unequal gene flow (Ward, 2006). We addressed this by comparing amplified fragment length polymorphisms after digesting genomic DNA with methylation-insensitive (i.e., AFLP; Vos et al., 1995) and methylation- sensitive restriction enzymes (MSAP; Reyna-López et al., 1997) for *S. canadensis*—an outcrossing species—analyzing plants grown from seeds and sampled along a European latitudinal gradient (Eckert et al., 2021).

We analyzed leaf tissue from offspring that originated from 25 populations (Eckert et al., 2021) and scored AFLP and MSAP patterns for each individual to answer three main questions:

(a) To what extent is genetic and epigenetic variation of this species explained by spatial genetic autocorrelation and isolation-by-distance (IBD) patterns along the sampled latitudinal gradient? We hypothesized that *S. canadensis* populations are epigenetically differentiated despite their high degree of admixture at microsatellite markers (Eckert et al., 2021), and that this reflects spatially autocorrelated genetic and epigenetic variation but not an IBD pattern. Similar to findings in clonal and apomictic species, this may show that outcrossing and admixed invasive plant species exhibit heritable epigenetic variation that is sensitive to spatial heterogeneity in the invasive range and expressed in the phenotype. IBD is characterized by continuously distributed populations where geographically restricted gene flow will lead to distance-based population-level kinship (Malecot, 1948; Wright, 1946). This assumption is, however, violated in the presence of spatial autocorrelation patterns that might have been generated via transport corridors, i.e., highways and railways, in the invasive range (Hansen & Clevenger, 2005). Also, IBD assumes genotype-independent spatial variation in fitness imposed by the environment (Heywood, 1991) and only locally occurring genetic drift (Hardy & Vekemans, 1999). In contrast, spatial population "networks" could have arisen from hybridization of previously isolated native lineages (Hastings et al., 2005) or in interaction with environmental stochasticity resulting in variation of individual fitness and spread rates (O'Reilly-Nugent et al., 2016). We were also interested whether (b) there is an effect of demethylation treatment on putative relationships between epigenetic and phenotypic variation. We expected that zebularine treatment would remove putative associations between epigenetic and phenotypic variation due to the induced loss of methylated loci during cell division. This may indicate that non-random heritable epigenetic changes in this species are at least partly involved in generating phenotypic variation in the invasive range. And finally, we asked (c) whether AFLP and MSAP-based genome scans (BAYESCAN, BAYESCENV, LFMM, and RDA) reveal markers that are potentially associated with climatic variation or spatial genetic autocorrelation patterns across the latitudinal gradient sampled. The former would indicate that epigenetic signatures might be involved in adaptive processes associated with climatic conditions of the sampled *S. canadensis* populations in the invasive European range. The latter would imply that adaptive epigenetic responses might be unevenly shaped in the outcrossing *S. canadensis* due to patchy habitat conditions in its invasive range along the latitudinal gradient in Central Europe.

Materials and Methods

Plant species

Solidago canadensis s.l. is a perennial Asteraceae native to North America and introduced to Europe in the 17th century (Aiton, 1813; Weber, 2000), and to several other continents (van Kleunen et al., 2019). This species reaches heights of over two meters and flowers in Europe from July to October (Schmeil et al., 1993). Multiple shoots re-sprout in the next spring from rhizomes

after the aboveground parts have died back in winter (Weber, 2000). Flowers are self-sterile (Melville & Morton, 1982; Schmid & Dolt, 1994), although some degree of autofertility has been found (Razanajatovo & van Kleunen, 2016). This species heavily relies on anemochory with seeds equipped with a feathery pappus (Tackenberg et al., 2003). *Solidago canadensis* s.l. is represented by several cytotypes in its native range in North America, but so far, only diploid individuals have been found in the invasive range in Central Europe (van Kleunen & Schmid, 2003). In its non-native range, *S. canadensis* is frequently found in ruderal and disturbed sites (van Kleunen & Schmid, 2003) and, due to its ability to outcompete local flora, has been classified as an invasive species in Central Europe (Freisetzungsverordnung AS 2008 4377, 2008; Bundesamt für Naturschutz, 2013).

Experimental set-up

Leaf samples were collected from plants grown in a common- garden experiment in the Botanical Garden of the University of Konstanz. The experiment is described in detail in Eckert et al. (2021). In brief, *S. canadensis* seeds were collected between 2014 and 2015 from 25 wild populations along a latitudinal gradient in Central Europe, with at least five mother plants per population. Information on exact sampling locations, elevation (m a.s.l.), estimated numbers of ramets in the source populations, and the sample sizes of maternal lines are given in Table A3.1. Two batches of seed subsamples were germinated per mother plant, one was treated with an aqueous solution of 25 µM of zebularine during germination and the other was mock-treated with water. Zebularine treatment results in non-specific and dose-dependent genome-wide cytosine demethylation (L. Zhou et al., 2002), whereby zebularine inhibits DNA methyltransferases leading to hypomethylation during mitosis (Baubec et al., 2009; Champion et al., 2010). Zebularine-derived hypomethylation (hereafter demethylation) has reportedly been shown to be heritable and to modify abiotic and biotic stress responses in different plant species without changing the underlying DNA sequence (Baubec et al., 2009; Verhoeven & van Gurp, 2012).

In total, 400 *S. canadensis* plants (206 control plants; 194 zebularine treated plants; 224 maternal lines) were grown in an (incomplete) randomized-block design for 2 years (2015–2016). For each individual, the following phenotypic traits were obtained: initial seedling size, phenology (as days until flowering), plant height, the number of ramets counted in spring of the second year, and total and reproductive-to-total aboveground biomass harvested at the end of the first year. Eckert et al. (2021) found latitudinal clines in all traits (even pronounced in some traits measured on zebularine-treated individuals) except for the number of ramets and the initial seedling size. Latitude of source populations was significantly correlated with climate variables derived from the WorldClim v2.0 database (Fick & Hijmans, 2017). Therefore, we restricted the phenotypic variables in our study to the ones that showed latitudinal variation in Eckert et al. (2021). We refer

to Eckert et al. (2021) for further details about the zebularine treatment, the corresponding common-garden experiment and the scoring of plant traits.

AFLP/MSAP analyses

For this study, we collected intact leaves from 395 surviving and healthy *S. canadensis* plants (Eckert et al., 2021) at the onset of flowering in August 2015, and stored them in dry silica gel at room temperature. Genomic DNA was extracted from 10 to 20 mg of leaf tissue using an extraction kit (E.Z.N.A. Plant DNA Kit, omega BIO-TEK Inc.) and about 15% of the samples were randomly chosen to serve as duplicates for later error rate analysis. To assess genetic and epigenetic variation, we performed AFLP and MSAP analysis using protocols detailed in Gáspár et al. (2019) and Schulz et al.(2014) (see also Method A3.1 for changes applied to these protocols). For AFLP analysis, both the restriction and ligation step were combined in one reaction using 500 ng of genomic DNA with the standard enzymes *Eco*RI and *Mse*I in the AFLP analysis. In each analysis, we used four primer combinations in the selective amplification step (see Table A3.2 for primer sequences) with the selective *Eco*RI primers labeled with the fluorescent dyes FAM, VIC, NED, and PET. The amplified products were measured on an ABI 3130 Genetic Analyzer (Applied Biosystems) using the GeneScan LIZ 500 sizing standard (Thermo Fisher Scientific).

For MSAP analyses, we followed the same procedures, except that *Mse*I was replaced in two parallel reactions by the isoschizomers *Hpa*II and *Msp*I, which differ in their sensitivity to methylated cytosines (Schulz et al., 2013). The combination of these enzymes with different sensitivity allows the distinction between a maximum of four methylation states for each locus, which is non-methylated if a signal is present in both cases, fully or partially methylated if a signal is present in either of these cases and ambiguous in its status if no signal is present at the specific locus and individual. Selective primer combinations were selected based on pre-testing and quality checking.

AFLP and MSAP scoring

After peak sizing of each fragment, each primer combination was analyzed separately and binned using GeneMapper Software v5.1 (Thermo Fisher Scientific). Only peaks with a minimum relative fluorescence unit (RFU) height of 10 within the range of 50–500 base-pairs (bp) were binned as peaks. Binned peaks did not exceed a width of 1.5 bp and peaks were ignored if they overlapped with shoulder peaks or had an odd shape indicating contamination. After peak quality assessment, the final AFLP dataset received an overall error rate of 3.92% on average based on 44 replicates. The final MSAP dataset received an overall error rate of 7.16% on average based on 40 replicates. Details of the genotyping approach are provided in Method A3.2.

To determine the methylation state per peak (hereafter locus), binary tables were analyzed using the MSAP_calc.R script by Schulz et al.(2013) and the scoring option Mix1, which for each locus distinguishes two epigenetic states, methylated (hereafter MSAP-m) and non-methylated loci (hereafter MSAP-n; Schulz et al., 2013). In total, 385 *S. canadensis* plants (199 control plants and 186 zebularine-treated plants, Table A3.1) were successfully genotyped with an initial number of 856 peaks and, after quality scoring, comprised a final number of 361 polymorphic AFLP loci. Additionally, 336 of these plants (172 control plants and 164 zebularine-treated plants) were successfully epigenotyped with an initial number of 1,396 peaks each and a final number of 187 polymorphic methylated MSAP- m loci and 182 non-methylated MSAP-n loci. Both datasets will be openly available in a public repository (Eckert et al., 2022).

Population structure and isolation-by-distance

To check for population structure along the sampled latitudinal gradient, we used the software STRUCTURE v2.3.4 (Pritchard et al., 2000) on AFLP data of control plants assuming K = 1–11 genetic clusters (Figure A3.1). For each STRUCTURE run, we applied the admixture model and considered the sampling location (LOCPRIOR parameter) with a burn-in of 1,000,000 and 500,000 repetitions, respectively (Porras-Hurtado et al., 2013). Additionally, we tested for isolation-by-distance using the Mantel test with n = 9,999 permutations correlating the geographical log-distance between populations with their corresponding genetic distance as the standardized Wright's F_{ST} (Rousset, 1997; Figure 3.1).

Spatial genetic neighborhood (MEMGENEs)

To infer the impact of uneven gene flow in the invasive range, we checked for spatial genetic and epigenetic patterns using Moran's eigenvector maps (MEM; Dray et al., 2006; Griffith & Peres-Neto, 2006) and the *mgQuick* function in the R package MEMGENE v1.0.1 (Galpern et al., 2014; Figure 3.1). To find the set of eigenvectors that are significantly associated with spatial genetic and epigenetic variation, respectively, we used the geographic coordinates of the source populations and Nei's unbiased between-population distance (Nei, 1977) calculated with GENALEX v6.5 (Peakall & Smouse, 2012). The *mgQuick* function calculates all Moran's eigenvectors based on the population coordinates and reduces them to a significant set using both forward and backward selection of positive and negative eigenvectors against genetic and epigenetic distance, respectively, via permutation tests. Explained genetic and epigenetic variation of each eigenvector is then assessed using redundancy analysis (RDA). Finally, *mgQuick* calculates MEMGENE axes, i.e., spatial genetic neighborhoods from eigenvectors based on a principal components analysis (PCA) of the fitted values from RDA representing orthogonal vectors 500 maximizing spatial autocorrelation. We checked significant MEMGENE axes separately for genetic (AFLP) and epigenetic (MSAP-m, MSAP-n) datasets using non-treated

control samples. To test the extent to which MEMGENE axes were associated with coordinates of source populations, we calculated a correlation matrix using Pearson's correlation coefficient. Details of MEMGENE calculations are provided in Figures A3.2, A3.3.

Effect of the demethylation treatment

To investigate whether the zebularine treatment has shifted the presence/absence patterns of AFLP, MSAP-m and MSAP-n loci per sample, we conducted a principal coordinates analysis (PCoA; also known as multi-dimensional scaling) coupled with a multivariate dispersion test (Anderson et al., 2006) based on 9,999 permutations using the *betadisper* function from the VEGAN v2.5-6 package (Figure 3.2). We expected that the zebularine treatment would not affect genetic variation (AFLP and MSAP-n loci) but reduce the number of MSAP-m loci present. To assess between-individual dissimilarity, we used the Sørensen-Dice coefficient (Dice, 1945; Sørensen, 1948) which counts pairwise presence signals as matches. We used the Sørensen-Dice coefficient because we expected that the zebularine treatment would reduce the presence of methylated markers due to its demethylating characteristic. To infer among-population variation against within-population variation, we conducted an analysis of molecular variance (AMOVA; Table 3.1) according to Excoffier et al. (1992) on control plants based on 9,999 permutations using the *poppr.amova* function from the R package POPPR v2.8.6. For both, MSAP-m and MSAP- n, we assessed—at the population level—the total number of loci present, the percentage of polymorphic loci, and the Shannon diversity H_S per population and treatment using the MSAP_CALC v1.3 R script (Schulz et al., 2013; see details in Figures A3.4, A3.5). For epigenetic diagnostics, we used all corresponding plants per population but also confined this analysis using only sibling pairs (maternal lines) present in both control and zebularine-treatment groups. To check whether the frequencies of epigenetic loci (MSAP- m, MSAP-n) changed due to the zebularine-treatment, we compared control plants vs. zebularine-treated individuals for each locus on a population level. Therefore, we calculated the $\log_2$-fold change ($\log_2$FC) which is common to evaluate the direction of gene expressions (e.g. McCarthy & Smyth, 2009) and visualized $\log_2$FCs per locus and population via heatmaps and boxplots (see details in Figure A3.6). For the calculation of $\log_2$FC, we used only maternal lines that were present in both control and zebularine-treated subsamples.

To check whether zebularine treatment affects the relationship between (epi)genetic and phenotypic population-level variation, we conducted (partial) Mantel tests with n = 9,999 permutations. All (partial) Mantel tests were conducted separately for control and zebularine-treated plants using only plants that were both successfully genotyped and epigenotyped (169 control plants; 159 zebularine-treated plants; Table 3.2). In partial Mantel tests comparing epigenetic (MSAP-m, MSAP-n) and phenotypic variation, we controlled for genetic variation

(AFLP) and spatial genetic neighborhoods from MEMGENE analysis. We applied Nei's fixation index (Nei, 1977) as (epi)genetic population-level distance and population-level Euclidean distance for both trait variation and spatial genetic autocorrelation data. Trait variation was assessed using the population-average empirical quantile dispersion coefficient (Bonett, 2006) based on plant height and flowering phenology in both years, first-year total biomass and first-year reproductive-to-total biomass ratio, respectively. In addition to the unadjusted *p*-values from (partial) Mantel tests, we also provide adjusted *p*-values based on the false discovery rate (Benjamini & Hochberg, 1995; Yekutieli & Benjamini, 1999), but only the unadjusted *p*-values were used for interpretation.

Genome-scan approaches

To screen for (epi)genetic loci of potential adaptive significance (hereafter referred to as outlier loci), we ran genome scans. As suggested by Meirmans (2015), we applied four complementary genome-scan methods that are commonly combined to minimize the risk of false positives: BAYESCAN v2.1 (Foll & Gaggiotti, 2008), BAYESCENV v1.1 (de Villemereuil & Gaggiotti, 2015), redundancy analysis (RDA; Capblancq et al., 2018) and latent factor mixed models (LFMM; Frichot et al., 2013). We ran all methods separately on genetic (AFLP; except RDA) and epigenetic (MSAP-m, MSAP-n) data using non-treated control samples. Detected putative outlier loci per dataset were pooled from each genome-scan approach and visualized as Venn diagrams (Venn, 1881; Figure 3.3). Conservatively, we only retained outlier loci for further statistical analyses that were detected by at least two approaches.

The applied approaches vary in their algorithm but also in whether they are capable of including environmental variables in their calculations. BAYESCAN uses logistic regression to estimate locus-specific and population-specific contributions to the multilocus F_{ST}, distinguishing between two selection models, diversifying and balancing selection (Figure A3.7). In contrast to BAYESCAN, both BAYESCENV and LFMM rely on environmental variables as a reference to check for loci of potential adaptive significance (Figures A3.8–13). BAYESCENV implements the same algorithm as BAYESCAN on a modified set of models, a neutral model, a locus-specific model, and the local adaptation model linked to the corresponding environmental variables of interest. LFMM corrects for confounding effects due to underlying population structure by applying admixture coefficient estimation as a first step. We used the same WorldClim 2.0 variables as in Eckert et al. (2021) but accounted for collinearity of these variables via PCA and retained only the first three principal components (PC1, PC2, PC3) for the above-mentioned genome-scan approaches according to the broken-stick criterion (Figure A3.8). To infer outlier loci that might be associated with spatial genetic neighborhoods of populations, we applied RDA on the detected MEMGENE axes as environmental predictors on both epigenetic datasets

(MSAP m, MSAP-n; Figure A3.14) following Forester et al. (2018). Details and settings of the different genome-scan approaches are provided in Method A3.3.

Validation of outlier loci

To infer the variation of outlier loci along the latitudinal climatic gradient and across spatial genetic neighborhoods, and whether these were affected by the zebularine treatment, we applied logistic mixed-effects models (LogMM). All models were fitted separately using the *glmer* function from the LME4 v1.1-23 package and incorporated the binary (presence/absence) information of each outlier locus as response variable. The sampled source population and maternal lines nested within populations were included as random-intercept factors to account for non-independence of plants from the same maternal lines within populations. For each model, the second-order Akaike information criterion, and the marginal and the conditional R^2 were calculated using the AICc-function and the *r.squaredGLMM* function, respectively, from the MUMIN v1.43.17 package. We provide details of the model parameters in Tables A3.3, A3.4.

Fixed factors of each fitted model depended on the environmental variables with which the outlier loci were detected (AFLP: loc58, loc286; MSAP-m: loc189, loc282; MSAP-n: loc135, loc176, loc222; see summary in Table A3.5). Models fitted with loci detected with genome-scan approaches that were based solely on PCs from a principal components analysis (PCA) derived from WorldClim 2.0 variables (WorldClim- PCA) included zebularine treatment, corresponding principal components (PCs) and their interaction as fixed factors. We provide details on dimensionality reduction using PCA based on WorldClim 2.0 variables in Figure A3.8. Models fitted with outlier loci detected with genome-scan approaches based on both PCs from WorldClim-PCA and spatial genetic neighborhoods included zebularine treatment, all three MEMGENE axes and their interaction as fixed factors. Because spatial autocorrelation could already point to variation in local climatic conditions (Herrera et al., 2016), PCs from WorldClim-PCA were not included in these latter models. To facilitate model convergence, fixed factors were z-transformed and shifted to positive space by adding a constant, i.e., the highest negative value plus 0.1. For each model, we checked the assumption that the log-odds of the response and each continuous fixed factor was linear applying the Box-Tidwell procedure (Box & Tidwell, 1962). In rare cases where linearity was violated, fixed factors were squared. Models showed no influential values, which was checked for each model separately using Cook's distance (Cook, 1986), and only moderate levels of multicollinearity among covariates, which was checked with the variance inflation factor (Dormann et al., 2013).

Fixed effects were tested for significance using likelihood- ratio tests (LRT) by comparing the full model to a model without interaction and the non-interaction model with models where single terms have been iteratively removed (Lewis et al., 2011). In addition to the unadjusted

p values from LRTs, we also provide adjusted *p*-values using the false discovery rate (Benjamini & Hochberg, 1995; Yekutieli & Benjamini, 1999), but interpretation was based only on the unadjusted *p*-values. Predicted marginal-effect values were visualized with the GGPLOT2 v3.3.0 package and all results were obtained using R v4.0.2 (R Core Team, 2020).

Results

Spatial genetic and epigenetic structuring

We found weak but significant spatial genetic structuring in the analyzed *S. canadensis* populations along the sampled latitudinal gradient. Based on coordinates of *S. canadensis* source populations, three MEMGENE axes (hereafter MEMGENE1, MEMGENE2, MEMGENE3) significantly explained 13.2% of the genetic variation underlying Moran's eigenvector maps (MEMs; n = 1,000 permutations; Figure 3.1c). On each MEMGENE axis, shared genetic neighborhoods among populations are given as similar population-level values and their corresponding signs (negative or positive; Figure A3.2). MEMGENE1 explained 48% of variation underlying MEMs and divided the analyzed populations into a southern (populations 1–13) and a northern genetic subgroup (populations 14–25). This corresponds to results of admixture coefficients estimation via LEA within the genome-scan framework (Figure A3.10 and Method A3.3) as well as to the STRUCTURE analysis where $K = 2$ was detected as the most probable number of genetic clusters according to Evanno et al. (2005) (see population-averaged cluster probabilities from STRUCTURE analysis in Figure 3.1a). MEMGENE1 significantly correlated to latitude of the sampled source populations ($R^2 = 0.94$; $p < 0.001$), whereas MEMGENE2 and MEMGENE3 axes were independent of the latitudinal gradient (Figure A3.3).

MEMGENE2 (29% of variation explained underlying MEMs) and MEMGENE3 (23% of variation explained underlying MEMs) split populations into multiple alternating subgroups where subgroups at the top of the sampled latitudinal gradient as well as in the southern part (MEMGENE2) or only in the southern part (MEMGENE3) split the remaining subgroup (Figure 3.1c). AMOVA showed that there was significant genetic differentiation among populations explaining around 8% of genetic variation (Table 3.1), but this was not reflected in IBD along the sampled latitudinal gradient (Figure 3.1b). Coordinates of source populations did not significantly explain any epigenetic variation in the MEMGENE analysis and no pronounced epigenetic structuring was found with LEA (Figures A3.11, A3.12). In contrast, AMOVA showed significant epigenetic differentiation between populations explaining 2.7% (MSAP-m) and 4.3% (MSAP-n), respectively, of epigenetic variation (Table 3.1).

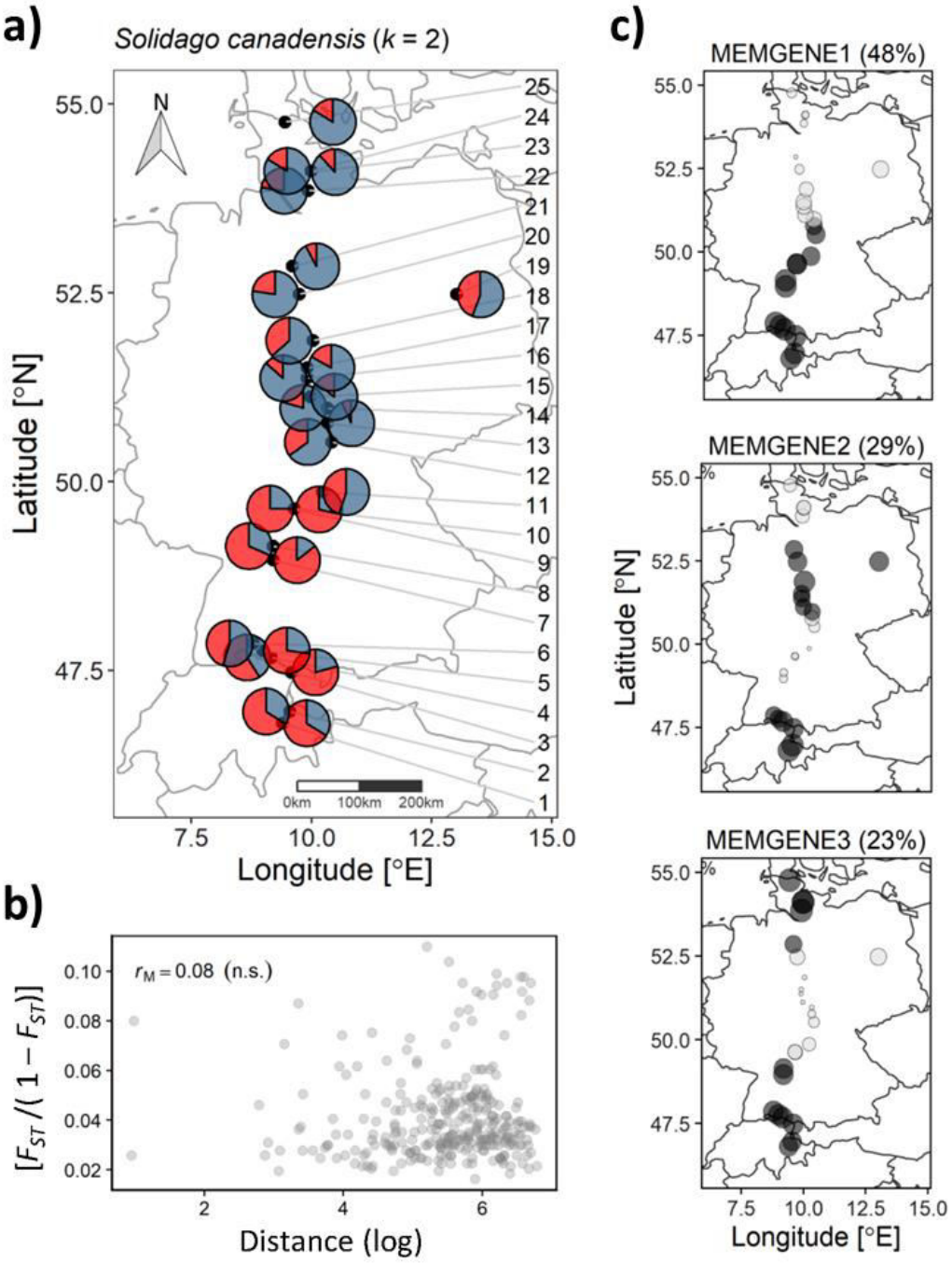


Figure 3.1: Genetic structuring and spatial genetic neighborhood of the 25 sampled populations of *S. canadensis* along the latitudinal gradient. (a) Genetic data (AFLP) was analyzed using the STRUCTURE software and the most probable clustering ($K = 2$) is given as a pie chart per population displaying the average probability per cluster. The population numbers (1–25) are denoted at the right side of the map with gray arrows pointing to their location. (b) Standardized pairwise genetic diversity [F_{ST} / (1 - F_{ST})] versus the pairwise log-distance between each population. The Mantel test was used to test for isolation-by-distance. Abbreviations: r_M – Mantel statistic; n.s. – not significant. (c) Significant spatial genetic autocorrelation axes (MEMGENE1, MEMGENE2, and MEMGENE3) and their corresponding percentage of spatial variation explained from Moran's eigenvector maps (see section *Spatial genetic neighborhood (MEMGENEs)*). Dark gray circles denote positive values and light gray circles denote negative values similar to principal components axes. Circles of similar size and shade denote populations with shared latent genetic neighborhood.

Table 3.1: Analysis of molecular variance from genetic (AFLP) and epigenetic (MSAP-m, MSAP-n) datasets. In total, 358 AFLP loci from 199 control plants, 184 MSAP-m loci and 169 MSAP-n loci both from 172 control plants were analyzed. Significance was assessed using permutation tests (n = 9,999) and significant *p*-values are given in bold. Abbreviations: df – degrees of freedom; SS – sum of squares; MS – mean squares; Estimate. – estimated variance; Var [%] – percentage of variance explained; φ – population differentiation statistic.

Source of variation		df	SS	MS	Estimate	Var. [%]	φ	*p*
AFLP	Between populations	24	1868.26	77.84	3.91	7.72	0.08	**<0.001**
	Within populations	174	8135.06	46.75	46.75	92.28		
	Total	198	10003.32	50.52	50.67	100		
MSAP-m	Between samples	24	782.86	32.62	0.76	2.71	0.03	**<0.001**
	Within samples	147	4026.11	27.39	27.39	97.29		
	Total	171	4808.97	28.12	28.15	100		
MSAP-n	Between samples	24	614.91	25.62	0.87	4.26	0.04	**<0.001**
	Within samples	147	2885.28	19.63	19.63	95.74		
	Total	171	3500.2	20.47	20.5	100		

Table 3.2: (Partial) Mantel tests conducted between phenotypic (Traits), genetic (AFLP) and epigenetic variation (MSAP-m; MSAP-n) separately for control and zebularine-treated plants. Tests were applied using population-based (n = 25) pairwise F_{ST} for (epi)genetic data and Euclidean distance for both trait and spatial genetic autocorrelation. Partial Mantel tests were applied only for epigenetic datasets controlling for genetic variation (AFLP) and spatial genetic autocorrelation (MEMGENE1, MEMGENE2, MEMGENE3). Significance was assessed based on n = 9,999 permutations and significant *p*-values are given in bold. Abbreviations: r_M – Mantel statistic; *p* – *p*-value; adj – adjusted *p*-value (false discovery rate).

Distance matrix			Control			Zebularine		
$Dist_X$	$Dist_Y$	$Dist_Z$	r_M	*p*	*adj*	r_M	*p*	*adj*
AFLP	Traits	-	-0.02	0.54	0.851	0.13	0.136	0.221
MSAP-m	AFLP	-	0.44	**<0.001**	**0.001**	0.14	0.119	0.221
MSAP-m	Traits	-	-0.13	0.832	0.851	0.1	0.216	0.236
MSAP-m	Traits	AFLP	-0.13	0.844	0.851	0.09	0.243	0.243
MSAP-m	Traits	MEMGENE1	-0.14	0.851	0.851	0.11	0.198	0.236
MSAP-m	Traits	MEMGENE2	-0.13	0.842	0.851	0.11	0.205	0.236
MSAP-m	Traits	MEMGENE3	-0.13	0.837	0.851	0.1	0.218	0.236
MSAP-n	AFLP	-	0.38	**0.002**	**0.014**	0.23	**0.037**	0.136
MSAP-n	Traits	-	-0.17	0.89	0.905	0.24	0.051	0.136
MSAP-n	Traits	AFLP	-0.17	0.9	0.905	0.22	0.063	0.136
MSAP-n	Traits	MEMGENE1	-0.17	0.905	0.905	0.24	0.055	0.136
MSAP-n	Traits	MEMGENE2	-0.17	0.892	0.905	0.24	0.051	0.136
MSAP-n	Traits	MEMGENE3	-0.17	0.895	0.905	0.24	0.054	0.136

Effect of the zebularine treatment

We found that more than one-third (37.9%; 66 loci) of all MSAP-m loci showed a fourfold decrease ($log_2FC < -2$) in occurence across zebularine-treated individuals compared to control plants, but also a fourfold increase ($log_2FC > 2$) was present in 22.9% loci (40 loci) pointing to a

bias for decreased methylation from the zebularine treatment. In addition, half of the MSAP-n loci (49.4%; 77 loci) showed a fourfold decrease in occurrence, whereas only 18.5% (29 loci) showed a fourfold increase. On average, up to 20.2 epigenetic loci per population at least halved in frequency whereas up to 15.9 epigenetic loci at least doubled in frequency (Figure A3.6). As expected, zebularine treatment did not affect genetic between-individual distance as revealed by PCoA and betadisper analyses (Figure 3.2a). Surprisingly, the overall between-individual distance for epigenetic loci was also not significantly reduced by the applied demethylation treatment (Figures 3.2b,c). Similarly, population-level descriptors of epigenetic diversity, i.e., the number of epigenetic loci present, the percentage of polymorphic epigenetic loci and the Shannon diversity index, were not significantly affected by the zebularine treatment (Figures A3.4, A3.5). Additionally, epigenetic population-level variation correlated with genetic variation as revealed by Mantel tests (Table 3.2). This relationship, however, vanished for MSAP-m but not for MSAP-n loci when looking at zebularine-treated plants of the same populations (Table 3.2). Population-level genetic and epigenetic diversity did not significantly reflect phenotypic population-level variation (Table 3.2). This was also the case for epigenetic population-level variation when controlling for genetic diversity and spatial genetic neighborhoods (Table 3.2).

Putative outlier loci

Our genome-scan approaches only revealed few outlier loci in *S. canadensis* populations that showed signs of adaptive significance. BAYESCAN detected only a single genetic outlier locus (label: loc185; size: 135 bp; marker sequence: ACG- CAT) but no epigenetic outlier loci (Figure 3.3a). BAYESCENV and LFMM, which relied on WorldClim 2.0 variables as environmental predictors derived from PCA (Figure A3.8), detected up to 15 outlier loci in AFLP (BAYESCENV: 8; LFMM: 9), MSAP-m (BAYESCENV: 4; LFMM: 15), and MSAP-n datasets (BAYESCENV: 3; LFMM: 9). From this pool of putative outlier loci, both approaches jointly detected six genetic and epigenetic outlier loci (AFLP: n = 2; MSAP-m: n = 1; MSAP-n: n = 0; Figures 3a–c). One of these jointly detected genetic loci [label: loc58; size: 58 bp;marker sequence: AAC-CCT] was significantly less probable in zebularine-treated plants as revealed by LogMM, although with an increasing probability along PC1 in both control and zebularine-treated plants (Figure 3.4a and Table 3.3). Any other jointly detected outlier locus was not significantly affected by the zebularine treatment and did not significantly vary with climatic variation among source populations (Table 3.3 and Table A3.3).

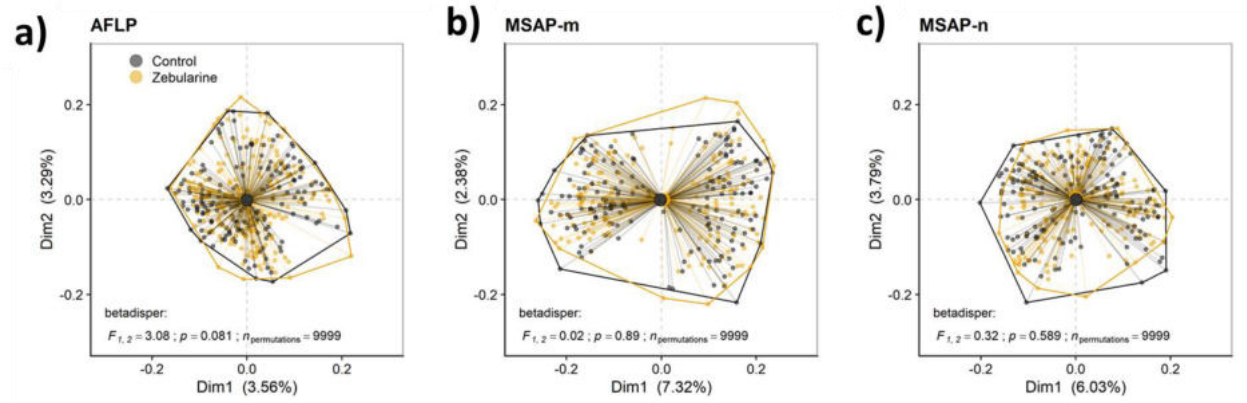


Figure 3.2: Biplots of principal coordinates analysis (PCoA) based on genetic (AFLP) and epigenetic (MSAP-m, MSAP-n) datasets from 25 *S. canadensis* populations. The first two PCoA axes (Dim1 and Dim2) denote the explained variances for (a) genetic loci (AFLP; loci: $n_{Control} = 358$; $n_{Zebularine} = 361$; samples: $n_{Control} = 199$; $n_{Zebularine} = 186$), (b) methylated epigenetic loci (MSAP-m; loci: $n_{Control} = 184$, $n_{Zebularine} = 187$; samples: $n_{Control} = 172$, $n_{Zebularine} = 164$), and (c) non-methylated epigenetic loci (MSAP-n; loci: $n_{Control} = 169$, $n_{Zebularine} = 181$; samples: $n_{Control} = 172$, $n_{Zebularine} = 164$) for both control (black) and zebularine-treated individuals (orange). The convex hulls display the border of the group dispersion with the group centroids. Dissimilarity between samples was determined using the Sørensen-Dice index (Dice, 1945; Sørensen, 1948). Multivariate homogeneity of group dispersion (betadisper) was used to infer differences in sample distance from the corresponding group centroid at the significance level of $p < 0.05$ using permutation tests ($n_{permutations} = 9{,}999$). Abbreviations: F – empirical F-distribution value in betadisper analysis with the corresponding degrees of freedom.

The RDA approach, which was applied only on epigenetic datasets (MSAP-m, MSAP-n) and relied on spatial genetic neighborhoods (MEMGENE axes; see Method A3.3) as environmental predictors, detected eight epigenetic outlier loci (Figures 3.3b,c). From this pool, a subset of four putative outlier loci, that were jointly detected by additional approaches to reduce false positives, was further analyzed (Table 3.4). One of these epigenetic outlier loci (label: loc135; size: 135 bp; marker sequence: AGG-CGA) was significantly less probable with increasing values of the spatial genetic neighborhoods (MEMGENE1), however, this was evident for control plants only but not for zebularine-treated individuals (Figure 3.4b). All other jointly detected putative epigenetic outlier loci were not significantly affected by either the zebularine treatment or spatial genetic neighborhoods of source populations (Table 3.4 and Table A3.4).

Discussion

Cytosine methylation has received growing attention in recent years as a potential epigenetic driver of adaptation (Banerjee et al., 2019; Richards & Pigliucci, 2021). In our study, we analyzed standard (AFLP) vs. methylation-sensitive (MSAP) amplified fragment length polymorphisms derived from 25 populations of the invasive plant species *S. canadensis* s.l. in Central Europe. We showed that *S. canadensis* populations in the invasive range formed spatial genetic neighborhoods, but that no spatial epigenetic patterns were evident.

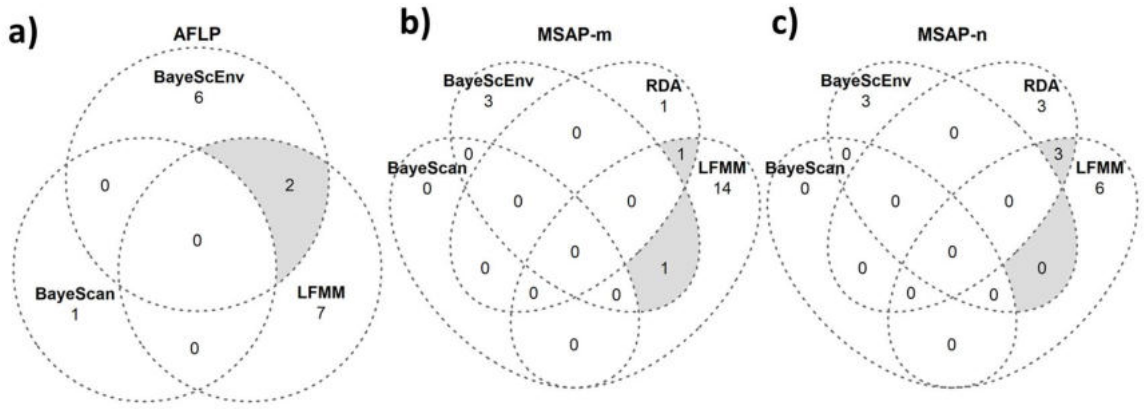


Figure 3.3: Venn diagrams from genome scans for outlier loci in *S. canadensis*. Outlier loci were detected using the genome-scan approaches BAYESCAN, BAYESCENV, LFMM and RDA separately on (a) genetic (AFLP) and (b, c) epigenetic (MSAP-m, MSAP-n) datasets. Details of these approaches are given in Method A3.3. Gray areas denote outlier loci that were jointly detected by different approaches and, to avoid false positives, only these loci were further analyzed in logistic mixed-effects models (Tables 3.3 and 3.4).

We also showed that among-population genetic variation was not correlated with epigenetic variation under zebularine treatment. Finally, we detected one genetic locus which increased in frequency along a climatic gradient, and another epigenetic locus which decreased in frequency along a spatial genetic autocorrelation gradient for control, but not for zebularine-treated plants. Our results thus point to some genetic adaptation processes in *S. canadensis* in the invasive range, but also indicate that these processes have not resulted in spatially structured epigenetic variation.

Spatial genetic and epigenetic neighborhoods

In contrast to clonal, apomictic or selfing species, outcrossing invasive species, such as *S. canadensis*, should be more affected in their genetic structuring at different spatial scales due to unequal gene flow and dispersal barriers (Ward, 2006). Spatial spread has been found to be often non-uniform, non-linear or to proceed from several locations simultaneously (Hastings et al., 2005). Based on Moran's eigenvector maps, we found that genetic variation, but not epigenetic variation reflects the spatial distribution of *S. canadensis* plants. Epigenetic variation has been linked to rapid phenotypic evolution (Zhang et al., 2013), which might be beneficial for outcrossing invasive species, e.g., in ruderal sites which are characterized by spatial heterogeneity and unstable environmental conditions creating potential for epigenetic memory and priming (Turgut-Kara et al., 2020).

Table 3.3: Summary of likelihood-ratio tests (LRT) from logistic mixed-effects models (LogMMs) applied to outlier loci detected jointly via the genome-scan approaches BAYESCENV and LFMM. Both approaches were applied seaparately to both genetic (AFLP) and epigenetic (MSAP-m, MSAP-n) datasets. Models included principal components from principal components analysis on WorldClim 2.0 variables (see Method A3.3) and zebularine treatment, and their interaction as fixed effects (details provided in Table A3.3). Population and maternal lines nested within population were added as random effects. Significant raw and adjusted *p*-values (false discovery rate) are given in bold. Abbreviations: X^2 – chi-square statistic; *p* – *p*-value; adj – adjusted *p*-value; PC1 – first principal component; PC2 – second principal component; PC3 – third principal component.

	AFLP						**MSAP-m**		
	loc58			loc286			loc189		
Explanatory variable	X^2	***p***	***adj***	X^2	***p***	***adj***	X^2	***p***	***adj***
Treatment	2.83	0.093	0.232	0.11	0.739	0.896	1.68	0.194	0.485
PC1	8.65	**0.003**	**0.015**	0.03	0.857	0.896	-	-	-
PC2	0.07	0.792	0.879	2.22	0.136	0.680	0.01	0.943	0.943
PC3	-	-	-	-	-	-	0.23	0.633	0.912
PC1 x Treatment	0.02	0.879	0.879	0.06	0.808	0.896	-	-	-
PC2 x Treatment	0.45	0.505	0.842	0.02	0.896	0.896	0.12	0.730	0.912
PC3 x Treatment	-	-	-	-	-	-	2.25	0.134	0.485

Inheriting the parental epigenome, however, was also suggested to being less beneficial after long-distance dispersal into a new range and advantageous only in environments where gene expression states were already fine-tuned to cope with local conditions (Verhoeven & Preite, 2014). Linking epigenetic diversity of the outcrossing species *S. canadensis* to spatial variation in the invasive range in Central Europe, we found that long-term spread within the invasive range did not result in spatial epigenetic structuring. The results of our study indicate that cytosine methylation appears to be less significant in adaptation processes of outcrossing invasive plant species that exhibit high levels of admixture. Our findings support the idea that, in contrast to clonal or apomictic non-native plant species that might exhibit a high degree of epigenetic differentiation (Verhoeven & Preite, 2014), in outcrossing and admixed invasive plant species epigenetic differentiation might rarely exceed genetic variation and thus will likely not result in population structuring. Spatial heterogeneity has been found to create and to increase time lags during invasion and to act as an environmental filter during the different invasion stages (Theoharides & Dukes, 2007). Rather than environmental selection on genotypic composition, the spatial genetic neighborhoods might also reflect that the analyzed *S. canadensis* populations vary in their stage of introduction or could be affected by bottleneck as well as by multiple introduction events at different spatial scales (Ward, 2006).

Table 3.4: Summary of likelihood-ratio tests (LRT) from logistic mixed-effects models (LogMMs) applied to outlier loci detected jointly via the genome-scan approaches LFMM and RDA. Models included spatial genetic autocorrelation variables (MEMGENE1, MEMGENE2, MEMGENE3) and zebularine treatment, and their interaction as fixed effects (details provided in Table A3.4). Population and maternal lines nested within population were added as random effects. Significant raw and adjusted *p*-values (false discovery rate) are given in bold. Abbreviations: X^2 – chi-square statistic; *p* – *p*-value; adj – adjusted *p*-value.

	MSAP-m			**MSAP-n**								
	loc282			loc176			loc222			loc135		
Explanatory variable	***X²***	***p***	***adj***	***X²***	***p***	***adj***	***X²***	***p***	***FDR***	***X²***	***p***	***adj***
Zebularine (Z)	1.17	0.279	0.873	0.04	0.846	0.846	0.81	0.368	0.638	0.81	0.368	0.798
MEMGENE1 (MG1)	0.14	0.712	0.873	1.65	0.198	0.549	1.86	0.173	0.605	1.86	0.173	0.605
MEMGENE2 (MG2)	0.63	0.426	0.873	0.14	0.711	0.830	0.56	0.456	0.638	0.56	0.456	0.798
MEMGENE3 (MG3)	2.10	0.147	0.873	0.44	0.507	0.710	0.08	0.782	0.782	0.08	0.782	0.912
MG1 x Z	0.23	0.629	0.873	1.16	0.281	0.549	0.73	0.393	0.638	4.60	**0.032**	0.224
MG2 x Z	0.01	0.930	0.930	2.12	0.145	0.549	0.30	0.583	0.680	0.01	0.922	0.922

Herrera et al. (2016) found that epigenetic between-individual similarity in *Helleborus foetidus*, a native plant species for which clonal propagation is exceptional, was greater than genetic similarity at the shortest distances. They pointed out that differences in local environmental features might play a similar or even greater role than spatial distance for epigenetic population structure, arguing for isolation-by-environment rather than IBD (Herrera et al., 2016). Our study focused on climatic variation and spatial autocorrelation along a latitudinal gradient, therefore, we did not include additional local site-specific features into our analyses. For example, both local soil properties and functional diversity have been found to affect the performance of *S. canadensis* in its invasive range in Central Europe (Czortek et al., 2020). Therefore, future studies on *S. canadensis* in Central Europe might more strongly emphasize local and site-specific conditions and their impact on epigenetic variation.

Ancestry estimation using the LEA framework indicated two epigenetic clusters, however, not structured along the latitudinal gradient under study. This is in line with (Lele et al., 2018), who analyzed genetic and epigenetic differentiation in the non-clonal plant species *Vitex negundo* var. *heterophylla* in its native range and found that adaptation to heterogeneous habitat conditions was mainly genetically driven. The results of this study, however, also indicated a weak connection of epigenetic diversity and adaptive phenotypes. In our study, we did not find a significant relationship between epigenetic population-level diversity and trait variation, but there was low but significant epigenetic population-level differentiation as revealed by AMOVA.

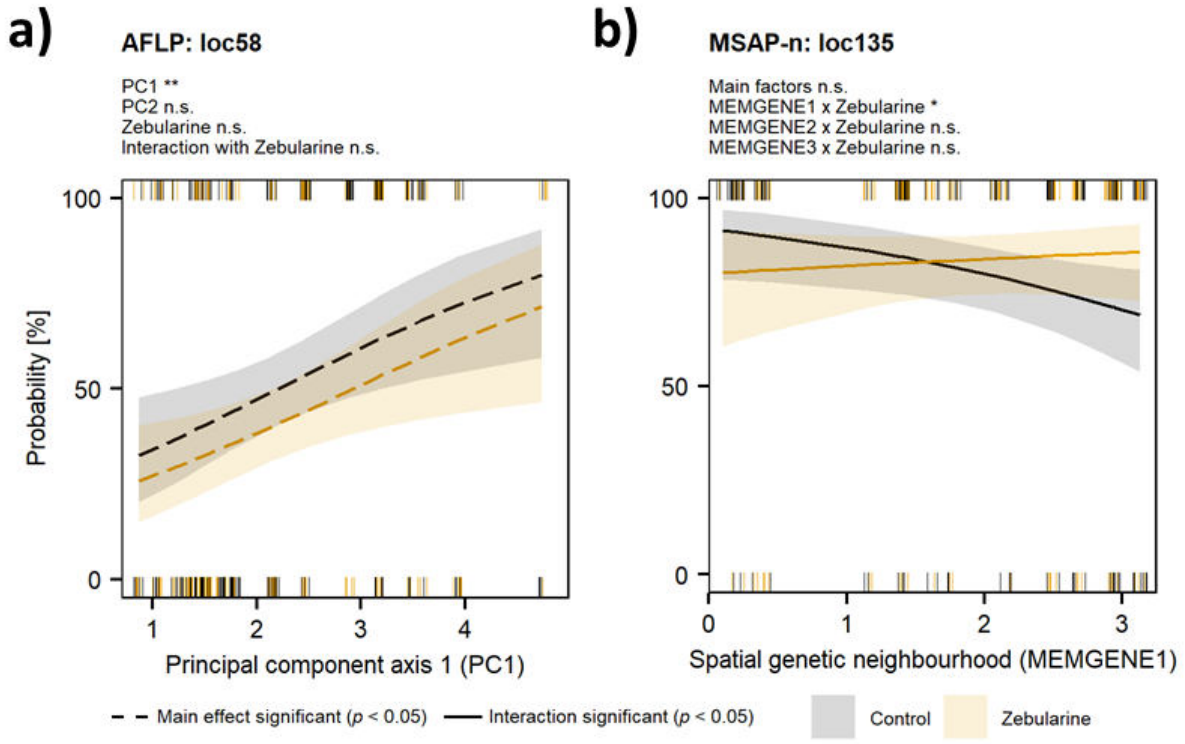


Figure 3.4: Probability of genetic (AFLP) and epigenetic (MSAP-n) outlier loci for *S. canadensis* along climatic (WorldClim-PCA) and spatial genetic autocorrelation (MEMGENE) gradients. Outlier loci (see Method A3.3) were analyzed with logistic mixed-effects models followed by likelihood-ratio tests (Tables 3.3 and 3.4; significance levels: **p < 0.01; *p < 0.05; n.s., not significant). Individual responses per group (control: black; zebularine treatment: orange) are given as rugs at the bottom (loci absent) and at the top (loci present). Predicted marginal-effect values are indicated by different line types (dashed—main effect significant; solid—effect of interaction with the zebularine treatment significant). (a) Genetic outlier locus (loc58; Table A3.3) analyzed with a model including principal components, zebularine treatment, and the interaction with zebularine treatment as fixed factors. (b) Epigenetic outlier locus (loc135; Table A3.4) analyzed with a model including MEMGENE variables, zebularine treatment, and the interaction with zebularine treatment as fixed factors.

This epigenetic population-level differentiation may be linked to adaptation involving traits that were not measured in this study and that could be correlated to local environmental features. Our results, however, speak against an adaptive role of spatial epigenetics in *S. canadensis* in the invasive range. The three significant spatial genetic autocorrelation axes found in our study, however, might indicate that adaptive dynamics in *S. canadensis*, if present, take place at different spatial scales simultaneously and in a non- linear fashion.

Besides evident spatial genetic neighborhoods, *S. canadensis* populations were divided along the latitudinal gradient by two genetic clusters as revealed by the STRUCTURE and LEA approaches although with no IBD dynamics. This finding is surprising given that Eckert et al. (2021) found an overall lack of genetic population structure along this gradient based on seven microsatellite markers. In contrast to microsatellites, AFLPs are dominant markers with no information about heterozygosity, but have been found to be more efficient in individual-based population-assignment when there is weak population structuring (Campbell et al., 2003). Yet,

population differentiation in *S. canadensis* based on AFLP analysis was weak and only a small amount of genetic variation could be explained by spatial patterns. While our results support the microsatellite analysis in Eckert et al. (2021), weak population differentiation is an unexpected result given the large latitudinal gradient and the long residence time of *Solidago canadensis* in Central Europe. Future approaches, however, might focus on more sophisticated molecular techniques such as SNPs (Manel et al., 2010) or spatial modeling based on large numbers of loci to map ecological adaptation (Fitzpatrick & Keller, 2015). The importance of including spatial heterogeneity into modeling plant invasion has been demonstrated in several studies (Herrera et al., 2016; Štajerová et al., 2017). Based on their results for *Helleborus foetidus*, Herrera et al. (2016) even argued that genetic and epigenetic differentiation might develop independently of each other leading to contrasting spatial patterns from spatially divergent selection. More studies are needed to assess whether this is the case for *S. canadensis*.

Effects of the demethylation treatment

To the best of our knowledge, our study is the first to analyze heritable epigenetic spatial structuring in non-clonal offspring of an invasive plant species by quantifying effects of experimental cytosine demethylation. Although the demethylation agent zebularine shows dose-dependent downsides, such as delayed height and root growth (Cho et al., 2011; Eckert et al., 2021), it has been useful to detect stress-related transgenerational epigenetic variation in clonal and apomictic plant species (Preite et al., 2015; Richards et al., 2012). In our study, we found one epigenetic locus that was affected by the zebularine treatment, i.e., the presence of this locus varied depending on spatial variation. Variation in epigenetic patterns using the AFLP/MSAP technique has already been demonstrated in different plant species in their natural habitat. For example, Schulz et al. (2014) have detected one epigenetic locus in *Viola elatior* whose probability varied with the amount of photosynthetic active radiation present. Additionally, changes in methylation related to temperature have been shown for different plant species, e.g., *Arabidopsis thaliana* (Finnegan, Genger, Kovac, et al., 1998) and *Festuca rubra* (Münzbergová et al., 2019). The underlying plant material for our study, however, was only sampled once, at the end of the first year of the common-garden experiment. Therefore, we cannot exclude that there were changes in methylation profiles of control plants resulting from the common-garden environment itself, e.g., epigenetic shifts due to microclimatic conditions in the common- garden environment. In general, the majority of the analyzed 369 epigenetic loci (MSAP-m: 187 loci; MSAP-n: 182 loci) remained nearly unchanged, when zebularine effects were compared within populations. When there were changes in frequency, they were bidirectional: Up to 44.1% of epigenetic loci, i.e., MSAP-m and MSAP-n loci combined, showed a fourfold decrease, but up to 21.3% showed a fourfold increase in frequency. This non- directional change might be attributed

to an increase in the level of transposable elements due to zebularine treatment, as found for *Arabidopsis thaliana* ecotypes (Konečná et al., 2021). With our approach, we could link the variation in two of these anonymous loci to climatic and spatial genetic variation, but more specific associations will need further analyses of the underlying mechanistic context, e.g., using next-generation approaches (Schield et al., 2016; Werner et al., 2020).

Although more epigenetic loci decreased than increased in mean frequency in zebularine-treated population subsets, the applied zebularine treatment did not result in a global change in the number of loci per population, percentage of polymorphic loci and Shannon diversity. Unfortunately, we did not sample at the beginning of the growing season, so the long-term stability of the applied zebularine treatment and, thus, persistent detectable changes remains to be tested. While we saw visible changes in plant phenotypes during the germination phase, the concentration applied during the germination phase might have been too low to cause persistent detectable changes. Hence, possible treatment differences, which may have been present at the beginning, may no longer have been visible in methylation profiles of later stages. In fact, plant DNA has been shown to be capable of compensating for experimental demethylation through repair mechanisms (Liu et al., 2015). If this is the case here, then this implies that experimental cytosine demethylation in *S. canadensis* can be compensated for within one growing season and, that cytosine methylation appears to be short-term only in this species.

Genetic and epigenetic population-level diversity in *S. canadensis* were strongly correlated. This is not surprising given that epigenetic variation is frequently found to be genetically controlled (Richards et al., 2017). In our study, however, we found that zebularine treatment decoupled genetic from epigenetic (MSAP-m) diversity. Still, there are plant species in which epigenetic variation may also exceed genetic variation or more strongly relate to a distinct environmental variable, e.g., grazing intensity in *Plantago lanceolata* populations in Germany (Gáspár et al., 2019). Although epigenetic variation might play a role in plastic responses to environmental challenges in the field in *S. canadensis*, the results of our study suggest that cytosine methylation most likely does not play a crucial role in transgenerational adaptive changes in its invasive range in Central Europe.

Solidago canadensis is often found in anthropogenically disturbed habitats (Weber, 2000). These habitats are often hotspots of continued introduction of non-natives and it seems that introduction rates for *S. canadensis* have not declined since this species has been introduced in Central Europe in the seventeenth century (Weber, 2000). Thus, variation in (epi)genotypes, i.e., variation in the presence of (epi)genetic loci, could also be due to multiple introductions and secondary spread pointing to unrelated genotypes. A similar scenario, has been assumed for the invasive hexaploid *S. canadensis* populations in China (Guo et al., 2016). We do not know the

donor populations of introduced *S. canadensis*, so direct comparisons with populations in their native North American range were not possible. Future studies should do intensive sampling in the native range and analyze genome-wide nucleotide variation to infer possible source populations (e.g., Vallejo-Marín et al., 2021). It would also be interesting to expand the latitudinal gradient in the invaded area in future studies to include aspects of land use and urbanization related to genetic and epigenetic effects at different scales (e.g., Gáspár et al., 2019).

Loci and epiloci under selection

Multiple studies have demonstrated elevational and latitudinal phenotypic clines in fitness-related traits for *S. canadensis* in the invasive range in Central Europe, and some of these clines persisted when offspring were grown in a common-garden environment (Eckert et al., 2021; Moran et al., 2017; Weber & Schmid, 1998). These phenotypic clines should thus be reflected at the genetic level, but possibly likewise at the epigenetic level. Applying three genome-scan approaches and subsequent logistic mixed-effects models, we were able to detect one genetic and one epigenetic locus putatively under selection. One of these genetic loci was significantly associated with climatic variation along the latitudinal gradient and one epigenetic locus was significantly correlated to spatial genetic variation. A comparably low number of two genetic outlier loci with signatures of selection were jointly detected for *Viola elatior* (Schulz et al., 2014) when applying multiple genome-scan approaches, though compared to a much higher number of 39 detected epigenetic outliers. Conversely, Herrera & Bazaga (2010) found that only 10 out of 23 candidate loci were significantly associated with epigenetic differentiation in *Viola cazorlensis* pointing to a link between genetic and epigenetic divergence. The low number of only two candidate outlier loci detected in our study might be attributed to ongoing introductions that maintain admixture in *S. canadensis* and, thus, introduce unrelated (epi)genotypes (Dlugosch et al., 2015; Verhoeven et al., 2011). In this case, the outlier loci would be false positives despite their detection by multiple genome-scan approaches. It could also be a sign of a yet cryptic differentiation, which could be obscured by already pre-existing (epi)genetic variation from the invasion process of this species. Therefore, it remains to be tested whether this might be due to adaptive processes or genetic drift. Likewise, putative non-neutral epigenetic changes might have triggered the Baldwin effect, i.e., plastic responses to environmental cues induced via epigenetic variation might eventually be replaced by genetic change (Bräutigam & Cronk, 2018; Simpson, 1953). This process is yet hypothetical and still needs to be tested in plants (Bräutigam & Cronk, 2018).

Our study of *S. canadensis* in Europe revealed the presence of one genetic locus associated with climatic variation and one epigenetic locus associated with spatial variation along the studied latitudinal gradient. Interestingly, not all spatial patterns detected with Moran's eigenvector maps and introduced into the genome screening using RDA resulted in the same, if

any, putative loci with signatures of selection. This could suggest that within the analyzed latitudinal gradient, small-scale heterogeneous dynamics and non-linear spatial corridors (Hansen & Clevenger, 2005) between populations might affect allele frequency more strongly than isolation-by-distance processes along the gradient under study. For example, (De Kort et al., 2020) used *Fragaria vesca* offspring collected as seeds from both a steep altitudinal (<2 km) and a wide spatial (>500 km) gradient and have grown individuals in a controlled environment to characterize DNA methylation signatures at different spatial scales. Their study demonstrated that epigenetic differentiation arises at varying spatial scales with CG methylation divergence more pronounced at the fine-scale altitudinal gradient. Since plant material for our analyses was collected at the end of the first growing season in the common-garden, we can only map one point in the lifetime of the plants. Many studies on epigenetic structuring among plant populations collected the samples directly in the field (see e.g., Bewick & Schmitz, 2015; De Kort et al., 2020; Gáspár et al., 2019; Herrera & Bazaga, 2010). Under field conditions, epigenetic structure, if present, could be directly affected by the local environment of the species. In our case, because we sampled in a common garden, the results would be due to transgenerational epigenetic inheritance. Still, it remains to be tested whether variation in the detected loci will be transgenerationally inherited and which underlying mechanistic processes these loci might trigger.

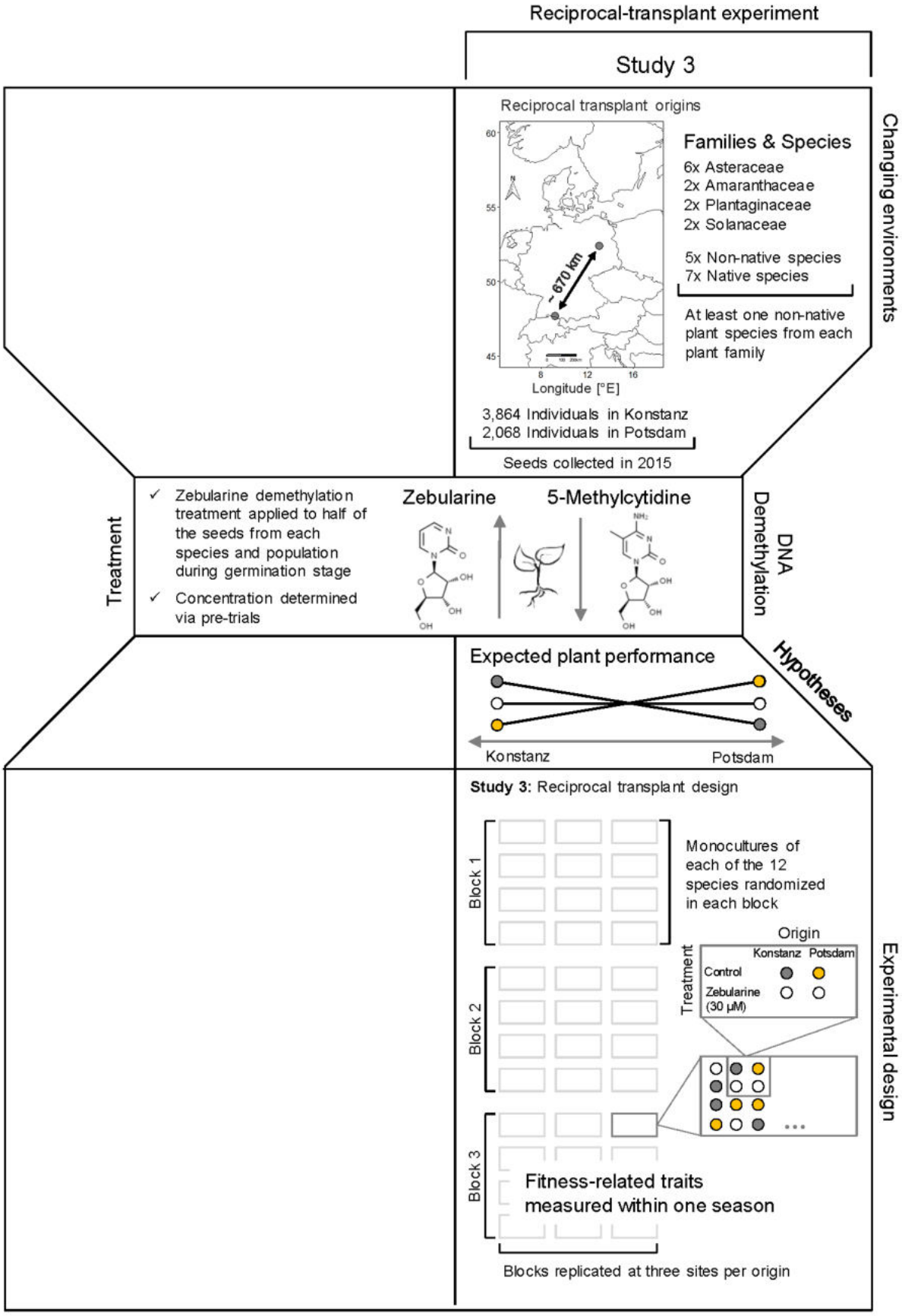
No genetic and epigenetic outlier loci were detected jointly by all three (for epigenetic loci four) genome-scan approaches. This could be because each of these approaches followed different strategies: BAYESCAN is able to detect stabilizing vs. diversifying selection if present (Foll & Gaggiotti, 2008), and BAYESCENV (de Villemereuil & Gaggiotti, 2015) and LFMM (Frichot et al., 2013) were developed to detect loci associated with environmental variables. Although the AFLP/MSAP approach is still useful for non-model organisms in detecting population structuring, it does not provide the regulatory context of the genomic sequence, i.e., linking gene-expression states to complex trait variation. Therefore, future approaches might overcome this limitation by focusing on more advanced methods of genome-wide association, such as QTL analysis (Long et al., 2011), bisulfite sequencing (Lu et al., 2020) or epiGBS (Schield et al., 2016; Werner et al., 2020).

Conclusions

Stochastic epigenetic changes, e.g., epimutations, were found to greatly exceed the frequency of DNA mutations providing a further source for phenotypic variation (van der Graaf et al., 2015). Transgenerational epigenetic variation in cytosine methylation has been related to phenotypic plasticity (K. J. F. Verhoeven & van Gurp, 2012; Y.-Y. Zhang et al., 2013) and has been suggested to be a promising mechanism in explaining the invasion success of introduced plant species (Banerjee et al., 2019). Still, only few studies analyzed cytosine methylation in genetically

diverse plant species when signals of population structure were weak (but see e.g., Herrera et al., 2019). We showed that genetic variation in the outcrossing invasive plant *S. canadensis* is non-linearly distributed across the analyzed latitudinal gradient. Moreover, artificial demethylation with zebularine decoupled genetic from epigenetic population-level distance and altered the frequency of one epigenetic locus. It is striking, however, that *S. canadensis* showed only few signatures of selection and a surprisingly low level of population differentiation in Central Europe. Epigenetic diversity was not linked to phenotypic variation, pointing to either developmental instability (Klingenberg, 2019) or unrelated but robust epigenotypes derived from ongoing introductions. Based on our results, we conclude that *S. canadensis* exhibits weak-to-absent transgenerational epigenetic variation in the invasive range.

Chapter 4 | Study 3



Chapter 4 | Study 3: No evidence for local adaptation and an epigenetic underpinning in native and non-native ruderal plant species in Germany

with Jasmin Herden, Marc Stift, Jasmin Joshi and Mark van Kleunen

Abstract

1. Many invasive species have rapidly adapted to different environments in their new ranges. This is surprising, as colonization is usually associated with reduced genetic variation. Heritable phenotypic variation with an epigenetic basis may explain this paradox.
2. Here, we assessed the contribution of DNA methylation to local adaptation in native and naturalized non-native ruderal plant species in Germany. We reciprocally transplanted offspring from natural populations of seven native and five non-native plant species between the Konstanz region in the south and the Potsdam region in the north of Germany. Before the transplant, half of the seeds were treated with the demethylation agent zebularine. We recorded survival, flowering probability, and biomass production as fitness estimates.
3. Contrary to our expectations, we found little evidence for local adaptation, both among the native and among the non-native plant species. Zebularine treatment had mostly negative effects on overall plant performance, regardless of whether plants were local or not, and regardless of whether they were native or non-native.
4. Synthesis. We conclude that local adaptation, at least at the scale of our study, plays no major role in the success of non-native and native ruderal plants. Consequently, we found no evidence yet for an epigenetic basis of local adaptation.

Introduction

Over the last centuries, human activities have led to the introduction of thousands of plant species across biogeographical barriers (van Kleunen et al., 2018). Of these, more than 13,000 have become naturalized (Pyšek et al., 2017; van Kleunen, Dawson, et al., 2015), and, occasionally, such naturalized species become invasive with negative ecological and socioeconomic impacts (Simberloff et al., 2013; Vilà et al., 2011; Vilà & Hulme, 2017). Understanding how invasive species cope with the abiotic and biotic environment in their new range is therefore both of fundamental and applied interest (Allendorf & Lundquist, 2003; Estoup et al., 2016; Schrieber & Lachmuth, 2017).

The adaptability of invasive species is surprising, since many non-native species go through genetic bottlenecks during introduction, which is likely to reduce genetic variation (Dlugosch & Parker, 2008; Hollingsworth & Bailey, 2000; Schrey et al., 2012; Zhang et al.,

2010). Nevertheless, there is evidence from comparisons between native and introduced populations that some invasive species have rapidly adapted to new environments (Joshi & Vrieling, 2005; Zhang et al., 2018). Moreover, common-garden studies revealed that trait expression of naturalized non-native plants often appears to follow altitudinal, climatic, or latitudinal clines (Agrawal et al., 2005; Alexander et al., 2012; Bhattarai et al., 2017; Kollmann & Bañuelos, 2004; Weber & Schmid, 1998; but see e.g. Colautti & Lau, 2015; Datta et al., 2017; Ebeling et al., 2011). Such clines imply that local populations of non-native species have been subject to divergent selection. Indeed, a number of common-garden and reciprocal transplant studies have found evidence for local adaptation in non-native species within their introduced range (Colautti & Barrett, 2013; Maron et al., 2004; Oduor et al., 2016). However, it remains unknown whether such patterns of rapid adaptation within the introduced ranges of invasive species are very common and whether they are achieved by genetic change alone.

Local adaptation could in theory also have an epigenetic basis (Bossdorf et al., 2008; Hawes et al., 2018), and this might be particularly important in the absence of genetic variation. Local adaptation through epigenetic modification might involve gene regulation via micro-RNAs, small interfering RNAs, histone modifications, or cytosine methylation (hereafter, DNA methylation; Henderson & Jacobsen, 2007; Nicotra et al., 2010; Rapp & Wendel, 2005). Of those different epigenetic mechanisms, DNA methylation is the most widely studied (Hawes et al., 2018; Kilvitis et al., 2014; Schrey et al., 2013). In plants, DNA methylation can occur at different sequence positions of cytosines (i.e. ^{m}CG, ^{m}CHG or ^{m}CHH; ^{m}C—5-methyl-cytosine, G—guanine, H—any other DNA base except guanine; van der Graaf et al., 2015) and is under control of a suite of cellular maintenance mechanisms (Kawashima & Berger, 2014; Niederhuth & Schmitz, 2017). Loss and gain of DNA methylation at specific sites is thought to be spontaneous (Johannes & Schmitz, 2019; van der Graaf et al., 2015), and epimutation rates appear to exceed mutation rates (Johannes & Schmitz, 2019). Most importantly, in angiosperms, DNA methylation can be transmitted transgenerationally, through both asexual and sexual reproduction (Henderson & Jacobsen, 2007; Kawashima & Berger, 2014) and thus produce heritable phenotypes (Cubas et al., 1999; Manning et al., 2006; Niederhuth & Schmitz, 2014; Wilschut et al., 2016). This implies that DNA methylation could be an epigenetic mechanism that allows for fast local adaptation.

Previous studies detected differentiated DNA methylation patterns across natural populations with the help of methylation-sensitive molecular markers. DNA methylation patterns have been linked to specific habitats of native and non-native plant species (Lira-Medeiros et al., 2010; Platt et al., 2015; Richards et al., 2012), disturbance (Herrera & Bazaga, 2016), and environmental stress (Herrera & Bazaga, 2011; Kooke et al., 2015; Robertson et al., 2017).

However, while these studies provide evidence for epigenetic differentiation, they cannot infer whether the observed patterns reflect local adaptation. Therefore, the next logical step would be for studies to experimentally modify DNA methylation in plants before testing their fitness under field conditions. However, to the best of our knowledge, such studies have not been done yet.

Here, we tested in a regional reciprocal transplant experiment whether treatment with the demethylation agent zebularine affects local adaptation in native and non-native ruderal plant species. Zebularine works as an inhibitor to DNA methyltransferases (Baubec et al., 2009; Griffin et al., 2016; Marquez, Barchi, et al., 2005; Marquez, Kelley, et al., 2005), which are an important part of the cellular maintenance mechanisms for DNA methylation (Baubec et al., 2009; Niederhuth & Schmitz, 2014). Importantly, zebularine does not induce genetic mutations (Bossdorf et al., 2008). Zebularine treatment during germination and the seedling stage in *Arabidopsis thaliana* was shown to result in hypomethylation of cytosine residues at all sites (e.g. reduction of total DNA methylation from 81.4% in untreated to 58.8% in treated plants after 80 µM zebularine; Baubec et al., 2009). This hypomethylation has the potential to erase transgenerationally transmitted methylation states conferring improved responses to drought (Herman et al., 2012), herbivory, and salt stress (Verhoeven et al., 2010).

We expected that epigenetic inheritance would contribute more to local adaptation in naturalized non-native species than in native species. This is because in contrast to native species, non-native species may have less genetic variation, as a consequence of genetic bottlenecks during introduction (Dlugosch & Parker, 2008), and have had less time, due to their recent introduction, to allow for local adaptation by genetic mechanisms.

To address this, we collected seeds from multiple maternal lines of seven native and five non-native short-lived ruderal species from two climatically and latitudinally different regions in Germany: the Konstanz region in southern Germany and the Potsdam region, situated c. 600 km to the northeast of Konstanz. Half of the seeds of each maternal line were treated with zebularine during germination. We then planted the zebularine- and non-zebularine-treated offspring from these two regions into three field sites in the Potsdam region and three field sites in the Konstanz region. We recorded survival, flowering probability, aboveground biomass, and reproductive biomass as fitness-related traits.

We asked three specific questions: (a) Do local plants outperform non-local plants of the same species (i.e. is there local adaptation *sensu* Kawecki & Ebert (2004)? If local plants show higher survival or flowering, or produced more biomass than non-local plants in transplant sites of both regions, this would indicate local adaptation. Based on previous meta-analyses of local adaptation in plants (Leimu & Fischer, 2008; Oduor et al., 2016), we expected to find evidence for

local adaptation in most study species. (b) Does the degree of local adaptation differ between native and non-native species? We expected local adaptation of similar strength and frequency in native and non-native species, in line with the meta-analysis results of (Oduor et al., 2016). (c) Do zebularine-treated plants show less evidence for local adaptation than control plants, and is this effect stronger for non-native than for native plant species? We expected local plants to outperform non-local plants under control conditions, but that zebularine treatment would weaken or remove this effect, especially in non-native plants. Such a finding would indicate that DNA methylations are a mechanism underlying local adaptation, particularly in non-native species.

Material and Methods

Species selection and seed collection

As study species for the reciprocal transplant experiment, we chose native and non-native species that are common throughout Germany and occur in the Konstanz (47.6779°N, 9.1732°E) and Potsdam (52.3906°N, 13.0645°E) regions according to the FloraWeb database (www.floraweb.de, Bundesamt für Naturschutz). To facilitate approximation of lifetime fitness, and facilitate interspecific comparisons, we specifically targeted short-lived (mainly annual) species from similar ruderal habitats. This habitat type was selected, because ruderal sites such as agricultural fields and fallow land in urban areas are especially rich in naturalized neophytes (Chytrý, Jarošík, et al., 2008; Chytrý, Maskell, et al., 2008), and the ruderal strategy is widely shared among naturalized non-native plants (Baker, 1974; Guo et al., 2018). To avoid confounding floristic status with taxonomy, we selected multiple confamilial groups that each contained at least one native and one naturalized non-native species. Using these criteria, we managed to collect viable seeds within a radius of 50 km around Konstanz and Potsdam for seven native and five naturalized non-native species, representing four families (Amaranthaceae, Asteraceae, Plantaginaceae, and Solanaceae; Table 4.1; species determined with Senghas & Seybold (1993) and Jäger et al., (2013). Seeds were collected from July to November 2015, and we aimed to collect seeds from at least 10 plants (maternal lines) per population. (See Table A3.1 for species, number of maternal lines and sampling locations, and Table A3.19 for native range and invasion history of non-native species.) Seeds were stored at room temperature in paper bags until sowing.

Pre-cultivation of study species and zebularine treatment

Before transplant into the common-garden field sites, we pre-cultivated plants in the botanical gardens of the University of Konstanz (for the Konstanz region) and the University of Potsdam (for the Potsdam region) during the second half of April and the first half of May 2016. For some species, the seeds were scarified with H_2SO_4 or soaked in water before sowing to promote germination (Table A3.2). Immediately before sowing, all seeds were surface sterilized in 5%

NaClO for 3 min and then rinsed three times in deionized water. To assure that all plants would be at a viable size at the start of the experiment, the sowing dates of species were adjusted to known germination speed (see Table A3.2 for details).

For each of the maternal seed lines (see Table A3.1 for the number of maternal lines used per species), we prepared two plastic petri dishes (diameter: 35 mm) with filter paper on the bottom. For the control treatment, the filter paper was moistened with 200 µl of deionized water, and for the demethylation treatment, it was moistened with 200 µl of a 35 µM aqueous solution of the demethylation agent zebularine (Sigma-Aldrich Corporation). The used concentration of zebularine, $C_9H_{12}N_2O_5$, a cytidine analogue, was chosen to be within the range of concentrations used by other studies, where they were shown to be effective without affecting plant survival (see Alonso et al., 2017; Verhoeven & van Gurp, 2012). Moreover, in a pilot study, we found that a concentration between 25 and 50 µM zebularine visibly slowed plant development, without affecting the viability of the plants (see Figure A3.1 for images of exemplary gradients of the zebularine trial). Depending on seed availability and size, we put 10–20 seeds in each petri dish. In total, we had 765 petri dishes in Konstanz and 768 petri dishes in Potsdam.

To prevent the seeds from drying out, we sealed the petri dishes with parafilm. Then, the petri dishes were randomly assigned to po- sitions in a phytochamber (11-hr light at 21°C and 13-hr dark at 16°C) and covered with a single layer of 80 g/m2 white paper to reduce condensation on the inside of the lids of the petri dishes. Although zebularine has a higher chemical stability than other methyltrans- ferase inhibitors (Cheng et al., 2003; L. Zhou et al., 2002), in an aque- ous solution, it degrades within a few days (Marquez, Barchi, et al., 2005; Marquez, Kelley, et al., 2005). Therefore, every second day, we transferred the seeds to new petri dishes with a freshly prepared zebularine solution or, in the case of the control treatment, with fresh water, until at least three seedlings had germinated.

For each of the 12 species, we transplanted all seedlings as soon as there were at least three seedlings in the majority of petri dishes of that respective species. For petri dishes that had fewer than three seedlings at that point (up to 8% of petri dishes within a species), we transplanted all available seedlings, resealed the petri dishes, and continued transferring remaining seeds to fresh dishes. We did this until three seedlings had germinated or until the 8 May 2016 (in Konstanz) or the 13 May 2016 (in Potsdam) (see Table A3.3 for the transplanting timeline).

We transplanted the seedlings to 7×7×6.5 cm pots filled with a peat-based substrate (Pikiererde Classic CL P, Einheitserdewerke Patzer). For each petri dish (i.e. maternal line by zebularine treatment combination), up to three pots were prepared. When there were more than three seedlings available, we planted up to three seedlings in a single pot, to increase the chance

that at least one of them would survive until transplanting in the field sites. The pots were randomly allocated to positions in a glasshouse. At least 1 week before planting at the field sites, plants were placed outside in a sun-protected place for acclimatization to field conditions.

Field sites and experimental set-up

Seeds of the 12 study species had been collected in different locations in the Konstanz and Potsdam regions (Table A3.1). As it was logistically not possible to reciprocally transplant the offspring of species between the exact locations were the seeds had been collected, we instead established three experimental field sites in Konstanz and three experimental field sites in Potsdam, where we planted all 12 species. These sites were agricultural fields or tilled grasslands (i.e. disturbed to mimic ruderal sites; see Table A3.4 for exact descriptions of the field sites).

Each field site was at least 100 m^2 and was divided into three blocks. Following a randomized block design per field site, we randomly allocated one-third of the maternal lines of each species to each block. For each maternal line, we planted, if possible, one control individual and one zebularine-treated individual into each of the three Konstanz and each of the three Potsdam field sites. To avoid interspecific competition, each block of a field site was subdivided into 12 plots, that is, one for each species. To avoid intraspecific competition within plots, we planted individual plants 30 cm apart in a 7×4 grid (1.7 m^2; see Figure A3.2 for an example), except for the larger *Datura stramonium*, which was planted 50 cm apart in a 5×3 grid in plots of 3.0 m^2. Although we aimed to have all maternal lines of each species represented with a control plant and a zebularine-treated plant in all six field sites, this was not possible for all maternal lines due to insufficient germination or survival of seedlings. In such cases, the number of complete treatment level pairs per maternal line was maximized, and these pairs were randomly assigned to field sites in each region (Konstanz, Potsdam). Leftover single plants of these maternal lines were randomly assigned to the remaining field sites.

Plants were transplanted into the three Konstanz field sites from 17 to 25 May 2016 (i.e. 4–5 weeks after sowing) and into the three Potsdam field sites from 5 to 13 June 2016 (i.e. 7–8 weeks after sowing). To avoid damaging the root systems during transplant, we did not remove the potting soil from the plants before planting. As some pots had up to three small individuals in a pot, we kept the largest individual and removed the others. Plants were watered twice a week during the first two weeks after transplant- ing, to reduce mortality and facilitate establishment. Additionally, because the summer of 2016 was unusually dry in Potsdam, we watered the plants there once or twice a week during episodes of severe drought (all field sites from the beginning of June to mid-July and the Gröben field site from mid-August to the end of September 2016). At the Konstanz field sites, we reduced mortality due to mollusk herbivory by sprinkling a molluscicide

(Schneckenkorn Spiess-Urania®G2, Spiess-Urania Chemicals GmbH) around the fields at the start of the experiment and at least once more during July–August 2016. At the Potsdam field sites, however, molluscicides were not required as slug and snail numbers there were low (Silvia Eckert, personal observation), probably due to the sandy soil and the unusually dry summer in 2016. We did not weed the plots, unless there was potential for confusion with experimental plants belonging to the same species.

Harvest and measurements

In the weeks before harvesting, we scored for each plant whether it flowered (or had flowered). We harvested all plants of a species in a specific field site as soon as at least 50% of all surviving plants had started to flower, and the first seeds were mature. In cases where seeds formed before 50% of the plants flowered (*Erigeron annuus*, *Erigeron canadensis*, and *Lactuca serriola*), we collected mature reproductive units from flowering plants to avoid losing reproductive biomass. At the end of the growing season (end of October 2016), we harvested all remaining plants on all field sites, regardless of the percentage of flowering plants. At harvest, we collected the above-ground biomass and separated it into reproductive and vegetative parts. Biomass was dried for at least 72 hr at 70°C in a drying oven and then weighed.

Statistical analyses

The final dataset used for analysis comprised 3,864 plant individuals, 2,068 from the Konstanz field sites and 1,796 from the Potsdam field sites. As measures of plant fitness or performance, we used survival, flowering probability, aboveground biomass, and reproductive biomass. From the analyses of flowering probability and reproductive biomass, we excluded 33 plants that had started flowering before planting in the Potsdam field sites (9 out of 97 *D. stramonium* plants, 5 out of 94 *Plantago major* plants, and 19 out of 114 *Senecio vulgaris* plants). Survival was analyzed for all plants (n = 3,729). Total aboveground biomass (n = 2,951) and flowering probability (n = 2,956) were analyzed for the surviving plants, and reproductive biomass was only analyzed for flowering plants (n = 2,293). We used a meta-analytical approach, which facilitates comparisons across species and field sites, to analyze effect sizes of differences between local and non-local plants. For explorative purposes, we also analyzed each species separately to test for effects of transplant region, zebularine treatment, and origin (see Method A3.1).

We used a meta-analytical approach to test (a) whether there was a general signature of local adaptation across all study species (see also Leimu & Fischer, 2008; Oduor et al., 2016), (b) whether this signature differed between native and naturalized non-native species, and (c) whether zebularine treatment had an effect on local adaptation. To fulfill the requirements for local adaptation, local populations in both tested regions must outperform the non-local populations

(Kawecki & Ebert, 2004). We calculated effect sizes for the meta-regressions such that positive values corresponded to a higher performance of the local populations (and negative values corresponded to a higher performance of non-local populations). Therefore, positive effect sizes in both regions would indicate local adaptation, whereas negative effect sizes would indicate local maladaptation (Leimu & Fischer, 2008).

All statistical analyses were done with R v3.4.1 (R Core Team, 2017) using RStudio v1.0.153 (RStudio Team, 2015). We used the *escalc* function in the METAFOR R package (Viechtbauer, 2010) to calculate effect sizes separately by species and zebularine treatment level. Effect sizes for the two continuous variables, total aboveground biomass and reproductive biomass, were calculated separately for each of the three blocks of a field site. For these two biomass variables, we calculated the effect sizes as standardized mean differences (SMDs) between the local and the non-local populations (Borenstein, 2009; Leimu & Fischer, 2008; Viechtbauer, 2010, 2016). Effect sizes for the two binomial variables, survival and flowering probability, were calculated separately for each field site (i.e. across the three blocks of a field site). For these two binomial variables, we calculated effect sizes as log-transformed odds ratios (LORs) from 2×2 contingency tables (Borenstein, 2009; Viechtbauer, 2010). We accounted for zeroes in the 2×2 contingency tables by using the default continuity correction of 0.5 in the METAFOR package (Viechtbauer, 2010). However, we also analyzed these data using an alternative continuity correction that is based on the ratio of sample sizes between the compared groups (Sweeting et al., 2004; see Methods A3.3 for more details). For the effect sizes (SMDs and LORs), we also calculated the corresponding variances (Borenstein, 2009; Viechtbauer, 2010, 2016). For the visualization of effect sizes in forest plots, effect sizes were summarized by species and zebularine treatments using the *rma.mv* function in the METAFOR R package (Viechtbauer, 2010, 2016). As random effects, we used fields and blocks (nested within field) for summarizing within regions (Figure A3.4 and Tables A3.9–12), and for summarizing across regions (Figure 4.1 and Tables A3.13–16), we used region, field sites (nested within region), and blocks (nested within field sites). A significant effect size would have 95% confidence intervals not overlapping with zero.

Table 4.1: The 12 ruderal study species used in our reciprocal transplant experiment between the Konstanz and Potsdam regions of Germany. Standardized species names were obtained from The Plant List (http://www.theplantlist.org/)

Family	Species	Status[a]	Growth form[b]	Life form[b]
Amaranthaceae	*Amaranthus retroflexus* L.	Non-native	Annual	Therophyte
	Chenopodium album L.	Native	Annual	Therophyte
Asteraceae	*Erigeron canadensis* L.	Non-native	Annual	Therophyte / hemicryptophyte
	Erigeron annuus (L.) Pers.	Non-native	Biennial	Hemicryptophyte
	Lactuca serriola L.	Native	Annual	Therophyte / hemicryptophyte
	Senecio vulgaris L.	Native	Annual	therophyte / hemicryptophyte
	Sonchus oleraceus (L.) L.	Native	Annual	Therophyte / hemicryptophyte
	Tripleurospermum inodorum (L.) Sch.Bip.	Native	Annual	Therophyte / hemicryptophyte
Plantaginaceae	*Veronica persica* Poir.	Non-native	Annual	Therophyte / hemicryptophyte
	Plantago major L.	Native	Perennial (plurienn-pollakanth)	Hemicryptophyte
Solanaceae	*Datura stramonium* L.	Non-native	Annual	Therophyte
	Solanum nigrum L.	Native	Annual	Therophyte

[a] Data on the native status of species were obtained from FloraWeb (Bundesamt für Naturschutz).

[b] Data on growth form and life form were obtained from the BiolFlor database (Kühn, Durka, & Klotz, 2004).

To test whether effect sizes were significantly affected by the native versus non-native status, and whether effect sizes significantly changed due to the zebularine treatment, we analyzed effect sizes of each fitness or performance variable (survival, aboveground biomass, flowering probability, and reproductive biomass) separately in mixed- effects meta-regression models with the *rma.mv* function. The models included region of the field site (Konstanz vs. Potsdam), floristic status of the species (native vs. non-native), and zebularine treatment (untreated vs. treated) as two-level factorial moderators, and their interactions. In addition, the models included field site, block nested within field site, plant family, and species nested within plant family as random effects. We aimed to use the full model whenever possible. However, in some cases, the full model did not converge, or profile likelihood plots indicated overparameterization. In such cases, we removed one or both of the outer random factors (i.e. plant family and field site) to get a converging model that was not overparameterized. Plots of distribution of the residuals, residuals versus fitted values, and qqplots indicated that the assumptions of the analysis were not violated. We obtained likelihood-ratio-test statistics and corresponding *p*-values for moderators and their interactions by step-wise model reduction (Table 4.3). Finally, to test for the global effect of a

fitness variable, we also analyzed effect sizes with meta-regression models with the *rma.mv* function without moderators, but the full set of random effects (unless there were problems with convergence or overparame terization of the models; Table 4.2).

Results

Overall, survival (80.3%) and flowering probability (78.4%) were high and most plants set seeds during the experiment. Survival ranged from 42.9% for the native *L. serriola* to 97.9% for the native *Senecio vulgaris* (Table A3.5). Flowering percentages ranged from 11.1% for the native *L. serriola* to 97.4% for the native *S. nigrum* (Table A3.7). Plants generally produced more biomass in the field sites of the Potsdam region than in the field sites of the Konstanz region (i.e. in three out of 12 species for aboveground biomass and in six out of 12 species in reproductive biomass) (Tables A3.6 and A3.8, Figures A3.5–16). Only *Plantago major* produced more biomass in the Konstanz than in the Potsdam region (Tables A3.6 and A3.8, Figure A3.14). However, survival (Table A3.5) and flowering (Table A3.7) did not significantly differ between the Konstanz and Potsdam transplant regions.

Overall evidence for local adaptation of the study species?

With global effect sizes (i.e. effect sizes averaged across all study species) not significantly different from zero for any of the performance traits, meta-regressions revealed no evidence for local adaptation (Table 4.2). In other words, local and non-local plants performed similarly. However, when effect sizes were summarized across both transplant regions for each individual species and treatment, we found a few significant effect sizes in the control treatment (Figure 4.1). One of those was a positive effect size for flowering probability in the non-native *D. stramonium* (Figure 4.1c), indicating superior performance of local plants in both regions. On the other hand, there were significantly negative effect sizes for aboveground biomass in the non-native *E. annuus* (Figure 4.1b) and for reproductive biomass in the native *Ch. album* (Figure 4.1d), indicating superior performance of non-local plants in both regions. Details on effect sizes of species in each of the two regions are provided in the Notes A3.2, Figure A3.4, and Tables A3.9–12, and the results of single-species analyses are provided in Notes A3.1, Tables A3.5–8, and Figures A3.5–16. Overall, both the meta-analytical approaches and the single-species analyses provide only scant evidence for local adaptation, but more evidence for local maladaptation.

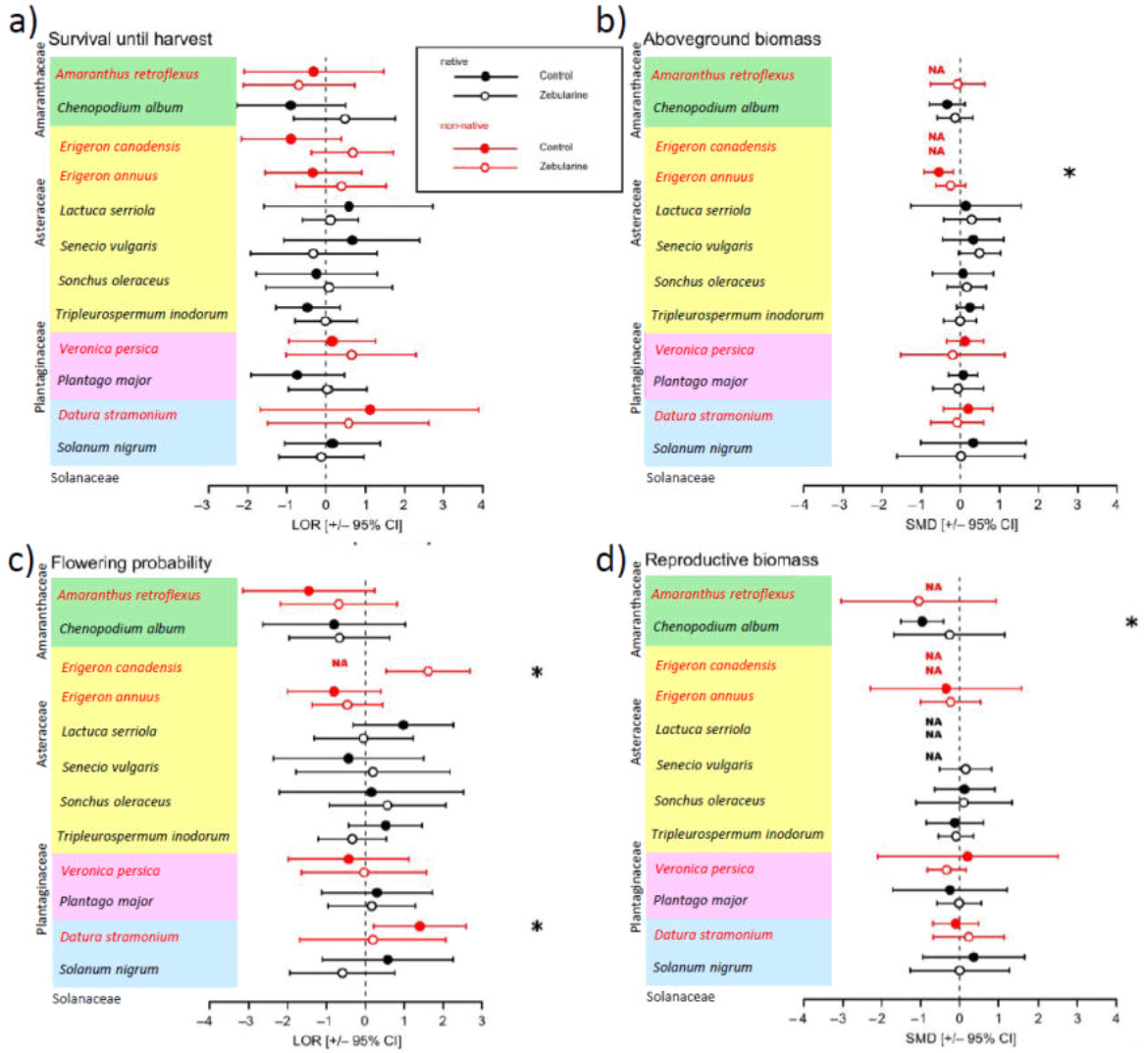


Figure 4.1: Forest plots with effect sizes summarized across regions. Effect sizes were calculated as the difference between local and nonlocal plants. Significantly positive across-region effect sizes indicate local adaptation and negative ones indicate local maladaptation. Stars denote effect sizes significantly different from 0 (i.e. 95% confidence intervals nonoverlapping with 0). NAs denote cases with insufficient data for effect size calculation in one or both regions (see Methods A3.2). Closed and open symbols stand for control and zebularine treatment, respectively. Natives are marked in black, and non-natives are marked in red. Survival with continuity correction based on the reciprocal of the opposite group size (a), aboveground biomass (b), flowering probability based on the reciprocal of the opposite group size (c), and reproductive biomass (d). LOR, log-transformed odds ratio; SMD, standardized mean difference.

Does the degree of local adaptation differ between native and non-native species?

There was no evidence for differences in local adaptation between native and non-native species (no significant effects of status in the meta-regression models in Table 4.3). However, in the meta-regression model for aboveground biomass, the region:status and region:status:zebularine treatment interactions were significant (Table 4.3). This reflects that in the Konstanz field sites all predicted effect sizes were close to zero, whereas in the Potsdam field sites the predicted effect

size of the zebularine- treated plants was positive for the natives and negative for the non-natives (Figure A3.3c).

Furthermore, in the meta-regression for reproductive biomass, the moderator region explained effect sizes significantly and the status had a marginally significant effect (Table 4.3). This reflects that in the Konstanz field sites the predicted effect sizes tended to be negative and that predicted effect sizes overall tended to be lower for non-native than for native species (Figure A3.3f). Thus, overall, the meta-analytical approach provides scant evidence for the importance of status (native vs. non-native) for the expression of local adaptation or maladaptation.

Table 4.2: Results of meta-regression for each fitness variable, without moderators, and random effects of blocks nested in field sites and species nested within plant family. The values are (in order) the continuity correction (CC) applied to the effect sizes of the respective model, the global effect size estimate of the model, the standard error (SE), and the corresponding Z- and *p*-values. Sample sizes of effect sizes were the same as specified for the respective fitness variable in mixed-effects meta-regressions (Table 4.3)

Fitness variable	Continuity correction (CC)	Effect size estimate ± SE	Z	*p*	Random effects structure
Survival	0.5	0.02 ± 0.11	0.164	0.87	~1 | Field
	+ $local_{CC}$ + $non\text{-}local_{CC}$	0.03 ± 0.11	0.325	0.745	~1 | Species
Aboveground biomass	NA	0.03 ± 0.09	0.307	0.759	~ Block | Field ~ 1 | Species
Flowering probability	0.5	0.01 ± 0.21	0.034	0.973	~1 | Field
	+ $local_{CC}$ + $non\text{-}local_{CC}$	0.07 ± 0.19	0.358	0.72	~ 1 | Species
Reproductive biomass	NA	-0.22±0.24	-0.916	0.36	~Block | Field ~species | Plant family

Effect of zebularine on local adaptation in natives and non-natives?

The single-species analyses allowed us to test the direct effect of zebularine on performance traits. These analyses showed that survival was completely unaffected by the zebularine treatment (Table A3.5) and that flowering was affected in only one species (Table A3.7, Figure A3.11). On the other hand, the zebularine treatment had significant negative effects on aboveground biomass production in seven of the 12 species and on reproductive biomass in seven of the 12 species (Tables A3.6 and A3.8, Figures A3.5–16). However, we found significant positive effects of zebularine treatment on aboveground and reproductive biomass in the native *Ch. album* (see Tables A3.6 and A3.8, Figure A3.6). So, zebularine treatment had significant effects on plants, but the effects depended on the fitness trait and on the species.

None of the meta-regression models for the four fitness variables revealed significant zebularine effects or status:zebularine interactions (Table 4.3). In other words, zebularine did not affect the magnitude of local adaptation, and this was the same for native and non-native species.

Table 4.3: Results of mixed effects meta-regression for each fitness variable, with transplant region (Konstanz or Potsdam), status (native or non-native), zebularine treatment and their interactions as moderators and random effects of blocks nested in field sites and species nested within plant family. The values are for each moderator in the stepwise model reduction, the $\chi 2$ value and the significance level of the likelihood ratio test. *p*-values lower than 0.05 are marked in bold. For survival and flowering probability, results for both types of continuity correction are shown (for details see Methods A3.3).

Fitness variable	**Survival**				**Aboveground biomass**		**Flowering probability**				**Reproductive biomass**	
Sample size	91		143		300		100		134		215	
Effect size	LOR		LOR		SMD		LOR		LOR		SMD	
comparison level	Field		Field		Block		Field		Field		Block	
Continuity correction	-0.5		+local$_{CC}$ +non-local$_{CC}$		NA		+0.5		+local$_{CC}$ +non-local$_{CC}$		NA	
Moderators	**$\chi 2$ (df = 1)**	***p***	**$\chi 2$ (df = 1)**	***p***	**$\chi 2$ (df = 1)**	***p***	**$\chi 2$ (df = 1)**	***p***	**$\chi 2$ (df = 1)**	***p***	**$\chi 2$ (df = 1)**	***p***
Region (R)	1.9	0.168	1.23	0.267	2.13	0.144	1.02	0.312	0.06	0.816	**8.68**	**0.003**
Status (S)	1.89	0.17	1.58	0.209	2.34	0.126	0.06	0.8	0.03	0.861	3.65	0.056
Zebularine treatment (Z)	2.22	0.136	1.65	0.199	0.02	0.882	0	0.952	0.01	0.929	0	0.963
R × S	1.37	0.241	1.18	0.277	**8.66**	**0.003**	0.84	0.359	0.5	0.479	0.35	0.552
R × Z	0.93	0.336	0.7	0.402	0.31	0.578	0.1	0.756	0.32	0.572	0.33	0.563
S × Z	0	0.986	0	0.944	0.95	0.331	2.31	0.129	2.43	0.119	0.54	0.462
R × S × Z	0.09	0.77	0.13	0.719	**4.39**	**0.036**	3.6	0.058	2.69	0.101	1.01	0.314
Random effects structure	~1|Field ~1|Species		~1|Field ~1|Species		~Block|Field ~1|Species		~1|Field ~1|Species		~1|Field ~1|Species		~Block|Field ~species|Plant family	

However, when effect sizes were summarized across both transplant regions for each individual species and treatment, we found a few significant changes in the effect sizes due to zebularine treatment (Figure 4.1). In the non-native *D. stramonium*, the significantly positive effect size for flowering in the control treatment disappeared in the zebularine treatment (Figure 4.1c). For the non-native *E. canadensis*, the effect size for flowering was significantly positive in the zebularine treatment (Figure 4.1c). However, because the corresponding effect size in the control treatment could not be calculated due to high mortality of *E. canadensis* plants in Potsdam (Figure A3.7), it is not clear whether this reflects a change in effect size or not. If we compare the *E. canadensis* flowering effect sizes for the Konstanz transplant region only (Figure A3.4c), it appears that the effect size was larger for the zebularine-treated than for the control plants (for details, see Notes A3.2 and Figure A3.4c). On the other hand, in the non-native *E. annuus*, the significantly negative effect size for aboveground biomass, indicating local maladaptation, disappeared in the zebularine treatment (Figure 4.1b). Similarly, the significantly negative effect size for reproductive biomass in the native *Ch. album* also disappeared in the zebularine treatment (Figure 4.1d). (Details on effect sizes of native and non-native species in each of the two regions are provided in the Notes A3.2, Figure A3.4, and Tables A3.9–12, and the results of single-species analyses are provided in Notes A3.1, Tables A3.5–8, and Figures A3.5–16.) Overall, the analyses revealed hardly any evidence for the influence of zebularine treatment on the expression of local adaptation or maladaptation in natives or non-natives.

Discussion

Our multi-species reciprocal transplant experiment of five naturalized non-native and seven native ruderal plant species between the Konstanz and Potsdam regions in Germany revealed no consistent differences in survival, growth, and reproduction between local and non-local plants. Treatment with the demethylation agent zebularine reduced performance (particularly biomass) of most species but showed no consistent effects on differences between local and non-local plants. So, our study revealed no clear evidence for local adaptation. Consequently, there were also no differences between native and non-native species in this respect, and no evidence for the role of epigenetic mechanism, such as DNA methylation, in rapid adaptation of ruderal plants.

Local adaptation of ruderal plants

Although our results could be interpreted as evidence for local adaptation in individual species with regard to certain fitness components (e.g. in *D. stramonium* with regard to flowering probability), overall our study revealed little evidence for local adaptation across all 12 species. For several species in our study (e.g. in *Ch. album* and *E. annuus*), non-local plants even performed better than local plants (see Figure 4.1), suggesting local maladaptation. These findings

are surprising given that several meta-analyses revealed that local adaptation is quite common, though not ubiquitous (Hereford, 2009; Hoeksema & Forde, 2008; Leimu & Fischer, 2008; Oduor et al., 2016). For example, Leimu & Fischer (2008) found that local plants outperformed non-local plants in 71% of the study sites and that this happened at both sites of a reciprocally transplanted pair of populations in 45% of the cases. Leimu & Fischer (2008) found, however, more evidence for local adaptation when the populations were large (>1,000 individuals) than when they were small, possibly because of larger evolutionary potential and lower inbreeding and drift in large populations. The fact that most populations that we sampled were relatively small might partly explain the limited evidence for local adaptation in our study.

We used a multi-species approach, which is powerful for detecting general patterns across species (van Kleunen et al., 2014). The results for the individual species should, however, be interpreted with caution, as our design merely included two populations for each of the 12 species (Table A3.1). Differences in performance between the two populations of a species, irrespective of whether the differences are in line with local adaptation or maladaptation, suggest that there is genetic (or epigenetic) differentiation (Tables A3.5–8, Figures A3.5–16). However, these differences could also have arisen due to random evolutionary processes, such as genetic drift, rather than due to adaptive evolution (Excoffier & Ray, 2008; Kawecki & Ebert, 2004). Furthermore, like in some previous studies on local adaptation (e.g. Colautti & Barrett, 2013), we could not transplant the species into the exact same locations where we had collected the seeds. Therefore, some field sites might by chance have been more similar to the collection locations of non-local seeds than to the collection locations of local seeds. So, even if local populations were adapted to the local conditions in the places where their seeds had been collected, they might not be adapted to the more regional environmental conditions of the field sites in their home region.

As ruderal plant species typically occur in recently disturbed but ephemeral open habitats, such as building sites and fallow land (Baker, 1974), they are likely to follow metapopulation dynamics (Bastin & Thomas, 1999; Schleicher et al., 2011). Therefore, we expected our study species to be adapted to their regional climatic, edaphic, and biotic conditions (Bucharova et al., 2017; Keller et al., 2000), and thus, that plants from the Potsdam region would outperform plants from the Konstanz region in the Potsdam field sites and *vice versa*. Konstanz and Potsdam are more than 600 km apart, and whereas Konstanz has a warm climate to temperate oceanic climate, Potsdam has a rather temperate continental climate (Peel et al., 2007). So, generally, in Konstanz, climatic conditions are milder and wetter (also see Table A3.18). For instance, on average, Konstanz has a 33% higher mean annual precipitation, 16% fewer frost days, and a four degrees-higher minimum temperature (Table A3.18). Furthermore, edaphic conditions clearly differed

between regions (Table A3.17): The soil samples in the Konstanz field sites had on average a higher water content (22.3% vs. 6.6%), a higher potential pH value (7.3 vs. 5.6), and a higher organic matter content (6.7% vs. 3.9%) than soil samples from Potsdam field sites (cf. Table A3.17). This probably reflects more loamy soils in the Konstanz region and more sandy soils in the Potsdam region. Differences in performance of several of our study species between the Konstanz and Potsdam transplant regions (Figures A3.5–16) further confirm the environmental differences between both regions. It is thus unlikely that the prevailing selective regimes between both regions were not sufficiently different to drive local adaptation.

Another explanation for the absence of local adaptation might be gene flow between northern and southern populations that is so high that local adaptation is impossible due to gene swamping (Kirkpatrick & Barton, 1997; Lenormand, 2002). This gene flow might also be partly facilitated by human impact, such as the transport of soil within the considered Central European range. Additionally, even though the non-native study species have been introduced to both regions more than a century ago (see Table A3.19), humans might still continue to facilitate genetic exchange between the native and non-native ranges, thus preventing local adaptation. Therefore, while we focused on ruderal species, because their short life cycle allows for better estimation of lifetime fitness, and because many successful non-native species are ruderals (Guo et al., 2018; Kalusová et al., 2017), future studies should also consider non-ruderal more specialist species occurring in spatially variable but stable environments (Kassen, 2002).

Previous studies have shown that plant populations can adapt to local environmental conditions already within a few centuries or even a decade (Carroll et al., 2007; Linhart & Grant, 1996). This appears to be the case not only in native but also in non-native species (see e.g. Colautti & Barrett, 2013; Maron et al., 2004). Our non-native study species have probably been present in the Konstanz and Potsdam regions for more than 100 years (Table A3.19), and therefore, local adaptation should have had time to arise. However, in principle, local adaptation-like patterns could also arise through several introduction events to different regions. For example, if cold-adapted genotypes of a non-native species are introduced to high latitudes and warm-adapted genotypes to low latitudes. As we did not find clear patterns of local adaptation, it is not clear to what extent pre-adaptation might have played a role in our non-native study species.

As evidence for local adaptation was largely absent from our study, there were also no obvious differences in this regard between the five non-native and the seven native species. Nevertheless, native-non-native status had a marginally non-significant effect on the reproductive biomass (p = 0.056, Table 4.3), as effect sizes tended to be higher for some of the natives (cf. Table A3.12, Figures A3.3f and A3.4d). However, as these differences were very small, and not

found for the other fitness components, we conclude that there were no clear differences in local adaptation between the native and non-native ruderal species.

Effects of the demethylation agent zebularine

One of the best-studied mechanisms of epigenetic inheritance in plants is DNA methylation (Hawes et al., 2018; Kilvitis et al., 2014; Schrey et al., 2013). Therefore, several studies have used demethylation agents, such as 5-azacytidine and zebularine, to study the role of DNA methylation in transgenerational plasticity (Herman & Sultan, 2016; Verhoeven & van Gurp, 2012) and inbreeding depression (Vergeer et al., 2012). However, to the best of our knowledge, our study is the first one to use a demethylation agent to test for a potential epigenetic mechanism behind rapid local adaptation.

Local adaptation through epigenetic mutations (epimutations) is expected to be faster than through genetic mutations, since epimutation rates are several orders of magnitudes higher than normal mutation rates (cf. Johannes & Schmitz, 2019). One would therefore expect epimutations (e.g. changes in DNA methylation) to precede mutational changes to the genome (Richards, 2006). Depending on genomic context, DNA methylation can, for example, result in prolonged epigenetic silencing (Cubas et al., 1999; Schmitz, Schultz, et al., 2013; Verhoeven et al., 2010), and subsequently, genetic mutations in the affected gene region would be hidden from purifying selection (Arnheim & Calabrese, 2009; Diez et al., 2014; Hwang & Green, 2004; Walsh & Xu, 2006). Therefore, adaptive methylation states may at the same time allow site-specific genetic mutations to accumulate that could at a later stage, when methylations are removed, provide the raw material for genetic change (Hughes, 2012; Rodin & Riggs, 2003).

Although the limited evidence for local adaptation in our study prevents us from making inferences about the role of DNA methylation in local adaptation, zebularine-treated plants overall had a lower biomass production than control plants (Tables A3.6 and A3.8, Figures A3.5-16). This reduced performance could reflect toxic side effects of zebularine (Baubec et al., 2009; Liu et al., 2015; Marquez, Barchi, et al., 2005; Marquez, Kelley, et al., 2005). However, it could also indicate that the zebularine treatment removed methylations of genes (see e.g. Cheng et al., 2003) that play a role in adaptation to a broad range of environmental conditions. Furthermore, some of the single-species analyses revealed significant interactions of zebularine treatment with region and origin (Tables A3.5–8, Figures A3.5–16). These genotype- and environment-specific effects of zebularine suggest that DNA methylation could still play a role in adaptation. Therefore, we conclude that more studies are needed on the potential role of DNA methylation and other epigenetic mechanisms in local adaptation.

Conclusions

Many studies in the last 70 years have conducted common-garden and reciprocal transplant studies to test for population differentiation and local adaptation (Carroll et al., 2007; Clausen et al., 1941, 1947; Hendry et al., 2007; Hiesey et al., 1942; Linhart & Grant, 1996). Furthermore, numerous studies have tested for maternal carry-over effects (Agrawal et al., 1999) and adaptive transgenerational plasticity (Colicchio, 2017; González et al., 2017; Groot et al., 2017; Herman et al., 2012). However, the potential ecological and evolutionary relevance of the epigenetic process gained attention only recently (Bossdorf et al., 2008; Hawes et al., 2018; Richards, 2011). Here, we studied whether DNA methylation can play a role in local adaptation, and particularly so in non-native species, which might have had limited genetic variation and limited time to adapt by genetic change (Dlugosch et al., 2015; Richards et al., 2012; Richards, 2006; Suarez & Tsutsui, 2008). Our study, however, revealed little evidence for local adaptation overall and therefore also could not reveal whether there is a role for epigenetic mechanisms in local adaptation. Possibly, our results reflect that the ruderal species on which we focused are general-purpose genotypes selected by the metapopulation dynamics in the ephemeral habitats in which they occur (Sultan & Spencer, 2002). To further assess the role of epigenetic mechanisms in local adaptation, we therefore call for studies that use species from more stable environments and preferably use study systems in which local adaptation in the invaded range has been demonstrated already (e.g. *Lythrum salicaria* or *Hypericum perforatum*; Colautti & Barrett, 2013; Maron et al., 2004). For these species, it might also be interesting to compare whether the relative adaptive importance of epigenetic mechanisms differs between the native and the invaded ranges, and to use recently developed molecular tools to study changes in the methylation states of genes (Paun et al., 2019; Schield et al., 2016). Finally, it remains to be tested whether other mechanisms of epigenetic inheritance than DNA methylation can play a role in local adaptation.

Chapter 5

General Discussion

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The number of naturalized plant species is ever-increasing due to intensified trade and tourism, and is facilitated by anthropogenic disturbance of natural ecosystems (Kalusová et al., 2017; McNeely, 2001; van Kleunen, Dawson, et al., 2015). Although there are many hypotheses that try to explain this phenomenon within the ecological framework (Enders et al., 2020; Enders et al., 2018), none has yet proven to be universally valid (Catford et al., 2009; Jeschke et al., 2012). Heritable epigenetic variation has been proposed to bridge the gap between random genetic variation and environmental changes (Richards, 2006). It has also been considered promising in explaining why distinct plant species are capable to adapt to climatic variation in novel environments within a few generations despite impoverished genetic variation after bottleneck events during introduction (Banerjee et al., 2019; Mounger et al., 2020). In particular, cytosine methylation has been perceived as an epigenetic response to stress (Annacondia et al., 2018; Boyko & Kovalchuk, 2008; Kinoshita & Seki, 2014), inbreeding depression (Vergeer et al., 2012), phenotypic plasticity (Kooke et al., 2015; Zhang et al., 2013), and population productivity (Latzel et al., 2013) with trait variation transgenerationally modified in the offspring (Becker & Weigel, 2012; Herman & Sultan, 2016; Jablonka & Raz, 2009). In the previous three chapters, I presented three studies dealing with cytosine methylation being placed in the context of (i) adaptive trait differentiation of two invasive goldenrod species along a climatic gradient in Central Europe (Eckert et al., 2021; Chapter 2 | Study 1), (ii) spatial and climatic variation in one of these goldenrod species, *S. canadensis*, mining for genetic and epigenetic loci putatively subject to selection (Eckert et al., 2022; Chapter 3 | Study 2), and (iii) local adaptation in ruderal non-native versus native plant species from four plant families (Herden et al., 2019; Chapter 4 | Study 3). In the following, based on my results, I will synthesize the role of cytosine methylation as a putative driver of adaptive processes in non-native plant species in Central Europe. Then I will relate these findings to the experimental demethylation strategy used in all three studies. And finally, I will point to the limitations but also highlight the implications of these studies for future research in invasion biology and biological conservation.

Cytosine methylation inconsistently shapes adaptive signatures

Cytosine methylation has been considered to facilitate populations that have undergone stages of genetic impoverishment during introduction (Hawes et al., 2018). However, studies on the transgenerational effect of cytosine methylation outside the framework of clonal (Bailey et al., 2009; Fuchs et al., 2018; Hollingsworth, 2000), apomictic (Morgado et al., 2017; Preite et al., 2015; Verhoeven & van Gurp, 2012) and model species such as *Arabidopsis thaliana* based on epigenetic recombinant inbred lines or distinct ecotypes (Dubin et al., 2015; Johannes et al., 2009;

Kawakatsu et al., 2016; Schmid et al., 2018), are still largely neglected (Bossdorf et al., 2008; Bossdorf & Zhang, 2011; but see Preite et al., 2015). Most studies focus on contrasting environments where a stressor is analysed in parental populations (Herrera & Bazaga, 2016; Lira-Medeiros et al., 2010) or modified under laboratory conditions (Dubin et al., 2015; Syngelaki et al., 2020) missing out climatic variability and demographic dynamics along environmental gradients (Lodge, 1993; Sakai et al., 2001). To the best of my knowledge, the two studies presented in Chapter 2–3 were the first to place experimental demethylation in offspring of invasive plant species into context with phenotypic variation along a latitudinal gradient (Eckert et al., 2021). Moreover, the study presented in Chapter 4 was the first to address local adaptation in the framework of experimental demethylation linking variation in cytosine methylation to putative divergent selection in offspring of ruderal (non-native) plant species (Herden et al., 2019). These approaches challenged the importance of cytosine methylation in the ecological-evolutionary context under more complex conditions (Bossdorf et al., 2008; Bossdorf & Zhang, 2011).

With the study presented in Chapter 2, I demonstrated for the invasive *Solidago canadensis* in Central Europe and its conspecific *S. gigantea* that latitudinal clines persist in offspring grown from seeds instead of rhizomes (Weber & Schmid, 1998). I showed that latitudinal clines can be present despite the lack of population structure (*S. canadensis*), but that population structure is not a prerequisite for this (*S. gigantea*). Surprisingly, instead of the *epigenetic memory* (Iwasaki & Paszkowski, 2014) being removed, distinct latitudinal clines in *S. canadensis* were slightly but significantly exaggerated in individuals treated with the cytosine demethylation agent zebularine. These findings show that the latitudinal clines detected in invasive *Solidago* spp. might most probably be mediated via genetic means. This is in line with Dubin et al. (2015) who found that variation in gene-body methylations in *A. thaliana*, when offspring was grown in contrasting temperature conditions, largely depends on the latitude of origin and, thus, on genetic differences. However, it is challenging to partition epigenetic from genetic components related to environmental variation or distinct trait variation when the plant species analysed exhibit a high degree of genetic admixture.

Using methylation-insensitive (AFLP) and methylation-sensitive (MSAP) amplified fragment length polymorphisms of leaf tissue from *S. canadensis* offspring, I showed that *S. canadensis* indeed exhibits genetic but also epigenetic loci putatively under divergent selection. Also, part of these loci varied in their presence along the latitudinal gradient under study, and genetic but not epigenetic variation was clustered on distinct non-linear spatial scales along the gradient. These findings are in line with Gáspár et al. (2019) who found weak but significant transmission of epigenetic variation related to environmental variation, i.e. land-use intensity, in

Plantago lanceolata L. Also, Schulz et al. (2014) detected one epigenetic locus in offspring of *Viola elatior* FR. that was associated with the habitat condition of its source populations, i.e. the mean percentage of transmitted photosynthetic-active radiation. However, Schulz et al. (2014) pointed out that MSAPs are not capable to distinguish whether the detected loci itself or underlying causative genetic states are putatively under selection albeit being useful to detect putatively adaptive variation related to environmental conditions.

In contrast to studies on invasive but mainly clonal species (Chwedorzewska & Bednarek, 2012; Guarino et al., 2019; Liu et al., 2018; Richards et al., 2012), the study presented in Chapter 3 did not detect any epigenetic population structure along the analysed gradient in the invasive *S. canadensis*. However, this study found weak but significant spatial genetic structuring albeit a surprisingly high degree of admixture, which confirmed results from the preceding study based on microsatellite markers (Chapter 2). Epigenetic diversity in populations may increase biomass production as well as resilience against competition and pathogen infection, as was found e.g. in *A. thaliana* (Latzel et al., 2013). Although no epigenetic population differentiation was detected in *S. canadensis*, epigenetic structuring might still be present at higher or lower spatial scales than those applied in this study (Pauchard & Shea, 2006). Also, selection pressure might act differently, depending on whether the anonymous epigenetic loci analyzed in this study might be located, e.g., within gene-body methylation sites (Muyle et al., 2021), regions of epimutations (Yao et al., 2021), or do not have phenotypic consequences at all (Hirsch et al., 2012).

The dynamic, however, between cytosine methylation and local adaptation, was in turn tested in a large-scale reciprocal transplant experiment (Herden et al., 2019) presented in Chapter 4. This study tested whether there is an overall pattern of divergent selection present in non-native ruderal plant species in Germany involving two contrasts, i.e. non-native versus native ruderals, and control plants versus individuals treated with experimental demethylation using zebularine. Although no clear effect of cytosine methylation on local adaptation was found, some of the offspring from ruderal (both non-native and native) species showed evidence of local maladaptation, i.e. non-local plants performed better than local ones. This was attributed to the low population size at the source locations and that it was not possible to translocate offspring between the exact sampling locations. Nevertheless, the findings are contrary to Leimu & Fischer (2008) who found that in most studies local plants outperformed non-local plants. As ruderal plant species predominantly occupy anthropogenically transformed habitats, the populations used in the analysis might be dispersed via transport corridors (Hansen & Clevenger, 2005) preventing divergent selection at the local scale. Indeed, methylation patterns have been found to act at

distinct spatial scales indicating that demographic history of repeated stress shapes the stability of *epigenetic memory* (De Kort et al., 2020).

Finally, non-native plant species experience a shifted set of biotic interactions in their new range, from competition for space, nutrients and water, but also attack by herbivores and pathogens (Mitchell et al., 2006; Traveset & Richardson, 2020; Waller et al., 2020). Here, cytosine methylation has been found to be associated with priming (Espinas et al., 2016; Pastor et al., 2013), i.e. sensitizing defence-related genes through distal sites to subsequent pathogen attack (Conrath et al., 2006). Priming experiences can be transmitted to the offspring generation (Espinas et al., 2016; Mauch-Mani et al., 2017). The seeds of the analysed plant species used in the studies in Chapters 2–4 were collected from wild populations. The offspring from the corresponding original populations may have brought adapted methylation patterns from past priming experiences at their source locations. Indeed, cytosine methylation seems to be involved in the interplay with herbivores in both *Solidago* spp. species as demonstrated in an unpublished study [see master thesis of Maxi Tomowski; University of Potsdam; unpublished].

Many ecological studies on cytosine methylation as a putative adaptive driver of plant invasions have been conducted on clonal and mainly vegetatively reproducing species (Dong et al., 2018; Guarino et al., 2019; Richards et al., 2012; Shi et al., 2019). This has led to cytosine methylation being characterised as a mechanism that can compensate for reduced or almost non-existent genetic variation (Dlugosch et al., 2015; Dlugosch & Parker, 2008; Mounger et al., 2020). In other words, this could mean that variation in cytosine methylation is predominantly important in clonal species (Douhovnikoff & Dodd, 2015) where genetic variation has limited contribution to adaptive processes in heterogeneous and changing environments. In contrast, this mechanism might be negligible in plant species with a high degree of admixture and meta-population dynamics in the invaded range. The two invasive *Solidago* species analysed in the studies presented in Chapters 2–3, as well as the ruderal (non-native) species analysed in the study presented in Chapter 4, exhibit a significant degree of genetic variation in the invaded range. This variation made them suitable to study cytosine methylation with respect to adaptive differentiation and local adaptation under more complex ecological conditions outside a laboratory framework using the common-garden and the reciprocal transplant approaches. In summary, the results from these studies extend the growing body of literature on cytosine methylation linked to adaptive processes in non-native plant species. All three studies, however, showed evidence that cytosine methylation is only to a very limited extent involved in the invasion success and, thus, in putative adaptive processes of the analysed genetically non-uniform plant species.

Experimental demethylation modulates phenotypes in a species-specific manner

All three studies in the Chapters 2–4 showed that it is challenging to investigate the contribution of cytosine methylation to adaptive processes, especially in the context of non-native plant species exhibiting a significant degree of genetic admixture. This is because demographic history is unknown in most cases (Prentis et al., 2009) but past events may shape the success of invasive species (Dostál et al., 2013; Mattingly & Orrock, 2013). And while most studies focus on *A. thaliana*, whose epigenome is easy to manipulate in the laboratory (Schmid et al., 2018; Schmitz, He, et al., 2013; Zhang et al., 2013), the analysis of non-model plants in semi-controlled environments, such as common gardens, is much more delicate although it places variation in cytosine methylation into ecological-evolutionary context.

Variation in cytosine methylation patterns is considered to be a time-dependent, transient and short-term effect (Walsh & Xu, 2006) that can, however, be transmitted to the offspring generation (Becker & Weigel, 2012). All three studies presented in Chapters 2–4 used genome-wide cytosine demethylation applying the demethylation agent zebularine (Baubec et al., 2009; Griffin et al., 2016). This was important to address trait variation in the offspring that were not attributed to the underlying genetic variation but most probably associated with variations in the cytosine methylation pattern (Bossdorf et al., 2008). Especially the study presented in Chapter 4 revealed that experimental demethylation leads to species-specific responses.

Griffin et al. (2016) demonstrated that zebularine-mediated demethylation might be sequence-specific, i.e. sequences containing methylated CHH might be more impacted than sequences containing CG methylation. Also, demethylation with zebularine upregulates transposable elements (Griffin et al., 2016), which in turn may directly affect the plants' genome and have been proposed to be subject to natural selection (Baduel & Colot, 2021). It could have been that the plastic response of plants under zebularine-mediated demethylation depended on the number of transposable elements and CHH snippets present in their genome. Because zebularine induces genome-wide demethylation without bias to the sequence context (Griffin et al., 2016), the effect size of the applied concentration might differ per species and future studies should incorporate that when planning demethylation treatments across multiple plant species, genera and families.

Implications and relevance for invasion biology research and conservation biology

The introduction rate of non-native species worldwide is considered to be increasing (McNeely, 2001; van Kleunen, Dawson, et al., 2015), most probably accelerated by the current climate crisis (Corlett & Westcott, 2013). Variation in cytosine methylation patterns might help in understanding why naturalized species become invasive within a few generations (Banerjee et al.,

2019; Hawes et al., 2018) or, in the case of apomictic and mainly vegetatively reproducing species, adapt despite the lack of genetic variation within a relatively short period of time (Douhovnikoff & Dodd, 2015).

With the studies presented in Chapter 2–3, I demonstrated that in invasive plant species with a high degree of genetic admixture, epigenetic structuring with regard to cytosine methylation may likewise be barely present, although latitudinal clines in fitness-related traits can be stable over at least one generation and within at least two years of measurement. Variation in plant traits, however, can be increased when cryptic genetic variation is released via demethylation (Kalisz & Purugganan, 2004). Especially, the study presented in Chapter 3 showed that signatures of genetic and epigenetic adaptive processes might be present but likely diluted by multiple introductions. Therefore, the findings of all three studies might help to understand the limitations of epigenetic effects with regard to cytosine methylation under more complex conditions. The results, however, might indicate that cytosine methylation might be more pronounced as an additional level of variation in clonal or apomictic species enhancing phenotypic differentiation (Douhovnikoff & Dodd, 2015; Keser et al., 2014; Shi et al., 2019).

The study in Chapter 4 demonstrated for five non-native and seven native ruderal plant species in Germany that cytosine methylation is likely not involved in local adaptation of these species (Herden et al., 2019). However, this study raises the question about what constitutes a local population in non-native plant species, and ruderal plant species in general, when dispersal corridors (Hansen & Clevenger, 2005) might provide extensive gene flow or gene swamping (Lenormand, 2002; Rius & Darling, 2014; van Kleunen, Röckle, et al., 2015). In line with this, the study on *S. canadensis* (Chapter 3) showed that genetic variation along the analysed latitudinal gradient is spatially clustered and not showing a classic isolation-by-distance pattern (Wright, 1946). Non-native, especially ruderal, species are predominantly found at heterogeneous sites with less predictable conditions (Hill et al., 2002). Additionally, the adaptive value of epigenetic stability is coupled with short-term responses to environmental cues and also dependent on the degree of environmental heterogeneity (Herman et al., 2014). Therefore, extrapolating the results from the three studies presented in this thesis should be done with caution: The inconsistent role of cytosine methylation in adaptive processes of invasive and ruderal (non-native) plant species might be due to extrinsic factors that destabilise population structures, e.g. by moving soil from fallow land to construction sites with completely transformed biotic and abiotic conditions. In other words, some form of spatial and temporal isolation in populations appears to be necessary to induce epigenetic differentiation. Therefore, studies in this context should incorporate the

confounding effects of transport corridors, spatial scales, habitat heterogeneity and demographic history.

Finally, an interesting finding from the study presented in Chapter 4 was, however, that some of the species showed population-level differences in performance indicative of (epi)genetic differentiation albeit with no clear direction. Although ruderal plant species are rarely a target of biodiversity conservation (but see Fischer et al., 2013, for a *ruderal meadow* approach), epigenetic variation could serve as a biomarker for demographic history in biological conservation and for purposes of population rescue. Rey et al. (2020) proposed the idea of *ecological populations*, i.e. genetically indistinguishable populations showing epigenetic shifts induced via environmental variation. Although there is still need for studies in this regard, this idea might help to refine the concept of *evolutionary significant units* in conservation biology, i.e. populations that are crucial for managing assisted gene flow and population rescue strategies (Eizaguirre & Baltazar-Soares, 2014; McMahon et al., 2014).

Limitations and suggestions for future research

Cytosine methylation is the most extensively studied epigenetic mechanism with respect to plant invasion (Banerjee et al., 2019; Hawes et al., 2018). However, all three studies presented in this thesis revealed a negligible role of cytosine methylation as an epigenetic driver of adaptive differentiation in two invasive goldenrod species (Chapters 2–3) and an inconsistent contribution to local adaptation in ruderal non-native plant species (Chapter 4). Nevertheless, the studies presented do not preclude the possibility that other epigenetic mechanisms besides genetic variation may be involved in the adaptive processes of the analysed plant species. For example, adenine methylation has been detected recently as a novel and transient epigenetic marker (Liang, Geng, et al., 2018) playing regulatory functions in *A. thaliana* (Liang, Shen, et al., 2018) and rice (C. Zhou et al., 2018). However, its impact on transgenerational phenotypic variation, especially with adaptive significance, in plants is largely unknown. In addition, cytosine methylation has been shown to interplay with other epigenetic mechanisms, such as histone modifications or small RNAs (Matzke & Mosher, 2014; Saze et al., 2012; Turck & Coupland, 2014), all of which have not been part of the studies presented in this thesis but could point to a possible direction for future studies.

In the study presented in Chapter 2, offspring of both *S. canadensis* and *S. gigantea* were grown in a common-garden environment and experienced the same climatic conditions (Eckert et al., 2021). This was important to quantify the epigenetic component of putative latitudinal clines in genetically non-uniform plants grown from seeds compared to offspring grown from rhizomes (Weber & Schmid, 1998). However, due to labelling issues, additional data from a

complementary common-garden experiment had to be dismissed. Therefore, one of the major drawbacks of the study presented in Chapter 2 is that the common-garden experiment was not replicated leaving the need to test the robustness of the obtained results. Common-garden replication has been found to be crucial in determining the source of trait variation in non-native plant species (Colautti & Barrett, 2013; Ebeling et al., 2011; Moloney et al., 2009), i.e. to distinguish transgenerational trait variation due to drift or pre-adaptation from traits that have been subject to divergent selection in the new range (Moloney et al., 2009).

In addition, the plant species analysed in Chapters 2–4 responded negatively to the demethylation treatment at the early stage: In the study in Chapter 2, experimental demethylation resulted in significant growth delay at the beginning of the experiment; in the study in Chapter 4, experimental demethylation induced an overall lower biomass product in non-native plants pointing to toxic side-effects. Herman & Sultan (2016) demonstrated genotype-specific effects due to experimental demethylation where parental drought effects on the offspring phenotype in *Polygonum persicaria* overall removed phenotypic variation for root length and leaf area, but enhanced seedling biomass in some of the analysed genotypes. In the studies presented in Chapter 2–3, we controlled for siblings in the populations analysed but the study presented in Chapter 4 did not involve genetic analyses. Therefore, future studies in that respect should incorporate an interdisciplinary perspective and join forces between ecological and (epi)genomic approaches (Craven et al., 2019; Holderegger & Wagner, 2008; Nabout et al., 2015).

Demethylation effects can stem from multiple sources, e.g. due to unleashed cryptic genetic variation (Gibson & Dworkin, 2004; Paaby & Rockman, 2014; Pecinka et al., 2013) but also activation of epigenetically silenced transposons (Matzke & Mosher, 2014; Saze et al., 2012; Stapley et al., 2015). Moreover, experimental demethylation induces repair mechanisms (Liu et al., 2015; Walsh & Xu, 2006) whose characteristics likewise will be species-specific. Threshold concentrations have been determined in all studies presented in this thesis, and doing this remains a crucial first step when applying experimental demethylation. Therefore, future studies should carefully estimate the concentration at which no sustainable growth disruptions occur that could introduce confounding effects with the measurements of interest.

The study presented in Chapter 3 expanded the analysis of latitudinal clines via genetic and epigenetic analyses of one of these invasive species, namely *S. canadensis* (Eckert et al., 2022). This was necessary to infer the stability of the demethylation treatment after one vegetation period but also a further opportunity to mine for adaptive signatures in this admixed species. However, a drawback of this study was that cytosine methylation patterns, i.e. methylation-sensitive polymorphism markers present per individual, have been scored at the end of the

common-garden experiment with no baseline from the start of the experiment for comparison. Methylation patterns may vary considerably during life stages (Herrera et al., 2021), but also do not necessarily correlate with methylation levels, i.e. the global amount of methylated cytosines still present after experimental demethylation (Alonso et al., 2016). Therefore, future studies on cytosine methylation should consider monitoring variation of methylation patterns during the life cycle of the analysed species.

All three studies in the Chapters 2–4 were conducted on offspring of wild populations of invasive and ruderal (non-native) plant species. Analysing the offspring generation in a common-garden and reciprocal transplant framework was an optimal context to infer the adaptive signature of trait variation utilising experimental demethylation. Demographic history, however, especially initial dispersal dynamics, in non-native plant species are often poorly understood or even unknown (Prentis et al., 2009; Puth & Post, 2005). In addition, invasive and non-native ruderal plant species predominantly colonize heterogeneous, disturbed and rudeal areas (Catford et al., 2012; Hill et al., 2002) where transport corridors can enable gene flow between distant sites (Gelbard & Belnap, 2003; Hansen & Clevenger, 2005). This could explain the lack of genetic and epigenetic population structure in the invasive *S. canadensis* (Chapters 2–3), but was a particular drawback in the study presented in Chapter 4. Although this study examined a respectable number of species for local adaptation (five non-native and seven native plant species in Germany), individual populations, including those of native ruderal species, may have been subject to underlying gene flow or gene swamping (Lenormand, 2002). Future studies should incorporate the demographic history of each population when designing experiments with non-native or invasive species, as this may introduce confounding effects (Dostál et al., 2013; Mattingly & Orrock, 2013).

In summary, the results of the studies presented in this thesis suggest that future studies on cytosine methylation related to plant invasion should consider four aspects in their experimental design: (i) Incorporating offspring grown from seeds (Chapter 2) to challenge cytosine methylation within a more complex context, but also account for multiple genotypes, as susceptibility to experimental demethylation has been found to be population- specific (Latzel et al., 2013) but also genotype-specific (Becker & Weigel, 2012; Groot et al., 2017; Herman & Sultan, 2016); (ii) Replicating common-garden and reciprocal transplant experiments to validate the robustness of experimental results (Moloney et al., 2009); (iii) Monitoring detrimental effects on trait variation due to experimental demethylation (Chapter 2) but also score variation in cytosine methylation throughout the time of the experiment when using a demethylation treatment, as this type of treatment is transient and methylation levels can be restored (Liu et al.,

2015; Walsh & Xu, 2006); (iv) Testing whether cytosine methylation may play a greater role in clonal species, in species that predominantly rely on vegetative propagation (Dodd & Douhovnikoff, 2016; Douhovnikoff & Dodd, 2015), and in specialised non-native species dependent on distinct habitat conditions that may isolate subpopulations (Herden et al., 2019; Oduor et al., 2016).

Overall conclusion

In this thesis, I have extended the knowledge of epigenetic mechanisms with respect to adaptive differentiation of invasive plant species along a latitudinal gradient and local adaptation of ruderal (non-native) species. In particular, the studies presented pointed out the importance of testing the effects of cytosine methylation under more complex conditions focusing on non-clonal plant species. The findings of these studies demonstrated that overall cytosine methylation provides an inconsistent paradigm in explaining adaptive processes in invasive and ruderal plant species, especially when a high degree of admixture is involved. The results presented in my thesis suggest that the invasion success of the studied non-native plant species is most likely not facilitated by variation in cytosine methylation. Although this needs to be tested explicitely in subsequent studies, genetic variation might most likely be maintained by general-purpose genotypes, standing genetic variation, admixture, multiple introductions and dispersal corridors.

Summary

The increasing introduction of non-native plant species may pose a threat to local biodiversity. However, the basis of successful plant invasion is not conclusively understood, especially since these plant species can adapt to the new range within a short period of time despite impoverished genetic diversity of the starting populations. In this context, DNA methylation is considered promising to explain successful adaptation mechanisms in the new habitat. DNA methylation is a heritable variation in gene expression without changing the underlying genetic information. Thus, DNA methylation is considered a so-called epigenetic mechanism, but has been studied in mainly clonally reproducing plant species or genetic model plants. An understanding of this epigenetic mechanism in the context of non-native, predominantly sexually reproducing plant species might help to expand knowledge in biodiversity research on the interaction between plants and their habitats and, based on this, may enable more precise measures in conservation biology.

For my studies, I combined chemical DNA demethylation of field-collected seed material from predominantly sexually reproducing species and rearing offsping under common climatic conditions to examine DNA methylation in an ecological-evolutionary context. The contrast of chemically treated (demethylated) plants, whose variation in DNA methylation was artificially reduced, and untreated control plants of the same species allowed me to study the impact of this mechanism on adaptive trait differentiation and local adaptation. With this experimental background, I conducted three studies examining the effect of DNA methylation in non-native species along a climatic gradient and also between climatically divergent regions.

The first study focused on adaptive trait differentiation in two invasive perennial goldenrod species, *Solidago canadensis* sensu latu and *S. gigantea* AITON, along a climate gradient of more than 1000 km in length in Central Europe. I found population differences in flowering timing, plant height, and biomass in the temporally longer-established *S. canadensis*, but only in the number of regrowing shoots for *S. gigantea*. While *S. canadensis* did not show any population structure, I was able to identify three genetic groups along this climatic gradient in *S. gigantea*. Surprisingly, demethylated plants of both species showed no change in the majority of traits studied. In the subsequent second study, I focused on the longer-established goldenrod species *S. canadensis* and used molecular analyses to infer spatial epigenetic and genetic population differences in the same specimens from the previous study. I found weak genetic but no epigenetic spatial variation between populations. Additionally, I was able to identify one genetic marker and one epigenetic marker putatively susceptible to selection. However, the results of this study reconfirmed that the epigenetic mechanism of DNA methylation appears to be hardly involved in adaptive processes within the new range in *S. canadensis*.

Finally, I conducted a third study in which I reciprocally transplanted short-lived plant species between two climatically divergent regions in Germany to investigate local adaptation at the plant family level. For this purpose, I used four plant families (Amaranthaceae, Asteraceae, Plantaginaceae, Solanaceae) and here I additionally compared between non-native and native plant species. Seeds were transplanted to regions with a distance of more than 600 kilometers and had either a temperate-oceanic or a temperate-continental climate. In this study, some species were found to be maladapted to their own local conditions, both in non-native and native plant species alike. In demethylated individuals of the plant species studied, DNA methylation had inconsistent but species-specific effects on survival and biomass production. The results of this study highlight that DNA methylation did not make a substantial contribution to local adaptation in the non-native as well as native species studied.

In summary, my work showed that DNA methylation plays a negligible role in both adaptive trait variation along climatic gradients and local adaptation in non-native plant species that either exhibit a high degree of genetic variation or rely mainly on sexual reproduction with low clonal propagation. I was able to show that the adaptive success of these non-native plant species can hardly be explained by DNA methylation, but could be a possible consequence of multiple introductions, dispersal corridors and meta-population dynamics. Similarly, my results illustrate that the use of plant species that do not predominantly reproduce clonally and are not model plants is essential to characterize the effect size of epigenetic mechanisms in an ecological-evolutionary context.

Zusammenfassung

Die zunehmende Eintragung nicht-heimischer Pflanzenarten kann eine Gefahr für die lokale Artenvielfalt darstellen. Die Grundlagen einer erfolgreichen pflanzlichen Ausbreitung sind jedoch nicht abschließend geklärt, zumal sich diese Arten innerhalb kurzer Zeit an das neue Verbreitungsgebiet anpassen können trotz anfänglich reduzierter genetischer Vielfalt der Startpopulationen. In diesem Kontext gilt DNA-Methylierung als vielversprechend, um erfolgreiche Anpassungsmechanismen im neuen Lebensraum zu erklären. Bei der DNA-Methylierung handelt es sich um eine vererbbare Variation der Genaktivität, ohne dass die zugrundeliegende genetische Erbinformation verändert wird. Damit gehört DNA-Methylierung zu den sogenannten epigenetischen Mechanismen, wurde jedoch vorwiegend bei sich klonal vermehrenden Pflanzenarten oder genetischen Modellpflanzen untersucht. Ein Verständnis dieses epigenetischen Mechanismus im Zusammenhang mit nicht-einheimischen, sich vorwiegend sexuell reproduzierenden Pflanzenarten erweitert das Wissen in der Biodiversitätsforschung zur Interaktion zwischen Pflanzen und ihrem Lebensraum und kann, darauf aufbauend, präzisere Maßnahmen in der Naturschutzbiologie ermöglichen.

Für meine Studien kombinierte ich die chemische DNA-Demethylierung von im Freiland gesammeltem Samenmaterial sich vorwiegend sexuell fortpflanzender Arten und die Aufzucht unter gemeinsamen klimatischen Bedingungen, um DNA-Methylierung im ökologisch-evolutionären Kontext zu untersuchen. Der Kontrast von chemisch behandelten (demethylierten) Pflanzen, deren Methylierungsvariation nun künstlich verringert war, und unbehandelten Kontrollpflanzen derselben Art ermöglichte mir die Auswirkung dieses Mechanismus auf adaptive Merkmalsvariationen und lokale Anpassung zu studieren. Vor diesem experimentellen Hintergrund führte ich drei Studien durch, um die Auswirkung von DNA-Methylierung bei nicht-einheimischen Pflanzenarten entlang eines klimatischen Gradienten und zwischen zwei klimatisch unterschiedlichen Regionen zu untersuchen.

Die erste Studie konzentrierte sich auf adaptive Merkmalsveränderungen bei Nachkommen von zwei invasiven, mehrjährigen Goldrutenarten, *Solidago canadensis* sensu latu und *S. gigantea* AITON, entlang eines Klimagradienten von mehr als 1000 km Länge in Zentraleuropa. Ich fand graduelle Unterschiede im Blühzeitpunkt, in der Pflanzenhöhe und der Biomasse bei der zeitlich länger etablierten *S. canadensis*, bei *S. gigantea* jedoch nur in der Anzahl der nachwachsenden Triebe. Während *S. canadensis* keinerlei Populationsstruktur aufwies, konnte ich bei *S. gigantea* drei genetische Gruppen entlang dieses Klimagradienten identifizieren. Überraschenderweise zeigten demethylierte Pflanzen beider Arten keine Veränderung in der überwiegenden Anzahl der untersuchten Merkmale. In der darauffolgenden zweiten Studie konzentrierte ich mich auf die länger etablierte Goldrutenart *S. canadensis* und

verwendete molekulare Analysen, um räumliche epigenetische und genetische Populationunterschiede aus den Exemplaren der vorhergehenden Studie abzuleiten. Ich fand schwache genetische aber keine epigenetische räumliche Variation zwischen den Populationen. Zusätzlich konnte ich einen genetischen und einen epigenetischen Marker identifizieren, welcher potentiell unter Selektion stehen könnte. Allerdings bestätigten die Ergebnisse dieser Studie erneut, dass DNA-Methylierung bei *S. canadensis* kaum in die Anpassung an das neue Verbreitungsgebiet involviert zu sein scheint.

Schließlich führte ich eine dritte Studie durch, in welcher ich Samen kurzlebiger Pflanzenarten reziprok zwischen zwei klimatisch unterschiedlichen Regionen in Deutschland transplantierte, um lokale Anpassung auf Ebene der Pflanzenfamilien zu untersuchen. Zu diesem Zweck nutze ich vier Pflanzenfamilien (Amaranthaceae, Asteraceae, Plantaginaceae, Solanaceae), wobei ich hier auch zwischen nicht-heimischen und heimischen Pflanzenarten verglich. Beide Regionen lagen mehr als 600 Kilometer voneinander entfernt und wiesen entweder ein gemäßigt-ozeanisches oder gemäßigt-kontinentales Klima auf. In dieser Studie zeigte sich für einige—sowohl nicht-einheimische als auch einhimische—Arten eine Fehlanpassung an die eigenen lokalen Bedingungen. In demethylierten Individuen der untersuchten Pflanzenarten wirkte sich die DNA-Methylierung widersprüchlich, aber artspezifisch auf das Überleben und die Biomasseproduktion aus. Die Ergebnisse dieser Studie unterstreichen, dass DNA-Methylierung einen vernachlässigbaren Beitrag zur lokalen Anpassung bei den untersuchten nicht-heimischen, aber auch einheimischen Arten leistete.

Zusammenfassend konnte ich mit dieser Arbeit festellen, dass DNA-Methylierung bei nicht-einheimischen Pflanzenarten eine untergeordnete Rolle sowohl bei der adaptiven Merkmalsvariation entlang von Klimagradienten als auch der lokalen Anpassung an klimatisch unterschiedliche Regionen spielt, wenn diese Pflanzenarten eine hohe genetische Vielfalt aufweisen und sich hauptsächlich sexuell vermehren. Ich konnte zeigen, dass der Anpassungserfolg dieser nicht-einheimischen Pflanzenarten kaum durch DNA-Methylierung erklärbar ist, sondern vielmehr eine mögliche Folge mehrfacher Eintragungen, von Ausbreitungskorridoren und Meta-Populationsdynamiken sein könnte. Die Ergebnisse dieser Studien verdeutlichen ebenso, dass die Verwendung von Pflanzenarten, die sich nicht überwiegend klonal vermehren und keine genetischen Modellpflanzen sind, unerlässlich ist, um die Effektstärke epigenetischer Mechanismen im ökologisch-evolutionären Kontext zu charakterisieren.

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Appendix | Study 1

Method A1.1: Zebularine pilot experiment

The zebularine concentration of 25 µM that we used in our common-garden experiment was based on results of a pilot experiment in which we exposed seeds of a single *S. gigantea* population from Konstanz (ID number 6 in Table A1.2) to a range of zebularine concentrations (0, 12.5, 25, 50, 100, and 200 µM). Verhoeven & van Gurp (2012) had used the same concentration range to establish the optimal concentration for *Taraxacum officinale*, a member of the same plant family (Asteraceae) that *Solidago* belongs to. At each concentration, we prepared two Petri-dishes lined with sterile filter paper by adding the required zebularine solution. We then placed sterilised seeds (same procedure as described in the main text) into one of the dishes, and non-sterilised seeds into the other. Thus, our pilot experiment allowed us to simultaneously assess potential side-effects of seed-sterilisation with bleach (which we did not observe), and of the different zebularine concentrations.

To ensure a constant exposure to the target concentration of zebularine, we transferred the seeds to a freshly prepared Petri-dish every other day until seeds had germinated. To assess seedling growth, we transplanted four seedlings from each dish to two separate pots with a peat-based growth medium (Pikiererde Classic, Einheitserdewerke Werkverband e.V.). Seedlings were grown under the same climate chamber and greenhouse conditions as described in the Methods of the main text. All seedlings were visibly affected above a concentration of 25 µM (Figure A1.10). Based on this, we decided to use the highest concentration (25 µM) at which development was not visibly affected.

Method A1.2: Evaluation of the demethylation treatment

We assessed the effect of zebularine treatment on plant growth (see Methods section in the main text) by measuring individual plant height on four occasions during the vegetation period (*S. canadensis*: 3, 5, 7, and 9 weeks after transfer to the common garden; *S. gigantea*: 2, 4, 6, and 8 weeks after transfer). As a starting point, we first calculated the log-averaged relative growth rate (RGR) on log-transformed plant height according to Hoffman & Poorter (2002) using the first and last census (*S. canadensis*: week 3 and 9; *S. gigantea*: week 2 and 8) as Δt (see equation 1). We found that control plants showed a lower growth rate (0.29 $cm \cdot cm^{-1} \cdot week^{-1}$) compared to zebularine-treated plants (0.31 $cm \cdot cm^{-1} \cdot week^{-1}$), but that control plants were already taller at the same time (Figure A1.1 and A1.2). So, zebularine-treated plants had a delayed height growth.

$$RGR = \frac{\overline{log(h_{t2})} - \overline{log(h_{t1})}}{\Delta t} \quad (1)$$

t – time (week since transplanting to common-garden)

h – plant height (cm) at time-point ti; transformed using the natural logarithm (log)

However, because equation (1) assumes that height growth is exponential, which is frequently not the case, and because non-linear growth models more precisely capture plant growth (discussed in Paine et al., 2011), we first fitted four common growth curves (i.e. linear, exponential, Gompertz [Gompertz, 1825] and logistic) to the non-transformed plant-height data and compared their fit using ΔAIC. We decided on a non-linear logistic mixed-effects model that used zebularine treatment as a fixed factor and accounted for individual plants as a random factor.

Because non-linear mixed-effects models are more challenging and are less likely to converge, we added individual plants only as a random-intercept term. Initial starting values were obtained using the *getInitial* function that evaluates parameter estimates of the specified non-linear growth function. Both fixed and random terms were correlated to the asymptote (hereafter 'Asym'; indicating the growth capacity of the logistic growth), the midpoint (hereafter 'xmid'; indicating the point at steepest growth in the linear growth phase), and the scaling factor controlling the steepness of the curve (hereafter 'scal'; Hunt, 1982; Paine et al., 2011; Table A1.16). We found that height growth was significantly affected by the zebularine treatment in both *S. canadensis* (X^2 = 21.45; $p < 0.001$) and *S. gigantea* (X^2 = 27.96; $p < 0.001$) using likelihood-ratio tests. We also found that zebularine-treated individuals of both species showed a significantly lower scaling, indicating a steeper growth curve, and for *S. gigantea* showed a significantly lower growth capacity (Table A1.16; Figure A1.1 and A1.2). All non-linear mixed-effects models were fitted using the software R v3.6.3 (R Core Team, 2020) and the R package NLME v3.1-147 (Pinheiro, Bates, & R Core Team, 2020).

Method A1.3: Evaluation of the latitudinal climate gradient

We checked the validity of the sampled latitude coordinates as a proxy for a climatic gradient by principal component regression (PCR; Jolliffe, 1982) based on principal component analysis (PCA; data scaled and centred) with data from the WorldClim 2.0 database (Fick & Hijmans, 2017) using all 19 bio-climatic variables (Table A1.7) plus solar irradiance [kJ m^{-2} d^{-1}] as a proxy for available photosynthetic active radiation (Meek, Hatfield, Howell, Idso, & Reginato, 1984) and variation in day length, and wind speed [m s^{-1}] as a proxy for wind-dispersal in pappus-bearing Asteraceae (Tackenberg, Poschlod, & Bonn, 2003). We analyzed the first two principal component (PC) axes that were significant according to the broken-stick criterion (MacArthur, 1957).

PC1 explained up to 43.7% of the variation in the climate data for both goldenrod species and PC2 explained up to 32.7% (Figure A1.7). In linear least-squares regression, latitude of sampled locations significantly correlated with PC1 scores for both goldenrod species (*S. canadensis*: $F_{1,23} = 128.3$, $p < 0.001$, $R^2 = 0.84$; *S. gigantea*: $F_{1,23} = 38.08$, $p < 0.001$, $R^2 = 0.62$; Figure A1.7) but not with PC2 scores (*S. canadensis*: $F_{1,24} = 1.49$, $p = 0.235$, $R^2 = 0.02$; *S. gigantea*: $F_{1,23} = 2.31$, $p = 0.143$, $R^2 = 0.05$). Loadings along the PC1 axis showed that for both *S. canadensis* and *S. gigantea* wind speed, variation of solar radiation, and the temperature of the driest quarter increased with latitude of sampled source populations, while precipitation-based climate variables increased with decreasing latitude. Although not significant in PCR, loadings along the PC2 axis for both *S. canadensis* and *S. gigantea* indicated that latitude was most strongly related to temperature-based climatic variables, such as seasonality and the temperature of the warmest and coldest months (Figure A1.7).

Method A1.4: Climate-based mixed-effects models

Because latitude was, for both *Solidago* species, strongly correlated with climate in principal components regression (Figure A1.7), significant effects of latitude might mainly reflect effects of climate. Therefore, we also ran separate analyses in which we replaced latitude with climatic variables as fixed factor in (generalized) linear mixed-effects models, i.e. we used the first two principal components derived from the principal component regression on the climate variables from the WorldClim 2.0 database (Fick & Hijmans, 2017; see Method A1.3, Table A1.7–9).

Additional fixed factors included initial seedling size in 2015, zebularine treatment and the interaction of zebularine treatment with either of the principal components. For both species, phenology data from 2015 for was analyzed using a Conway-Maxwell-Poisson distribution with a log-lambda link-function as a generalization of the Poisson family and phenology data from 2016 was analyzed using a zero truncated negative binomial distribution with a log link-function in generalized linear mixed effects models (GLMM). For *S. gigantea*, the number of ramets was also analyzed with a zero truncated negative-binomial distribution in GLMMs. For both species, reproductive to total biomass data was analysed using a beta distribution with a logit link-function in GLMMs. Data was transformed using either square-root transformation, ordered quantile normalization (Peterson & Cavanaugh, 2019) or Yeo-Johnson transformation (Yeo & Johnson, 2000) to counter variance heterogeneity.

Models included block, source population, and maternal line nested within population as random-intercept factors to account for non-independence of plants in the same block and for non-independence of plants from the same maternal lines nested within populations. All random effects were kept in the models (Barr, Levy, Scheepers, & Tily, 2013) despite boundary (singular)

fit as they were part of the experimental design. Models were fitted using the fitme function from the package SPAMM v3.2.0, the *glmmTMB* function from the package GLMMTMB v1.0.1, and the *(g)lmer* function from the package LME4 v1.1-23. The contribution of fixed model terms was tested via likelihood ratio tests to assess overall model fit (Pinheiro & Bates, 2009; Zuur, Ieno, Walker, Saveliev, & Smith, 2009; Lewis, Butler, & Gilbert, 2011). Marginal and conditional R^2 were calculated (not available for Conway-Maxwell-Poisson, beta, and zero-truncated distributions) using the *r.squaredGLMM* function from the package MUMIN v1.43.17. Assumptions were checked using model diagnostics (Bolker et al., 2009; Zuur, Ieno, & Elphick, 2010). Results were visualized using the R packages GGPLOT2 v3.3.2, SJPLOT v2.8.4, COWPLOT v1.0.0.9000, GGPUBR v0.3.0, GGPLOTIFY v0.0.5, GGTHEMES v4.2.0, and DOTWHISKER v0.5.0. All analyses were performed in R v3.6.3 (R Core Team, 2020).

We found that the mixed models confirmed the results of the models for *S. canadensis*, which included latitude as a fixed factor (Table A1.8 und A1.9). Contrary to the latitude-based models, mixed models with climate-based PC scores for *S. gigantea* revealed climatic clines in flowering phenology in both years, plant height in 2015, and reproductive-to-total biomass ratio in 2015 (Table A1.8 and A1.9).

Table A1.1. Information on sampled *Solidago canadensis* source populations. Seed sampling sites, population ID, latitude and longitude given as Northern [°N] and Eastern [°E] coordinates in decimal degrees, elevation [m a.s.l.] of source locations, estimated source population size based on the number of shoots, number of collected maternal lines, and experimental sample size of maternal lines per control and zebularine treatment for *S. canadensis* s.l. populations along a latitudinal South-North gradient in Central Europe (47–54°N). Sampled plants were at least three meters apart to reduce the chance of sampling the same clone multiple times. In total, seeds of 384 individuals from 48 populations were collected in November 2014 until March 2015 and stored in paper bags. After excluding populations with few maternal lines, with few seeds and/or low germination success, 25 populations representing 224 maternal lines in 400 individuals were transplanted in the common-garden study. After replacement of dead plants with buffer plants to avoid changes in the micro-climatic regime and depending on survival, 395 healthy plants were measured in 2015 and 386 in 2016. Information is sorted by latitude of source population from South to North. NA—information not available; ZEB—zebularine-treated individuals.

S. canadensis		**Coordinates**		**Elevation**	**Estimated**	**Maternal**	**Sample size**	
	ID	**°N**	**°E**	**[m a.s.l.]**	**shoots**	**lines**	**Control**	**ZEB**
Rhäzüns (CH)	1	46.80153	9.39882	656.0	1000	14	10	9
Landquart (CH)	2	46.95745	9.55380	521.3	1000	19	8	9
Rheineck (CH)	3	47.47613	9.57967	401.3	30	8	7	6
Konstanz (D)	4	47.67258	9.16095	399.0	1000+	10	9	9
Radolfzell (D)	5	47.76432	8.98473	434.8	100	10	10	10
Engen (D)	6	47.85763	8.79533	539.5	100	10	10	6
Pleidelsheim (D)	7	48.96087	9.19872	189.4	1000	10	10	9
Heilbronn (D)	8	49.14628	9.19737	159.8	80–100	10	8	6
Tauberbischofsheim (D)	9	49.63172	9.65733	179.6	15–20	5	10	7
Tauberbischofsheim (D)	10	49.64210	9.64187	200.3	15000	10	10	8
Volkach (D)	11	49.86363	10.22380	196.7	200	11	10	10
Rödelmaier (D)	12	50.52465	10.42545	310.5	150	10	7	5
Breitungen (D)	13	50.77387	10.32783	259.5	600	10	9	10
Eisenach (D)	14	50.97552	10.32697	225.7	30–50	9	5	5
Hoheneiche (D)	15	51.12350	9.97555	197.3	1000+	12	8	10
Wollrode (D)	16	51.36967	9.91143	242.6	500	10	10	10
Kassel (D)	17	51.50665	9.91338	154.8	300	10	10	10
Bad Gandersheim (D)	18	51.86658	10.03582	147.4	40	9	6	9
Potsdam (D)	19	52.47797	13.01649	37.2	NA	8	8	7
Kaltenweide (D)	20	52.47997	9.74547	47.7	300	10	9	9
Walsrode (D)	21	52.84990	9.60088	38.2	40–50	10	6	4
Nützen (D)	22	53.85155	9.92888	20.3	1000+	10	6	7
Neumünster (D)	23	54.09808	9.98737	25.4	20–30	9	4	5
Neumünster (D)	24	54.11280	9.99352	29.1	800	10	9	7
Flensburg (D)	25	54.76182	9.44657	37.0	100–150	10	7	7

Table A1.2. Information on sampled Solidago gigantea source populations. Seed sampling sites, population ID, latitude and longitude given as Northern [°N] and Eastern [°E] coordinates in decimal degrees, elevation [m a.s.l.] of source locations, population ID, estimated source population size based on the number of shoots, number of collected maternal lines, and experimental sample size of maternal lines per control and zebularine treatment for S. gigantea L. populations along a latitudinal South-North gradient in Central Europe (47–60°N). Sampled plants were at least one meter apart to reduce the chance of sampling the same clone multiple times. In total, seeds of 308 individuals from 33 populations were collected in November 2014 until March 2015 and stored in paper bags. After excluding populations with few maternal lines, few seeds and/or low germination success, 24 populations representing 189 maternal lines in 336 individuals were transplanted in the common garden study. After replacement of dead plants with buffer plants to avoid changes in the micro climatic regime and depending on survival, 326 healthy plants were measured in 2015 and 322 in 2016. Information is sorted by latitude of source population from South to North. NA—information not available; ZEB—zebularine-treated individuals.

S. gigantea		**Coordinates**		**Elevation**	**Estimated**	**Maternal**	**Sample size**	
	ID	**°N**	**°E**	**[m a.s.l.]**	**ramets**	**lines**	**Control**	**ZEB**
Landquart (CH)	1	46.95745	9.55380	521.3	1000	13	5	5
Eschen (LI)	2	47.24987	9.52010	436.2	40	13	8	7
Kempthal (CH)	3	47.43787	8.70233	516.2	1000+	10	10	10
Rheineck (CH)	4	47.47613	9.57967	401.3	30	8	7	6
Eriskirch (DE)	5	47.62711	9.54661	414.0	1000+	15	9	9
Konstanz (DE)	6	47.68845	9.16977	417.3	1000+	10	10	10
Engen (DE)	7	47.80753	8.73595	498.3	1000	10	10	10
Leutkirch (DE)	8	47.82122	10.00264	660.0	1000+	15	8	5
Oberkirchberg (DE)	9	48.33053	10.00931	518.6	100	8	5	0
Giengen an der Brenz (DE)	10	48.60247	10.25050	455.7	1000+	15	10	9
Schorndorf (DE)	11	48.81967	9.54897	251.0	100	15	6	5
Reinshof (DE)	12	51.49524	9.90886	156.0	600–700	10	10	9
Bad Gandersheim (DE)	13	51.86658	10.03582	147.4	30	8	6	3
Hannover (DE)	14	52.43957	9.72320	54.6	800	10	10	7
Potsdam (DE)	15	52.47797	13.01649	37.2	NA	8	5	3
Elstal (DE)	16	52.53250	13.01368	49.5	NA	8	6	6
Berlin (DE)	17	52.54594	13.11629	31.3	NA	8	8	8
Walsrode (DE)	18	52.84990	9.60088	38.2	40–50	10	6	3
Brockel (DE)	19	53.10658	9.50535	37.7	100	10	3	1
Scheeßel (DE)	20	53.16800	9.49907	26.0	NA	10	8	6
Hamburg (DE)	21	53.51463	9.89840	4.8	600	10	9	10
Nützen (DE)	22	53.85155	9.92888	20.3	1000+	10	10	9
Schleswig (DE)	23	54.53125	9.56970	16.3	800	10	10	9
Vedyxa (SE)	24	59.85300	17.78513	40.0	80–150	10	5	2

Table A13: Microsatellite markers used for genotyping. Control plants of both S. canadensis and S. gigantea used in the common-garden study (see Methods section in the main text) were genotyped using seven and nine microsatellite markers, respectively. Leaf samples were collected at the end of the vegetation period in the first year (August 2015) and stored in bags with silica beads for subsequent analyses. Microsatellite markers were selected based on studies that published markers suitable for the Solidago genus (Beck et al., 2014; Wieczorek & Geber, 2002). For S. canadensis, the markers Sg6, Sg12 and SS19D were excluded during peak annotation due to poor quality. Markers Sg1 and Sg12 were monomorphic and were therefore excluded from statistical analyses of both species. For details on DNA extraction and laboratory analyses see Methods section in the main text.

Marker	Core motif	Forward primer sequence (5'—3')	Reverse primer sequence (5'—3')	Reference
Sg1	(TTGG)	GCGTACTTATTAAATTGATTTCTATAACCG	ACAGATGGCTTCCATGATCG	Beck et al., 2014
Sg2	(AATG)	TCTAAACTGTAAGTCTTTGATGAAACC	GCCGTCAATCCTTACAATCC	Beck et al., 2014
Sg6	(AAAT)	TTTACCTTTGAATTGCGGC	GTTTAGTACCAATCAACCATGGGC	Beck et al., 2014
Sg8	(AAAG)	TCCCTCTTTATTCTTTCAACAAACC	GTTTAACACCAACATTGCAATCCC	Beck et al., 2014
Sg9	(AATG)	GACGTGGCTAAATTAAGGTGTACG	GTTTGCAACGTAATCCACCTCC	Beck et al., 2014
Sg10	(ATCT)	CGTTTGTTCTTTGTCCCTTTCC	GTTTCTATACCTCGTGCGTGTCGG	Beck et al., 2014
Sg12	(AAAT)	CTAGAAGATGTGGATTGACCAGC	GTTTCAAATGAGTCAGTCGGTGCC	Beck et al., 2014
SS4F	(CTT)7	AGCTTTTCTTCGCCATTTCCTTCC	AATTTGGTTACTGGGTTTTCTTGA	Beck et al., 2014
SS19C	(GAT)11(GAC)(GAT)4	TTAATTGAAAACCCAGATG	ACAAACCGATAGTGATACG	Wieczorek & Geber, 2002
SS19D	(CA)2(GA)(CA)(GA)(CA)8	CATTTGCCTTCAAACCATGA	CAATTGACACATCATTCGCC	Wieczorek & Geber, 2002
SS20E	(TA)4(TG)12	CACACAGACACTCAAAGCTTCA	ACCCGCCCTAAAAATAAAGA	Wieczorek & Geber, 2002

Table A1.4: Locus-specific summary statistics of microsatellite analyses per species. Control individuals of both *S. canadensis* (n = 203) and *S. gigantea* (n = 175) populations sampled along a latitudinal gradient in Central Europe (Table A1.1 and A1.2) were genotyped (Table A1.3) from dried leaf tissue. Abbreviations: H_{exp}—expected heterozygosity; H_{obs}—observed heterozygosity (calculated for *S. canadensis*); 1-D—Simpson's diversity index (calculated for *S. gigantea*); E—evenness; HWE_p—*p* value denoting significant deviation (*p* value < 0.05) from Hardy-Weinberg equilibrium, F_{IS}—inbreeding coefficient (Wright, 1922). The HWE_p statistic was calculated as a χ^2-test based on Monte Carlo permutations (n = 1000) of alleles for *S. canadensis* and based on expected genotype frequencies for *S. gigantea*. The frequency of null alleles was calculated using bootstrap estimation (Brookfield, 1996). Population-specific summary statistics are available in the Table A1.5. For *S. gigantea*, the frequency of null alleles and the inbreeding coefficient could not be calculated due to ambiguous genotypes.

Marker	**Alleles [n]**	**H_{exp}**	**H_{obs}**	**Evenness**	**HWE_p**	***F_{IS}***	**Null alleles [%]**
S. canadensis							
Sg2	12	0.84	0.75	0.80	**0.013**	0.03	4.54
Sg8	5	0.44	0.43	0.52	0.088	-0.07	0.83
Sg9	5	0.61	0.53	0.78	**0.004**	0.06	5.67
Sg10	5	0.63	0.59	0.84	0.219	0.01	2.84
SS4F	4	0.40	0.38	0.79	0.434	-0.01	2.76
SS19C	5	0.62	0.50	0.82	**<0.001**	0.10	9.68
SS20E	5	0.50	0.32	0.73	**<0.001**	0.27	12.73
	Alleles [n]	**H_{exp}**	**1-D**	**Evenness**	**HWE_p**		
S. gigantea							
Sg2	22	0.91	0.90	0.74	**<0.001**		
Sg6	10	0.68	0.68	0.53	1.000		
Sg8	10	0.59	0.59	0.62	0.641		
Sg9	7	0.74	0.74	0.74	0.119		
Sg10	9	0.79	0.79	0.83	1.000		
SS4F	10	0.76	0.76	0.79	1.000		
SS19C	16	0.83	0.83	0.71	1.000		
SS19D	34	0.93	0.92	0.69	**<0.001**		
SS20E	9	0.76	0.76	0.76	1.000		

Table A1.5: Population-specific summary statistics of microsatellite analyses per species. Control individuals (*S. canadensis*: n = 203; *S. gigantea*: n = 175) were genotyped from leaf tissue using seven and nine microsatellite markers, respectively (Table A1.3). For each population (ID; Table A1.1 and A1.2), the number of alleles μ, allelic richness A_R, private alleles A_P, expected heterozygosity H_{exp}, observed heterozygosity H_{obs} for *S. canadensis*, Simpson index of observed genetic diversity 1-D for *S. gigantea*, and the inbreeding coefficient F_{IS} (Wright, 1922) were averaged per population. Locus-specific summary statistics are given in Table A1.4. For the tetraploid *S. gigantea*, H_{obs} and F_{IS} could not be calculated due to ambiguous genotypes.

Population	**ID**	**Alleles [μ]**	**A_R**	**A_P**	**H_{exp}**	**H_{obs}**	***F_{IS}***
S. canadensis							
46.8015	1	3.00	2.15	0	0.40	0.41	-0.03
46.9575	2	3.43	2.68	1	0.53	0.47	0.06
47.4761	3	3.14	2.68	0	0.58	0.49	0.16
47.6726	4	3.00	2.53	0	0.54	0.44	0.11
47.7643	5	3.29	2.72	0	0.55	0.49	0.07
47.8576	6	3.86	2.92	0	0.60	0.49	0.17
48.9609	7	3.43	2.71	0	0.55	0.47	0.17
49.1463	8	2.71	2.44	0	0.56	0.55	-0.03
49.6317	9	2.86	2.36	0	0.49	0.43	0.16
49.6421	10	3.29	2.67	2	0.56	0.46	0.16
49.8636	11	3.71	2.88	0	0.56	0.51	0.07
50.5247	12	2.57	2.40	0	0.56	0.63	-0.18
50.7739	13	2.57	2.24	2	0.51	0.35	0.30
50.9755	14	2.29	2.11	0	0.47	0.51	-0.10
51.1235	15	3.14	2.44	0	0.51	0.54	-0.05
51.3697	16	2.71	2.33	0	0.45	0.47	-0.02
51.5067	17	2.86	2.27	0	0.45	0.48	-0.05
51.8666	18	3.43	2.87	0	0.62	0.74	-0.19
52.4780	19	3.43	2.64	0	0.54	0.48	0.11
52.4800	20	3.14	2.63	0	0.55	0.44	0.09
52.8499	21	3.43	2.85	0	0.60	0.60	0.05
53.8516	22	2.86	2.49	0	0.50	0.47	0.06
54.0981	23	2.29	2.14	0	0.45	0.50	-0.12
54.1128	24	3.29	2.76	0	0.58	0.52	0.14
54.7618	25	3.14	2.51	1	0.51	0.55	-0.12
	ID	**Alleles [μ]**	**A_R**	**A_P**	**H_{exp}**	**1-D**	
S. gigantea							
46.9575	1	4.89	4.12	0	0.76	0.64	
47.2499	2	5.78	4.60	0	0.79	0.75	
47.4379	3	5.44	4.17	4	0.69	0.72	
47.4761	4	5.00	4.20	2	0.77	0.69	
47.6271	5	6.22	4.58	2	0.76	0.77	
47.6885	6	6.33	4.42	2	0.74	0.74	
47.8075	7	6.00	4.57	2	0.76	0.80	
47.8212	8	5.56	4.20	4	0.72	0.73	
48.3305	9	3.22	2.94	0	0.61	0.50	
48.6025	10	4.44	3.70	1	0.70	0.69	
48.8197	11	4.33	3.89	0	0.76	0.67	
51.4952	12	5.00	3.97	1	0.70	0.71	

Continuation of **Table A1.5**

Population	ID	Alleles [µ]	A_R	A_P	H_{exp}	1-D
S. gigantea						
51.8666	13	4.56	3.79	2	0.72	0.64
52.4396	14	6.22	4.65	0	0.76	0.79
52.4780	15	4.22	3.75	2	0.71	0.67
52.5325	16	4.22	3.66	0	0.72	0.54
52.5459	17	5.67	4.29	0	0.72	0.73
52.8499	18	4.22	3.76	2	0.72	0.69
53.1066	19	4.33	4.00	0	0.79	0.62
53.1680	20	5.67	4.53	2	0.78	0.77
53.5146	21	6.56	4.95	0	0.81	0.80
53.8516	22	6.56	4.92	0	0.80	0.82
54.5313	23	4.44	3.67	10	0.66	0.61
59.8530	24	4.00	3.66	3	0.74	0.55

Table A1.6: Results of (generalized) linear mixed effects models using latitude as a covariate. Models used maximum likelihood and were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g], and the number of ramets in spring 2016 for both *S. canadensis* and *S. gigantea*, respectively. Fixed effects included initial size (number of first true leaves × the length × the width of the longest true leaf; scaled and centered; not used if response variable), latitude (decimal degrees of sampled source locations along South-North gradient in Central Europe; scaled and centered), zebularine treatment (zebularine-treated versus control) and the interaction of latitude and zebularine treatment. Block (i.e. position in the common garden), source population and maternal lines where used as random intercept effects with maternal lines nested within source populations. All random-effect components were kept in the models (Barr et al., 2013). Depending on data structure, generalized mixed models (GLMM) either incorporated a zero-truncated log-link Poisson distribution (tP), a (zero truncated; tNB1) negative-binomial distribution (NB1) for count data (days to flowering and number of ramets), or a logit-link beta distribution (B) for proportional data (reproductive-to-total aboveground biomass ratio). Data was transformed using either ordered quantile normalization (OQN; Peterson and Cavanaugh, 2019), Yeo-Johnson transformation (YJ; Yeo & Johnson, 2000) or Box-Cox transformation (BC; Box & Cox, 1964) to meet assumptions of residual normality. Default settings of model optimizers have been adjusted in cases where models did not converge. The p values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943) whereas *p* values for LMMs were estimated using Satterthwaite-approximation (Satterthwaite, 1946). R^2_m—marginal pseudo- R^2 (without random effects) and R^2_c—conditional pseudo-R^2 (including random effects; Nakagawa & Schielzeth, 2013); AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

Model parameters	**Initial size**	**Phenology**	**Phenology**	**Height**	**Height**	**Total biomass**	**Biomass ratio**	**Ramets**
	2015	**2015**	**2016**	**2015**	**2016**	**2015**	**2015**	**2016**
S. canadensis								
Sample size	395	388	381	384	381	390	384	395
Transformation	YJ	OQN	–	–	OQN	–	–	OQN
Distribution (Link)	N	N	tP (log)	N	N	N	B (logit)	N
Fixed factors in (generalized) linear mixed-effects models								
Intercept								
Estimate	0.20	-0.10	4.20	158.67	0.07	103.33	-0.88	0.02
SE	0.10	0.10	0.02	2.11	0.11	2.29	0.04	0.09
z/t	2.06	-1.01	212.54	75.33	0.69	45.04	-19.89	0.18
p	**0.051**	0.322	**<0.001**	**<0.001**	0.500	**<0.001**	**<0.001**	0.860
Initial (seedling) size 2015								
Estimate	–	-0.35	-0.03	0.63	0.04	13.08	-0.01	-0.07
SE	–	0.04	0.01	0.90	0.05	1.50	0.02	0.05
z/t	–	-8.15	-3.42	0.70	0.84	8.75	-0.42	-1.28
p	–	**<0.001**	**<0.001**	0.486	0.403	**<0.001**	0.677	0.200
Latitude (L)								
Estimate	-0.13	-0.45	-0.07	-3.20	-0.27	-1.48	0.10	0.10
SE	0.09	0.09	0.02	1.89	0.09	2.10	0.03	0.09
z/t	-1.55	-4.86	-3.61	-1.69	-2.95	-0.71	2.77	1.09
p	0.129	**<0.001**	**<0.001**	0.100	**0.005**	0.482	**0.006**	0.281
Zebularine (Z)								
Estimate	-0.42	0.18	-0.01	-1.88	-0.16	-3.54	-0.05	-0.03
SE	0.09	0.07	0.01	1.47	0.09	2.68	0.04	0.09
z/t	-4.69	2.43	-0.99	-1.27	-1.77	-1.32	-1.14	-0.29

Continuation of **Table A1.6**

Model parameters	Initial size 2015	Phenology 2015	Phenology 2016	Height 2015	Height 2016	Total biomass 2015	Biomass ratio 2015	Ramets 2016
p	**<0.001**	**0.016**	0.324	0.204	**0.078**	0.187	0.254	0.769
S. canadensis								
L x Z								
Estimate	0.00	0.04	-0.00	-3.06	0.05	-5.40	-0.01	0.05
SE	0.09	0.07	0.01	1.44	0.09	2.63	0.04	0.09
z/t	-0.01	0.61	-0.26	-2.13	0.62	-2.05	-0.31	0.55
p	0.995	0.540	0.797	**0.035**	0.533	**0.042**	0.760	0.581
Random factors and goodness-of-fit								
SD Block	0.12	0.07	0.01	2.47	0.15	2.69	0.08	0.03
SD Population	0.30	0.38	0.08	7.54	0.31	2.95	0.10	0.30
SD Maternal lines	0.27	0.27	0.08	8.79	0.33	11.39	0.04	0.44
SD Residuals	0.87	0.70	–	13.67	0.83	25.58	–	0.83
R^2_m	0.06	0.28	NA	0.08	0.07	0.22	NA	0.02
R^2_c	0.24	0.50	NA	0.47	0.30	0.36	NA	0.31
AICc	1093.34	937.30	2911.04	3271.73	1043.65	3725.92	-803.04	1099.32
S. gigantea								
Sample size	326	288	314	286	313	322	284	326
Transformation	YJ	–	–	BC	YJ	–	–	–
Distribution (Link)	N	tP (log)	tNB1 (log)	N	N	N	B (logit)	NB1(log)
Fixed factors in (generalized) linear mixed-effects models								
Intercept								
Estimate	0.24	4.47	4.12	0.00	-0.02	104.30	-1.22	3.39
SE	0.10	0.02	0.03	0.12	0.11	3.31	0.07	0.03
z/t	2.44	218.50	146.54	0.00	-0.13	31.51	-17.74	103.71
p	**0.020**	**<0.001**	**<0.001**	0.998	0.895	**<0.001**	**<0.001**	**<0.001**
Initial (seedling) size 2015								
Estimate	–	-0.04	-0.03	0.04	0.08	14.21	-0.01	0.00
SE	–	0.01	0.01	0.06	0.06	1.79	0.03	0.02
z/t	–	-5.22	-2.67	0.77	1.39	7.92	-0.21	0.01
p	–	**<0.001**	**0.008**	0.439	0.164	**<0.001**	0.833	0.990
Latitude (L)								
Estimate	-0.04	0.02	0.00	0.12	-0.07	-5.29	-0.05	-0.06
SE	0.09	0.02	0.03	0.11	0.10	3.16	0.06	0.03
z/t	-0.38	0.86	-0.03	1.13	-0.74	-1.67	-0.85	-2.14
p	0.709	0.392	0.978	0.267	0.465	0.103	0.397	**0.032**
Zebularine (Z)								
Estimate	-0.58	0.01	0.00	-0.08	-0.04	-5.50	-0.09	0.00
SE	0.10	0.01	0.02	0.11	0.11	3.43	0.06	0.03
z/t	-5.95	0.94	-0.18	-0.71	-0.42	-1.60	-1.46	0.02
p	**<0.001**	0.345	0.853	0.476	0.675	0.110	0.145	0.985
L x Z								
Estimate	0.04	-0.01	0.02	-0.02	0.06	0.61	0.00	0.01
SE	0.10	0.01	0.02	0.10	0.10	3.32	0.06	0.03
z/t	0.45	-0.47	0.84	-0.23	0.61	0.18	-0.04	0.37
p	0.650	0.636	0.398	0.819	0.543	0.854	0.969	0.711

Continuation of **Table A1.6**

Model parameters	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
Random factors and goodness-of-fit								
SD Block	–	0.02	0.00	0.13	0.13	0.00	0.00	0.04
SD Population	0.34	0.08	0.12	0.44	0.36	11.99	0.26	0.09
SD Maternal lines	0.28	0.03	0.09	0.17	0.31	0.00	0.19	–
SD Residuals	0.85	–	–	0.86	0.87	28.99	–	–
R^2_m	0.08	NA	NA	0.02	0.01	0.21	NA	0.16
R^2_c	0.28	NA	NA	0.26	0.25	0.32	NA	0.70
AICc	892.93	2165.05	2454.93	789.52	884.28	3128.40	-519.54	2357.03

Table A1.7: Bio-climatic variables used in principal component regression. In total, 21 bio-climatic variables from the WorldClim 2.0 database (Fick & Hijmans, 2017) were used in the principal component regression (PCR; Figure A1.7). The coefficient of variation (CV) from monthly-averaged solar radiation [kJ m^{-2} day^{-1}] was calculated to incorporate a variable that accounts for the variability in day length. Labels are given according to the WorldClim 2.0 database and according to the abbreviations used in the principal component analysis (PCR).

PCR ID	WorldClim ID	Variable	Unit	Explanation
T_A	BIO1	Mean Annual Temperature	°C	Mean monthly temperatures averaged over one year
$T_{D\text{-}range}$	BIO2	Mean Diurnal Range in Temperature	°C	Mean of monthly temperature ($max_{Temp} - min_{Temp}$)
T_{iso}	BIO3	Temperature Isothermality	BIO2 / BIO7 x 100	Quantifies day-to-night fluctuation compared to summer-winter fluctuation
T_{season}	BIO4	Temperature Seasonality	SD°C x 100	Variability in mean monthly temperatures
$T_{M\text{-}warmest}$	BIO5	Maximum Temperature of Warmest Month	°C	
$T_{M\text{-}coldest}$	BIO6	Minimum Temperature of Coldest Month	°C	
$T_{A\text{-}range}$	BIO7	Annual Temperature Range	BIO5 – BIO6	
$T_{Q\text{-}wettest}$	BIO8	Mean Temperature of Wettest Quarter	°C	A quarter is defined as a period of three month (¼ of the year)
$T_{Q\text{-}driest}$	BIO9	Mean Temperature of Driest Quarter	°C	
$T_{Q\text{-}warmest}$	BIO10	Mean Temperature of Warmest Quarter	°C	
$T_{Q\text{-}coldest}$	BIO11	Mean Temperature of Coldest Quarter	°C	
P_A	BIO12	Annual Precipitation	mm	Mean monthly precipitation averaged over one year
$P_{M\text{-}wettest}$	BIO13	Precipitation of Wettest Month	mm	
$P_{M\text{-}driest}$	BIO14	Precipitation of Driest Month	mm	
P_{season}	BIO15	Precipitation Seasonality	CV	Variability of monthly precipitation as coefficient of variation (CV)
$P_{Q\text{-}wettest}$	BIO16	Precipitation of Wettest Quarter	mm	
$P_{Q\text{-}driest}$	BIO17	Precipitation of Driest Quarter	mm	
$P_{Q\text{-}warmest}$	BIO18	Precipitation of Warmest Quarter	mm	
$P_{Q\text{-}coldest}$	BIO19	Precipitation of Coldest Quarter	mm	Monthly averaged data with coast-distance as covariate
$Wind_{speed}$	–	Average Wind Speed	m s^{-1}	
$Radiation_{solar}$	–	Variation in Solar Radiation	CV	Monthly averaged [kJ m^{-2} day^{-1}] MODIS satellite data with cloud cover as a covariate converted to coefficient of variation

Table A1.8: Summary of likelihood-ratio tests for (generalized) linear mixed effects models using climate data instead of latitude. Models were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g] and the number of ramets in spring 2016 [n] for both S. canadensis and S. gigantea, respectively. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centred; not included if response variable), the first two principal components (PC1 and PC2) from principal component regression using bio-climatic variables (Table A1.7; Figure A1.7), zebularine treatment and the interaction of PC1 and PC2 with the zebularine treatment. Contribution of each fixed effect was evaluated via iterative elimination of fixed effects comparing the interaction to the full model and the main effects to the model without interactions (Pinheiro & Bates, 2009; Zuur et al., 2009). All p values were adjusted (padj) for multiple comparisons (Benjamini & Hochberg, 1995). Significant p values ($p < 0.05$) are given in bold. $\chi 2$—deviance of the likelihood-ratio test.

Response variable		**Initial size 2015**			**PC1**			**PC2**			**Zebularine (Z)**			**PC1 x Z**			**PC2 x Z**		
	n	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}
S. canadensis																			
Initial size 2015	395	–	–	–	3.23	0.072	0.217	0.01	0.918	0.975	20.83	**<0.001**	**<0.001**	0.00	0.975	0.975	0.60	0.438	0.875
Phenology 2015	388	55.01	**<0.001**	**<0.001**	19.33	**<0.001**	**<0.001**	1.02	0.313	0.492	5.86	**0.015**	**0.036**	0.64	0.422	0.492	0.65	0.420	0.492
Phenology 2016	381	12.01	**0.001**	**0.002**	12.82	<0.001	0.002	0.54	0.462	0.778	0.95	0.329	0.768	0.02	0.885	0.885	0.35	0.556	0.778
Height 2015	384	0.30	0.586	0.586	9.54	0.002	0.014	0.32	0.571	0.586	1.63	0.202	0.282	5.05	**0.025**	0.057	3.02	0.082	0.144
Height 2016	381	0.56	0.454	0.754	10.42	**0.001**	**0.009**	0.09	0.768	0.768	3.16	0.076	0.264	0.52	0.471	0.754	0.38	0.539	0.754
Total biomass	390	66.87	**<0.001**	**<0.001**	7.24	0.007	0.025	2.19	0.139	0.195	1.67	0.197	0.230	4.01	**0.045**	0.106	0.06	0.812	0.812
Biomass ratio	384	0.17	0.684	0.798	10.85	**<0.001**	**0.007**	0.01	0.943	0.943	1.33	0.248	0.723	0.26	0.608	0.798	1.03	0.310	0.723
Ramets 2016	395	1.70	0.193	0.675	3.21	0.073	0.512	0.00	0.956	0.969	0.11	0.736	0.969	0.13	0.718	0.969	0.00	0.969	0.969
S. gigantea																			
Initial size 2015	326	–	–	–	0.81	0.369	0.737	0.00	0.996	0.996	32.23	**<0.001**	**<0.001**	0.15	0.699	0.839	1.14	0.286	0.737
Phenology 2015	288	30.86	**<0.00**	**<0.00**	7.99	**0.005**	**0.016**	6.93	**0.008**	**0.020**	1.12	0.290	0.344	1.95	0.163	0.285	0.35	0.556	0.556
Phenology 2016	314	7.04	**0.008**	0.056	1.12	0.289	0.337	5.47	**0.019**	0.068	0.07	0.798	0.798	2.15	0.142	0.295	1.57	0.211	0.295
Height 2015	286	0.69	0.407	0.836	4.97	**0.026**	0.180	0.30	0.584	0.836	0.47	0.495	0.836	0.00	0.978	0.978	0.28	0.597	0.836
Height 2016	313	2.03	0.154	0.871	0.07	0.799	0.871	0.03	0.871	0.871	0.16	0.687	0.871	0.15	0.699	0.871	0.81	0.368	0.871
Total biomass	322	56.36	**<0.00**	**<0.00**	1.57	0.210	0.490	0.36	0.550	0.770	2.49	0.114	0.400	0.09	0.760	0.778	0.45	0.504	0.770
Biomass ratio	284	0.05	0.815	0.815	4.87	**0.027**	0.191	1.23	0.268	0.626	2.12	0.145	0.508	0.56	0.455	0.638	0.59	0.442	0.638
Ramets 2016	326	0.00	0.955	0.978	3.18	0.075	0.522	0.61	0.434	0.978	0.00	0.978	0.978	0.09	0.765	0.978	0.10	0.751	0.978

Table A1.9: Results of (generalized) linear mixed effects models using climate data. Models used maximum likelihood and were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g] and the number of ramets in spring 2016 [n] for both *S. canadensis* and *S. gigantea*, respectively. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centred; not used if response variable), the first two principal components (PC1 and PC2) from principal component regression using bio-climatic variables from WorldClim 2.0 database (Fick & Hijmans, 2017; Table A1.7; Figure A1.7), zebularine treatment (zebularine-treated versus control) and the interaction of each principal component with zebularine treatment. Block (i.e. position in the common garden), source population and maternal lines where used as random-intercept effects with maternal lines nested in source populations. All random-effect components were kept in the models (Barr et al., 2013). Depending on the dispersion and data structure, generalized mixed models (GLMM) were based either on a log-lambda-link Conway-Maxwell-Poisson distribution (CMP; Shmueli, Minka, Kadane, Borle, & Boatwright, 2005), a zero-truncated log-link negative-binomial distribution (tNB1) for count data (days to flowering and number of ramets), or a logit-link beta distribution (B) for proportional data (reproductive-to-total biomass ratio). Data was transformed either using ordered-quantile normalization (OQN; Peterson & Cavanaugh, 2019), square-root transformation (SQRT), or Yeo-Johnson transformation (YJ; Yeo & Johnson, 2000) to meet assumptions of residual normality and to counter variance heterogeneity (Bartlett, 1936). Default settings of model optimizers have been adjusted in cases where models did not converge. The *p* values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943) whereas *p* values for LMMs were estimated using Satterthwaite-approximation (Satterthwaite, 1946). R^2_m—marginal pseudo- R^2 (without random effects) and R^2_c—conditional pseudo- R^2 (including random effects; Nakagawa & Schielzeth, 2013); AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

Model parameters	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
S. canadensis								
Sample size	395	388	381	384	381	390	384	395
Transformation	YJ	–	–	–	OQN	–	–	SQRT
Distribution (Links)	N	CMP (loglambda)	tNB1 (log)	N	N	N	B (logit)	N
Fixed factors in (generalized) linear mixed effects models								
Intercept								
Estimate	0.19	14.11	4.12	158.47	0.05	103.03	-0.88	3.99
SE	0.10	0.02	0.03	1.99	0.10	2.26	0.04	0.05
z/t	1.94	607.47	164.46	79.56	0.49	45.67	-20.33	74.27
p	0.064	–	**<0.001**	<0.**001**	0.626	**<0.001**	**<0.001**	**<0.001**
Initial size 2015								
Estimate	–	-0.09	-0.03	0.60	0.04	12.99	-0.01	-0.04
SE	–	0.01	0.01	0.90	0.05	1.49	0.02	0.03
z/t	–	-7.82	-2.64	0.67	0.72	8.73	-0.40	-1.32
p	–	–	**0.008**	0.505	0.475	**<0.001**	0.688	0.190
Principal component 1 (PC1)								
Estimate	-0.05	-0.04	0.00	-1.46	-0.11	-0.68	0.04	0.03
SE	0.03	0.01	0.01	0.64	0.03	0.72	0.01	0.02
z/t	-1.63	-5.27	0.59	-2.29	-3.54	-0.94	3.27	1.47
p	0.110	–	0.556	**0.029**	**0.001**	0.351	**0.001**	0.150

Continuation of **Table A1.9**

Model parameters	Initial size 2015	Phenology 2015	Phenology 2016	Height 2015	Height 2016	Total biomass 2015	Biomass ratio 2015	Ramets 2016
S. canadensis								
Principal component 2 (PC2)								
Estimate	0.01	0.01	0.03	-0.86	0.02	1.03	0.01	-0.00
SE	0.03	0.01	0.01	0.71	0.03	0.80	0.01	0.02
z/t	0.30	0.60	2.68	-1.22	0.57	1.29	0.53	-0.03
p	0.769	–	**0.007**	0.232	0.574	0.202	0.600	0.979
Zebularine (Z)								
Estimate	-0.41	0.05	0.00	-2.29	-0.15	-3.76	-0.04	-0.02
SE	0.09	0.02	0.02	1.46	0.09	2.69	0.04	0.05
z/t	-4.63	2.45	0.05	-1.56	-1.72	-1.40	-1.10	-0.32
p	**<0.001**	–	0.961	0.120	0.087	0.16	0.273	0.753
PC1 x Z								
Estimate	0.00	0.01	0.01	-1.14	0.02	-1.87	-0.01	0.01
SE	0.03	0.01	0.01	0.50	0.03	0.93	0.01	0.02
z/t	0.03	0.80	1.48	-2.28	0.72	-2.01	-0.51	0.36
p	0.975	–	0.140	**0.024**	0.470	**0.046**	0.608	0.719
PC2 x Z								
Estimate	-0.03	0.01	-0.01	0.98	-0.02	-0.25	-0.02	-0.00
SE	0.04	0.01	0.01	0.56	0.03	1.04	0.02	0.02
z/t	-0.78	0.81	1.26	1.75	-0.62	-0.24	-1.02	-0.04
p	0.438	–	0.208	0.082	0.540	0.813	0.310	0.969
Random-factors and goodness-of-fit								
SD Maternal line	0.27	0.01	0.10	8.94	0.33	11.36	0.05	0.28
SD Population	0.30	0.01	0.10	6.84	0.29	1.54	0.09	0.17
SD Block	0.12	<0.001	0.00	2.40	0.15	2.78	0.07	0.00
SD Residuals	0.87	–	–	13.52	0.83	25.57	–	0.50
R^2_m	0.07	NA	NA	0.11	0.09	0.22	NA	0.03
R^2_c	0.24	NA	NA	0.49	0.31	0.36	NA	0.32
AICc	1096.72	2511.68	2450.12	3269.10	1044.54	3727.22	-802.58	715.59
S. gigantea								
Sample size	326	288	314	286	313	322	284	326
Transformation	YJ	–	–	–	YJ	–	–	–
Distribution (Link)	N	CMP (loglambda)	tNB1 (log)	N	N	N	B (logit)	tNB1 (log)
Fixed factors in (generalized) linear mixed effects models								
Intercept								
Estimate	0.24	6.23	4.12	143.15	-0.02	103.86	-1.23	3.38
SE	0.10	0.02	0.03	2.03	0.11	3.36	0.06	0.03
z/t	2.49	268.95	164.46	69.66	-0.19	30.88	-19.50	102.72
p	**0.018**	–	**<0.001**	**<0.001**	0.853	**<0.001**	**<0.001**	**<0.001**
Initial size 2015								
Estimate	–	-0.05	-0.03	0.88	0.09	14.18	-0.01	0.00
SE	–	0.01	0.01	1.00	0.06	1.80	0.03	0.02

Continuation of **Table A1.9**

Model parameters	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
z/t	–	-5.59	-2.64	0.88	1.49	7.87	-0.23	-0.04
p	–	–	**0.008**	0.380	0.138	**<0.001**	0.819	0.966
Principal component 1 (PC1)								
Estimate	-0.03	0.02	0.00	1.33	0.00	-1.41	-0.05	-0.02
SE	0.03	0.01	0.01	0.62	0.03	1.12	0.02	0.01
z/t	-1.01	3.38	0.59	2.13	0.08	-1.27	-2.39	-1.76
p	0.322	–	0.556	**0.041**	0.934	0.215	**0.017**	0.078
Principal component 2 (PC2)								
Estimate	-0.02	0.03	0.03	0.61	0.01	-0.45	-0.02	0.01
SE	0.04	0.01	0.01	0.85	0.05	1.50	0.03	0.01
z/t	-0.43	2.85	2.68	0.71	0.19	-0.30	-0.78	0.83
p	0.672	–	**0.007**	0.479	0.851	0.767	0.433	0.405
Zebularine (Z)								
Estimate	-0.59	0.02	0.00	-1.12	-0.03	-5.00	-0.08	0.00
SE	0.10	0.02	0.02	1.89	0.11	3.49	0.06	0.03
z/t	-6.02	1.05	0.05	-0.60	-0.24	-1.43	-1.26	0.06
p	**<0.001**	–	0.961	0.553	0.814	0.153	0.207	0.954
PC1 x Z								
Estimate	0.01	-0.01	0.01	0.02	0.01	0.33	0.01	0.00
SE	0.03	0.01	0.01	0.59	0.03	1.08	0.02	0.01
z/t	0.39	-1.40	1.48	0.03	0.39	0.31	0.75	0.30
p	0.699	–	0.140	0.978	0.700	0.760	0.456	0.765
PC2 x Z								
Estimate	0.06	-0.01	-0.01	-0.52	-0.05	-1.20	-0.02	-0.01
SE	0.05	0.01	0.01	0.98	0.05	1.80	0.03	0.02
z/t	1.07	-0.59	-1.26	-0.53	-0.90	-0.67	-0.77	-0.32
p	0.286	–	0.208	0.593	0.367	0.504	0.439	0.751
Random-factors and goodness-of-fit								
SD Maternal lines	0.28	0.01	0.10	2.78	0.31	0.00	0.18	<0.001
SD Population	0.33	0.01	0.10	6.94	0.36	12.37	0.22	0.09
SD Block	0.00	<0.001	<0.001	2.13	0.13	0.00	<0.001	0.04
SD Residuals	0.85	–	–	14.83	0.87	28.97	–	–
R^2_m	0.09	NA	NA	0.06	0.01	0.21	NA	NA
R^2_c	0.28	NA	NA	0.26	0.25	0.33	NA	NA
AICc	897.32	2094.55	2450.12	2419.45	888.24	3133.20	-521.44	2364.40

Table A1.10: Summary of likelihood-ratio tests for (generalized) linear mixed effects models with the northernmost (Swedish; ID 24) *S. gigantea* population excluded. Models were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g] and the number of ramets in spring 2016 [n]. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centred; not included if response variable), latitude of sampled source populations [°N], zebularine treatment and the interaction of latitude with zebularine treatment. Contribution of each fixed effect was evaluated via iterative elimination of fixed effects comparing the interaction to the full model and the main effects to the model without interactions (Pinheiro & Bates, 2009; Zuur et al., 2009). All *p* values were adjusted (p_{adj}) for multiple comparisons using the method of Benjamini and Hochberg (Benjamini & Hochberg, 1995). Significant p values ($p < 0.05$) are given in bold. $\chi 2$—deviance of the likelihood-ratio test.

S. gigantea		**Initial size**			**Latitude (L)**			**Zebularine (Z)**			**L x Z**		
	n	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}	χ^2	p	p_{adj}
Initital size 2015	319	–	–	–	1.03	0.310	0.310	29.95	**<0.001**	**<0.001**	1.16	0.282	0.310
Phenology 2015	281	24.30	**<0.001**	**<0.001**	7.79	**0.005**	**0.011**	0.83	0.362	0.362	1.33	0.249	0.332
Phenology 2016	307	5.63	**0.018**	**0.071**	2.41	0.120	0.241	0.03	0.874	0.874	0.53	0.468	0.624
Height 2015	280	0.74	0.391	0.654	2.84	0.092	0.368	0.47	0.491	0.654	0.17	0.682	0.682
Height 2016	306	1.95	0.163	0.651	0.06	0.811	0.811	0.23	0.628	0.811	0.18	0.673	0.811
Total biomass 2015	315	58.00	**<0.001**	**<0.001**	2.69	0.101	0.134	2.88	0.090	0.134	0.08	0.784	0.784
Biomass ratio 2015	277	0.10	0.753	0.984	2.71	0.100	0.282	2.17	0.141	0.282	0.00	0.984	0.984
Ramets 2016	319	0.07	0.794	0.990	2.35	0.125	0.501	0.00	0.990	0.99	0.03	0.869	0.990

Table A1.11: Results of (generalized) linear mixed effects models excluding the northernmost (Swedish; ID 24) population for *S. gigantea*. Models used maximum likelihood and were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g], and the number of ramets in spring 2016. Fixed effects included initial size (number of first true leaves × the length × the width of the longest true leaf; scaled and centred; not used if response variable), latitude (decimal degrees of sampled source locations along South-North gradient in Central Europe; scaled and centered), zebularine treatment (zebularine-treated maternal lines versus their control) and the interaction of latitude and zebularine treatment. Block (i.e. position in the common garden), source population and maternal lines where used as random-intercept effects with maternal lines nested within source populations. All random effect components were kept in the models (Barr et al., 2013). Depending on data structure, generalized mixed models (GLMM) either incorporated a zero-truncated log-link Poisson distribution (tP), a (zero-truncated; tNB1) negative-binomial distribution (NB1) for count data (days to flowering and number of ramets), or a logit-link beta (B) distribution for proportional data (reproductive to total aboveground biomass ratio). Data was transformed using either Box Cox transformation (BC; Box & Cox, 1964) or Yeo Johnson transformation (YJ; Yeo & Johnson, 2000) to meet assumptions of residual normality. Default settings of model optimizers have been adjusted in cases where models did not converge. The *p* values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943) whereas *p* values for LMMs were estimated using Satterthwaite-approximation (Satterthwaite, 1946). R^2_m—marginal pseudo- R^2 (without random effects) and R^2_c—conditional pseudo- R^2 (including random effects; Nakagawa & Schielzeth, 2013); AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
Sample size	319	281	307	280	306	315	277	319
Transformation	YJ	–	–	BC	YJ	YJ	–	–
Distribution (Link)	N	tP (log)	tNB1 (log)	N	N	N	B (logit)	NB1(log)
Fixed factors in (generalized) linear mixed-effects models								
Intercept								
Estimate	0.23	4.47	4.13	0.00	-0.01	0.07	-1.23	3.39
SE	0.10	0.02	0.03	0.13	0.12	0.10	0.07	0.03
z/t	2.35	259.27	156.27	0.02	-0.11	0.68	-17.74	101.57
p	**0.025**	**<0.001**	**<0.001**	0.984	0.918	0.500	**<0.001**	**<0.001**
Initial (seedling) size								
Estimate	–	-0.04	-0.03	0.05	0.08	0.41	-0.01	0.00
SE	–	0.01	0.01	0.06	0.06	0.05	0.04	0.02
z/t	–	-4.87	-2.42	0.90	1.37	7.98	-0.31	0.25
p	–	**<0.001**	**0.015**	0.368	0.171	**<0.001**	0.756	0.803
Latitude (L)								
Estimate	-0.14	0.05	0.03	0.20	-0.04	-0.14	-0.11	-0.04
SE	0.10	0.02	0.03	0.12	0.11	0.10	0.07	0.03
z/t	-1.39	3.23	1.29	1.75	-0.39	-1.40	-1.52	-1.45
p	0.173	**0.001**	0.197	0.089	0.698	0.171	0.127	0.146
Zebularine (Z)								
Estimate	-0.56	0.01	-0.00	-0.07	-0.05	-0.17	-0.10	0.00

Continuation of **Table A1.11**

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
SE	0.10	0.01	0.02	0.11	0.11	0.10	0.06	0.03
z/t	-5.75	0.97	-0.18	-0.69	-0.49	-1.70	-1.48	0.02
p	**<0.001**	0.334	0.587	0.494	0.624	0.090	0.140	0.987
L x Z								
Estimate	0.10	-0.02	0.01	-0.04	0.04	-0.03	-0.00	0.01
SE	0.10	0.01	0.02	0.11	0.10	0.09	0.06	0.03
z/t	1.08	-1.15	0.73	-0.41	0.42	-0.28	-0.02	0.17
p	0.283	0.249	0.467	0.682	0.673	0.784	0.984	0.869
Random factors and goodness-of-fit								
SD Block	0.00	0.02	0.00	0.13	0.13	0.00	0.00	0.04
SD Population	0.33	0.06	0.11	0.43	0.36	0.35	0.25	0.09
SD Maternal lines	0.28	0.03	0.09	0.16	0.30	0.00	0.18	0.00
SD Residuals	0.85	–	–	0.86	0.87	0.82	–	–
R^2_m	0.09	NA	NA	0.04	0.01	0.22	NA	0.10
R^2_c	0.27	NA	NA	0.27	0.25	0.34	NA	0.69
AICc	875.85	2102.78	2399.10	770.82	864.88	813.04	-504.72	2314.24

Table A1.12: Summary of likelihood-ratio tests for (generalized) linear mixed effects models in which populations were divided into two geographical subgroups based on the gap in the latitudinal transect. Models were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g] and the number of ramets in spring 2016 [n]. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centred; not included if response variable), zebularine treatment, subgroup (southern subgroup: population 1—11; northern subgroup: population 12—23; see Table A1.2 for population IDs) and the interaction of zebularine treatment and subgroup. Contribution of each fixed effect was evaluated via iterative elimination of fixed effects comparing the interaction to the full model and the main effects to the model without interactions (Pinheiro & Bates, 2009; Zuur et al., 2009). All p values were adjusted (p_{adj}) for multiple comparisons (Benjamini & Hochberg, 1995). Significant p values ($p < 0.05$) are given in bold. $\chi 2$—deviance of the likelihood-ratio test.

S. gigantea		**Initial size**			**Zebularine (Z)**			**Subgroup (S)**			**Z x S**		
	n	$\chi 2$	p	p_{adj}	$\chi 2$	p	p_{adj}	$\chi 2$	p	p_{adj}	$\chi 2$	p	p_{adj}
Initital size 2015	319	–	–	–	1.03	0.310	0.310	29.95	**<0.001**	**<0.001**	1.16	0.282	0.310
Phenology 2015	281	24.30	**<0.001**	**<0.001**	7.79	**0.005**	**0.011**	0.83	0.362	0.362	1.33	0.249	0.332
Phenology 2016	307	5.63	**0.018**	**0.071**	2.41	0.120	0.241	0.03	0.874	0.874	0.53	0.468	0.624
Height 2015	280	0.74	0.391	0.654	2.84	0.092	0.368	0.47	0.491	0.654	0.17	0.682	0.682
Height 2016	306	1.95	0.163	0.651	0.06	0.811	0.811	0.23	0.628	0.811	0.18	0.673	0.811
Total biomass 2015	315	58.00	**<0.001**	**<0.001**	2.69	0.101	0.134	2.88	0.090	0.134	0.08	0.784	0.784
Biomass ratio 2015	277	0.10	0.753	0.984	2.71	0.100	0.282	2.17	0.141	0.282	0.00	0.984	0.984
Ramets 2016	319	0.07	0.794	0.990	2.35	0.125	0.501	0.00	0.990	0.99	0.03	0.869	0.990

Table A1.13: Results of (generalized) linear mixed effects models using subgroups of *S. gigantea* populations divided by the gap in the latitudinal transect as fixed factor (see Figure 2.1 in main text). Models used maximum likelihood and were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g], and the number of ramets in spring 2016. Fixed effects included initial size (number of first true leaves × the length × the width of the longest true leaf; scaled and centred; not used if response variable), zebularine treatment (zebularine-treated versus control), subgroup (southern subgroup: population 1—11; northern subgroup: population 12—23; see Table A1.2 for population IDs) and the interaction of zebularine treatment and subgroup. Position of block in the common garden, source population and maternal lines where used as random-intercept effects with maternal lines nested within source populations. All random-effect components were kept in the models (Barr et al., 2013). Depending on data structure, generalized mixed models (GLMM) either incorporated a log-link Poisson distribution (P), a gamma distribution (G) for count data (days to flowering and number of ramets), or a logit-link beta distribution (B) for proportional data (reproductive-to-total aboveground biomass ratio). Data was transformed using either scaling (to a mean of zero and a standard deviation of one; SCALED), ordered-quantile normalization (OQN; Peterson & Cavanaugh, 2019), or Yeo-Johnson transformation (YJ; Yeo & Johnson, 2000) to meet assumptions of residual normality. Default settings of model optimizers have been adjusted in cases where models did not converge. The *p* values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943) whereas *p* values for LMMs were estimated using Satterthwaite-approximation (Satterthwaite, 1946). R^2_m—marginal pseudo-R^2 (without random effects) and R^2_c—conditional pseudo- R^2 (including random effects; Nakagawa & Schielzeth, 2013); AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
Sample size	326	288	314	286	313	322	284	326
Transformation	SCALED	–	OQN	SCALED	YJ	SCALED	–	–
Distribution (Link)	N	P (log)	N	N	N	N	B (logit)	G (log)
Fixed factors of (generalized) linear mixed effects models								
Intercept								
Estimate	0.33	4.42	-0.12	-0.19	-0.02	0.19	-1.12	3.44
SE	0.14	0.03	0.19	0.17	0.16	0.14	0.10	0.06
z/t	2.36	172.94	-0.66	-1.14	-0.11	1.39	-11.74	59.63
p	**0.024**	**<0.001**	0.518	0.261	0.914	0.18	**<0.001**	**<0.001**
Initial (seedling) size 2015								
Estimate	–	-0.04	-0.13	0.05	0.08	0.40	-0.01	-0.00
SE	–	0.01	0.05	0.06	0.06	0.05	0.03	<0.001
z/t	–	-5.22	-2.54	0.83	1.41	7.84	-0.25	-0.10
p	–	**<0.001**	**0.012**	0.406	0.159	**<0.001**	0.803	0.920
Subgroups (S)								
Estimate	-0.23	0.10	0.31	0.39	-0.01	-0.26	-0.21	-0.10
SE	0.19	0.03	0.26	0.22	0.20	0.19	0.13	0.06
z/t	-1.18	2.89	1.21	1.75	-0.02	-1.38	-1.59	-1.65
p	0.246	**0.004**	0.236	0.089	0.981	0.177	0.112	0.100
Zebularine (Z)								
Estimate	-0.58	0.02	-0.14	-0.08	-0.07	-0.17	-0.09	0.00

Continuation of **Table A1.13**

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
SE	0.14	0.02	0.12	0.15	0.15	0.14	0.09	0.05
z/t	-4.17	1.30	-1.19	-0.52	-0.48	-1.27	-1.09	-0.01
p	**<0.001**	0.194	0.237	0.605	0.632	0.205	0.276	0.995
S x Z								
Estimate	0.11	-0.02	0.20	0.01	0.05	0.03	-0.00	0.00
SE	0.20	0.03	0.17	0.21	0.20	0.19	0.12	0.07
z/t	0.54	-0.84	1.23	0.03	0.26	0.17	-0.03	0.03
p	0.593	0.401	0.221	0.976	0.793	0.864	0.979	0.974
Random factors and goodness-of-fit								
SD Population	0.34	0.07	0.55	0.42	0.36	0.35	0.25	0.09
SD Maternal line	0.25	0.03	0.47	0.16	0.30	0.00	0.19	0.00
SD Block	0.00	0.02	0.00	0.12	0.13	0.00	0.00	0.04
SD Residuals	0.87	–	0.70	0.86	0.87	0.82	–	–
R^2_m	0.08	0.18	0.06	0.04	0.01	0.20	NA	0.02
R^2_c	0.25	0.47	0.54	0.26	0.24	0.33	NA	0.12
AICc	898.22	2158.52	830.50	786.89	884.87	835.91	-521.56	2369.09

Table A1.14: Summary of likelihood-ratio tests for (generalized) linear mixed effects models using membership to genetic cluster for *S. gigantea* as a fixed factor. Models were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total aboveground biomass ratio and total aboveground biomass in 2015 [g] and the number of ramets in spring 2016 [n] including control plants only. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centered; not used if response variable), latitude (decimal degrees of sampled source locations along South North gradient in Central Europe; scaled and centred), and the cluster membership of each plant according to discriminant analysis of principal components (Jombart, Devillard, & Balloux, 2010; see Methods section in the main text). Contribution of each fixed effect was evaluated via iterative elimination of fixed effects comparing the interaction to the full model and the main effects to the model without interactions (Pinheiro & Bates, 2009; Zuur et al., 2009). All *p* values were adjusted ($p_{adjusted}$) for multiple comparisons using the method of Benjamini and Hochberg (Benjamini & Hochberg, 1995). Significant *p* values ($p < 0.05$) are given in bold. χ2—deviance of the likelihood-ratio test.

S. gigantea		**Initial size 2015**			**DAPC cluster**			**Latitude**		
	n	χ^2	***p***	$p_{adjusted}$	χ^2	***p***	$p_{adjusted}$	χ^2	***p***	$p_{adjusted}$
Initial size 2015	174	–	–	–	1.53	0.466	0.466	1.20	0.274	0.466
Phenology 2015	158	**12.38**	**<0.001**	**<0.001**	5.41	0.067	0.100	0.09	0.762	0.762
Phenology 2016	171	3.41	0.065	0.194	2.46	0.292	0.438	0.00	0.995	0.995
Height 2015	157	1.26	0.262	0.546	1.40	0.498	0.546	0.36	0.546	0.546
Height 2016	171	2.21	0.137	0.205	4.25	0.119	0.205	0.37	0.542	0.542
Total biomass 2015	174	**24.32**	**<0.001**	**<0.001**	1.78	0.410	0.410	0.96	0.328	0.410
Biomass ratio 2015	158	0.32	0.571	0.663	2.92	0.233	0.663	0.19	0.663	0.663
Ramets 2016	174	2.82	0.093	0.279	2.21	0.332	0.394	0.73	0.394	0.394

Table A1.15: Results of (generalized) linear mixed effects models using membership to genetic cluster for *S. gigantea* individuals as a fixed factor. Models used maximum likelihood and were conducted on the response variables initial (seedling) size obtained in 2015, phenology (days to flowering) and plant height [cm], both measured in 2015 and 2016, reproductive-to-total biomass ratio and total biomass in 2015 [g] and the number of clonal ramets in spring 2016 [n]. Fixed effects included initial size (number of first true leaves × length × width of the longest true leaf; scaled and centered), latitude (decimal degrees of sampled source locations along South North gradient in Central Europe; scaled and centered; not used if response variable), and the cluster membership of each plant in discriminant analysis of principal components (DAPC; Jombart et al., 2010) based on microsatellite analysis (see Methods section in the main text). Position of block in the common garden and source population were included as random-intercept variance components and kept in the models (Barr et al., 2013). Depending on the dispersion and data structure, generalized mixed models (GLMM) were based on either a log-link gamma (G) distribution, or (zero-truncated; tNB1) negative binomial (NB1; NB2) distribution for the response variables initial size, days to flowering, and number of ramets, and logit-link beta distribution (B) for proportional data (reproductive-to-total aboveground biomass ratio). Data has been transformed using Box-Cox transformation (BC; Box & Cox, 1964) to meet assumptions of residual normality. Default settings of model optimizers have been adjusted in cases where models did not converge. The *p* values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943) whereas *p* values for LMMs were estimated using Satterthwaite-approximation (Satterthwaite, 1946). R^2_m—marginal pseudo- R^2 (without random effects) and R^2_c —conditional pseudo- R^2 (including random effects; Nakagawa & Schielzeth, 2013); AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
Sample size	174	158	171	157	171	174	158	174
Transformation	–	–	–	–	BC	BC	–	–
Distribution (Link)	G (log)	tNB2 (log)	tNB1 (log)	N	N	N	B (logit)	NB2(log)
Fixed factors of (generalized) linear mixed effects models								
Intercept								
Estimate	3.83	4.43	4.11	139.15	-0.13	-0.06	-1.06	3.42
SE	0.10	0.03	0.04	3.30	0.17	0.17	0.11	0.05
z/t	37.60	156.53	101.20	42.20	-0.74	-0.33	-9.47	71.08
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.464	0.740	**<0.001**	**<0.001**
Initial size 2015								
Estimate	–	-0.04	-0.03	1.52	0.11	0.35	0.03	-0.04
SE	–	0.01	0.01	1.30	0.07	0.07	0.05	0.02
z/t	–	-3.55	-1.85	1.17	1.52	5.12	0.57	-1.69
p	–	**<0.001**	0.06	0.244	0.131	**<0.001**	0.570	0.091
DAPC cluster 2								
Estimate	0.03	0.07	0.04	4.17	0.31	0.17	-0.25	-0.02
SE	0.12	0.04	0.05	4.04	0.21	0.21	0.14	0.06
z/t	0.22	1.87	0.76	1.03	1.45	0.80	-1.72	-0.30
p	0.822	0.062	0.448	0.304	0.151	0.423	0.086	0.766
DAPC cluster 3								
Estimate	0.19	0.00	-0.04	1.04	-0.10	-0.09	-0.23	-0.12
SE	0.18	0.05	0.07	5.80	0.32	0.30	0.21	0.10

Continuation of **Table A1.15**

S. gigantea	**Initial size 2015**	**Phenology 2015**	**Phenology 2016**	**Height 2015**	**Height 2016**	**Total biomass 2015**	**Biomass ratio 2015**	**Ramets 2016**
z/t	1.06	0.06	-0.57	0.18	-0.31	-0.30	-1.09	-1.27
p	0.291	0.951	0.571	0.858	0.758	0.763	0.276	0.204
Latitude								
Estimate	-0.07	0.01	-0.00	1.46	-0.07	-0.12	0.04	-0.03
SE	0.07	0.02	0.03	2.41	0.12	0.13	0.08	0.03
z/t	-1.10	0.30	-0.01	0.61	-0.62	-0.98	0.44	-0.86
p	0.273	0.763	0.995	0.547	0.540	0.330	0.662	0.390
Random factors and goodness-of-fit								
SD Population	0.15	0.07	0.11	7.47	0.18	0.36	0.22	0.04
SD Block	0.09	0.02	0.00	2.50	0.17	0.00	0.04	0.00
SD Residuals	–	–	–	14.53	0.94	0.84	–	–
R^2_m	0.01	NA	NA	0.03	0.05	0.15	NA	<0.001
R^2_c	0.11	NA	NA	0.25	0.11	0.29	NA	<0.001
AICc	1609.86	1208.59	1347.44	1329.71	489.54	470.62	-273.33	1245.29

Table A1.16: Results of non-linear logistic mixed effects models conducted on plant height growth as response variable of both *S. canadensis* and *S. gigantea*. Models used maximum likelihood, and zebularine treatment was included as a fixed factor. Individual plants were used as a random intercept factor. Both fixed and random factors were correlated to the asymptote (indicating the growth capacity of the logistic growth; Asym), the midpoint (indicating steepest growth in the linear growth phase; xmid), and the scaling factor controlling the steepness of the curve (scal). Initial starting values for each non-linear model were obtained using the *getInitial*() function. The *p* values for GLMMs were obtained using asymptotic Wald tests (Wald, 1943). AICc—Akaike information criterion corrected for small sample sizes (Cavanaugh, 1997). Significant *p* values ($p < 0.05$) are given in bold.

Height growth 2015	***S. canadensis*** (n = 393)		***S. gigantea*** (n = 326)	
	Full model	**Zebularine factor excluded**	**Full model**	**Zebularine factor excluded**
Fixed factors in (generalized) non-linear mixed effects model				
Asym (Intercept)				
Estimate	161.75	158.84	127.03	122.93
SE	2.49	1.79	2.40	1.81
df	1174	1177	973	976
t	64.96	88.93	52.83	67.91
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Asym (Zebularine)				
Estimate	-5.91	–	-9.56	–
SE	3.57	–	3.63	–
df	1174	–	973	–
t	-1.66	–	-2.63	–
p	0.098	–	**0.009**	–
xmid (Intercept)				
Estimate	6.97	7.03	5.71	5.78
SE	0.05	0.04	0.07	0.06
df	1174	1177	973	976
t	130.41	182.94	79.35	105.00
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**
xmid (Zebularine)				
Estimate	0.14	–	0.15	–
SE	0.08	–	0.11	–
df	1174	–	973	–
t	1.78	–	1.33	–
p	0.075	–	0.182	–
scal (Intercept)				
Estimate	2.03	1.99	1.88	1.84
SE	0.03	0.02	0.02	0.02
df	1174	1177	973	976
t	75.05	103.02	84.85	110.60
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**
scal (Zebularine)				
Estimate	-0.08	–	-0.10	–
SE	0.04	–	0.03	–
df	1174	–	973	–
t	-1.97	–	-2.96	–
p	**<0.050**	–	**0.003**	–
Random-factors on the individual level and goodness-of-fit				
SD Asym	29.91	30.00	27.40	27.54
SD xmid	0.52	0.52	0.73	0.75
SD scal	0.29	0.30	–	–
SD Residual	2.58	2.58	3.06	3.08
AICc	10262.67	10278.02	8698.28	8720.16

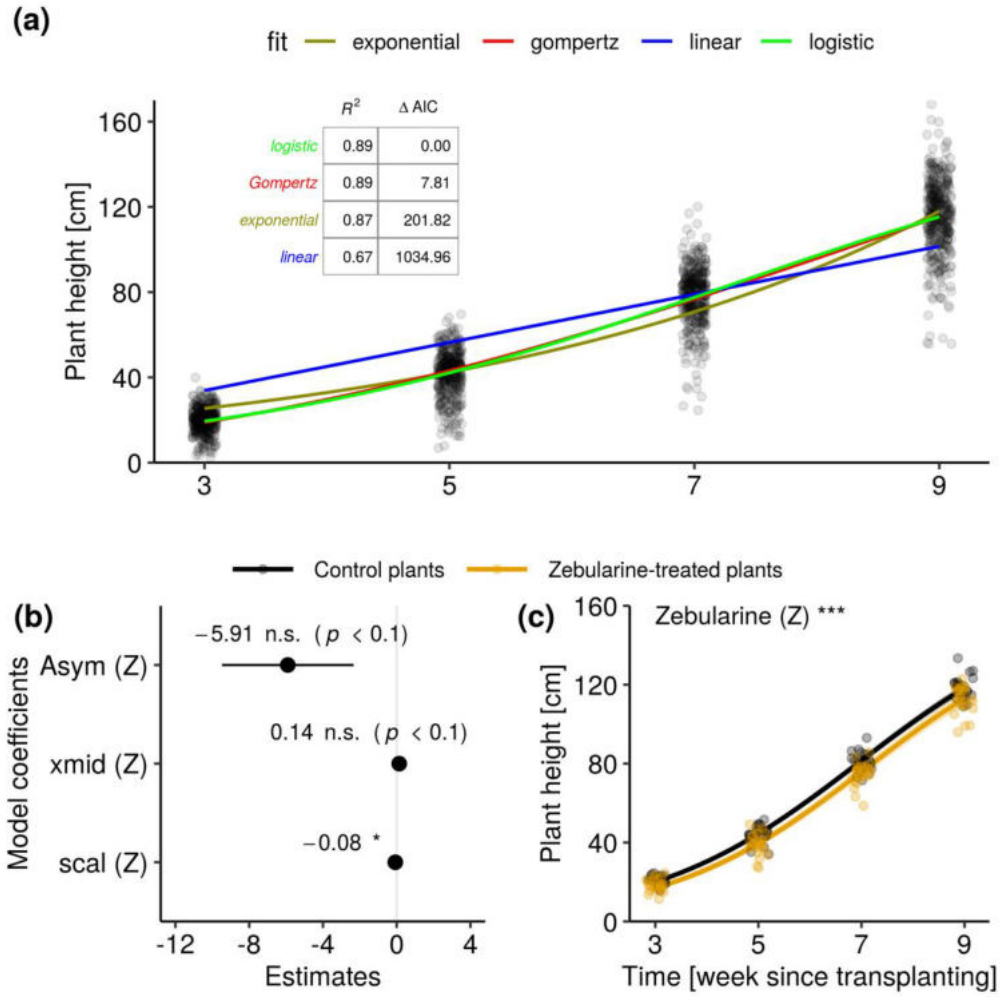


Figure A1.1: Plant height growth for *S. canadensis* measured on week 3–9 after transplanting to the common-garden experiment in 2015. (a) Linear and non-linear curves fitted to the raw height growth data over time to evaluate height growth via ΔAIC. Note that points indicating plant growth per week have been jittered to increase visibility. (b) Estimates and (c) predicted marginal-effect values of a non-linear logistic mixed-effects model used to evaluate the zebularine treatment. The model analyzed the asymptotic growth capacity of the height growth (Asym), the point at steepest height growth in the linear growth phase (xmid) and the scaling factor controlling the steepness of the non-linear growth curve (scal). Model parameters are given in Table A1.16. A likelihood-ratio test showed a significant Zebularine-treatment effect (X^2 = 21.45; p < 0.001). Points show population mean values per treatment, and have been jittered to increase visibility. Confidence bands were estimated using the delta method (Doob, 1935).

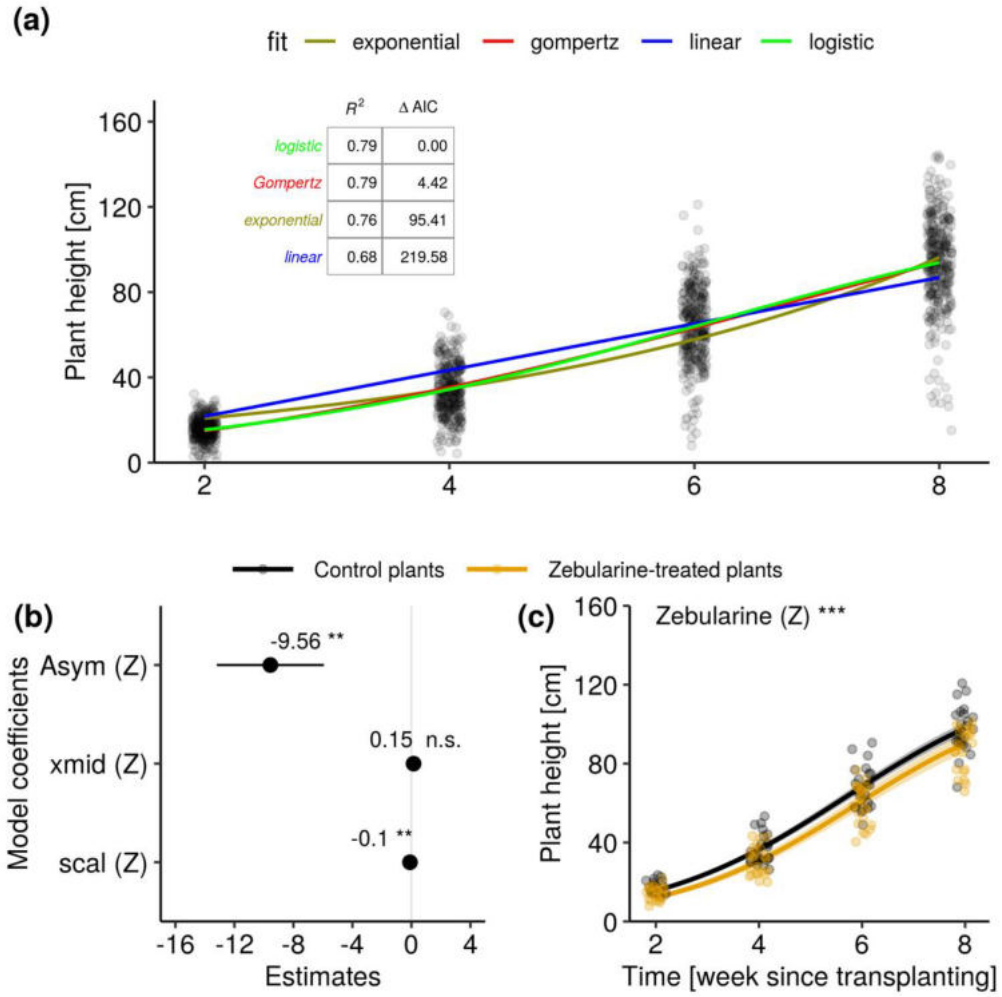


Figure A1.2: Plant height growth for *S. gigantea* measured on week 2–8 after transplanting to the common-garden experiment in 2015. (a) Linear and non-linear curves fitted to the raw height growth data over time to evaluate height growth via ΔAIC. Note that points indicating plant growth per week have been jittered to increase visibility. (b) Estimates and (c) predicted marginal-effect values of a non-linear logistic mixed-effects model used to evaluate the zebularine treatment. The model analyzed the asymptotic growth capacity of the height growth (Asym), the point at steepest height growth in the linear growth phase (xmid) and the scaling factor controlling the steepness of the non-linear growth curve (scal). Model parameters are given in Table A1.16. A likelihood-ratio test showed a significant zebularine-treatment effect (X^2 = 27.96; p < 0.001). Points show population mean values per treatment, and have been jittered to increase visibility. Confidence bands were estimated using the delta method (Doob, 1935).

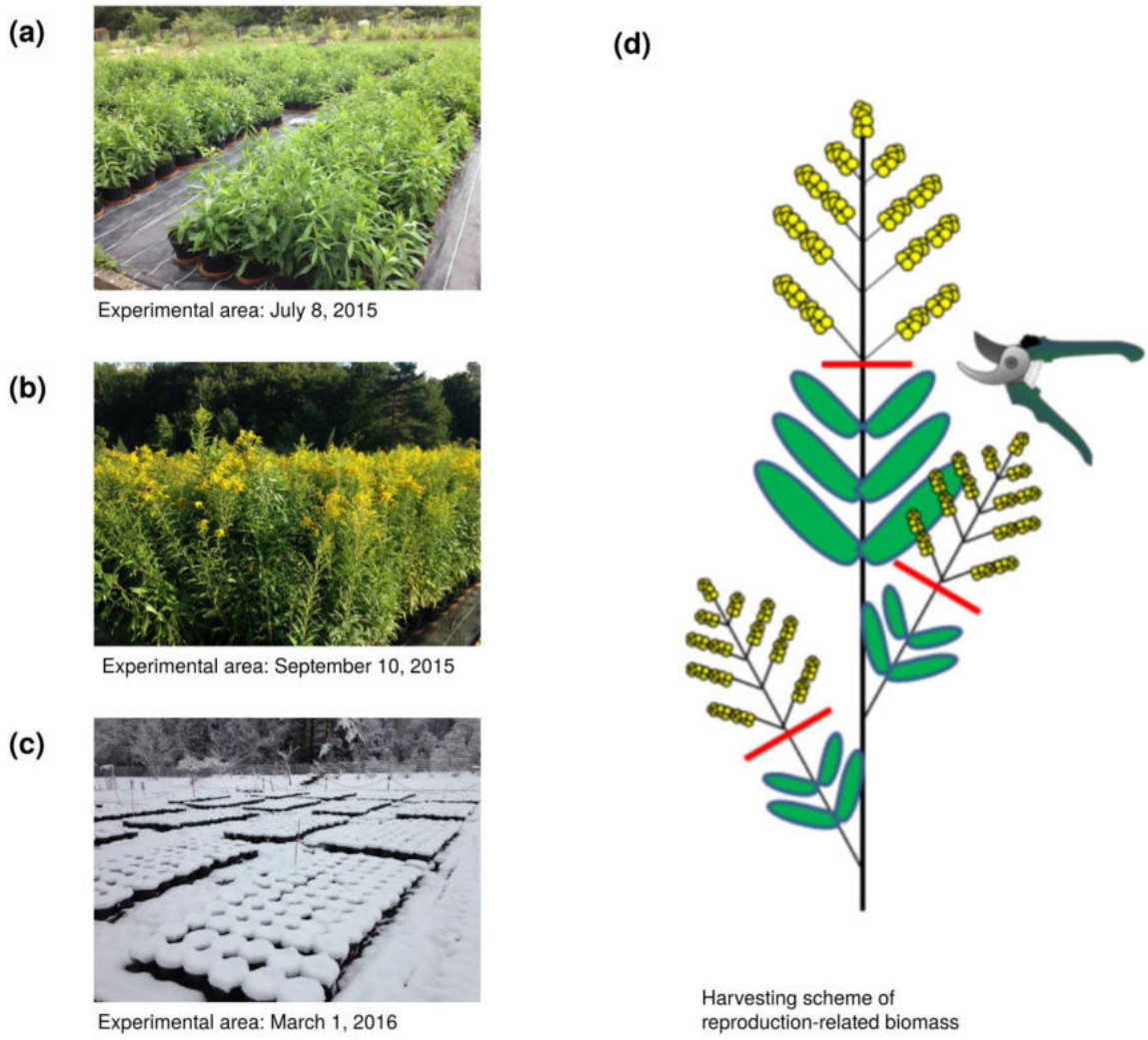


Figure A1.3: Experimental area and plant harvesting scheme. The common-garden experiment contained both *S. canadensis* and *S. gigantea* plants in an incomplete randomized block design with eight blocks per species with half of the plants treated with the demethylation agent zebularine (see Methods section in the main text). Blocks consisted of up to 55 plants for *S. canadensis* and up to 46 plants for *S. gigantea* with plants grown in 7L pots containing peat-based soil. Plants were measured for flowering phenology and performance-related traits for two years (2015–2016) whereas aboveground biomass was harvested in the first year (2015) only. Images of experimental area: Mark van Kleunen. Experimental area (a) at the start of the experiment in July 2015, (b) at the end of the first vegetation period in September 2015, and (c) at the beginning of the second vegetation period in March 2016. (d) Harvesting scheme of reproduction-related biomass. Inflorescences were harvested in autumn 2015, dried, reduced to flowers-only biomass and weighed to calculate reproductive-to-total aboveground biomass ratio. Drawing: Marc Stift.

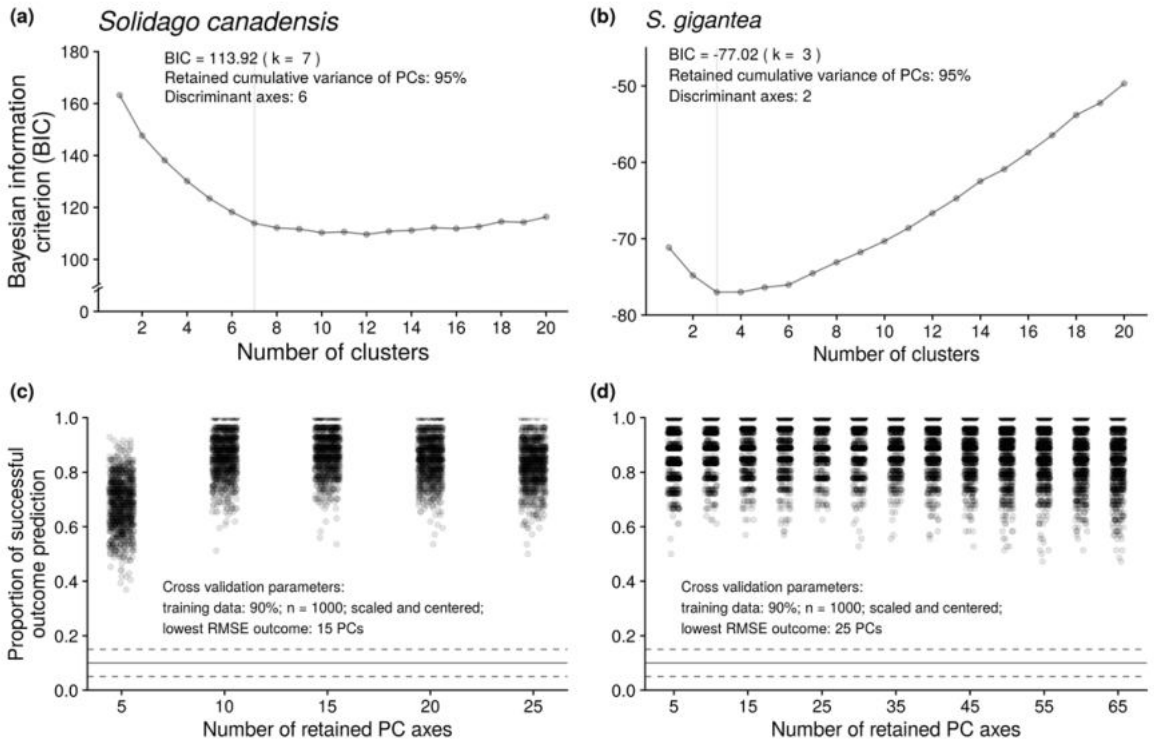


Figure A1.4: Diagnostics of discriminant analyses of principal components based on microsatellite data. Genotyped control plants were used to infer population structuring of both goldenrod species via discriminant analysis of principal components (DAPC; Jombart et al., 2010). Data dimensionality was first reduced via principal components analysis (PCA; see Method A1.3), then a two-step analysis involved successively repeating *k*-means clustering (Jain, 2010) using a pre-defined number retained PC axes, that account for at least 95% of the cumulative explained variance (i.e. at least 70 PCs), a pre-defined number of $k = 2–20$ clusters, and all retained discriminant axes. The optimal *k* was selected based on the highest increase in difference between successive values of the Bayesian information criterion (BIC; Schwarz, 1978) followed by cross-validation via successively repeating DAPCs with an increasing number of retained PC axes while keeping all other parameters constant (n = 1000). DAPCs used 90% of the data as training data to assess the proportion of successful prediction outcome of the remaining 10% whereby calculating a root mean square error (RMSE) that indicates successful re-assignment. The number of PCs with the lowest RMSE was used for the final DAPC. BIC of repeated DAPCs versus the number of *k*-means clusters for **(a)** *S. canadensis* (optimal number of clusters: $k = 7$) and **(b)** *S. gigantea* (optimal number: $k = 3$). Proportion of successful outcome prediction versus the number of retained PC axes refining the number of retained PC axes for the final DAPC for **(c)** *S. canadensis* (final number: 15 PCs) and **(d)** *S. gigantea* (final number: 25 PCs). Note that the proportion of successful outcome prediction is shown with random noise added to avoid overplotting.

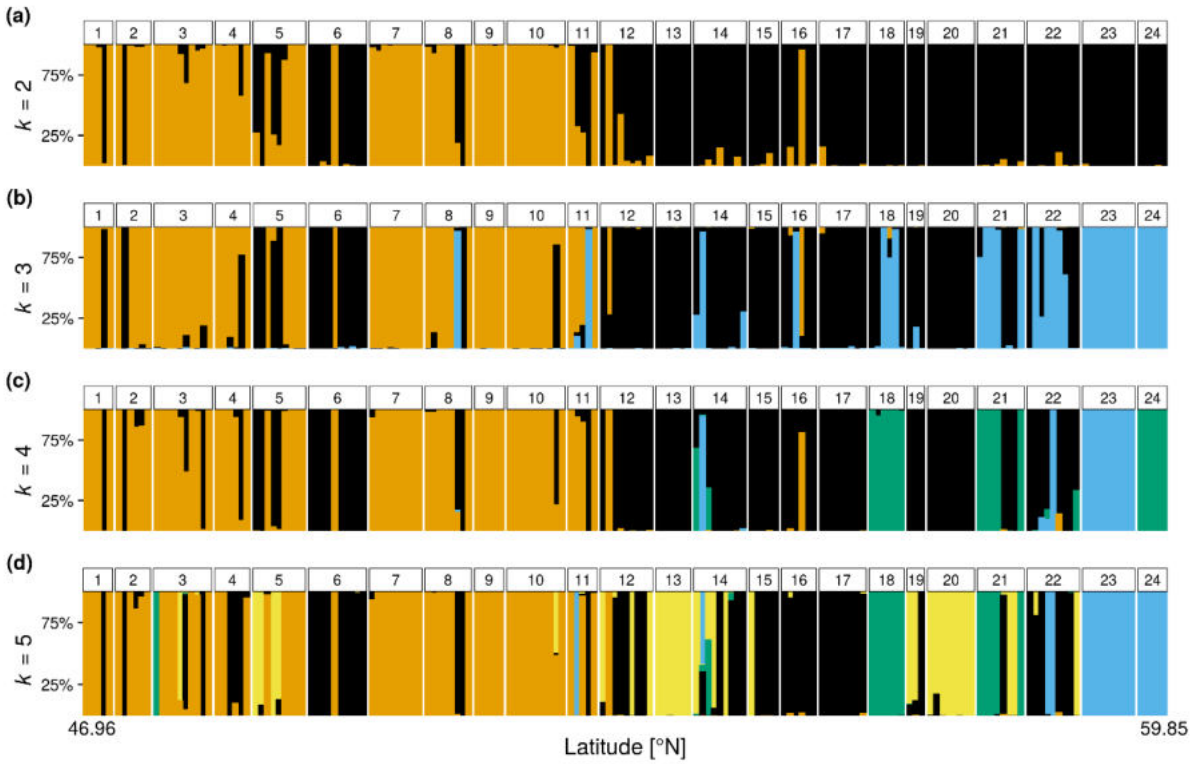


Figure A1.5: Variation of *k*-means clusters in discriminant analysis of principal components for *S. gigantea* (DAPC; Jombart et al., 2010). Analysis was performed with k = 2–5 to follow the stratification process of *S. gigantea* microsatellite data by displaying *k*-means clustering at the lower bound of the Bayesian information criterion (BIC) around k = 3 (Figure A1.4). Data is based on 174 control plants that were genotyped with nine microsatellite markers (Table A1.3–5) representing all 24 populations. Stacked bar plots display the probability [%] of membership to each cluster according to DAPC. Note that colours represent the same cluster across figures. Membership probability to **(a)** k = 2 genetic clusters (BIC =-74.81; conserved variance (Var.) = 49%; reassignment probability (prob.) = 94–100%; pre-defined/retained PCs (d.pc/r.pc) = 70/20), **(b)** k = 3 genetic clusters (BIC = -77.02; Var. = 56%; prob. = 91–100%; d.pc/r.pc = 70/25), **(c)** k = 4 genetic clusters (BIC = -77.00; Var. = 49%; prob. = 79–100%; d.pc/r.pc = 70/25), and **(d)** k = 5 genetic clusters (BIC = 76.38; Var. = 74%; prob. = 87–100%; d.pc/r.pc = 70/40).

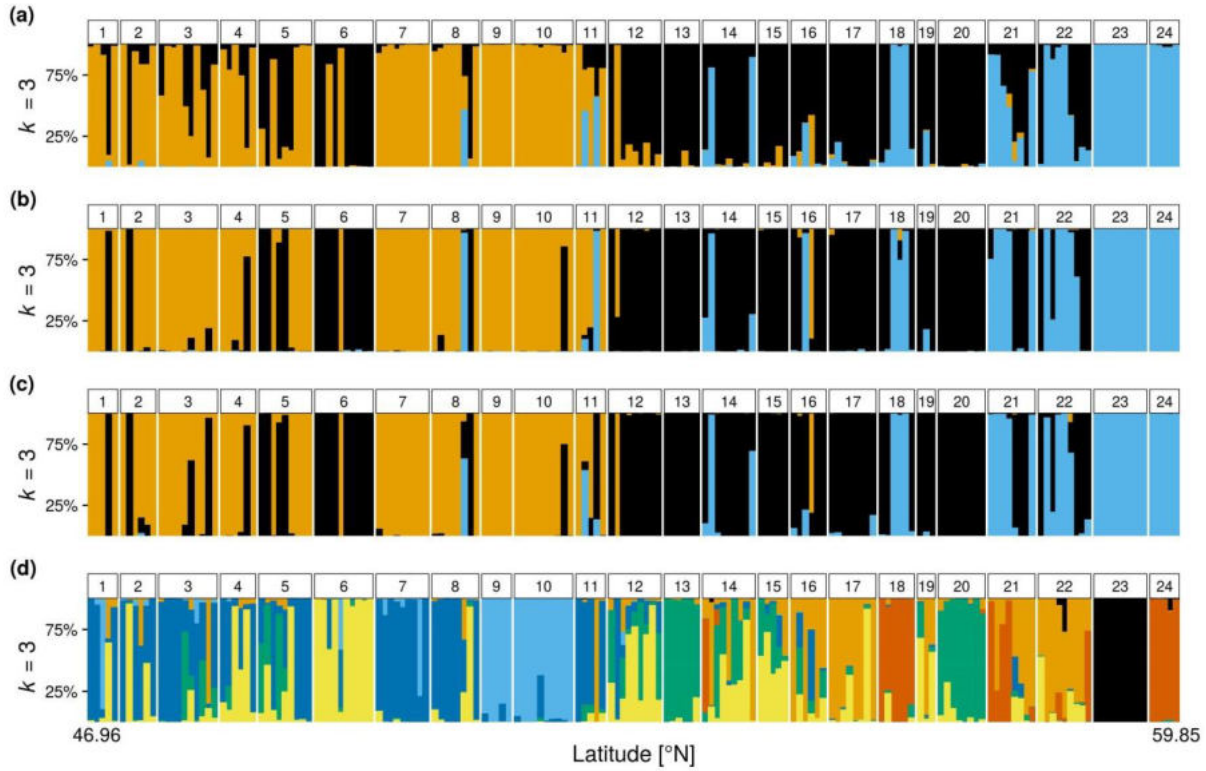


Figure A1.6: Clustering sensitivity to the number retained principal components in discriminant analysis of principal components for *S. gigantea* (DAPC; Jombart et al., 2010). Analysis was repeated with a varying number of pre-defined principal components (PC) to assess the robustness of the clustering results. Data is based on 174 genotyped control plants (Table A1.3–5) representing all 24 populations. Stacked bar plots display the probability [%] of membership to each cluster for each plant. Colours represent the same genetic cluster across figures, except in the last subfigure. Membership probability to *k*-clusters based on **(a)** 120 pre-defined PCs representing at least 99% of the variation in the data (conserved variation (Var.) = 30%; reassignment probability (prob.) = 91–97%; cross-validated number of retained PCs (r.pc) = 10), **(b)** 70 PCs representing at least 95% of the variation (Var. = 56%; prob. = 91–100%; r.pc= 25), **(c)** 35 PCs representing at least 75% of the variation (Var. = 56%; prob. = 91–100%; r.pc = 25), and **(d)** 18 PCs representing at least 50% of the variation (Var. = 34%; prob. = 75–95%; r.pc = 12). Note that the number of *k*-clusters cannot be reproduced below a cumulative explained variance of 50%.

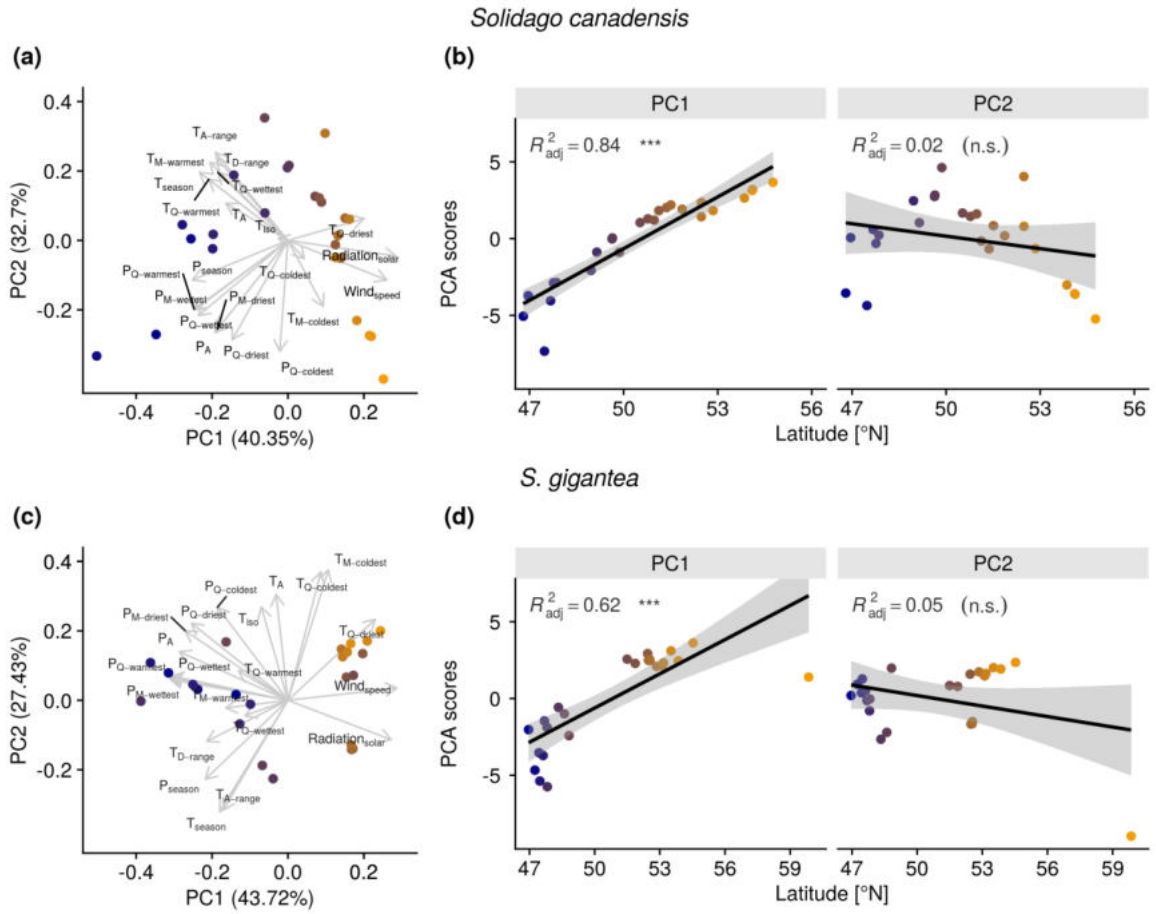


Figure A1.7: Principal component regression of climate data against latitude. The climate data contains coefficients of variation, ranges and average values of 21 climate variables measured in the years 1970–2000. The validity of the sampled latitudinal gradient was assessed via principal component regression (Jolliffe, 1982) of extracted data from WorldClim 2.0 database (Fick & Hijmans, 2017) correlated against latitude of sampled source populations of both goldenrod species used in this study (Table A1.1 and A1.2). For the abbreviation of each WorldClim 2.0 variable and its explanation see Table A1.7. **(a)** Biplot of principal component analysis (PCA) of *S. canadensis* populations. Principal component 1 (PC1) explained 40.35% of the variation in the climatic data along the gradient whereas principal component 2 (PC2) explained 32.70% of the variation in the data. **(b)** Scatter plots of simple linear least-squares regression of PC1 and PC2 against latitude of *S. canadensis* populations displaying adjusted R^2 (PC1: *** $p < 0.001$, PC2: n.s.—not significant). **(c)** Biplot of principal component analysis (PCA) of *S. gigantea* populations. PC1 explained 43.72% of the variation in the climatic data along the gradient whereas PC2 explained 27.43% of the variation in the data. **(d)** Scatter plots of simple linear least-squares regression of PC1 and PC2 against latitude of *S. gigantea* populations displaying adjusted R^2 (PC1: *** $p < 0.001$, PC2: n.s.—not significant). Please note that the color gradient for the respective populations in figure (a) and (c) represents their position along the latitudinal gradient shown in panel (b) and (d).

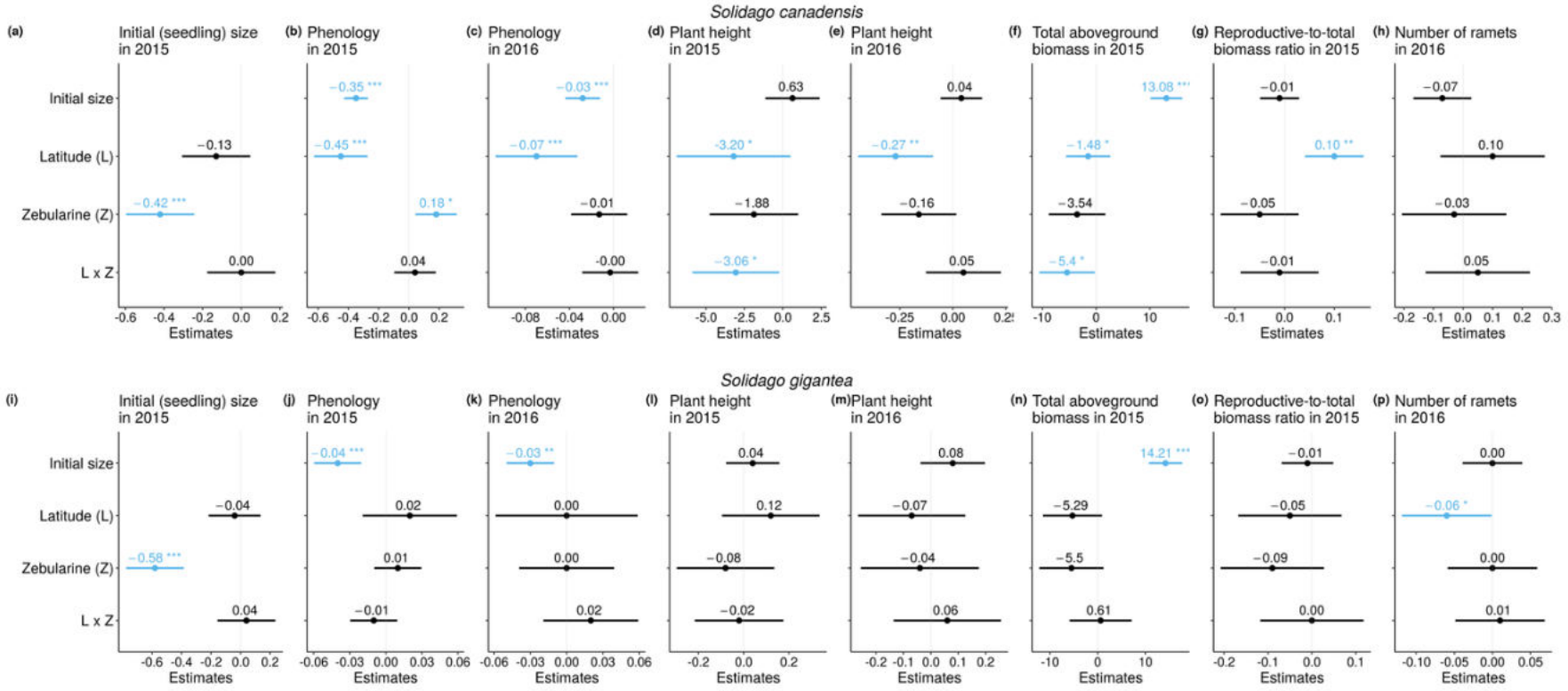


Figure A1.8: Forest plots of (generalized) linear mixed effects models. Results are displayed for each response variable per species (see Figures 2.3 and 2.4; Table A1.6). Blue-coloured effect sizes indicate significant contribution of fixed factors to model fit (Likelihood-ratio tests; significance level p value < 0.05; Table 2.1 in the main text). Odds ratios and standard errors from generalized linear mixed effects models as well as estimates and their standard deviations from linear mixed effects models for (a–h) *S. canadensis* and (i–p) *S.*

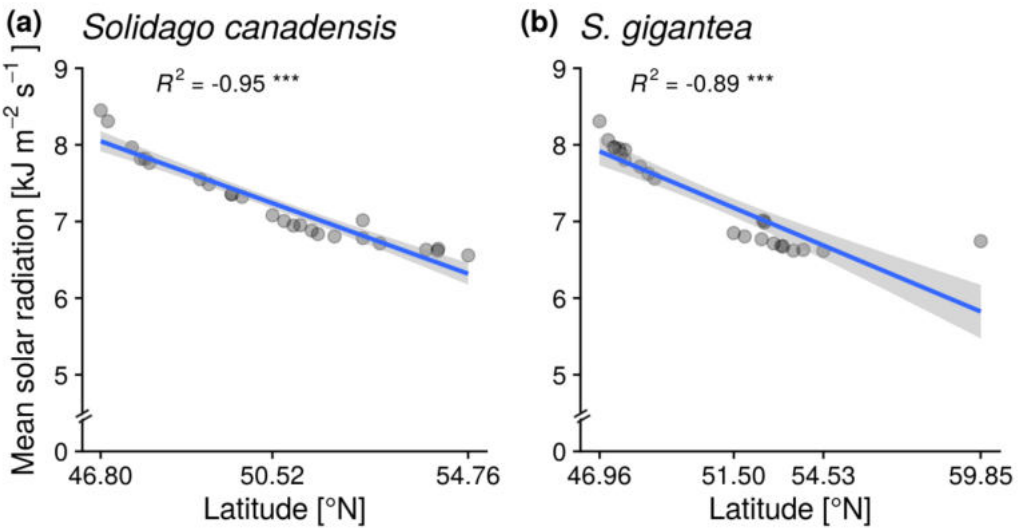


Figure A1.9: Changes in day length correlated with the analyzed latitudinal gradients of both *Solidago* species. Day length data was obtained from the WorldClim 2.0 database (Fick & Hijmans, 2017). Pearson product-moment correlation of mean solar radiation [kJ m^{-2} s^{-1}] against latitude [°N] for **(a)** *S. canadensis* (R^2 = -0.95; r_{23} = -15.11; $p < 0.001$) and **(b)** *S. gigantea* (R^2 = -0.89; r_{22} = -9.20; $p < 0.001$).

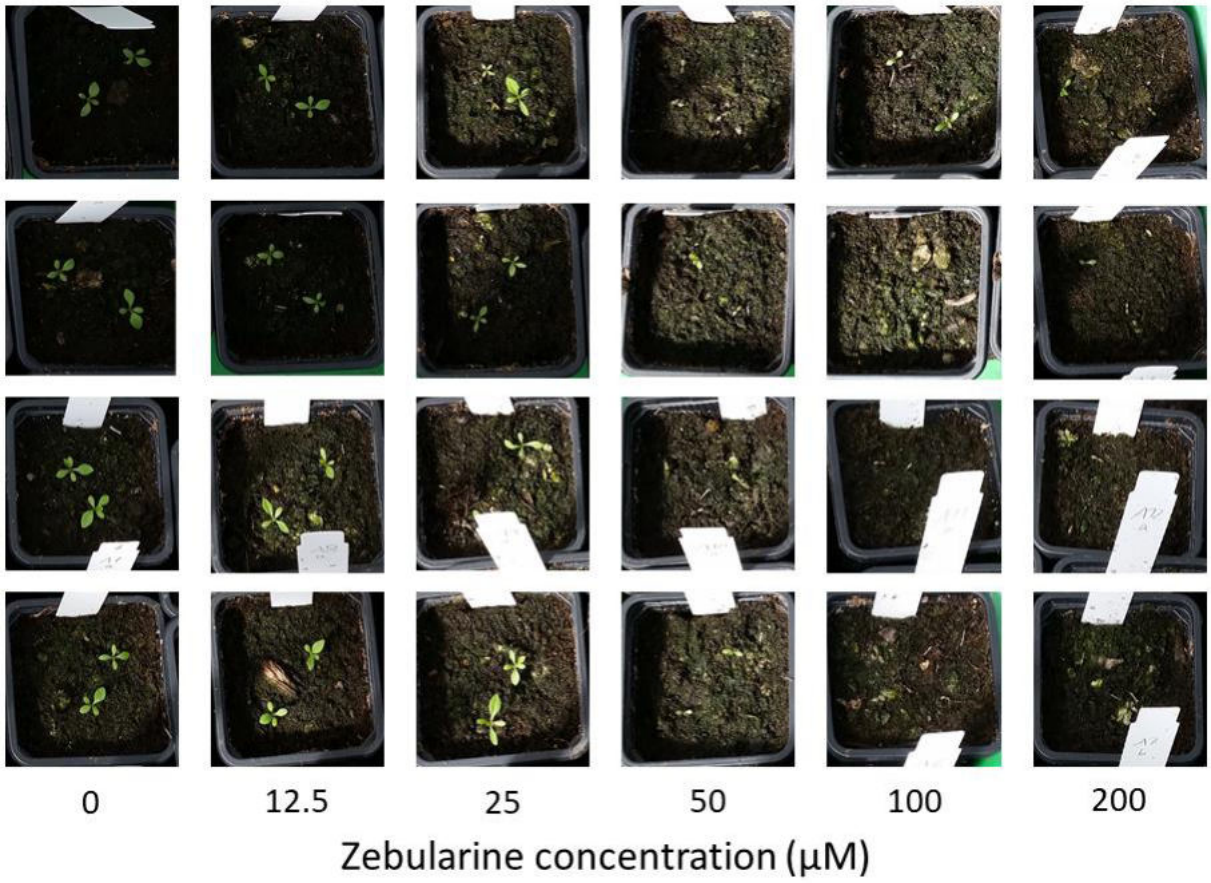


Figure A1.10: Pilot experiment conducted to determine the optimal concentration of zebularine for the common-garden experiment. Seeds from a single *S. gigantea* population (ID number 6 in Table A1.2) were germinated under different zebularine concentrations (12.5, 25, 50, 100 and 200 µM) or mock-treated for comparison (0 µM; see Method A1.1). Zebularine was applied on filter paper and refreshed every other day to avoid depletion. Seedlings were grown under the same growth chamber and greenhouse conditions as described in the Methods section of the main manuscript.

Appendix | Study 2

Method A2.1

Changes applied on the MSAP protocol

- In the restriction/ligation step, the reaction mixture contained 50 pmol/µl of the *Hpa*II/*Msp*I adapter instead of the *Mse*I adapter, 10 U /µl of the *Hpa*II/*Msp*I enzymes instead of the *Mse*I enzyme. The extracted DNA was mixed with 5.2 µl instead of 5 µl of the reaction mixture and the mixture was diluted at a ratio of 1:3 instead of 1:5.
- In the pre-selective amplification step, 30 ng/µl of *Hpa*II/*Msp*I pre-selective primer were used instead of the *Mse*I pre-selective primer and the PCR product was diluted at a ratio of 1:6 instead of 1:10.
- In the selective amplification step, the *Hpa*II/*Msp*I pre-selective primers were used instead of the *Mse*I selective primer and the PCR program was updated to the following: 2 min at 95 °C; 10 cycles (20 sec at 94 °C; 30 sec at 66 °C, decrease of 1 °C per cycle, 2 min at 72 °C); 20 cycles (20 sec at 94 °C; 30 sec at 56 °C; 2 min at 72 °C); 30 min at 60 °C.
- The agarose gel concentration was updated to 1.5 % and, for the second gel, 10 µl of the PCR product was used instead of 6 µl.
- In order to select appropriate primers, we tested 16 primers per dye for AFLP and 10 primers per dye for MSAP analysis and chose the corresponding primers based on visual inspection of peak quality in the resulting electropherograms after sequencing.

Method A2.2

Details of the AFLP/MSAP genotyping approach

Peak heights from binned data were exported and the peak quality for each bin was assessed manually using a custom Excel file. For this, we set a minimum peak height for each bin as a threshold to accept a peak as present. This was assisted by two error rates in defining the optimal threshold for ambiguous cases, the overall error rate (defined as the number of mismatches per number of sample-replicate pairs) and a confined error rate (defined as the number of mismatches per number of comparisons; Gáspár et al., 2019). Additionally, non-polymorphic peaks were defined according to Lynch & Milligan (1994) and ignored in further analyses. For AFLP scoring, peaks that showed an overall error rate lower than 5% or at least a three-fold higher number of sample-replicate comparisons compared to the number of mismatches were included in the AFLP binary table for subsequent statistical analyses. Samples from both MSAP enzyme combinations, *Hpa*II + *Eco*RI and *Msp*I + *Eco*RI, were binned jointly to assure consistent peak detection across

both enzyme combinations. However, peak quality was assessed separately to take into account the different distributional characteristics of the scored signals based on their methylation status. Corresponding peaks from both sets were selected such that the scoring of at least one of the two peaks was below the error threshold. Therefore, the MSAP error rates were relaxed because the peak quality differed between the isoschizomer measurements.

Method A2.3

Details of genome-scan approaches

- **BAYESCAN** determines the evidence of whether a locus is under selection by calculating posterior odds (PO), i.e., comparing the model with selection to the neutral model. Following Jeffreys (1939) and Pérez-Figueroa et al. (2010), we checked for false positives and retained loci as outliers when exceeding a threshold of $\log_{10}(PO) > 1.3$. This follows the scale of Jeffreys (1939), were loci with values exceeding this threshold are interpreted as having *substantial evidence* for selection (Figure A2.7). We ran BAYESCAN using 100,000 iterations with 10 repetitions and a burn-in of 50,000 following 20 pilot runs.
- A principal components analysis (**PCA**) was used to account for collinearity of WorldClim 2.0 environmental variables applied on BAYESCENV and latent factor mixed models (LMM). The first three principal components were retained for further analyses according to the broken-stick criterion (MacArthur, 1957; Figure A2.8). Given the first three variables with the highest relative [%] above-average contribution (> 4.76%; Figure S8), PC1 had the highest negative association with solar radiation (10.1%) and wind speed (8.4%), and the highest positive association with precipitation seasonality (BIO15; 7.9%). PC2 had the highest negative associations with precipitation-related variables, i.e., precipitation of both the coldest (BIO19; 12.7%) and the driest quarter (BIO17; 10.1%), and annual precipitation (BIO12; 8.6%). PC3 had the highest positive association with temperature-related variables, i.e., mean temperature of the coldest quarter (BIO11; 30.4%), annual mean temperature (BIO1; 18.4%), and minimum temperature of coldest month (BIO6; 17.1%). Together, all three PCs explained 87.4% of climatic variation along the sampled latitudinal gradient (PC1: 40.3%; PC2: 32.7%; PC3: 14.4%).
- In **BAYESCENV**, allele frequency and environmental variation are considered associated for a given locus if the posterior probability of the neutral model is lower than that of the local adaptation model. Following Aguirre-Liguori et al., (2017), we used the posterior error probability (PEP) calculated by BAYESCENV for both models and defined any locus as outlier whose PEP was lower for the local adaptation model compared to the neutral

model (Figure A2.9). We ran BAYESCENV using a thinning interval of 20, a final sample size of 5,000, and a burn-in of 50,000 following 20 pilot runs.

- For **LFMM**, we first ran an admixture coefficient estimation (**LEA**) on a range of K = 1-11 ancestral populations using 20 repetitions and 100,000 iterations. We chose the final K based on the cross-entropy criterion and according to the LEA package vignette, i.e., either a K at the minimum of the cross-entropy values (i.e., MSAP-n and MSAP-m) or, if there was no clear minimum value for the cross-entropy, at the beginning of the plateau (i.e., AFLP; Frichot & François, 2015; Figures A2.10–12). The final K was then used in LFMMs to assess outlier loci associated with environmental variables using the *lfmm*-function with 10 repetitions, 50,000 iterations, and a burn-in of 5,000. LFMM computes p-values for each locus indicating whether a given locus is associated with the environmental variable of interest. A final K = 2 was applied for AFLPs (Figure A2.10). For both MSAP-m and MSAP-n data, however, a K = 1 was used because no pronounced population structuring was visible along the sampled latitudinal gradient using the *snmf*-function (Figures A2.11 and A2.12). To correct for multiple testing, we used the false discovery rate (FDR) and applied a threshold of 95% where loci were defined as outliers when $\log_{10}(\text{FDR}) > 1.3$ (Figure A2.13).
- **RDA** was found to detect loci even under moderate-to-weak selection (Forester et al., 2018). The significance of the global model was assessed using the *anova.cca*-function in the VEGAN v2.5-6 package ($p < 0.05$ based on n = 9,999 permutations) and the first three axes were retained in the analysis according to the broken-stick criterion (MacArthur, 1957). Loci loading with a 3-times standard deviation from the center of the loadings distribution of each retained RDA axis (corresponding to a two-tailed p-value of $p < 0.001$) were defined as outlier loci (Figures 3.3 and A2.14). The proportion of the variance explained by the environmental predictors (adjusted R^2) was 0.3% for the MSAP-m dataset and 0.4% for the MSAP-n dataset, respectively.

Table A2.1: Details of sampled *Solidago canadensis* source populations. Information includes seed-sampling sites, population IDs, coordinates given in decimal degrees, elevation [m a.s.l.], estimated population sizes based on the estimated number of shoots at the sampling sites, and the number of sampled maternal lines along the latitudinal south-north gradient in Central Europe (47–54°N). Additionally, the number of individuals per population is given that were used for genotyping (AFLP) and epigenotyping (MSAP-m, MSAP-n). Abbreviations: NA – information not available; AFLP – Amplified fragment length polymorphisms; MSAP – Methylation-sensitive amplified polymorphisms.

S. canadensis	**ID**	**Coordinates**		**Elevation**	**Estimated**	**Maternal**	**AFLP**	**MSAP**
		°N	**°E**	**[m a.s.l.]**	**shoots**	**lines**		
Rhäzüns (CH)	1	46.80153	9.39882	656.0	1000	19	19	17
Landquart (CH)	2	46.95745	9.55380	521.3	1000	17	17	14
Rheineck (CH)	3	47.47613	9.57967	401.3	30	13	13	12
Konstanz (D)	4	47.67258	9.16095	399.0	1000+	18	18	16
Radolfzell (D)	5	47.76432	8.98473	434.8	100	20	19	18
Engen (D)	6	47.85763	8.79533	539.5	100	16	16	14
Pleidelsheim (D)	7	48.96087	9.19872	189.4	1000	19	19	17
Heilbronn (D)	8	49.14628	9.19737	159.8	80–100	14	14	13
Tauberbischofsheim (D)	9	49.63172	9.65733	179.6	15–20	15	15	13
Tauberbischofsheim (D)	10	49.64210	9.64187	200.3	15000	18	18	15
Volkach (D)	11	49.86363	10.22380	196.7	200	19	18	14
Rödelmaier (D)	12	50.52465	10.42545	310.5	150	12	12	10
Breitungen (D)	13	50.77387	10.32783	259.5	600	19	18	15
Eisenach (D)	14	50.97552	10.32697	225.7	30–50	10	9	10
Hoheneiche (D)	15	51.12350	9.97555	197.3	1000+	18	17	14
Wollrode (D)	16	51.36967	9.91143	242.6	500	19	19	17
Kassel (D)	17	51.50665	9.91338	154.8	300	18	18	15
Bad Gandersheim (D)	18	51.86658	10.03582	147.4	40	15	15	13
Potsdam (D)	19	52.47797	13.01649	37.2	NA	15	15	14
Kaltenweide (D)	20	52.47997	9.74547	47.7	300	18	17	16
Walsrode (D)	21	52.84990	9.60088	38.2	40–50	8	8	7
Nützen (D)	22	53.85155	9.92888	20.3	1000+	12	12	11
Neumünster (D)	23	54.09808	9.98737	25.4	20–30	9	9	6
Neumünster (D)	24	54.11280	9.99352	29.1	800	16	16	12
Flensburg (D)	25	54.76182	9.44657	37.0	100–150	14	14	13

Table A2.2: Oligo-sequences from AFLP/MSAP analyses. Combination of selective primers applied in the AFLP analysis: E1+*Mse*I_2, E2+*Mse*I_2, E3+*Mse*I_3, E4+*Mse*I_1. Combination of selective primers applied in the MSAP analysis: E1+*H*_1/*M*_1, E2+*H*_2/*M*_2, E3+*H*_3/*M*_2 and E4+*H*_3/*M*_3.

Oligo name	Sequence ('5–'3)
Adapters in the restriction/ligation step	
*Eco*RI	CTCGTAGACTGCGTACC
	AATTGGTACGCAGTCTAC
*Mse*I	GACGATGAGTCCTGAG
	TACTCAGGACTCAT
*Hpa*II / *Msp*I	GACGATGAGTCTAGAA
	CGTTCTAGACTCATC
Pre-selective primers	
*Eco*RI	GACTGCGTACCAATTCA
*Mse*I	GATGAGTCCTGAGTAAC
*Hpa*II (H) / *Msp*I (M)	ATCATGAGTCCTGCTCGG
Selective primers	
*Eco*RI-FAM (E1)	FAM-GACTGCGTACCAATTC-ACT
*Eco*RI-VIC (E2)	VIC-GACTGCGTACCAATTC-ACA
*Eco*RI-NED (E3)	NED-GACTGCGTACCAATTC-ACC
*Eco*RI-PET (E4)	PET-GACTGCGTACCAATTC-AGC
*Mse*I_1	GATGAGTCCTGAGTAA-CAGT
*Mse*I_2	GATGAGTCCTGAGTAA-CTGT
*Mse*I_3	GATGAGTCCTGAGTAA-CTAT
*H/M*_1	ATCATGAGTCCTGCTCGGTCAGT
*H/M*_2	ATCATGAGTCCTGCTCGGTCTGT
*H/M*_3	ATCATGAGTCCTGCTCGGTCTAT

Table A2.3: Model coefficients from logistic mixed-effects models based on climate-related principle components. Models included principal components axes (PC1, PC2, PC3; Table S5; Figure S8) and zebularine-treatment as fixed effects and population as well as maternal lines nested within populations as random effects. PCs were determined via the broken-stick criterion. Outlier loci were derived from genome-scan approaches outlined in the main manuscript (see Figure 3.3). Statistical significance of explanatory variables was assessed in likelihood-ratio tests (Table 3.3). Abbreviations: *SE* – Standard error of model estimate; *z* – model-based z-score; *p* – the *p*-value for each model term (significant *p*-values given in bold).

Locus	**Term**	***Estimate***	***SE***	***z***	***p***
loc58 (FAM-AAC-CCT-58; AFLP)	*Intercept*	-1.27	0.65	-1.94	0.052
	Zebularine	0.04	0.75	0.05	0.958
	PC1	0.55	0.20	2.76	**0.006**
	PC2	0.03	0.20	0.15	0.884
	PC1 x Zebularine	-0.03	0.22	-0.15	0.879
	PC2 x Zebularine	-0.15	0.22	-0.67	0.504
	SD Maternal lines	0.00			
	SD Population	0.60			
	AICc = 512.97; R^2m = 0.07; R^2c = 0.15				
loc286 (FAM-AAC-CCT-286; AFLP)	*Intercept*	0.64	0.79	0.81	0.416
	Zebularine	-0.14	0.73	-0.19	0.848
	PC1	0.02	0.26	0.06	0.955
	PC2	-0.35	0.27	-1.31	0.191
	PC1 x Zebularine	0.06	0.24	0.24	0.807
	PC2 x Zebularine	-0.03	0.24	-0.13	0.895
	SD Maternal lines	0.76			
	SD Population	0.97			
	AICc = 510.44; R^2m = 0.02; R^2c = 0.29				
loc189 (VIC-ACG-CAT-189; MSAP-m)	*Intercept*	2.53	1.06	2.39	**0.017**
	Zebularine	-1.76	1.10	-1.60	0.110
	PC2	-0.04	0.32	-0.13	0.894
	PC3	-0.11	0.30	-0.36	0.719
	PC2 x Zebularine	0.12	0.34	0.34	0.731
	PC3 x Zebularine	0.49	0.34	1.46	0.144
	SD Maternal lines	0.76			
	SD Population	0.91			
	AICc = 308.02; R^2m = 0.01; R^2c = 0.18				

Table A2.4: Model coefficients from logistic mixed-effects models based on spatial genetic neighborhoods. Models included spatial genetic variation (MEMGENE1, MEMGENE2, MEMGENE3; Table A2.5; Figure A2.2) and zebularine-treatment as fixed effects and population as well as maternal lines nested within populations as random effects. MEMGENEs were determined via *mgQuick*-function in the MEMGENE v1.0.1 R-package. Outlier loci were derived from genome-scan approaches outlined in the main manuscript (see Figure 3.3). Statistical significance of explanatory variables was assessed in likelihood-ratio tests (Table 3.4). Abbreviations: *SE* – Standard error of model estimate; *z* – model-based z-score; *p* – the *p*-value for each model term (significant *p*-values given in bold).

Locus	**Term**	***Estimate***	***SE***	***z***	***p***
loc282 (FAM-AAC-CCT-282; MSAP-m)	*Intercept*	1.27	0.82	1.55	0.122
	Zebularine	0.41	0.99	0.41	0.681
	MEMGENE1 (MG1)	0.14	0.25	0.56	0.575
	MEMGENE2 (MG2)	-0.18	0.25	-0.70	0.483
	MEMGENE3 (MG3)	0.26	0.25	1.04	0.297
	MG1 x Zebularine	-0.16	0.34	-0.48	0.631
	MG2 x Zebularine	0.03	0.31	0.09	0.930
	MG3 x Zebularine	0.10	0.32	0.32	0.748
	SD Maternal lines	0.71			
	SD Population	0.60			
	AICc = 325.73; *R2m* = 0.02; *R2c* = 0.13				
loc176 (FAM-AAC-CCT-176; MSAP-n)	*Intercept*	-0.80	0.67	-1.19	0.233
	Zebularine	0.87	0.70	1.25	0.213
	MEMGENE1 (MG1)	-0.56	0.34	-1.64	0.101
	MEMGENE2 (MG2)	0.25	0.23	1.08	0.281
	MEMGENE3 (MG3)	0.34	0.33	1.05	0.294
	MG1 x Zebularine	0.42	0.39	1.08	0.282
	MG2 x Zebularine	-0.36	0.25	-1.45	0.148
	MG3 x Zebularine	-0.36	0.35	-1.01	0.314
	SD Maternal lines	0.59			
	SD Population	0.73			
	AICc = 462.46; R^2m = 0.02; R^2c = 0.20				
loc222 (PET-AGG-CGA-222; MSAP-n)	*Intercept*	0.60	0.51	1.18	0.237
	Zebularine	-0.97	0.74	-1.32	0.188
	MEMGENE1 (MG1)	-0.21	0.15	-1.37	0.171
	MEMGENE2 (MG2)	0.02	0.15	0.15	0.883
	MEMGENE3 (MG3)	-0.06	0.16	-0.36	0.719
	MG1 x Zebularine	0.20	0.23	0.86	0.392
	MG2 x Zebularine	0.12	0.22	0.55	0.583
	MG3 x Zebularine	0.37	0.23	1.61	0.107
	SD Maternal lines	0.00			
	SD Population	0.00			
	AICc = 470.90; *R2m* = 0.03; *R2c* = 0.03				

Continuation of **Table A2.4**

Locus	**Term**	***Estimate***	***SE***	***z***	***p***
loc135 (PET-AGG-CGA-135; MSAP-n)	*Intercept*	2.83	0.76	3.74	0.000
	Zebularine	-1.26	0.95	-1.32	0.188
	MEMGENE1 (MG1)	-0.51	0.23	-2.26	0.024
	MEMGENE2 (MG2)	-0.10	0.20	-0.50	0.615
	MEMGENE3 (MG3)	-0.12	0.24	-0.52	0.601
	MG1 x Zebularine	0.65	0.31	2.10	0.036
	MG2 x Zebularine	-0.03	0.28	-0.10	0.922
	MG3 x Zebularine	0.15	0.30	0.49	0.624
	SD Maternal lines	0.75			
	SD Population	0.25			
	AICc = 359.00; R^2m = 0.03; R^2c = 0.12				

Table A2.5: Jointly detected outlier loci and their applied corresponding genome-scan approaches. For each marker, the applied fluorescence dye and the cutting sequence are given. Environmental variables that helped detecting outlier loci, are given in bold and were included as fixed factors in logistic mixed-effects models (see Tables A2.3 and A2.4). Details of the genome-scan approaches are given in the main manuscript. Abbreviations: bp – base pair size of outlier locus.

Marker	**Locus ID**	**Dye**	**Sequence**	**bp**	**Genome scan approach**	**Environmental variables**
AFLP	loc58	FAM	AAC-CCT	58	BayeScEnv and LEA	**PC1, PC2,** PC3
	loc286	FAM	AAC-CCT	286	BayeScEnv and LEA	**PC1, PC2,** PC3
MSAP-m	loc189	VIC	ACG-CAT	189	BayeScEnv and LEA	PC1, **PC2, PC3**
	loc282	FAM	AAC-CCT	282	RDA and LEA	**MEMGENE1, MEMGENE2, MEMGENE3**
MSAP-n	loc135	PET	AGG-CGA	135	RDA and LEA	**MEMGENE1, MEMGENE2, MEMGENE3**
	loc176	FAM	AAC-CCT	176	RDA and LEA	**MEMGENE1, MEMGENE2, MEMGENE3**
	loc222	PET	AGG-CGA	222	RDA and LEA	**MEMGENE1, MEMGENE2, MEMGENE3**

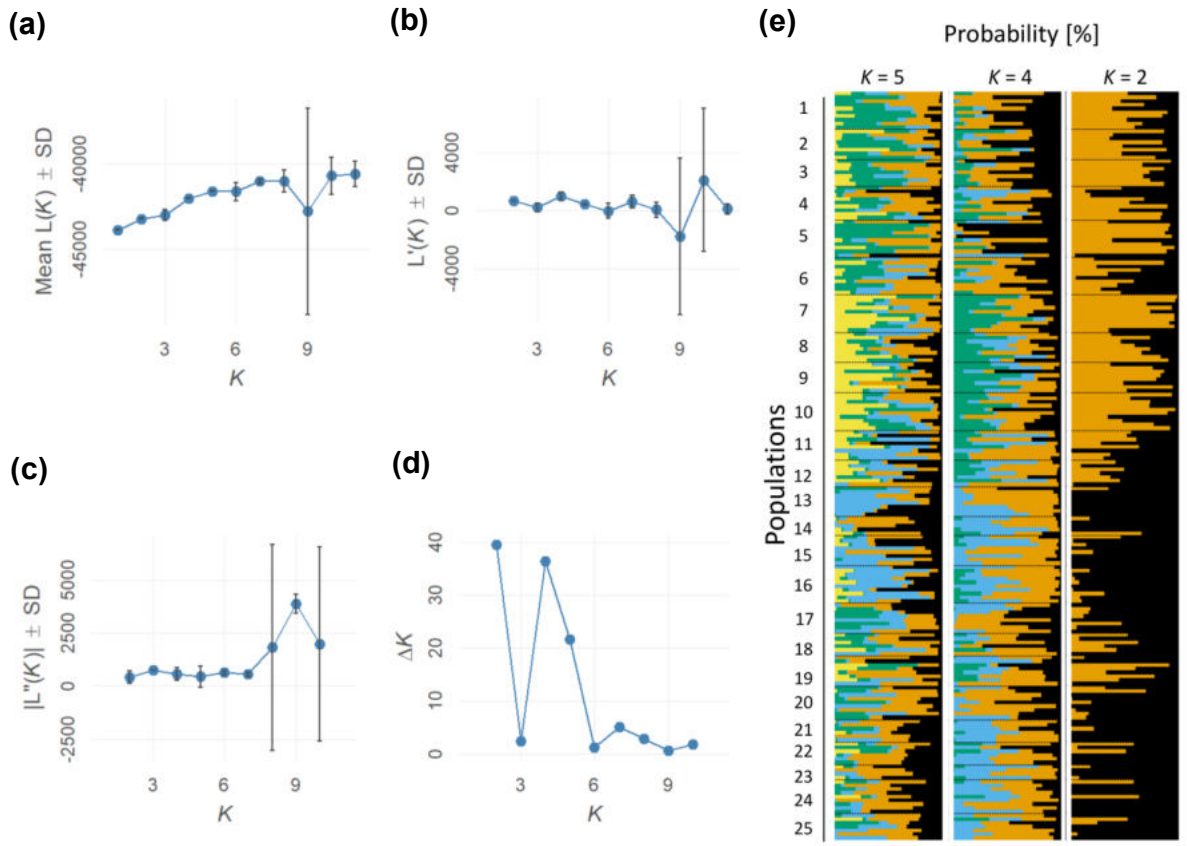


Figure A2.1: STRUCTURE analysis of AFLP loci. STRUCTURE analysis was applied using the admixture model with LOCPRIOR parameter, a burn-in of 1,000,000 and 500,000 repetitions. Diagnostics were based on Evanno et al., (2005), i.e., (**A**) the likelihood distribution as the mean L(*K*) (± SD), (**B**) the rate of change of the likelihood distribution as L'(*K*) = L(*K*) – L(*K*-1), (**C**) the absolute values of the second-order rate of change of the likelihood distribution as |L''(*K*)| = |L'(*K*+1) – L'(*K*)|, and (**D**) Δ*K* from Δ*K* = m| L''(*K*)| / s[L(*K*)], for each simulation of *K*. Based on these diagnostics, (**E**) *K* = 5, *K* = 4, and *K* = 2 were estimated as the most probable number of genetic clusters present in *S. canadensis* populations along the latitudinal gradient.

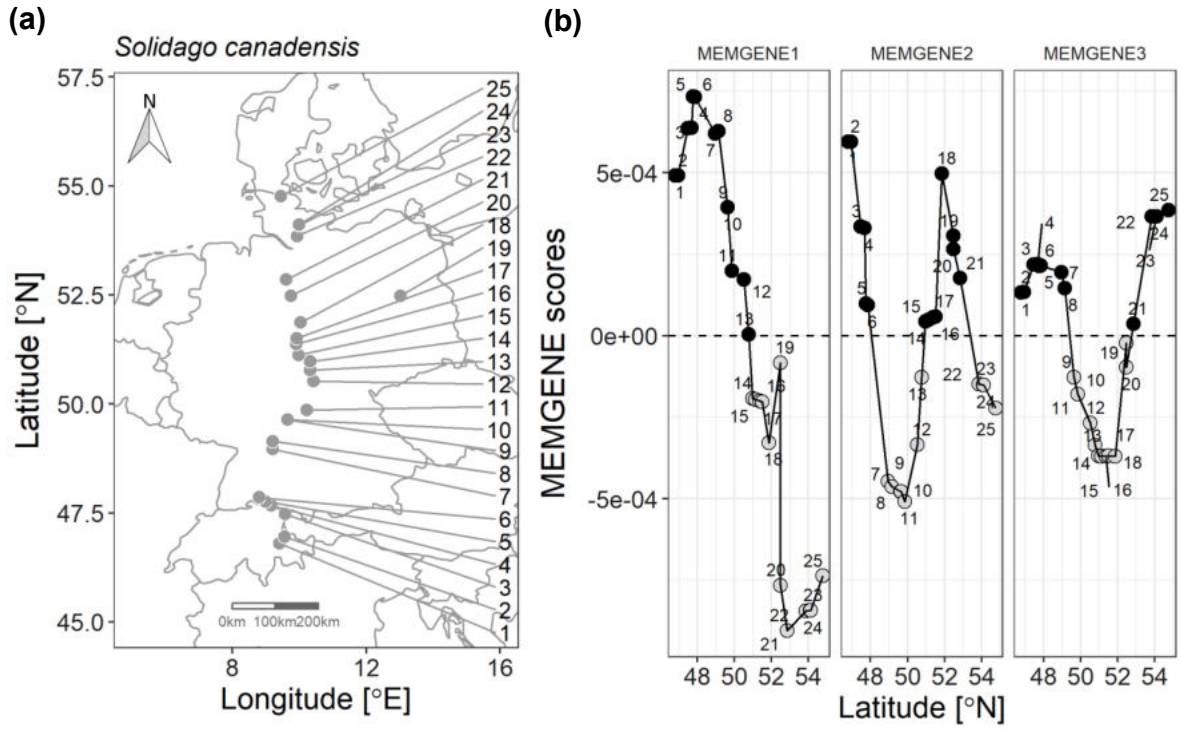


Figure A2.2: Spatial genetic neighborhoods based on Moran's eigenvector maps (MEM). Three autocorrelation (spatial neighborhoods) axes were significantly associated with genetic variation in redundancy analysis using MEMs and transformed to MEMGENE axes using principal components analysis. MEMGENE selection based on **(a)** population coordinates (longitude and latitude in decimal degrees) and **(b)** similar size and color of circles represent similar MEMGENE values for the corresponding populations. In total, MEMGENE analysis of spatial genetic neighborhoods explained 13.2% of genetic variation.

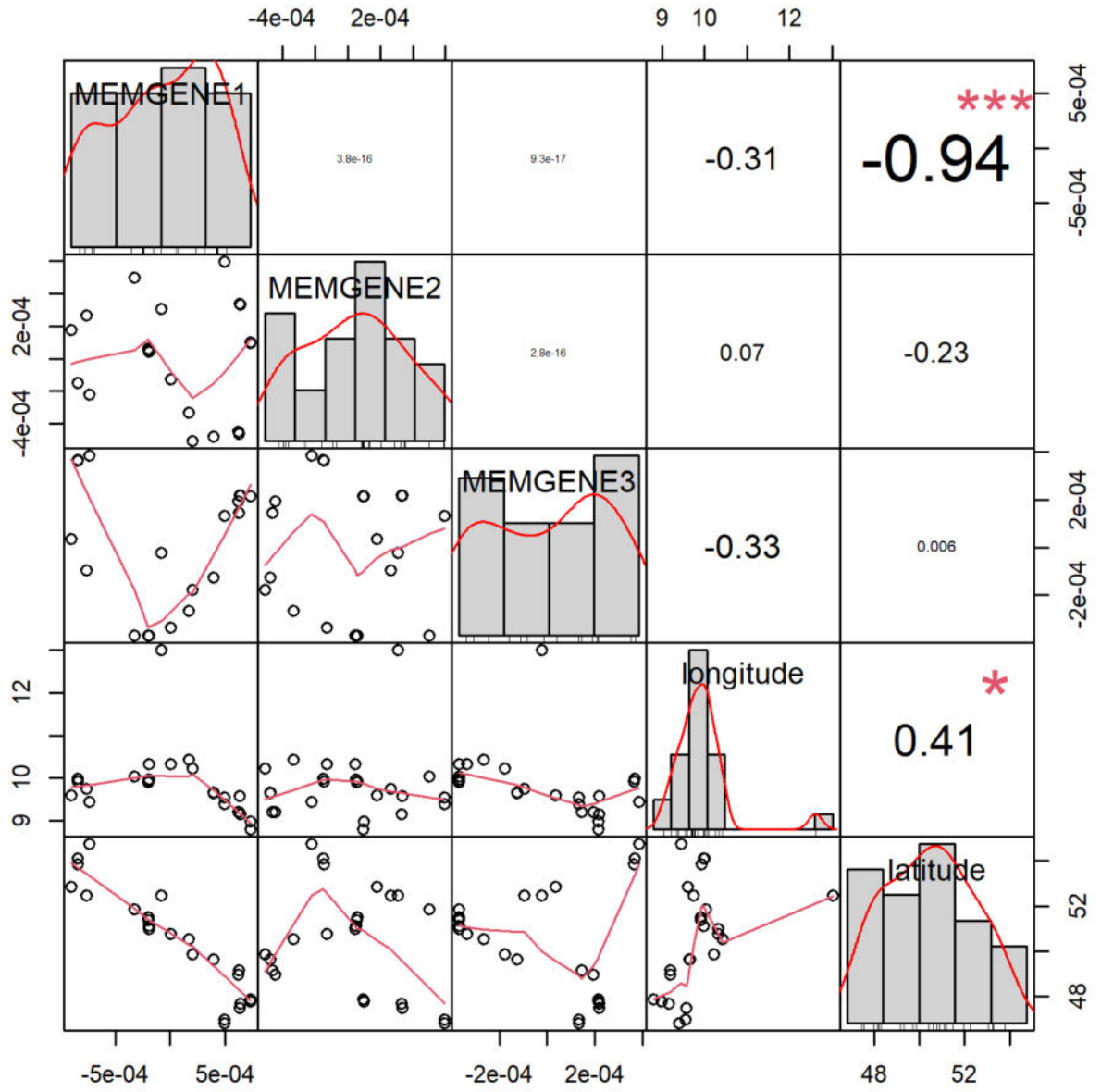


Figure A2.3: Correlation matrix of MEMGENE axes versus population coordinates. The strength and direction of each correlation is given as R^2 and was estimated using the Pearson correlation coefficient. Significance level: $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***. Latitude and longitude values are given as decimal degrees.

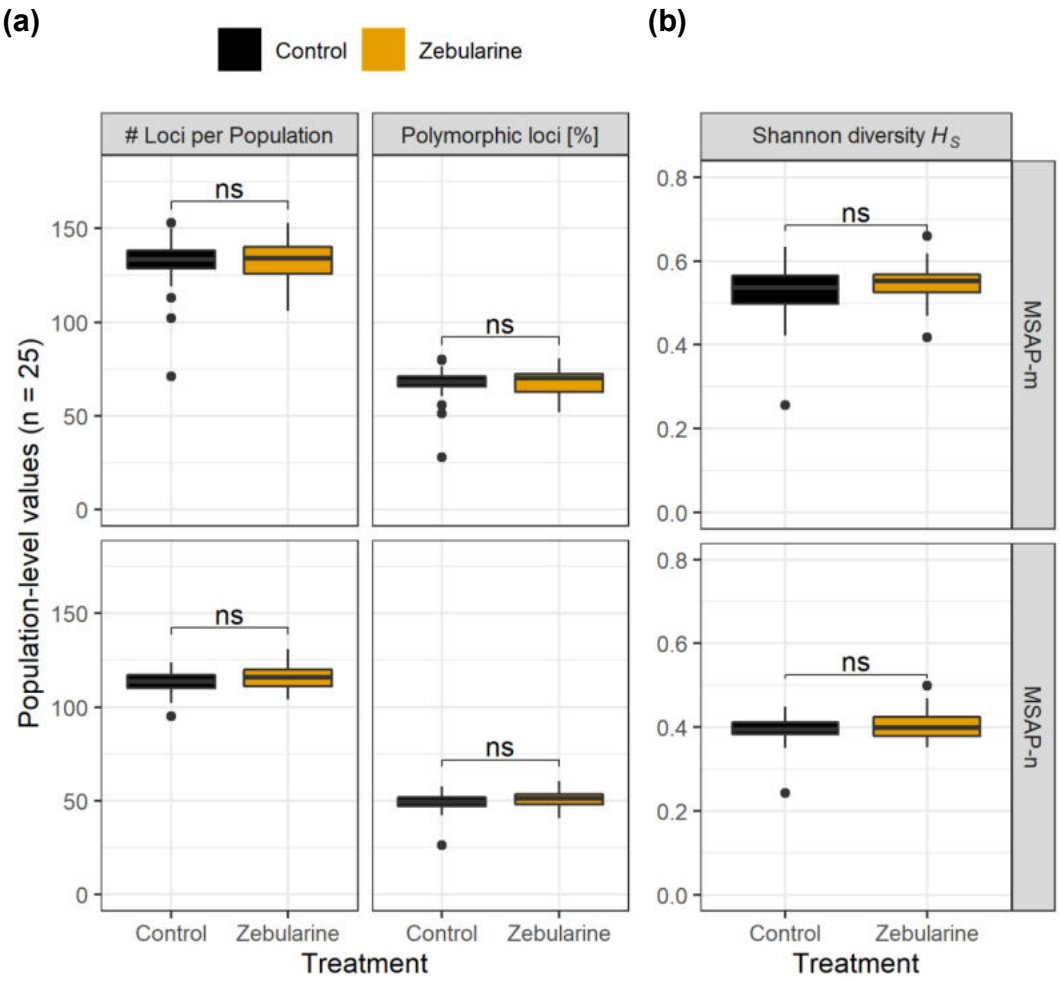


Figure A2.4: MSAP population-level diagnostics using all available samples. Population-level paired *t*-test comparing control versus zebularine-treated individuals was conducted separately on the sets of methylated (MSAP-m) and non-methylated (MSAP-n) loci for (**A**) the total number of loci and the percentage of polymorphic loci, (**B**) the Shannon information criterion (H_S). Significance level: $p < 0.05$. Abbreviation: ns – not significant.

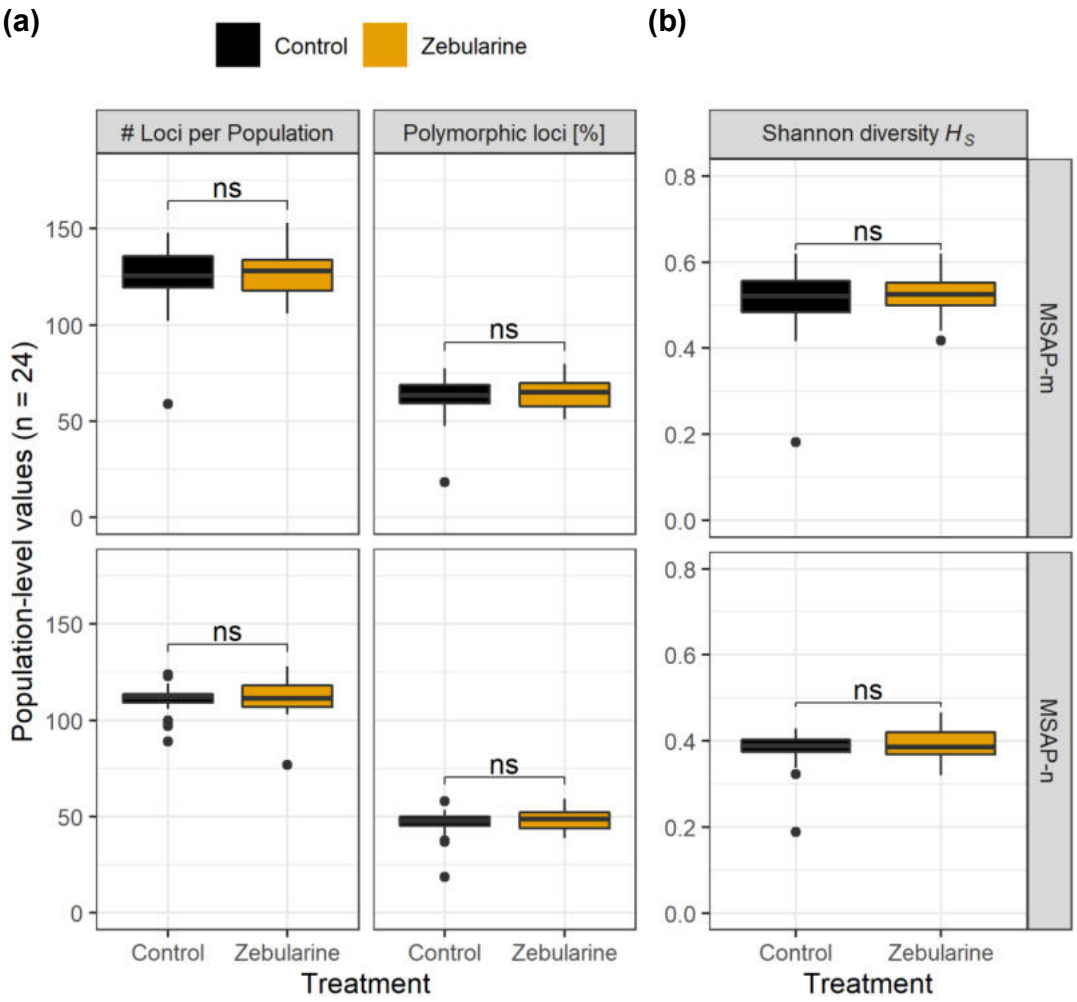


Figure A2.5: MSAP population-level diagnostics using only samples from siblings present in control and zebularine treatment groups. Population-level paired *t*-test comparing control versus zebularine-treated individuals was conducted separately on the sets of methylated (MSAP-m) and non-methylated (MSAP-n) loci for **(a)** the total number of loci and the percentage of polymorphic loci, **(b)** the Shannon information criterion (H_S). Please note that population 21 was not included because no individual of this population was present in both treatment groups. Significance level: $p < 0.05$. Abbreviation: ns – not significant.

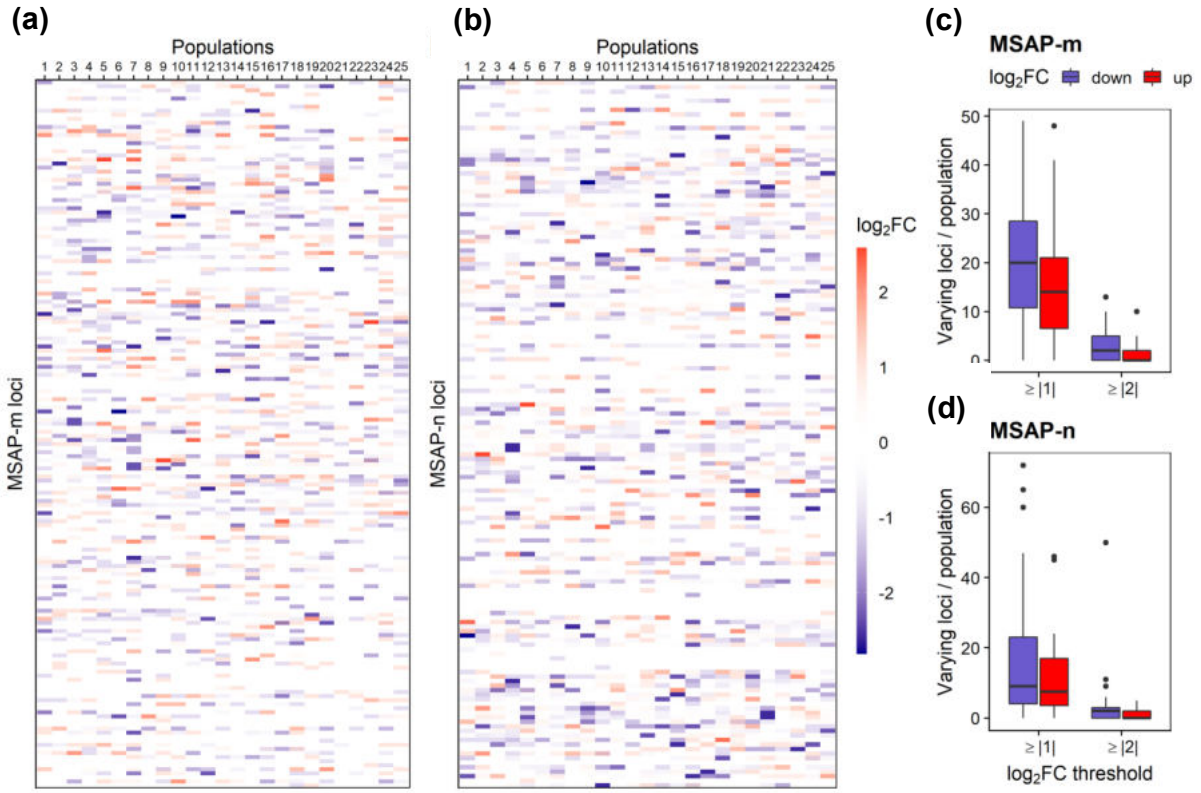


Figure A2.6: Heatmaps of population-level change (log_2FC) in MSAP loci due to the zebularine treatment. The increase and decrease, respectively, are shown color-coded as log_2FC per population separately for (**A**) methylated (MSAP-m) and (**B**) non-methylated (MSAP-n) loci. Positive values denote that loci occur more frequently in zebularine-treated individuals compared to control plants and vice versa. A log_2FC value of 2 (or -2) means that the corresponding locus occurs four times more (or less) frequent in the population-level zebularine subgroup. Both **(C)** MSAP-m and (**D**) MSAP-n datasets were filtered separately for loci changing in frequency per population, see box-and-whisker plots. For comparison, loci were filtered for two log_2FC thresholds, i.e., two-fold variance ($log_2FC \geq |1|$) and four-fold variance ($log_2FC \geq |2|$).

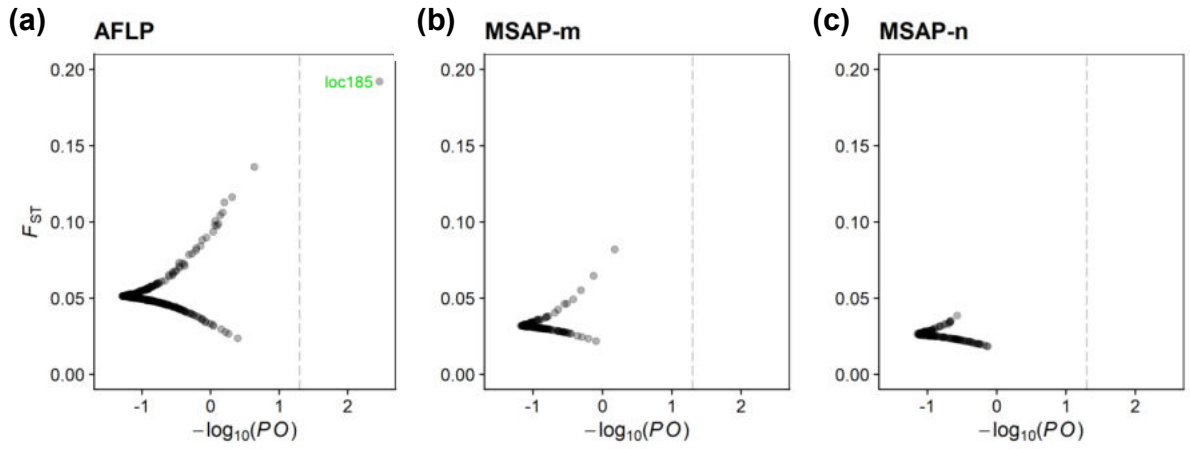


Figure A2.7: BAYESCAN analysis of AFLP/MSAP loci. Outlier screening was conducted separately for (**A**) AFLP, (**B**) MSAP-m, and (**C**) MSAP-n datasets. BAYESCAN was run with 100,000 iterations, 10 repetitions and a burn-in of 50,000 following 20 pilot runs (see Method A2.2). The dashed line marks the threshold of 1.3 for the false discovery rate.

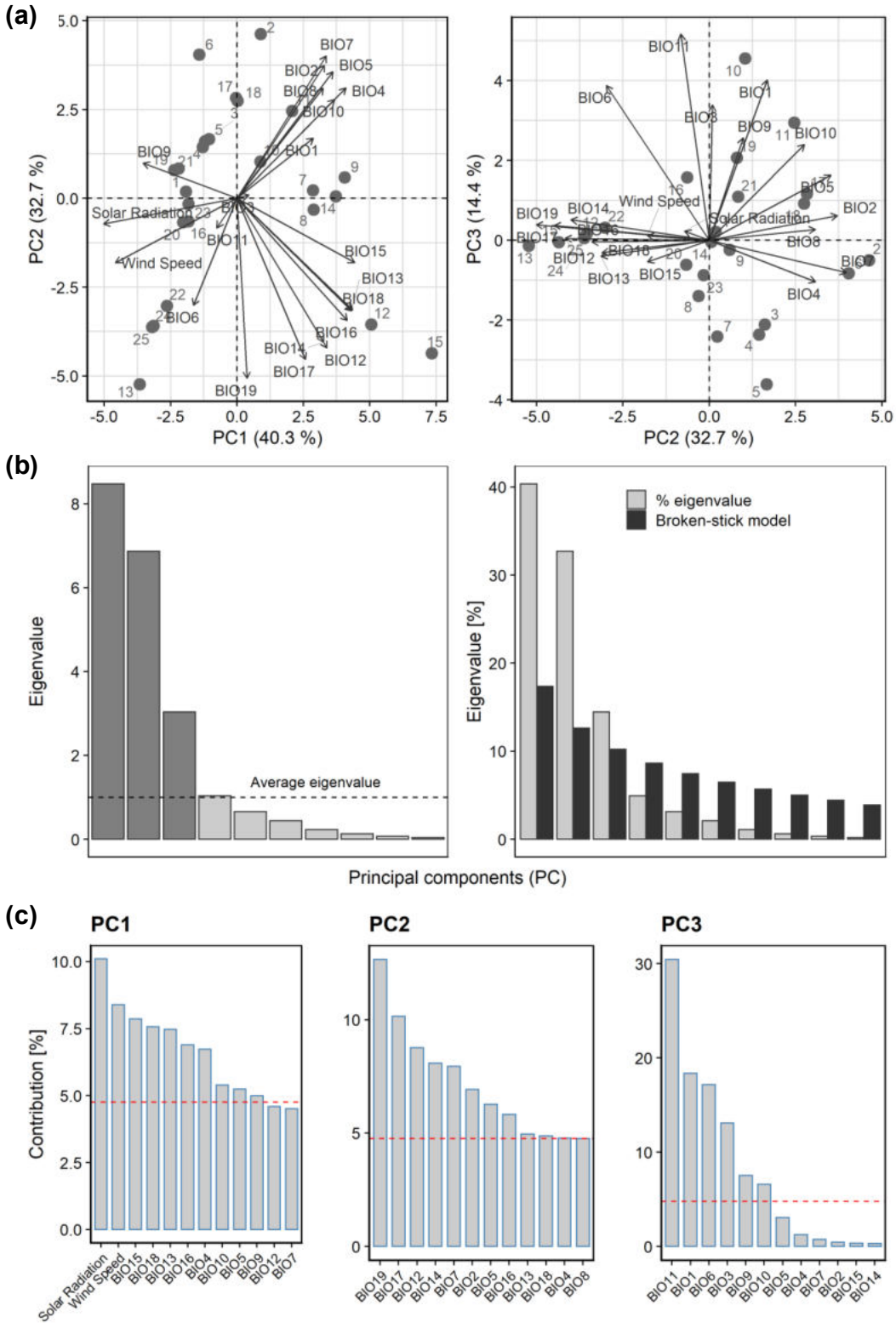


Figure A2.8: Principal components analysis (PCA) of WorldClim 2.0 climate variables. **(a)** Biplots of first-versus-second (PC1, PC2) and second-versus-third (PC2, PC3) PCA axes. Numbers denote the population IDs (see Table A2.1). **(b)** Eigenvalues of PCs, their corresponding percentage of explained variation [%] against the broken-stick criterion, and **(c)** the WorldClim 2.0 climate variables with highest loadings on each PC axis.

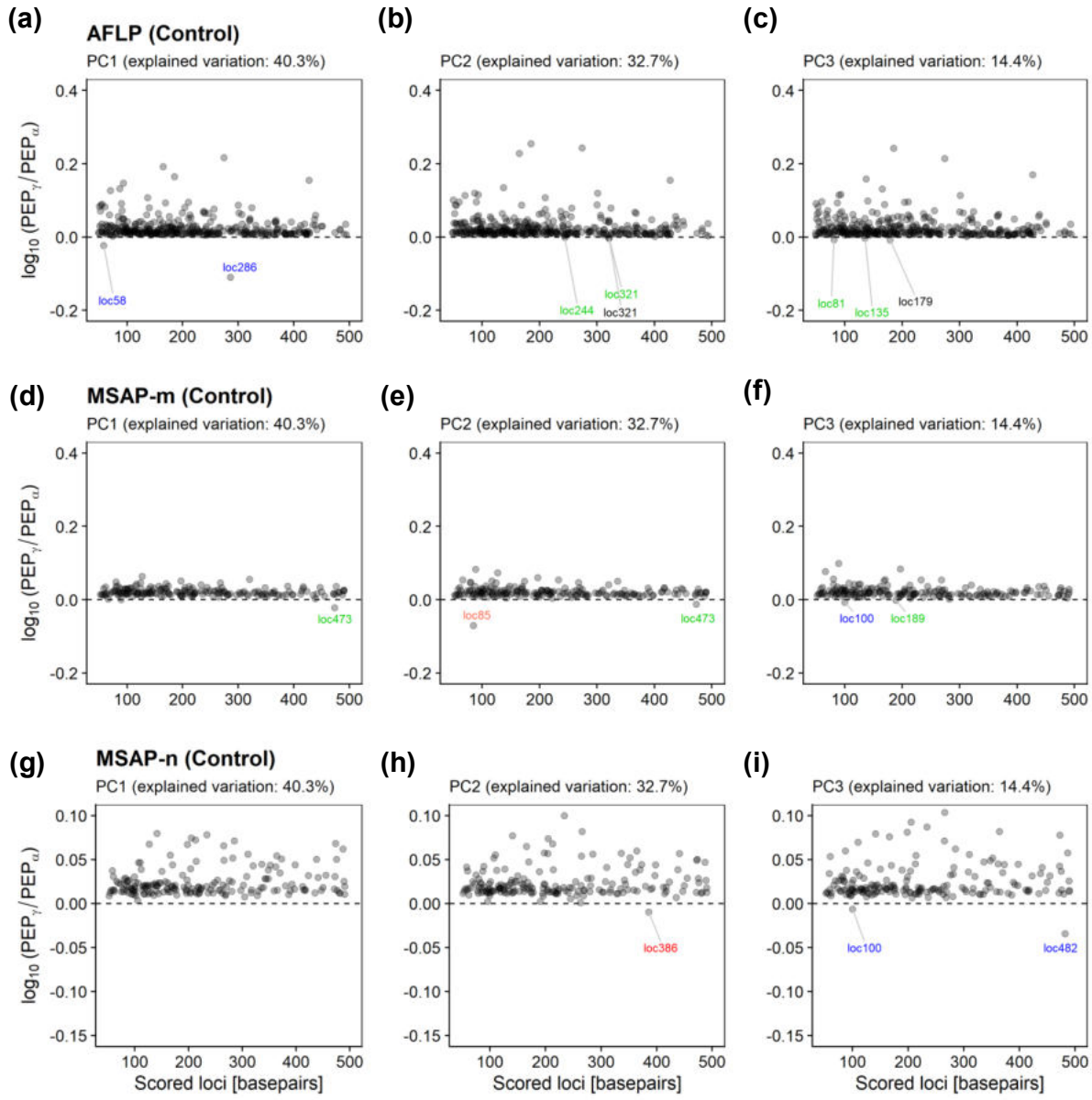


Figure A2.9: BAYESCENV analysis of AFLP/MSAP loci. Colors of loci IDs correspond to the fluorescent dyes from the GENEMAPPER software. Outlier screening was conducted using three principal component axes (**A, D, G**: PC1, **B, E, H**: PC2, **C, F, I**: PC3) separately based on principal components analysis of WorldClim 2.0 variables (Figure A2.8). BAYESCENV analysis was applied separately on (**A-C**) AFLP, (**D-F**) MSAP-m, and (**G-I**) MSAP-n datasets (see Method A2.2). In total, the applied PCs explained 87.1% of climatic variation among sampling locations of *S. canadensis* source populations. Only non-treated control plants were used for analysis and detected outlier loci were pooled for subsequent statistical analyses.

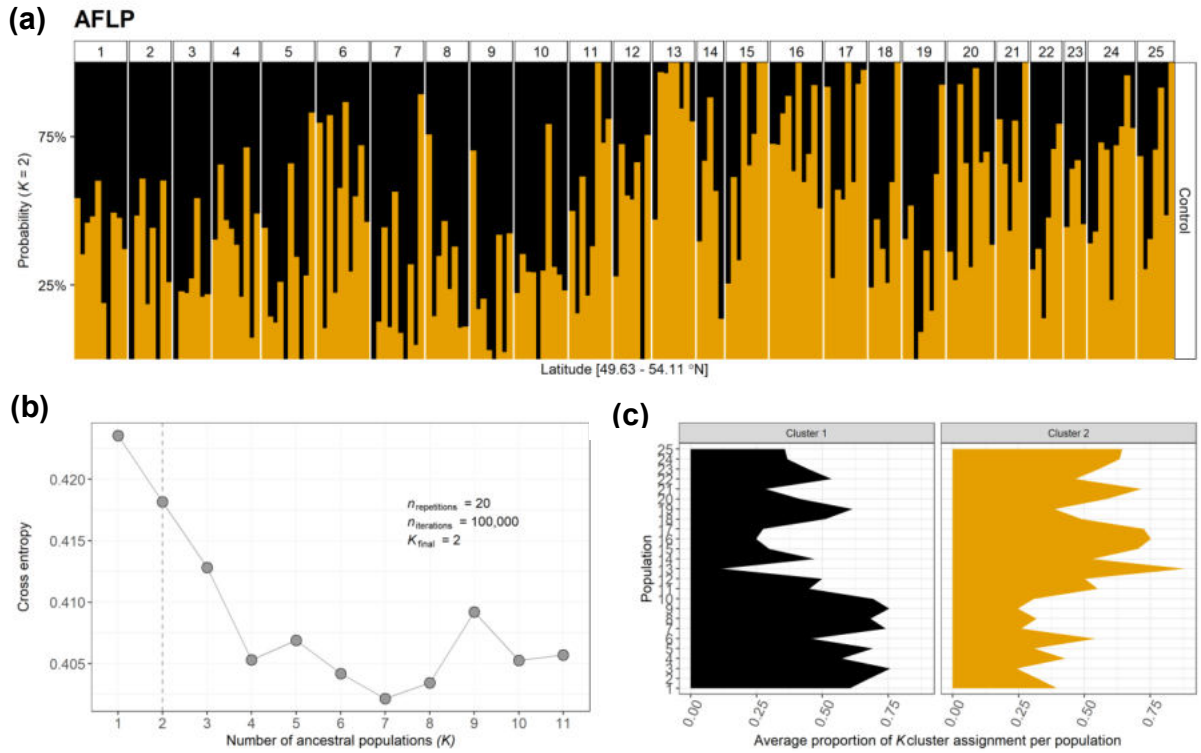


Figure A2.10: LEA analysis of genetic (AFLP) population structure. (**A**) Membership proportions of *K*=2 estimated genetic clusters along the latitudinal gradient under study, (**B**) cross-entropy used for selecting the number of *K* clusters used in subsequent outlier screening with LFMM (Figure A2.13 and Method A2.2), (**C**) distribution of the averaged population-level proportion of each *K* cluster assignment along the latitudinal gradient. Only non-treated control plants were used to analyze genetic population structure.

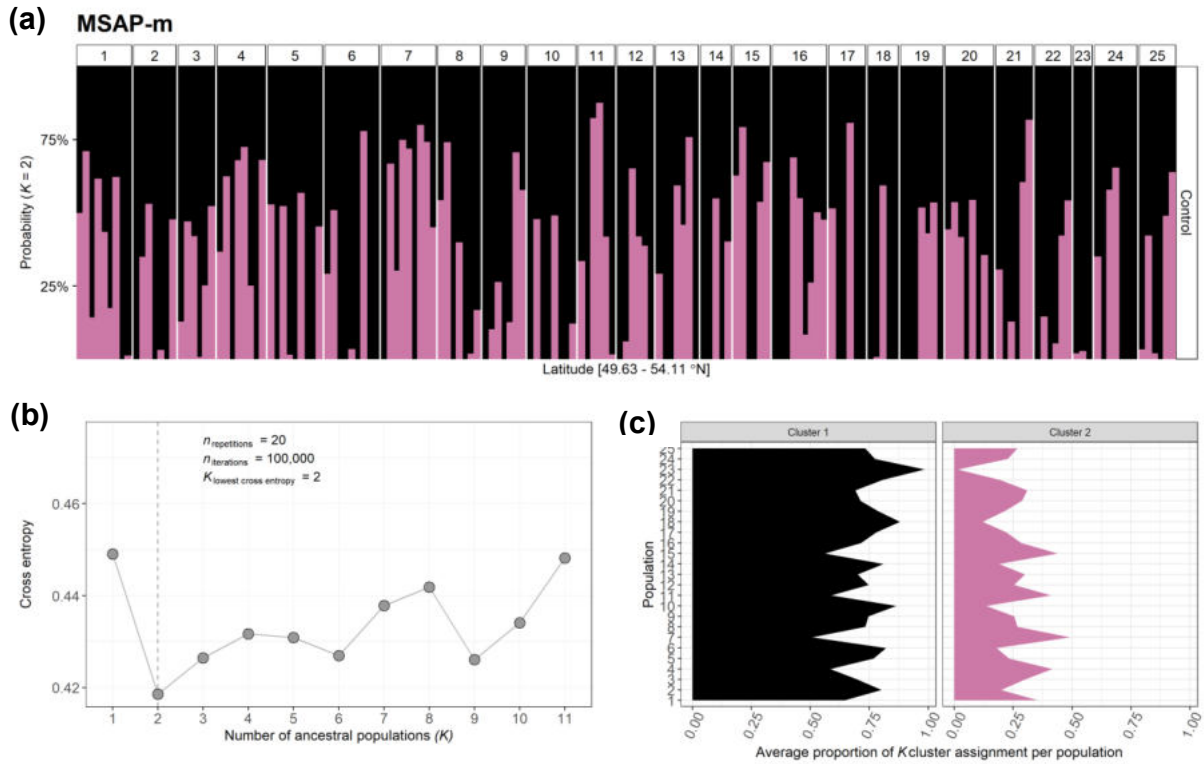


Figure A2.11: LEA analysis of epigenetic (MSAP-m) population structure. (**A**) Membership proportions of *K*=2 estimated genetic clusters along the latitudinal gradient under study, (**B**) cross-entropy used for selecting the number of *K* clusters, (**C**) distribution of the averaged population-level proportion of each *K* cluster assignment along the latitudinal gradient. Only non-treated control plants were used to analyze genetic population structure. Because no pronounced population structure was found with LEA, a final *K* =1 was used in subsequent outlier screening with LFMM (Figure A2.13 and Method A2.2).

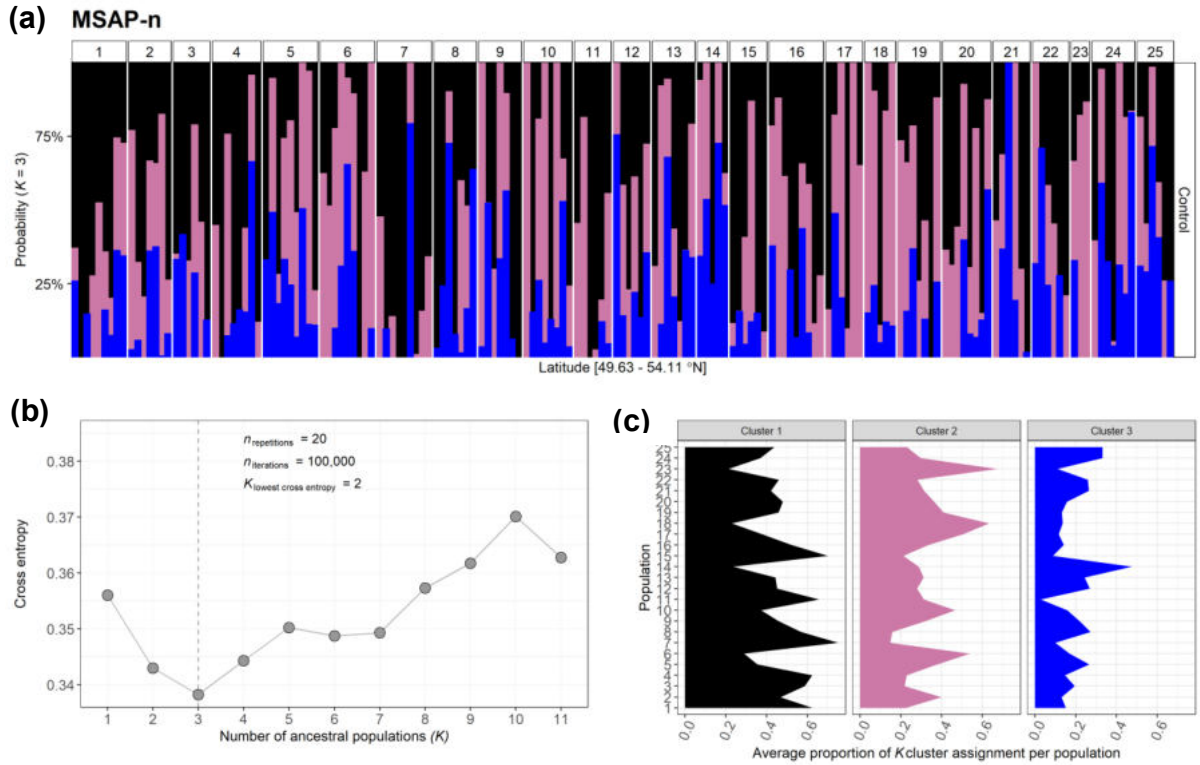


Figure A2.12: LEA analysis of epigenetic (MSAP-n) population structure. (**A**) Membership proportions of *K*=3 estimated genetic clusters along the latitudinal gradient under study, (**B**) cross-entropy used for selecting the number of *K* clusters, (**C**) distribution of the averaged population-level proportion of each *K* cluster assignment along the latitudinal gradient. Only non-treated control plants were used to analyze genetic population structure. Because no pronounced population structure was found with LEA, a final *K* =1 was used in subsequent outlier screening with LFMM (Figure A2.13 and Method A2.2). Please note that the colors do not represent the same or similar clusters as in Figure A2.11 and were chosen only to facilitate visualization in cases of color-deficiency.

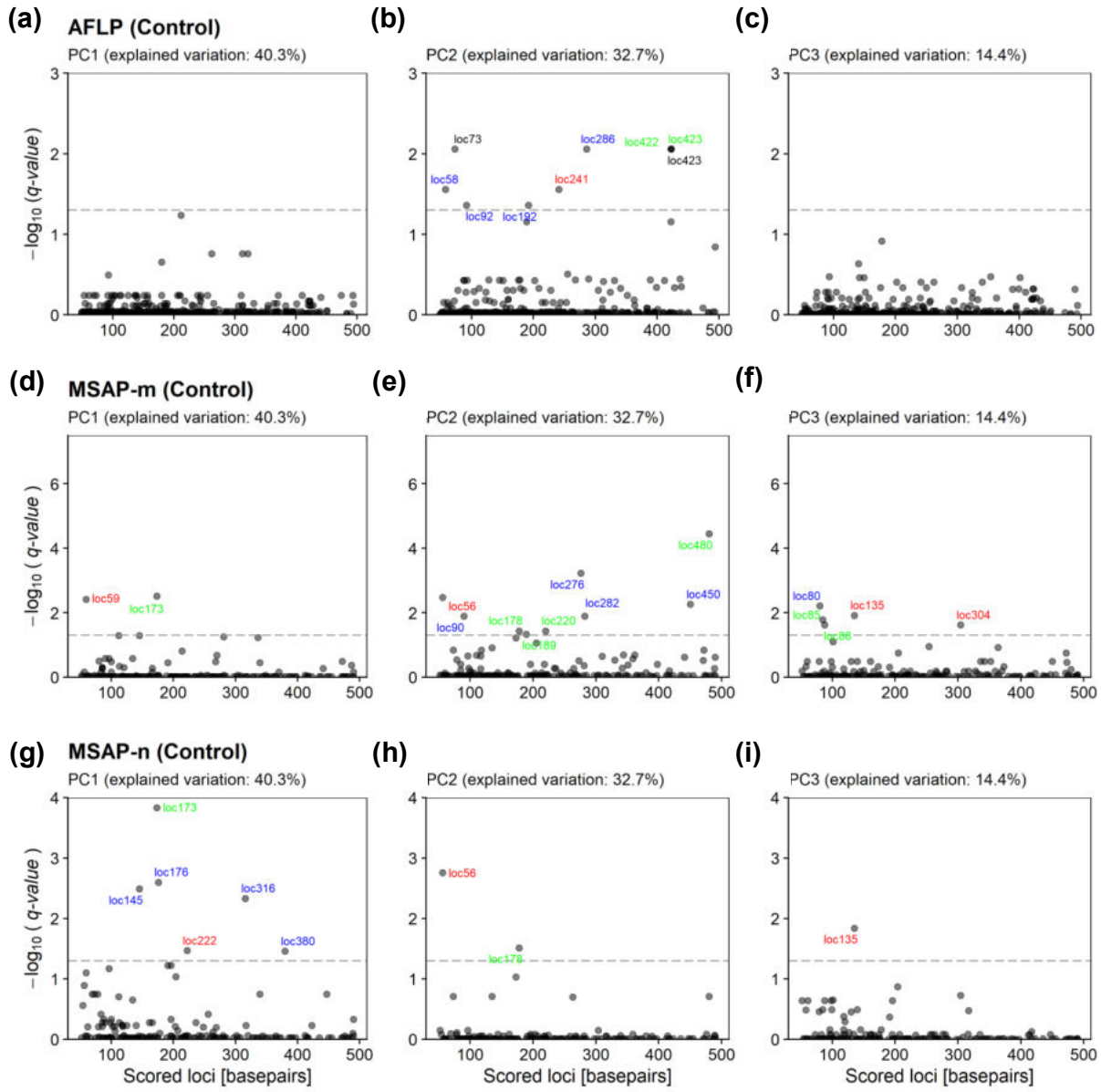


Figure A2.13: LFMM analysis of AFLP/MSAP loci. Colors of loci IDs correspond to the fluorescent dyes represented in the GENEMAPPER software. Outlier screening was conducted using three principal component axes (**A, D, G**: PC1, **B, E, H**: PC2, **C, F, I**: PC3) separately based on principal components analysis of WorldClim 2.0 variables (Figure A2.8 and Method A2.2). LFMM analysis was applied separately on (**A-C**) AFLP, (**D-F**) MSAP-m, and (**G-I**) MSAP-n datasets. In total, the applied PCs explained 87.1% of climatic variation among sampling locations of *S. canadensis* source populations. Only non-treated control plants were used for analysis and detected outlier loci were pooled for subsequent analyses.

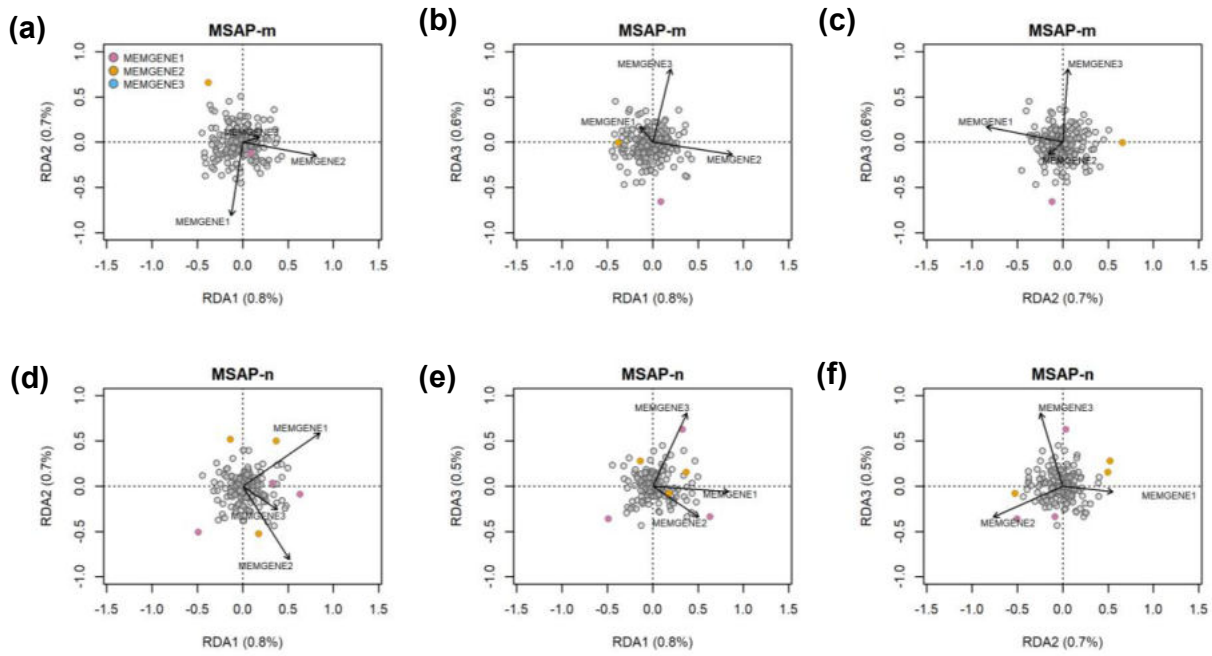


Figure A2.14: Redundancy analysis (RDA) of MSAP loci. RDA was conducted on methylated MSAPs (MSAP-m: **A**-**C**) and non-methylated MSAP (MSAP-n: **D**-**F**) separately using spatial genetic neighborhoods from MEMGENE analysis(Figure A2.2 and Method A2.2). Outlier loci were colored based on their highest loading on corresponding MEMGENE variables. Biplots showing the percentage of explained variation of (**A, D**) the first-versus-second (**B, E**) first-versus-third, and (**C, F**) second-versus-third RDA axis separately for MSAP-m (RDA1: 0.8%; RDA2: 0.7%; RDA3: 0.6%) and MSAP-n RDA1: 0.8%; RDA2: 0.7%; RDA3: 0.5%).

Appendix | Study 3

Methods A3.1: Single-species analyses of fitness parameters

We tested for each species separately the effects of region of the field sites, zebularine treatment, origin and their interactions. In all models, we included as fixed terms the factors region of the field sites (Konstanz vs. Potsdam), zebularine treatment (untreated vs. treated), origin of maternal lines (Konstanz region vs. Potsdam region) and their two- and three-way interactions. As random terms, we included field site and block nested within field to account for non-independence of plants in the same block and field site, and maternal lines to account for non-independence of offspring from the same mother plant.

We analyzed aboveground biomass and reproductive biomass with linear mixed models, implemented in the R package LME4 (Bates, Mächler, Bolker, & Walker, 2015). Both biomass variables were power transformed (see Tables A3.5 and A3.7) to fulfil the assumptions of normality of the residuals and to reduce heterogeneity of variance. We analyzed survival probability (0, 1) and flowering probability (0, 1) with generalized linear mixed models with a binomial distribution with the MCMCGLMM package (Hadfield, 2010), since generalized mixed models with the R package 'lme4' (Bates et al., 2015) often did not converge. There was nearly complete survival and flowering in certain field sites (a phenomenon known as quasi-complete separation; see e.g. Albert & Anderson, 1984; Rainey, 2016; Sauter & Held, 2016). The reason for this is that, in such cases, the likelihood function often has no maximum. Therefore, instead of a probabilistic approach, we used a Markov chain Monte Carlo (MCMC) estimation approach implemented in the MCMCGLMM package (Hadfield, 2010) following a worked example provided at https://ms.mcmaster.ca/~bolker/R/misc/foxchapter/bolker_chap.html (last accessed on 28 March 2019). Priors for random effects, observation-level variance and the fixed effects were specified as recommended in the worked example. We used the logit link (family = categorical), a burn-in period of at least 100,000 iterations, and thinned at least every 900 iterations until we had an effective sample size of ~10,000 draws. We checked that draws were not affected by autocorrelation (non-independence between successive samples in the chain) and that the model had converged (no trend in the time series, i.e. traces of the chains are stationary). The effect of each fixed effect was estimated using the calculated 95% credible interval of their posterior distribution and the computed probability that such an effect is larger than zero (PMCMC).

Methods A3.2: Effect size calculation in meta-regression

The number of local and non-local plants represented in each effect size differed between block-level effect sizes (1-7 plant individuals) and field-level effect sizes (1-21). Unfortunately, in some species, there were considerable amounts of missing data for all field sites of a transplant region,

due to either very low survival (e.g. in the non-native Erigeron canadensis in Potsdam) or very few flowering plants (e.g. in the native Lactuca serriola in both Konstanz and Potsdam) (see Figs. A3.5-16). But to calculate an effect size, we needed data for at least one plant individual in each group (local vs. non-local) for the log odds ratio, and at least two individuals in each group (local vs. non-local) for the standardized mean difference (SMD). Therefore, for some species, we sometimes could not calculate effect sizes and summarize them at the region-level (see Figure A3.4), or, when effect sizes for one of the two regions were missing entirely, we could not summarize them across regions (see Figure 4.1). Missing effect sizes are indicated with NA in figures.

Methods A3.3: Effect sizes corrected with the ratio of sample sizes of the compared groups

For the analyses of the binomial variables (survival, flowering probability), a zero in one of the cells of a 2×2 contingency table can be a problem for effect size calculation (e.g. the odds ratio). Therefore, routinely a so-called continuity correction is added to every cell of a contingency table that has at least one zero. The most commonly used continuity correction value is 0.5 (Sweeting, Sutton, & Lambert, 2004; Viechtbauer, 2010), and the *escalc* function in the metafor package (Viechtbauer, 2010) by default adds this value to tables with zeroes. However, when sample sizes between the local and non-local groups become increasingly unbalanced, a continuity correction of 0.5 can lead to erroneous effect sizes, if events are rare (e.g. in less than 1% of all cases; Sweeting et al., 2004).

Our data showed both high rates of survival and flowering (i.e. rare events of dead or non-flowering plants), and occasionally huge sample size differences (with a maximum ratio of 1:16 between local and non-local plants). Therefore, we calculated a second set of effect sizes, corrected with the ratio of sample sizes of the compared groups, as proposed by Sweeting et al. (2004). Based on the ratio R of sample sizes between both groups, with R = nnon-local/nlocal, a continuity correction (non-local_{CC} or local_{CC}) is added to the cells of the respective group. The non-local_{CC} is calculated as $\text{non-local}_{CC} = R/(1+R)$, and the local_{CC} for the treatment group as $\text{local}_{CC} = 1/(1+R)$. (It follows that the sum of both non-local_{CC} and local_{CC} is 1.) In cases with balanced sample sizes (i.e. at a R of 1:1), both the standard continuity correction in the metafor package (Viechtbauer, 2010) and the method by Sweeting et al. (2004) give the same results. Meta-regressions with both methods gave very similar results (see Table 4.3).

Finally, the method by Sweeting et al. (2004) allows to include tables with two zeroes in events or non-events (i.e. complete flowering or complete non-flowering) in the analysis with metafor (Viechtbauer, 2010), whereas the standard method does not. Effect sizes of zero do not contribute significantly to the overall result when odds ratios are used (Friedrich, Adhikari, &

Beyene, 2007), but it helped to better visualize effect sizes of zero in forest plots (Figs. 4.1 and A3.4).

Methods A3.4: Soil parameter measurements in field sites

To assess the soil parameters in the six field sites, we randomly collected 11-12 soil samples (each at least 300 cm3) per field site at the end of July 2016 down to a depth of 15 – 22 cm. The 72 soil samples were stored in plastic bags at 8°C. For each sample, we determined dry-to-fresh mass ratio, organic matter after loss-on-ignition, plant-available phosphate according to Olsen et al. (1954), plant-available ammonium, total phosphate content, total nitrogen content, relative carbon content, relative nitrogen content, pH-value and C:N ratio. The averaged ratio of dry-to-fresh mass was determined by weighing two replicate subsamples of 15 – 82 g per soil sample and drying it for 48 h at 105°C in a drying chamber (M 115, Binder GmbH, Tuttlingen, Germany). The percentage of organic matter was obtained as the mean percentage weight loss of two subsamples (subsample weight: 170 – 490 mg), incinerated for 2h at 550°C in a muffle furnace (Carbolite LHT, Carbolite Gero Limited, Hope Valley, UK).

Plant-available phosphate (mg P L^{-1} dry mass) was extracted using three subsamples (2.5 g of fresh weight), following Olsen et al. (1954), and concentration was determined photometrically at 880 nm (DR/2000, Hach Lange GmbH, Düsseldorf, Germany) and results averaged over three replicated measurements per subsample. Plant-available ammonium (mg NH4 kg-1 dry mass) was determined using three subsamples (5 g of fresh weight) diluted each in 50 ml of 2M KCl (99.5 % p.a., Roth, Karlsruhe, Germany), shaken for 30 min (Level 2/9, REAX 2, Heidolph, Schwabach, Germany) and filtered with a glass fibre filter (MG160, ⌀ 150mm, Qty 50, glass-micro fibre discs, Sartorius, Göttingen, Germany). Concentration was determined photometrically at 425 nm (DR/2000, Hach Lange GmbH, Düsseldorf, Germany) adding one drop of mineral stabilizer solution (Hach Lange GmbH, Karlsruhe, Germany), one drop of polyvinyl alcohol dispersing agent (Hach Lange GmbH, Düsseldorf, Germany) and 0.2 ml of Nessler's reagent (Hach Lange GmbH, Düsseldorf, Germany). The measurement was replicated three times for each of the three subsamples and the results were averaged per soil sample. The amounts of total nitrogen and total phosphate were obtained according to Kneis et al. (2006) and Heinze et al. (2016), using 250 mg of dried soil and chemical digestion for 15 min at 450°C (Digesdahl digestion apparatus, Hach Lange GmbH, Düsseldorf, Germany), and applying a mixture of 4 mL of H2SO4+ first (95 %, AnalaR NORMAPUR, VWR, Radnor/Pennsylvania, USA) for 8 min and 15 ml of H2O2 second (30 %, AnalaR NORMAPUR, VWR, Radnor/Pennsylvannia, USA) for 10 min. Samples were heated at 100°C in a water quench (using pumice stones) for 15 min, brought to pH 2 – 2.5 with 67.5% KOH (VWR, Radnor/Pennsylvania, USA), and filled with double-distilled H2O up to 100 mL. Concentrations were then determined photometrically at 425 nm for

total nitrogen and 880 nm for total phosphate (DR/2000, HACH, Düsseldorf, Germany). Both total nitrogen (g N kg^{-1} dry mass) and total phosphate (g P kg^{-1} dry mass) were extracted using two subsamples and measurements were replicated three times per subsample. Actual pH was measured on subsamples of 5 g fresh mass at a soil:water ratio of 1:5 (WTW 325 pH meter, Germany) using double-distilled H2O after 30 min of dilution (Heinze et al., 2016). Subsamples were in the same ratio diluted in 0.01M CaCl2- to measure potential pH. To measure C:N ratio, subsamples of 40 – 70 mg were sieved to a grain size of 1 mm and ground in a mixer mill (25 Hertz, 3 min, MM 200, Retsch, Haan, Germany) for 3 min. Samples were then transferred to aluminium cartridges (HE 25208000, 10 x 10, HEKAtech GmbH, Wegberg, Germany) and measured with a C:N analyzer (EA3000 CHNS-O, Eurovector Srl, Pavia, Italia).

Notes A3.1: Results of the single species analyses

Survival

Of the 3729 plants included in our survival analyses, 2993 (80.3%) survived until harvest, and survival ranged from 42.9% for the native *Lactuca serriola* to 97.9% for the native *Senecio vulgaris*. The single-species analyses showed that survival probabilities were for all 12 study species similar in the Konstanz and Potsdam transplant regions and not affected by the zebularine treatment (Table A3.5). For the non-native *Datura stramonium*, plants from the Konstanz origin had a significantly higher survival than those from the Potsdam origin (significant origin effect in in Table A3.5, Fig. A3.15), but this effect was only visible in the Konstanz transplant region (significant region:origin interaction Table A3.5, Fig. A3.15). In the Potsdam transplant region, on the other hand, the local plants of the native *L. serriola* had a higher survival probability than the non-local ones, whereas in the Konstanz transplant region this difference was absent (significant region:origin interaction in Table A3.5, Fig. A3.9).

Aboveground biomass

For the subset of plants that survived until harvest, the single-species analyses showed that three of the 12 species, i.e. the non-native *Amaranthus retroflexus* and *D. stramonium*, and the native *Chenopodium album*, produced significantly more aboveground biomass in the Potsdam transplant region than in the Konstanz transplant region, whereas the reverse was true for the native Plantago major (Table A3.6, Figs. A3.5-6 and A3.14-15). The origin effects also varied among species: for the non-native *D. stramonium* and *Veronica persica*, and the native *S. vulgaris*, plants from the Konstanz origin produced more biomass than those from the Potsdam origin (Table A3.6, Figs. A3.10, A3.13 and A3.15), whereas the reverse was true for the non-native *Erigeron annuus*, and the native *L. serriola* and *Solanum nigrum* (Table A3.6, Figs. A3.8, A3.9 and A3.16). There were significant region:origin interactions for aboveground biomass in

five of the 12 species (Table A3.6). However, only for one of those five species, the native *S. vulgaris*, the local plants produced more biomass than the non-local plants, both in the Konstanz and Potsdam transplant regions (Fig. A3.10). For the other four species, the non-native *A. retroflexus*, *D. stramonium* and *E. annuus*, and the native *L. serriola*, the non-local plants tended to produce more aboveground biomass than the local plants, at least in one of the two transplant regions (Figs. A3.6, A3.8, A3.9, and A3.15). So, the single-species analyses of aboveground biomass production provided more evidence for local maladaptation than adaptation. The zebularine treatment had negative effects on biomass production of seven of the species (see Table A3.6 and Figs. A3.8-14) and a positive effect for one of them (see Table A3.6 and Fig. A3.6), but it did not significantly affect the expression of local adaptation or maladaptation (no significant region:treatment:origin interaction in Table A3.6).

Flowering probability

Of the 2953 plants included in our flowering analyses, 2316 (78.4%) flowered until harvest. All 12 species flowered, but the flowering percentage ranged from 11.1% for the native *L. serriola* to 97.4% for the native *S. nigrum* (Table A3.7). The single-species analyses showed that for all 12 study species flowering probabilities were similar in the Konstanz and Potsdam transplant regions (Table A3.7). For two of the non-native species, *A. retroflexus* and *E. annuus*, plants from the Potsdam origin were more likely to flower than those from the Konstanz origin (Table A3.7, Figs. A3.5 and A3.8). For *A. retroflexus* the origin effect was most pronounced in the Konstanz transplant region, whereas in the Potsdam transplant region plants from Konstanz tended to be more likely to flower than the ones from Potsdam (significant region:origin interaction in Table A3.7, Fig. A3.5). On the other hand, for the non-native *D. stramonium* local plants were more likely to flower than the non-local plants (significant region:origin interaction in Table A3.7, Fig. A3.15). The zebularine treatment had a significantly negative effect on flowering of the native *Sonchus oleraceus* (Table A3.7, Fig. 4.11). Interestingly, the flowering advantage of local over non-local plants in *D. stramonium* tended to disappear in the zebularine treatment, as indicated by a marginally non-significant (P = 0.052) region:origin:zebularine interaction (Table A3.7, Fig. A3.15). So, overall, the single-species analyses of flowering probability provided some scant evidence for both local maladaptation and local adaptation, and showed that the latter might be mediated by DNA methylation.

Reproductive biomass

For the subset of plants that flowered, the single-species analyses showed that six of the 12 species, i.e. the non-native *A. retroflexus*, *D. stramonium*, *E. canadensis* and *V. persica*, and the native *Ch. album* and *L. serriola*, produced significantly more reproductive biomass in the Potsdam transplant region than in the Konstanz transplant region, whereas the reverse was true for

the native *P. major* (Table A3.8, Figs. A3.5-7, A3.9, A3.13-15). The origin effects also varied among species: for the native *Ch. album*, plants from the Konstanz origin tended to produce more reproductive biomass than those from the Potsdam origin (Table A3.8, Fig. A3.6), whereas the reverse was true for the non-native *A. retroflexus* and *V. persica* and the native *S. nigrum* (Table A3.8, Figs. A3.5, A3.13 and A3.16). There were significant region:origin interactions for reproductive biomass in five of the 12 species (Table A3.8). However, only for one of those five species, the native *S. vulgaris*, the local plants produced more reproductive biomass than the non-local plants, both in the Konstanz and Potsdam transplant regions (Fig. A3.10). For three other species, the non-native *A. retroflexus* and *E. annuus* and the native *Ch. album*, the non-local plants tended to produce more reproductive biomass than the local plants, at least in one of the two transplant regions (Figs. S5- A3.6, A3.8). For a fifth species, the non-native *E. canadensis*, local plants produced more reproductive biomass than non-local plants in Konstanz transplant region, but data is missing for local plants at Potsdam transplant region (Fig. A3.8). So, the single-species analyses of reproductive biomass provided partial evidence for local adaptation but particularly for local maladaptation. The zebularine treatment had negative effects on biomass production of seven of the 12 species (Table S8, Figs. A3.7, A3.10-14 and A3.16) and a positive effect for one of them (Table A3.8, Fig. A3.6), but it did not significantly affect the expression of local adaptation or maladaptation (no significant region:treatment:origin interaction in Table A3.8).

Notes A3.2: Results of the effect sizes summarized at the region-level

Survival

There were significant positive effect sizes for the native species *Lactuca serriola* in the control treatment but not in the zebularine treatment in the Potsdam field sites (Fig. A3.4A). For the non-native species *Veronica persica* and *Datura stramonium*, there were significant positive effect sizes in both the control and the zebularine treatments in the Konstanz field sites (Fig. A3.4A).

Aboveground biomass

Besides the negative effect size for *Erigeron annuus*, which was only apparent in the Konstanz transplant region despite its prominence in across-region effect sizes (Fig. 4.1), there were also a negative effect sizes for the non-native *V. persica* in the zebularine treatment of the Potsdam transplant region and for the native *Solanum nigrum* in the zebularine treatment of the Konstanz transplant region (Fig. A3.4B). Moreover, there were positive biomass effect sizes for the native Senecio vulgaris in the control treatment of the Konstanz transplant region and for the native *S. nigrum* in both treatments of the Potsdam transplant region (Fig. A3.4B).

Flowering probability

When the flowering effect sizes were plotted for each transplant region separately (Fig. A3.4C), it appears that at least in the Konstanz transplant region the positive effect size for *Erigeron canadensis* that was found for across-region effect sizes (Fig. 4.1) was not present in the control treatment. These plots also revealed a significant negative flowering effect size for the non-native *E. annuus* in the control treatment of the Konstanz transplant region, and significantly positive flowering effect sizes for the native *Sonchus oleraceus* in both treatments of the Konstanz transplant region (Fig. A3.4C).

Reproductive biomass

When the reproductive biomass effect sizes were plotted for each transplant region separately (Fig. A3.4D), however, the negative effect size for *Chenopodium album* which was prominent in the across-region effect sizes (Fig. 4.1) was only present in the Konstanz transplant region, and for both treatments. These plots also revealed significant negative reproductive biomass effect sizes for the non-native *Amaranthus retroflexus* in both treatments of the Konstanz transplant region, for the non-native *E. annuus* in the control treatment of the Potsdam transplant region, and for the non-native *V. persica* in the control treatment of the Konstanz transplant region (Fig. A3.4D). There was, however, a significant positive reproductive biomass effect sizes for the native Plantago major in the control treatment of the Konstanz transplant region (Fig. A3.4D). For the native *S. nigrum*, the reproductive biomass effect size was negative, and significantly so in the zebularine treatment in the Konstanz transplant region, whereas they were significantly positive in the Potsdam transplant region (Fig. A3.4D).

Table A3.1: Number of used maternal lines per species and sampling region. K – Konstanz transplant region; P – Potsdam transplant region. Species names of the natives are in black, and the ones of the non-natives are in red.

Population	Species	Source	Maternal lines	Location	Latitude	Longitude
	Amaranthaceae					
1	*Amaranthus retroflexus*	K	17		47.76131	8.820415
2		P	10		52.49144	12.98392
3	*Chenopodium album*	K	19		47.71661	8.908667
4		P	20		52.40323	13.02458
	Asteraceae					
5	*Erigeron canadensis*	K	20		47.67347	9.1538
6		P	20		52.40306	12.97156
7	*Erigeron annuus*	K	17		47.69225	9.177502
8		P	10		52.3923	13.06947
9	*Lactuca serriola*	K	18		47.65978	9.132178
10		P	20		52.41968	13.05278
11	*Senecio vulgaris*	K	18		47.68695	9.072934
12		P	11		52.40367	13.0241
13	*Sonchus oleraceus*	K	13		47.67347	9.1538
14		P	10		52.41968	13.05278
15	*Tripleurospermum inodorum*	K	17		47.67964	9.139306
16		P	20		52.39775	12.97966
	Plantaginaceae					
17	*Veronica persica*	K	17		47.72857	9.161455
18		P	16		52.40833	13.01942
19	*Plantago major*	K	17		47.69106	9.187332
20		P	18		52.40811	13.02132
	Solanaceae					
21	*Datura stramonium*	K	15		47.76201	8.802124
22		P	19		52.36363	12.96103
23	*Solanum nigrum*	K	21		47.70683	9.166862
24		P	9		52.40291	13.02453

Diese Stelle enthält persönliche Daten. Sie ist deshalb nicht Bestandteil der Online-Veröffentlichung.

This section contains personal data. It is therefore not part of the online publication.

Table A3.2: Notes on species-specific treatments (pre-sowing treatment + sowing date [day.month.year]). Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Species	Status	Pre-sowing treatment	Sowing date
Amaranthaceae	*Amaranthus retroflexus*	non-native	–	22.04.2016 (Konstanz)
			–	22.04.2016 (Potsdam)
	Chenopodium album	native	Chemical scarification	22.04.2016 (Konstanz)
		native	(3 min in 96 % H_2SO_4)	24.04.2016 (Potsdam)
Asteraceae	*Erigeron canadensis*	non-native	–	20.04.2016 (Konstanz)
			–	22.04.2016 (Potsdam)
	Erigeron annuus	non-native	–	20.04.2016 (Konstanz)
			–	22.04.2016 (Potsdam)
	Lactuca serriola	native	–	24.04.2016 (Konstanz)
			–	26.04.2016 (Potsdam)
	Senecio vulgaris	native	–	24.04.2016 (Konstanz)
			–	26.04.2016 (Potsdam)
	Sonchus oleraceus	native	–	20.04.2016 (Konstanz)
			–	22.04.2016 (Potsdam)
	Tripleurospermum inodorum	native	–	24.04.2016 (Konstanz)
			–	26.04.2016 (Potsdam)
Plantaginaceae	*Veronica persica*	non-native	–	18.04.2016 (Konstanz)
			–	19.04.2016 (Potsdam)
	Plantago major	native	Soaked in ddH_2O for 48 h	20.04.2016 (Konstanz)
				21.04.2016 (Potsdam)
Solanaceae	*Datura stramonium*	non-native	Soaked in ddH_2O for 24 h	19.04.2016 (Konstanz)
				20.04.2016 (Potsdam)
	Solanum nigrum	native	Soaked in ddH_2O for 24 h	19.04.2016 (Konstanz)
				20.04.2016 (Potsdam)

Table A3.3: Notes on time-line of the experiment (transplanting, planting and harvesting) per species and region. Dates are in the format day.month.year. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Species	Status	Transplanting date	Planting date	Harvesting date
Amaranthaceae	*Amaranthus retroflexus*	non-native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Chenopodium album	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
Asteraceae	*Erigeron canadensis*	non-native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Erigeron annuus	non-native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Lactuca serriola	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Senecio vulgaris	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Sonchus oleraceus	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Tripleurospermum inodorum	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
Plantaginaceae	*Veronica persica*	non-native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Plantago major	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
Solanaceae	*Datura stramonium*	non-native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
	Solanum nigrum	native	27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)
			27.-30.04.2016 (Konstanz)	17.-25.05.2016 (Konstanz)	23.-30.06.2016 (Konstanz)

Table A3.4: Description of the field sites in the Konstanz region (black) and the Potsdam region (blue).

Site ID	Site	Latitude [°N]	Longitude [°E]	Address	Environmental gradient	Plant cover at start [%]	Plant cover at end [%]	Plant cover forbs [%][a]	Plant cover grasses [%][a]
MQ	Potsdam Marquard	52.46647	12.95647	*Diese Stelle enthält persönliche Daten. Sie ist deshalb nicht Bestandteil der Online-Veröffentlichung. This section contains personal data. It is therefore not part of the online publication.*	Soil moisture (lower part of field contrastingly wetter)	0	100	70	30
DB	Potsdam Botanical Garden	52.40769	13.0224		Shading	0	100	90	10
GR	Ludwigsfelde/Gröben	52.28219	13.16803		Shading	0	25	20	80
Boga	Konstanz/Botanical Garden	47.69216	9.179243		Soil moisture (upper part of field slightly drier than lower part)	~1	100	30	50
Hättli	Konstanz/Hättelihof	47.68384	9.19343		Soil moisture	~2	75	35	40
Uni	Konstanz/University	47.69081	9.186349		Shading (e.g. only full sun exposure 9:00 – 17: 00, Mid-August), soil msoisture	~5-10	100	55	10

[a]Plant cover of forbs and grasses was scored in August 2016 (i.e. at the end of the growing season)

Table A3.5: Results of generalized linear mixed models for survival. Analysis followed the prior specification by Bolker and Hadfield (see Methods A3.1) for the analysis of datasets with quasi- or complete separation (i.e. complete 0s or 1s in one of the subgroups). We accounted for the random effects of maternal lines and blocks nested within field sites. Significant *P*-values at the $P < 0.05$ threshold are marked in bold. CI is the 95% credible interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Species	***Amaranthus retroflexus***				***Chenopodium album***			
Sample size n	214 plants (95.3% surviving)				282 plants (87.9% surviving)			
Ratio of non-event to event	10 dead		204 surviving		34 dead		248 surviving	
Status	non-native				native			
Plant family	Amaranthaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	**3.98**	**1.74**	**6.45**	**0.009**	4.15	-0.39	8.17	0.086
Region [R]	0.48	-2.39	3.89	0.784	-1.13	-5.37	3.70	0.581
Zebularine treatment [Z]	-0.50	-2.32	1.34	0.589	-1.42	-4.07	1.30	0.281
Origin [O]	3.12	-0.37	6.70	0.062	-0.54	-3.58	2.30	0.707
R×Z	0.13	-3.00	3.26	0.935	1.09	-1.70	3.95	0.462
R×O	-1.29	-5.49	2.88	0.534	-0.68	-3.74	2.43	0.663
Z×O	0.07	-3.86	4.09	0.977	-1.34	-4.52	1.51	0.392
R×Z×O	-2.52	-7.11	1.79	0.260	2.32	-1.18	5.84	0.200
Species	***Veronica persica***				***Plantago major***			
Sample size n	321 plants (73.2% surviving)				324 plants (88.9% surviving)			
Ratio of non-event to event	86 dead		235 surviving		36 dead		288 surviving	
Status	non-native				native			
Plant family	Plantaginaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	PMCMC	Posterior mean	L-95% CI	U-95% CI	PMCMC
Intercept	2.59	-2.56	7.25	0.3	4.49	1.5	7.4	0.016
Region [R]	-1.25	-6.42	4.04	0.627	-0.65	-3.96	2.84	0.658
Zebularine treatment [Z]	-1.56	-4.41	1.15	0.257	-0.11	-2.24	2.18	0.916
Origin [O]	-0.23	-3.11	2.64	0.878	-0.24	-2.53	2.06	0.826
R×Z	1.45	-1.49	4.45	0.335	-1.7	-4.3	0.66	0.171
R×O	0.86	-2.1	3.81	0.583	-1.47	-4.09	1.03	0.251
Z×O	-2.78	-5.83	0.08	0.063	0.55	-2.39	3.52	0.72
R×Z×O	1.59	-1.62	4.84	0.328	1.58	-1.62	4.77	0.329

(**Table A3.5** continued from previous page)

Species	***Datura stramonium***				***Solanum nigrum***			
Sample size n	282 plants (80.9% surviving)				356 plants (92.7% surviving)			
Ratio of non-event to event	54 dead		228 surviving		26 dead		330 surviving	
Status	non-native				native			
Plant family	Solanaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	PMCMC	Posterior mean	L-95% CI	U-95% CI	PMCMC
Intercept	3.27	0.61	5.76	0.033	4.78	1.27	8.26	0.032
Region [R]	-0.72	-3.88	2.58	0.601	-1.94	-5.72	2.14	0.308
Zebularine treatment [Z]	0.19	-1.59	2.05	0.849	-0.48	-2.96	2.15	0.72
Origin [O]	-3.46	-5.2	-1.84	<1e-04	0.65	-2.31	3.64	0.68
R×Z	-0.38	-2.51	1.69	0.728	0.98	-1.8	3.63	0.482
R×O	2.92	0.92	4.91	0.004	-0.06	-3.17	3.02	0.986
Z×O	0.45	-1.65	2.68	0.692	-1.24	-4.63	1.89	0.45
R×Z×O	-0.58	-3.22	2.02	0.674	0.24	-3.27	3.61	0.899
Species	***Erigeron canadensis***				***Erigeron annuus***			
Sample size n	313 plants (75.1% surviving)				317 plants (80.1% surviving)			
Ratio of non-event to event	235 surviving		63 dead		254 surviving		235 surviving	
Status	non-native				non-native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	PMCMC	Posterior mean	L-95% CI	U-95% CI	PMCMC
Intercept	0.97	-4.37	6.35	0.712	1.5	-3.74	6.18	0.53
Region [R]	-0.63	-6.18	4.91	0.822	-0.71	-5.97	4.48	0.777
Zebularine treatment [Z]	0.69	-1.24	2.61	0.479	-0.33	-2.37	1.71	0.749
Origin [O]	0.25	-2.84	3.14	0.856	0.76	-1.65	3.13	0.532
R×Z	0.73	-1.82	3.5	0.595	-1.68	-4.54	0.92	0.223
R×O	-0.74	-4.88	3.68	0.737	-1.16	-3.95	1.87	0.432
Z×O	-4.12	-7.28	-1.07	0.005	0.66	-2.34	3.53	0.666
R×Z×O	3.39	-0.94	7.8	0.131	0.79	-2.63	4.29	0.65

(**Table A3.5** continued from previous page)

Species	***Lactuca serriola***				***Senecio vulgaris***			
Sample size n	427 plants (42.9% surviving)				287 plants (97.9% surviving)			
Ratio of non-event to event	244 dead		183 surviving		6 dead		281 surviving	
Status	native				native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	PMCMC	Posterior mean	L-95% CI	U-95% CI	PMCMC
Intercept	-0.55	-3.67	2.35	0.699	3.97	-2.44	9.42	0.235
Region [R]	-2.05	-5.93	1.8	0.296	0.51	-4.5	5.69	0.869
Zebularine treatment [Z]	-0.54	-1.59	0.46	0.313	1.62	-1.72	5.26	0.361
Origin [O]	1.06	-0.09	2.17	0.058	-0.45	-4.18	3.55	0.802
R×Z	1.97	0.29	3.77	0.024	-1.68	-5.47	2.02	0.376
R×O	1.94	0.09	3.7	0.033	1.29	-3.13	5.75	0.557
Z×O	-0.77	-2.2	0.68	0.291	0.32	-4.1	4.27	0.883
R×Z×O	-0.98	-3.24	1.26	0.389	-1.55	-6.23	2.94	0.505
Species	***Sonchus oleraceus***				***Tripleurospermum inodorum***			
Sample size n	398 plants (78.4% surviving)				398 plants (78.4% surviving)			
Ratio of non-event to event	8 dead		200 surviving		86 dead		312 surviving	
Status	native				native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	PMCMC	Posterior mean	L-95% CI	U-95% CI	PMCMC
Intercept	3.37	-3.33	9.33	0.35	3.61	-0.54	7.26	0.096
Region [R]	0.51	-4.94	5.98	0.86	-1.84	-6.19	2.82	0.4
Zebularine treatment [Z]	-0.22	-4.49	3.91	0.928	1.24	-1.16	3.82	0.306
Origin [O]	0	-4.58	4.56	0.993	0.52	-1.7	2.61	0.638
R×Z	-1.59	-5.82	2.59	0.456	-1.49	-4.2	1.03	0.249
R×O	-0.89	-5.42	3.65	0.694	-1.26	-3.61	1.16	0.3
Z×O	0.78	-3.78	5.38	0.74	-1.34	-4.15	1.48	0.352
R×Z×O	0.37	-4.33	4.86	0.867	1.51	-1.51	4.58	0.323

Table A3.6: Results of linear mixed models for complete aboveground biomass. Data was power transformed to fulfil assumption of normality and homogeneity of variance in the residuals for the linear mixed models. We accounted for the random effects of maternal lines and blocks nested within field sites. Significant *P*-values at the $P < 0.05$ threshold are marked in bold. Species names of the natives are in black, and the ones of the non-natives are in red.

Species	***Amaranthus retroflexus***		***Chenopodium album***		***Veronica persica***		***Plantago major***	
No. of fields	6		6		6		6	
No. of blocks	18		18		16		18	
Maternal lines	27		30		31		33	
Sample size n	204 plants		244 plants		231 plants		281 plants	
Data trans-formation (y^x)	x=0.1		x=0.1		x=0.1		x=0.1	
Status	non-native		native		non-native		native	
Plant family	Amaranthaceae				Plantaginaceae			
Moderators	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*
Region [R]	**4.05**	**0.044**	**7.07**	**0.008**	0.01	0.924	**3.86**	**0.049**
Zebularine treatment [Z]	2.55	0.111	**5.87**	**0.015**	**15.66**	**<1e-03**	**12.25**	**<1e-03**
Origin [O]	1.33	0.249	0.58	0.447	**5.41**	**0.020**	1.22	0.269
R×Z	2.14	0.143	**4.21**	**0.040**	**3.98**	**0.046**	0.35	0.555
R×O	**5.65**	**0.017**	3.68	0.055	0.56	0.455	0.00	0.963
Z×O	1.75	0.186	0.04	0.841	**12.64**	**<1e-03**	**7.01**	**0.008**
R×Z×O	0.73	0.394	0.72	0.396	3.41	0.065	1.81	0.178
Species	***Datura stramonium***		***Solanum nigrum***		***Erigeron canadensis***		***Erigeron annuus***	
No. of fields	6		6		5		5	
No. of blocks	18		18		15		15	
Maternal lines	28		30		38		35	
Sample size n	216 plants		327 plants		233 plants		250 plants	
Data trans-formation (y^x)	x=0.1		x=0.1		x=0.1		x=0.1	
Status	non-native		native		non-native		non-native	
Plant family	Solanaceae				Asteraceae			
Moderators	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*
Region [R]	**6.39**	**0.011**	3.30	0.069	0.10	0.750	0.43	0.510
Zebularine treatment [Z]	0.31	0.575	2.83	0.093	1.57	0.211	**6.48**	**0.011**
Origin [O]	**11.65**	**<1e-03**	**11.01**	**<1e-03**	0.15	0.696	**4.39**	**0.036**
R×Z	0.11	0.741	1.50	0.220	1.01	0.315	0.08	0.775
R×O	**5.59**	**0.018**	2.55	0.110	1.58	0.209	**10.84**	**<1e-03**
Z×O	0.06	0.800	1.07	0.301	0.32	0.575	0.71	0.398
R×Z×O	0.67	0.413	1.41	0.234	NA	NA	2.23	0.136

(**Table A3.6** continued from previous page)

Species	***Lactuca serriola***		***Senecio vulgaris***		***Sonchus oleraceus***		***Tripleurospermum inodorum***	
No. of fields	6		6		6		6	
No. of blocks	16		18		18		17	
Maternal lines	38		29		19		34	
Sample size n	182 plants		279 plants		197 plants		307 plants	
Data trans-formation (y^x)	x=0.1		x=0.1		x=0.1		x=0.1	
Status	native		native		native		native	
Plant family	Asteraceae							
Moderators	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*	χ^2 (d.f. = 1)	*P*
Region [R]	0.29	0.589	0.34	0.562	0.38	0.540	0.10	0.752
Zebularine treatment [Z]	**10.93**	**<1e-03**	**26.71**	**<1e-06**	**5.78**	**0.016**	**9.70**	**0.002**
Origin [O]	**7.73**	**0.005**	**5.67**	**0.017**	1.66	0.197	0.92	0.337
R×Z	0.11	0.743	**18.23**	**<1e-04**	0.12	0.734	**14.62**	**<1e-03**
R×O	**6.87**	**0.009**	**4.46**	**0.035**	0.05	0.830	0.17	0.678
Z×O	0.60	0.438	0.82	0.366	1.10	0.295	0.00	0.994
R×Z×O	0.00	0.997	0.09	0.763	0.18	0.675	1.01	0.314

Table A3.7: Results of generalized linear mixed models for flowering probability. Analysis followed the prior specification by Bolker and Hadfield (see Methods A3.1) for the analysis of datasets with quasi- or complete separation (i.e. complete 0s or 1s in one of the subgroups). We accounted for the random effects of maternal lines and blocks nested within field sites. CI is the 95% credible interval. Significant *P*-values at the $P < 0.05$. Species names of the natives are in black, and the ones of the non-natives are in red.

Species	***Amaranthus retroflexus***				***Chenopodium album***			
Sample size n	204 plants (94.1% flowering)				248 plants (89.9% flowering)			
Ratio of non-event to event	12 non-flowering		192 flowering		25 non-flowering		223 flowering	
Status	non-native				native			
Plant family	Amaranthaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	**2.67**	**0.14**	**5.26**	**0.047**	3.30	-0.67	7.19	0.108
Region [R]	1.70	-1.75	5.28	0.310	0.99	-3.28	5.50	0.663
Zebularine treatment [Z]	1.52	-0.26	3.36	0.075	-1.40	-3.12	0.22	0.092
Origin [O]	**2.71**	**-0.08**	**5.39**	**0.030**	3.10	-0.10	6.61	0.059
R×Z	-1.00	-4.44	2.26	0.556	1.13	-1.41	3.69	0.386
R×O	**-4.08**	**-7.81**	**-0.26**	**0.035**	-0.53	-4.81	3.72	0.789
Z×O	0.82	-3.13	4.90	0.706	-1.75	-5.15	1.56	0.297
R×Z×O	-0.65	-5.10	3.69	0.790	-1.74	-6.25	2.97	0.460
Species	***Veronica persica***				***Plantago major***			
Sample size n	235 plants (95.3% flowering)				283 plants (93.6% flowering)			
Ratio of non-event to event	11 non-flowering		224 flowering		18 non-flowering		265 flowering	
Status	non-native				native			
Plant family	Plantaginaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	3.65	-1.35	7.78	0.133	4.96	-0.42	9.50	0.095
Region [R]	-0.14	-4.70	4.66	0.925	-1.40	-5.71	3.64	0.510
Zebularine treatment [Z]	-0.59	-2.62	1.49	0.578	-0.52	-3.62	2.43	0.742
Origin [O]	2.67	-0.31	5.98	0.076	2.68	-0.91	6.95	0.168
R×Z	-1.30	-4.36	1.64	0.399	-0.68	-3.94	2.60	0.659
R×O	-1.50	-5.29	2.18	0.428	-0.19	-4.11	3.69	0.936
Z×O	1.33	-2.8	5.21	0.524	-0.16	-4.3	3.78	0.929

(**Table A3.7** continued from previous page)

Species	***Datura stramonium***				***Solanum nigrum***			
Sample size n	213 plants (81.7% flowering)				327 plants (97.4% flowering)			
Ratio of non-event to event	39 non-flowering		174 flowering		39 non-flowering		174 flowering	
Status	non-native				native			
Plant family	Solanaceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	**1.83**	**0.35**	**3.70**	**0.046**	**5.38**	**1.05**	**9.00**	**0.038**
Region [R]	0.49	-1.74	2.71	0.644	-1.16	-4.85	3.07	0.470
Zebularine treatment [Z]	0.28	-0.99	1.61	0.692	-0.02	-2.80	2.89	0.991
Origin [O]	-1.00	-2.71	0.76	0.278	2.59	-1.18	6.71	0.187
R×Z	0.65	-1.27	3.07	0.546	0.74	-2.43	3.91	0.643
R×O	**3.49**	**0.76**	**6.30**	**0.008**	0.17	-4.03	4.24	0.934
Z×O	-0.73	-2.80	1.40	0.508	-0.86	-5.04	3.40	0.676
R×Z×O	-3.24	-6.52	0.26	0.052	-2.51	-6.88	1.70	0.254
Species	***Erigeron canadensis***				***Erigeron annuus***			
Sample size n	235 plants (50.6% flowering)				254 plants (44.9% flowering)			
Ratio of non-event to event	116 non-flowering		119 flowering		140 non-flowering		114 flowering	
Status	non-native				non-native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	-0.19	-4.45	3.56	0.930	-0.70	-3.87	2.64	0.631
Region [R]	-1.02	-5.76	3.81	0.674	-0.36	-4.61	3.90	0.864
Zebularine treatment [Z]	0.74	-0.30	1.85	0.177	-0.11	-1.20	1.00	0.843
Origin [O]	-0.95	-2.51	0.56	0.219	**1.72**	**0.59**	**3.03**	**0.004**
R×Z	-0.80	-3.21	1.74	0.516	-0.18	-2.61	2.18	0.872
R×O	2.92	-0.92	6.93	0.140	-1.81	-3.98	0.51	0.114
Z×O	**-2.26**	**-3.94**	**-0.51**	**0.007**	-0.72	-2.27	0.75	0.363
R×Z×O	NA	NA	NA	NA	0.76	-2.17	3.75	0.615

(**Table A3.7** continued from previous page)

Species	***Lactuca serriola***				***Senecio vulgaris***			
Sample size n	181 plants (11.1% flowering)				265 plants (95.1% flowering)			
Ratio of non-event to event	161 non-flowering		20 flowering		13 non-flowering		252 flowering	
Status	native				native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	-3.68	-7.6	0.69	0.084	**4.71**	**1.27**	**7.96**	**0.032**
Region [R]	-0.21	-4.7	4.2	0.936	-0.82	-4.16	3.10	0.575
Zebularine treatment [Z]	-0.88	-3.38	1.66	0.494	-0.52	-2.72	1.70	0.641
Origin [O]	-1.75	-5.04	1.32	0.269	1.56	-1.76	4.81	0.357
R×Z	-1.49	-4.81	2.27	0.405	-0.96	-3.52	1.70	0.459
R×O	0.78	-3.04	4.36	0.658	-1.67	-5.50	2.18	0.397
Z×O	1.79	-1.29	4.84	0.243	2.60	-1.67	7.12	0.252
R×Z×O	-1.58	-5.35	2.46	0.433	1.63	-2.97	6.86	0.535
Species	***Sonchus oleraceus***				***Tripleurospermum inodorum***			
Sample size n	199 plants (76.9% flowering)				312 plants (84.0% flowering)			
Ratio of non-event to event	46 non-flowering		153 flowering		50 non-flowering		262 flowering	
Status	native				native			
Plant family	Asteraceae							
Moderators	Posterior mean	L-95% CI	U-95% CI	*P*MCMC	Posterior mean	L-95% CI	U-95% CI	*P*MCMC
Intercept	**3.65**	**0.72**	**6.31**	**0.014**	2.74	0.20	5.51	0.056
Region [R]	0.10	-2.64	3.10	0.956	0.55	-2.48	3.95	0.750
Zebularine treatment [Z]	**-1.76**	**-3.69**	**-0.01**	**0.048**	-0.81	-2.19	0.39	0.234
Origin [O]	-1.77	-5.22	1.95	0.312	-0.61	-2.12	0.74	0.396
R×Z	-1.57	-4.14	0.76	0.212	-0.32	-2.30	1.57	0.742
R×O	0.49	-2.25	3.20	0.725	1.19	-1.15	3.66	0.344
Z×O	-0.31	-2.86	2.25	0.806	1.55	-0.65	3.25	0.116
R×Z×O	1.38	-2.00	4.56	0.414	-1.55	-4.43	1.33	0.288

Table A3.8: Results of linear mixed models for reproductive biomass. Data was power transformed to fulfil assumption of normality and homogeneity of variance in the residuals for the linear mixed models. We accounted for the random effects of maternal lines and blocks nested within field sites. Significant *P*-values at the $P < 0.05$ threshold are marked in bold. Species names of the natives are in black, and the ones of the non-natives are in red.

Species	***Amaranthus retroflexus***		***Chenopodium album***		***Veronica persica***		***Plantago major***	
No. of fields	6		6		6		6	
No. of blocks	18		18		16		18	
Maternal lines	27		30		31		33	
Sample size n	192 plants		223 plants		220 plants		258 plants	
Data trans-formation (y^x)	x=0.1		x=0.1		x=0.1		x=0.1	
Status	non-native		native		non-native		native	
Plant family	Amaranthaceae				Plantaginaceae			
Moderators	$\chi 2$ (d.f. =1)	*P*	$\chi 2$ (d.f. =1)	*P*	$\chi 2$ (d.f. =1)	*P*	$\chi 2$ (d.f.=1)	*P*
Region [R]	**4.42**	**0.036**	**11.04**	**<1e-03**	3.24	0.072	**5.97**	**0.015**
Zebularine treatment [Z]	0.17	0.681	**7.98**	**0.005**	**14.99**	**<1e-03**	**7.55**	**0.006**
Origin [O]	**22.18**	**<1e-05**	**9.98**	**0.002**	**8.44**	**0.004**	2.21	0.137
R×Z	3.37	0.066	0.17	0.680	0.57	0.451	1.15	0.283
R×O	**12.58**	**<1e-03**	**7.67**	**0.006**	1.30	0.254	0.42	0.517
Z×O	1.03	0.309	0.01	0.910	**6.07**	**0.014**	**5.38**	**0.020**
R×Z×O	1.69	0.194	0.49	0.486	1.39	0.238	0.41	0.521
Species	***Datura stramonium***		***Solanum nigrum***		***Erigeron canadensis***		***Erigeron annuus***	
No. of fields	6		6		4		5	
No. of blocks	18		18		12		13	
Maternal lines	27		30		34		34	
Sample size n	172 plants		315 plants		118 plants		112 plants	
Data trans-formation (y^x)	x=0.2		x=0.2		x=0.1		x=0.2	
Status	non-native		native		non-native		non-native	
Plant family	Solanaceae				Asteraceae			
Moderators	$\chi 2$ (d.f. =1)	*P*	$\chi 2$ (d.f.=1)	*P*	$\chi 2$ (d.f.=1)	*P*	$\chi 2$ (d.f.=1)	*P*
Region [R]	**5.80**	**0.016**	**5.85**	**0.016**	**5.85**	**0.016**	2.30	0.129
Zebularine treatment [Z]	1.50	0.221	**6.84**	**0.009**	**6.84**	**0.009**	0.41	0.523
Origin [O]	3.13	0.077	1.40	0.237	1.40	0.237	3.51	0.061
R×Z	0.01	0.919	0.53	0.467	0.53	0.467	0.32	0.574
R×O	1.27	0.259	**4.78**	**0.029**	**4.78**	**0.029**	**6.06**	**0.014**
Z×O	1.21	0.272	0.59	0.442	0.59	0.442	1.29	0.256
R×Z×O	1.73	0.188	NA	NA	NA	NA	1.35	0.245

(**Table A3.8** continued from previous page)

Species	***Lactuca serriola***		***Senecio vulgaris***		***Sonchus oleraceus***		***Tripleurospermum inodorum***	
No. of fields	5		6		6		6	
No. of blocks	9		18		18		17	
Maternal lines	10		29		17		34	
Sample size n	20 plants		252 plants		152 plants		259 plants	
Data trans-formation (y^x)	x=0.1		x=0.2		x=0.1		x=0.2	
Status	native		native		native		native	
Plant family	Asteraceae							
Moderators	$\chi2$ (d.f. =1)	*P*	$\chi2$ (d.f. =1)	*P*	$\chi2$ (d.f. =1)	*P*	$\chi2$ (d.f. =1)	*P*
Region [R]	**3.84**	**0.050**	0.12	0.729	0.52	0.470	0.31	0.579
Zebularine treatment [Z]	3.24	0.072	**16.10**	**<1e-04**	**5.92**	**0.015**	**4.03**	**0.045**
Origin [O]	0.95	0.330	0.12	0.730	0.48	0.489	2.14	0.144
R×Z	1.39	0.239	**8.37**	**0.004**	2.39	0.122	**9.40**	**0.002**
R×O	1.00	0.317	**5.68**	**0.017**	0.00	0.977	0.10	0.753
Z×O	2.66	0.103	0.09	0.764	0.01	0.929	1.91	0.167
R×Z×O	1.88	0.171	0.03	0.854	0.06	0.807	1.68	0.195

Table A3.9: Estimates and confidence intervals of survival effect sizes summarized at the region-level, in Fig. A3.4A). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. K – Konstanz transplant region; P – Potsdam transplant region; CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	K	CON	-0.724	-2.928	1.480
				ZEB	-1.093	-3.270	1.085
			P	CON	0.453	-2.542	3.448
				ZEB	-0.407	-2.259	1.445
	native	*Chenopodium album*	K	CON	0.000	-2.415	2.415
				ZEB	1.034	-0.600	2.667
			P	CON	-1.331	-3.009	0.347
				ZEB	-0.300	-2.229	1.628
Asteraceae	non-native	*Erigeron canadensis*	K	CON	-0.864	-2.168	0.441
				ZEB	0.777	-0.402	1.957
			P	CON	-1.513	-7.330	4.303
				ZEB	0.303	-1.836	2.441
		Erigeron annuus	K	CON	-0.331	-2.076	1.413
				ZEB	-0.332	-2.049	1.386
			P	CON	-0.335	-2.057	1.387
				ZEB	0.855	-0.533	2.242
	native	*Lactuca serriola*	K	CON	-0.467	-1.487	0.552
				ZEB	-0.148	-0.894	0.598
			P	CON	**1.666**	**0.438**	**2.893**
				ZEB	0.720	-0.738	2.179
		Senecio vulgaris	K	CON	0.673	-1.483	2.829
				ZEB	0.000	-2.422	2.422
			P	CON	0.622	-2.285	3.529
				ZEB	-0.565	-2.720	1.591
		Sonchus oleraceus	K	CON	0.000	-2.526	2.526
				ZEB	0.000	-2.519	2.519
			P	CON	-0.385	-2.332	1.561
				ZEB	0.248	-2.235	2.730
		Tripleurospermum inodorum	K	CON	-0.363	-2.066	1.340
				ZEB	0.855	-1.152	2.862
			P	CON	-0.499	-1.424	0.425
				ZEB	-0.161	-1.019	0.696

(**Table A3.9** continued from previous page)

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Plantaginaceae	non-native	*Veronica persica*	K	CON	0.000	-2.322	2.322
				ZEB	**1.538**	**0.013**	**3.063**
			P	CON	0.235	-1.237	1.707
				ZEB	-0.149	-1.412	1.115
	native	*Plantago lanceolata*	K	CON	0.090	-1.732	1.911
				ZEB	0.095	-1.936	2.125
			P	CON	-1.176	-2.389	0.037
				ZEB	0.015	-1.124	1.153
Solanaceae	non-native	*Datura stramonium*	K	CON	**2.525**	**0.968**	**4.081**
				ZEB	**1.653**	**0.255**	**3.050**
			P	CON	-0.302	-1.925	1.322
				ZEB	-0.442	-1.555	0.671
	native	*Solanum nigrum*	K	CON	0.000	-2.494	2.494
				ZEB	0.487	-1.616	2.590
			P	CON	0.208	-1.178	1.594
				ZEB	-0.333	-1.587	0.922

Table A3.10: Estimates and confidence intervals of aboveground biomass effect sizes summarized at the region-level, in Fig. A3.4B). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. Single block effect sizes are highlighted in red. K – Konstanz transplant region; P – Potsdam transplant region; CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	K	CON	-0.391	-1.360	0.578
				ZEB	0.130	-0.834	1.094
			P	CON	NA	NA	NA
				ZEB	-0.548	-1.556	0.460
	native	*Chenopodium album*	K	CON	-0.286	-0.755	0.183
				ZEB	-0.171	-0.680	0.339
			P	CON	-1.049	-3.317	1.219
				ZEB	0.144	-1.342	1.630
Asteraceae	non-native	*Erigeron canadensis*	K	CON	-0.108	-0.605	0.389
				ZEB	-0.079	-0.857	0.700
			P	CON	NA	NA	NA
				ZEB	NA	NA	NA
		Erigeron annuus	K	CON	**-0.581**	**-1.050**	**-0.112**
				ZEB	-0.325	-0.836	0.185
			P	CON	-0.505	-1.148	0.137
				ZEB	-0.027	-0.785	0.731
	native	*Lactuca serriola*	K	CON	-0.521	-1.086	0.043
				ZEB	-0.050	-0.666	0.567
			P	CON	0.924	-0.079	1.927
				ZEB	0.677	-0.047	1.401
		Senecio vulgaris	K	CON	**0.630**	**0.141**	**1.119**
				ZEB	0.551	-0.215	1.316
			P	CON	-0.204	-1.193	0.786
				ZEB	0.388	-0.573	1.348
		Sonchus oleraceus	K	CON	-0.308	-0.953	0.338
				ZEB	-0.047	-0.702	0.607
			P	CON	0.487	-0.264	1.238
				ZEB	0.459	-0.322	1.240
		Tripleurospermum inodorum	K	CON	0.220	-0.230	0.670
				ZEB	0.146	-0.267	0.558
			P	CON	0.291	-0.298	0.880
				ZEB	-0.274	-1.090	0.542

(**Table A3.10** continued from previous page)

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Plantaginaceae	non-native	*Veronica persica*	K	CON	-0.033	-0.460	0.394
				ZEB	0.433	-0.069	0.935
			P	CON	0.507	-0.357	1.372
				ZEB	**-0.922**	**-1.789**	**-0.055**
	native	*Plantago major*	K	CON	0.390	-0.050	0.830
				ZEB	-0.359	-0.909	0.192
			P	CON	-0.496	-1.094	0.102
				ZEB	0.329	-0.382	1.040
Solanaceae	non-native	*Datura stramonium*	K	CON	0.644	-0.285	1.573
				ZEB	0.276	-0.415	0.966
			P	CON	-0.033	-0.582	0.516
				ZEB	-0.413	-1.066	0.240
	native	*Solanum nigrum*	K	CON	-0.348	-0.810	0.114
				ZEB	**-0.819**	**-1.302**	**-0.336**
			P	CON	**1.008**	**0.278**	**1.739**
				ZEB	**0.876**	**0.177**	**1.574**

Table A3.11: Estimates and confidence intervals of flowering probability effect sizes summarized at the region-level, in Fig. A3.4C). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. Single field effect sizes are highlighted in red. K – Konstanz transplant region; P – Potsdam transplant region; CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	K	CON	-1.644	-3.686	0.398
				ZEB	-0.733	-2.915	1.449
			P	CON	-1.037	-4.073	1.998
				ZEB	-0.642	-2.722	1.438
	native	*Chenopodium album*	K	CON	-1.318	-3.437	0.801
				ZEB	-0.713	-2.128	0.701
			P	CON	0.705	-2.916	4.327
				ZEB	-0.427	-3.578	2.725
Asteraceae	non-native	*Erigeron canadensis*	K	CON	0.457	-0.418	1.333
				ZEB	**1.698**	**0.474**	**2.923**
			P	CON	NA	NA	NA
				ZEB	0.775	-3.139	4.689
		Erigeron annuus	K	CON	**-1.272**	**-2.163**	**-0.381**
				ZEB	-0.635	-1.841	0.572
			P	CON	-0.020	-1.607	1.567
				ZEB	0.131	-1.647	1.909
	native	*Lactuca serriola*	K	CON	1.317	-0.262	2.897
				ZEB	0.154	-1.335	1.643
			P	CON	0.288	-1.947	2.524
				ZEB	-0.576	-3.781	2.630
		Senecio vulgaris	K	CON	-0.370	-2.718	1.977
				ZEB	-0.592	-2.910	1.726
			P	CON	-0.573	-3.968	2.822
				ZEB	1.488	-1.588	4.564
		Sonchus oleraceus	K	CON	**1.289**	**0.006**	**2.572**
				ZEB	**1.334**	**0.081**	**2.588**
			P	CON	-1.139	-2.916	0.639
				ZEB	-0.199	-1.461	1.063
		Tripleurospermum inodorum	K	CON	0.552	-0.516	1.620
				ZEB	-0.636	-1.782	0.511
			P	CON	0.413	-1.576	2.402
				ZEB	0.083	-1.260	1.425

(**Table A3.11** continued from previous page)

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Plantaginaceae	non-native	*Veronica persica*	K	CON	-0.695	-2.850	1.459
				ZEB	-0.737	-3.009	1.534
			P	CON	-0.145	-2.383	2.092
				ZEB	0.681	-1.595	2.957
	native	*Plantago major*	K	CON	0.000	-2.309	2.309
				ZEB	-0.471	-2.653	1.711
			P	CON	0.485	-1.329	2.299
				ZEB	0.392	-0.921	1.706
Solanaceae	non-native	*Datura stramonium*	K	CON	1.222	-0.287	2.731
				ZEB	1.061	-0.238	2.359
			P	CON	1.694	-0.226	3.613
				ZEB	-0.864	-2.613	0.886
	native	*Solanum nigrum*	K	CON	0.000	-2.494	2.494
				ZEB	-0.365	-2.791	2.062
			P	CON	1.056	-1.221	3.333
				ZEB	-0.693	-2.314	0.928

Table A3.12: Estimates and confidence intervals of reproductive biomass effect sizes summarized at the region-level, in Fig. A3.4D). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. Single block effect sizes are highlighted in red. K – Konstanz transplant region; P – Potsdam transplant region; CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	K	CON	**-1.792**	**-2.408**	**-1.175**
				ZEB	**-1.994**	**-2.844**	**-1.143**
			P	CON	NA	NA	NA
				ZEB	0.577	-1.976	3.131
	native	*Chenopodium album*	K	CON	**-1.025**	**-1.627**	**-0.423**
				ZEB	**-0.885**	**-1.689**	**-0.081**
			P	CON	-0.377	-2.544	1.789
				ZEB	0.647	-1.151	2.444
Asteraceae	non-native	*Erigeron canadensis*	K	CON	0.242	-0.606	1.090
				ZEB	0.021	-1.042	1.084
			P	CON	NA	NA	NA
				ZEB	NA	NA	NA
		Erigeron annuus	K	CON	0.399	-1.929	2.728
				ZEB	0.057	-0.814	0.928
			P	CON	**-1.695**	**-3.067**	**-0.323**
				ZEB	-0.833	-2.977	1.311
	native	*Lactuca serriola*	K	CON	NA	NA	NA
				ZEB	NA	NA	NA
			P	CON	NA	NA	NA
				ZEB	NA	NA	NA
		Senecio vulgaris	K	CON	0.039	-0.441	0.519
				ZEB	-0.037	-0.858	0.785
			P	CON	NA	NA	NA
				ZEB	0.594	-0.582	1.770
		Sonchus oleraceus	K	CON	-0.185	-1.365	0.995
				ZEB	-0.135	-3.043	2.773
			P	CON	0.363	-0.657	1.383
				ZEB	0.336	-2.084	2.755
		Tripleurospermum inodorum	K	CON	-0.471	-0.944	0.003
				ZEB	-0.066	-0.609	0.477
			P	CON	0.278	-0.327	0.882
				ZEB	-0.354	-1.553	0.846

(**Table A3.12** continued from previous page)

Plant family	Status	Species	Region	Treatment	predicted	ci.lb	ci.ub
Plantaginaceae	non-native	*Veronica persica*	K	CON	**-0.895**	**-1.478**	**-0.311**
				ZEB	-0.316	-0.862	0.229
			P	CON	1.496	-0.037	3.029
				ZEB	-0.366	-1.858	1.127
	native	*Plantago major*	K	CON	**0.472**	**0.032**	**0.913**
				ZEB	-0.236	-0.698	0.225
			P	CON	-0.938	-1.908	0.033
				ZEB	0.361	-0.371	1.092
Solanaceae	non-native	*Datura stramonium*	K	CON	-0.019	-2.424	2.385
				ZEB	0.995	-0.746	2.736
			P	CON	-0.109	-0.734	0.516
				ZEB	-0.075	-1.041	0.891
	native	*Solanum nigrum*	K	CON	-0.293	-0.754	0.167
				ZEB	**-0.638**	**-1.116**	**-0.160**
			P	CON	**1.053**	**0.385**	**1.722**
				ZEB	**0.668**	**0.094**	**1.241**

Table A3.13: Estimates and confidence intervals of across-region summarized effect sizes for survival (Fig. 4.1a). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	CON	-0.310	-2.085	1.464
			ZEB	-0.695	-2.105	0.716
	native	*Chenopodium album*	CON	-0.898	-2.276	0.480
			ZEB	0.469	-0.823	1.761
Asteraceae	non-native	*Erigeron canadensis*	CON	-0.895	-2.167	0.378
			ZEB	0.666	-0.366	1.699
		Erigeron annuus	CON	-0.333	-1.559	0.893
			ZEB	0.374	-0.768	1.516
	native	*Lactuca serriola*	CON	0.569	-1.585	2.723
			ZEB	0.111	-0.594	0.815
		Senecio vulgaris	CON	0.655	-1.077	2.387
			ZEB	-0.315	-1.925	1.295
		Sonchus oleraceus	CON	-0.242	-1.784	1.300
			ZEB	0.069	-1.539	1.677
		Tripleurospermum inodorum	CON	-0.468	-1.281	0.344
			ZEB	-0.004	-0.793	0.784
Plantaginaceae	non-native	*Veronica persica*	CON	0.153	-0.951	1.257
			ZEB	0.638	-1.011	2.287
	native	*Plantago major*	CON	-0.733	-1.916	0.450
			ZEB	0.034	-0.960	1.027
Solanaceae	non-native	*Datura stramonium*	CON	1.112	-1.677	3.901
			ZEB	0.561	-1.490	2.612
	native	*Solanum nigrum*	CON	0.159	-1.053	1.371
			ZEB	-0.118	-1.195	0.960

Table A3.14 Estimates and confidence intervals of across-region summarized effect sizes for aboveground biomass (Fig. 4.1b). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	CON	NA	NA	NA
			ZEB	-0.068	-0.762	0.627
	native	*Chenopodium album*	CON	-0.338	-0.791	0.114
			ZEB	-0.136	-0.590	0.317
Asteraceae	non-native	*Erigeron canadensis*	CON	NA	NA	NA
			ZEB	NA	NA	NA
		Erigeron annuus	CON	**-0.552**	**-0.923**	**-0.182**
			ZEB	-0.251	-0.630	0.128
	native	*Lactuca serriola*	CON	0.139	-1.271	1.550
			ZEB	0.288	-0.422	0.999
		Senecio vulgaris	CON	0.328	-0.458	1.113
			ZEB	0.485	-0.051	1.020
		Sonchus oleraceus	CON	0.066	-0.712	0.843
			ZEB	0.161	-0.340	0.663
		Tripleurospermum inodorum	CON	0.244	-0.093	0.580
			ZEB	-0.009	-0.423	0.405
Plantaginaceae	non-native	*Veronica persica*	CON	0.117	-0.355	0.588
			ZEB	-0.193	-1.521	1.135
	native	*Plantago major*	CON	0.071	-0.297	0.440
			ZEB	-0.058	-0.702	0.587
Solanaceae	non-native	*Datura stramonium*	CON	0.198	-0.431	0.827
			ZEB	-0.082	-0.752	0.589
	native	*Solanum nigrum*	CON	0.327	-1.013	1.667
			ZEB	0.011	-1.621	1.642

Table A3.15: Estimates and confidence intervals of across-region summarized effect sizes for flowering probability (Fig. 4.1c). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	CON	-1.455	-3.149	0.239
			ZEB	-0.685	-2.191	0.820
	native	*Chenopodium album*	CON	-0.802	-2.631	1.027
			ZEB	-0.665	-1.956	0.625
Asteraceae	non-native	*Erigeron canadensis*	CON	NA	NA	NA
			ZEB	**1.614**	**0.537**	**2.691**
		Erigeron annuus	CON	-0.802	-2.002	0.398
			ZEB	-0.463	-1.365	0.440
	native	*Lactuca serriola*	CON	0.975	-0.315	2.264
			ZEB	-0.048	-1.318	1.221
		Senecio vulgaris	CON	-0.436	-2.366	1.495
			ZEB	0.192	-1.784	2.168
		Sonchus oleraceus	CON	0.156	-2.217	2.530
			ZEB	0.570	-0.933	2.072
		Tripleurospermum inodorum	CON	0.521	-0.420	1.462
			ZEB	-0.333	-1.205	0.539
Plantaginaceae	non-native	*Veronica persica*	CON	-0.431	-1.983	1.121
			ZEB	-0.030	-1.637	1.578
	native	*Plantago major*	CON	0.300	-1.126	1.726
			ZEB	0.163	-0.963	1.288
Solanaceae	non-native	*Datura stramonium*	CON	**1.401**	**0.218**	**2.584**
			ZEB	0.192	-1.685	2.068
	native	*Solanum nigrum*	CON	0.576	-1.106	2.257
			ZEB	-0.592	-1.940	0.756

Table A3.16: Estimates and confidence intervals of across-region summarized effect sizes for reproductive biomass (Fig. 4.1d). Significant effect sizes at the $P < 0.05$ threshold are marked in bold. CON – control treatment; ZEB – zebularine treatment; predicted – summarized effect size; ci.lb – lower 95% confidence interval; ci.ub – upper 95% confidence interval. Species names of the natives are in black, and the ones of the non-natives are in red.

Plant family	Status	Species	Treatment	predicted	ci.lb	ci.ub
Amaranthaceae	non-native	*Amaranthus retroflexus*	CON	NA	NA	NA
			ZEB	-1.051	-3.039	0.936
	native	*Chenopodium album*	CON	**-0.957**	**-1.505**	**-0.409**
			ZEB	-0.259	-1.682	1.163
Asteraceae	non-native	*Erigeron canadensis*	CON	NA	NA	NA
			ZEB	NA	NA	NA
		Erigeron annuus	CON	-0.345	-2.284	1.594
			ZEB	-0.230	-1.002	0.542
	native	*Lactuca serriola*	CON	NA	NA	NA
			ZEB	NA	NA	NA
		Senecio vulgaris	CON	NA	NA	NA
			ZEB	0.159	-0.513	0.830
		Sonchus oleraceus	CON	0.129	-0.643	0.900
			ZEB	0.111	-1.125	1.348
		Tripleurospermum inodorum	CON	-0.121	-0.853	0.611
			ZEB	-0.092	-0.541	0.358
Plantaginaceae	non-native	*Veronica persica*	CON	0.202	-2.101	2.504
			ZEB	-0.329	-0.829	0.170
	native	*Plantago major*	CON	-0.247	-1.714	1.221
			ZEB	-0.008	-0.577	0.560
Solanaceae	non-native	*Datura stramonium*	CON	-0.098	-0.685	0.489
			ZEB	0.233	-0.679	1.145
	native	*Solanum nigrum*	CON	0.363	-0.944	1.670
			ZEB	0.005	-1.274	1.284

Table A3.17: Soil parameters at field sites. For each transplant field site, the average of 11-12 soil samples is given (collected in four species plots per block). Samples were collected in July/August 2016. BoGa – Botanical Garden of Konstanz. Hättli – Konstanz/Hättelihof. Uni – University of Konstanz. DB – Potsdam/Botanical Garden. GR – Ludwigsfelde/Gröben (close to Potsdam). MQ – Potsdam-Marquardt. WeightRatio – dry-to-fresh mass ratio. Npercent – relative [%] nitrogen content of dry mass. Cpercent – relative [%] carbon content of dry mass. CNratio – carbon-to-nitrogen ratio of dry mass. pHcurrent – pH value in ddH_2O. pHpotential – pH value in $CaCl_2$. Phosphate – total phosphate content [g kg^{-1} dry mass]. Nitrogen – total nitrogen content [g kg^{-1} dry mass]. Ammonium – plant-available ammonium [mg kg^{-1} dry mass]. PhosphatePA – plant-available phosphate content [mg L^{-1} solved dry mass]. OrganicMatter – relative [%] organic matter in dry mass after loss-on-ignition. (See Methods A3.4 for a detailed description of sampling and soil analysis.)

Region	**Konstanz**			**Potsdam**		
Field site	**BoGa**	**Hättli**	**Uni**	**DB**	**GR**	**MQ**
WeightRatio	0.75	0.81	0.77	0.90	0.97	0.88
Npercent [% dry mass]	0.26	0.21	0.20	0.14	0.09	0.13
Cpercent [% dry mass]	5.95	3.51	5.49	1.58	1.07	1.96
Cnratio	23.84	20.27	29.33	11.60	11.58	14.99
pHcurrent	7.99	7.81	8.05	5.91	5.62	7.03
pHpotential	7.34	7.24	7.35	5.42	5.01	6.49
Phosphate [g kg^{-1} dry mass]	1.18	0.67	0.64	0.53	0.41	1.18
Nitrogen [g kg^{-1} dry mass]	2.65	1.97	2.02	1.66	0.97	1.55
Ammonium [mg kg^{-1} dry mass]	3.35	1.60	1.20	2.83	2.02	2.73
PhosphatePA [mg L^{-1} solved dry mass]	2.41	0.59	0.70	2.87	0.13	9.03
OrganicMatter [% dry mass]	6.45	5.73	7.86	4.19	3.02	4.55
Nr. of samples	12	11	12	12	12	12

Table A3.18: Climatic differences Data of Konstanz and Potsdam weather stations averaged over years 1975 to 2015. (Based on data available from the German weather service, Deutscher Wetterdienst DWD, 2019. See below for data sources.)

Climate variable	Konstanz weather station	Potsdam weather station
latitude[a]	47.67° N	52.38° N
longitude[a]	9.18° E	13.05° E
maximum day length	16.0[d]	16.8[e]
minimum temperature [°C][c]	-10.06	-14.00
maximum temperature [°C][c]	33.3	34.53
average air temperature [°C][b]	9.82	9.30
sun hours per year[b]	1701.47	1740.02
annual precipitation [mm][b]	850.86	582.79
nr of frost days (< 0°C) per year[c]	69.90	83.00
nr. of hot (≥ 30 °C) days per year[c]	10.63	10.78

Data sources:

[a]https://www.dwd.de/DE/leistungen/klimadatendeutschland/stationsuebersicht.html (accessed 17 March 2019).

[b]Values based on monthly data by the German weather service (Deutscher Wetterdienst, DWD) https://www.dwd.de/DE/leistungen/klimadatendeutschland/klarchivtagmonat.html. (accessed 18 March 2019)

[c]Values based on daily data by the German weather service (Deutscher Wetterdienst, DWD) https://www.dwd.de/DE/leistungen/klimadatendeutschland/klarchivtagmonat.html. (accessed 18 March 2019)

[d]https://sunrisesunset.de/sonne/deutschland/konstanz/ (accessed 18 March 2019).

[e]https://sunrisesunset.de/sonne/deutschland/potsdam/ (accessed 18 March 2019).

Table A3.19 First records of the non-native study species in Germany and in the states of Germany in which Konstanz (Baden-Württemberg) and Potsdam (Brandenburg, but close to Berlin) are.

Species	Plant family	Native range	First record in Germany[a]	First record in Baden-Württemberg	First records in Berlin (BE), Brandenburg (BR) or Berlin- Brandenburg (BB)
Amaranthus retroflexus	Amaranthaceae	Tropical Americas (Costea, Weaver & Tardif, 2011)	1815	1820; maybe even earlier (1813) (Sebald, Philippi, & Seybold, 1993)	1859 (BB) (Ascherson, 1859)i
Erigeron annuus	Asteraceae	North America (Frey, 2003)	Eighteenth century	1562?, but definitely in the 18th century(Sebald, Phillippi, Seybold, & Wörz, 1996)	1859 (BE) (Ascherson, 1859)
Erigeron canadensis	Asteraceae	North America (Weaver, 2011))	1700	1728 (Ulm) (Sebald, Phillippi, et al., 1996)	1787 (BE) and 1859 (BR) (Ascherson, 1859; Willdenow, 1787)
Veronica persica	Plantaginaceae	Caucasus region (Fischer, 1987)	1805	1805 (Karlsruhe) (Sebald, Seybold, Philippi, Kleinsteuber, & Lange, 1996)	1850 (BR) and 1859 (BE) (Ascherson, 1859)
Datura stramonium	Solanaceae	most likely the Americas (Weaver & Warwick, 1984)	1584	1728 (Ulm) (Sebald, Seybold, et al., 1996)	1787 (BE) and 1859 (BR) (Ascherson, 1859; Willdenow, 1787)

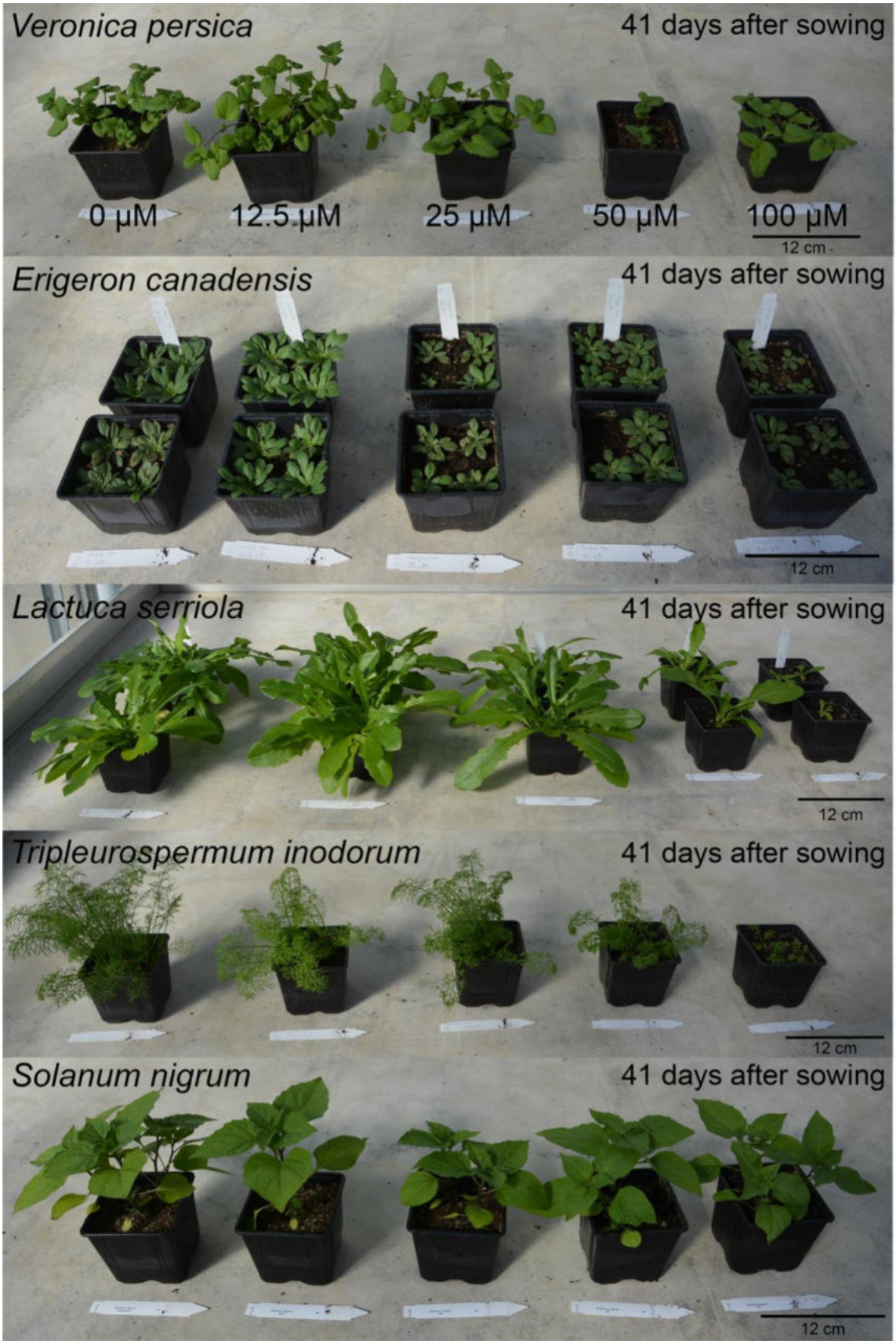


Figure A3.1: Zebularine concentration trial. Exemplary gradients of zebularine treatment during germination for study species. From left to right: 0 µM (control), 12.5 µM, 25 µM, 50 µM and 100 µM. Based on these gradients we decided on a concentration between 25 and 50 µM zebularine.

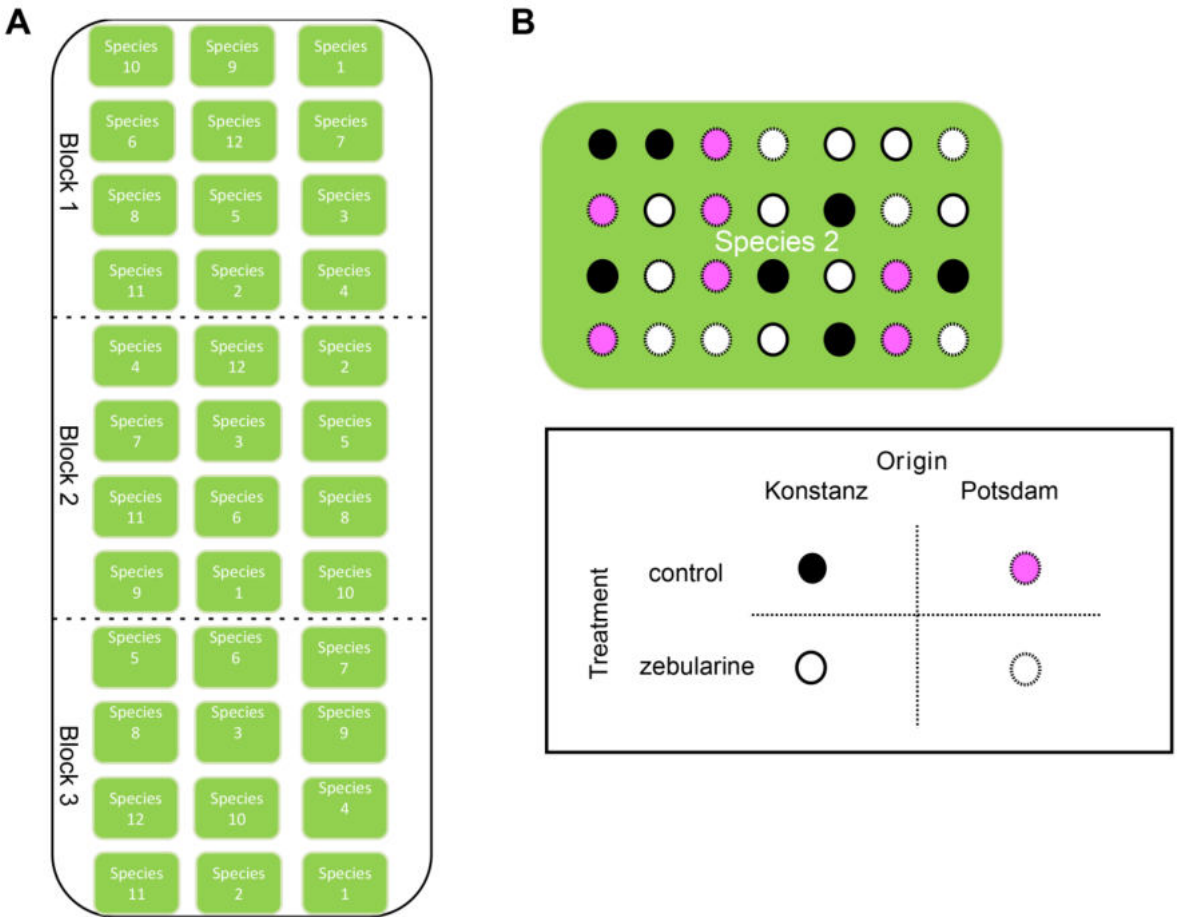


Figure A3.2: Layout of field sites. As an example, the positions of blocks and species plots at the Botanical Garden Konstanz field site are shown (A). An example of random allocation of control and zebularine-treated local and non-local plants to positions within a species plot (B).

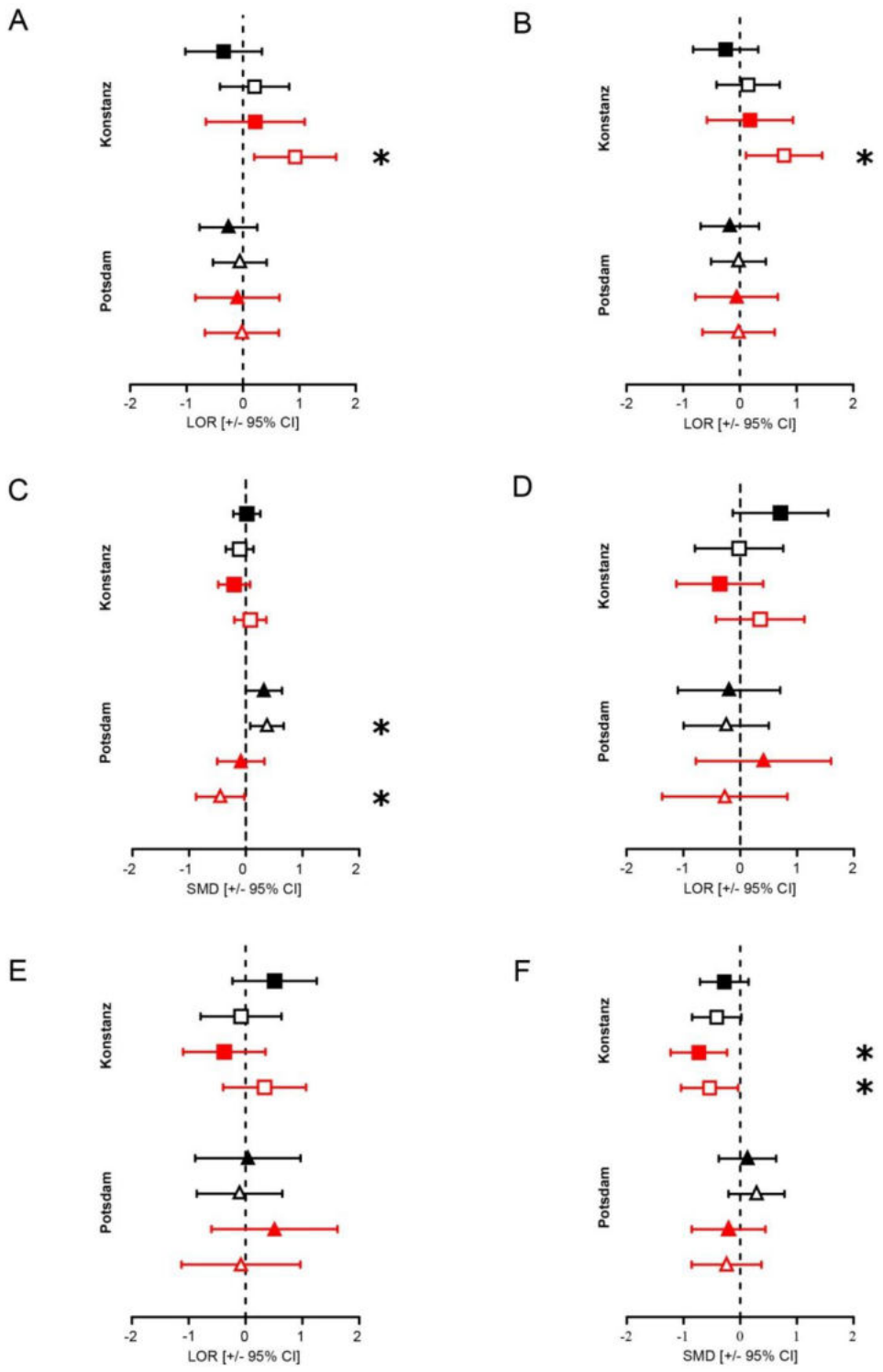
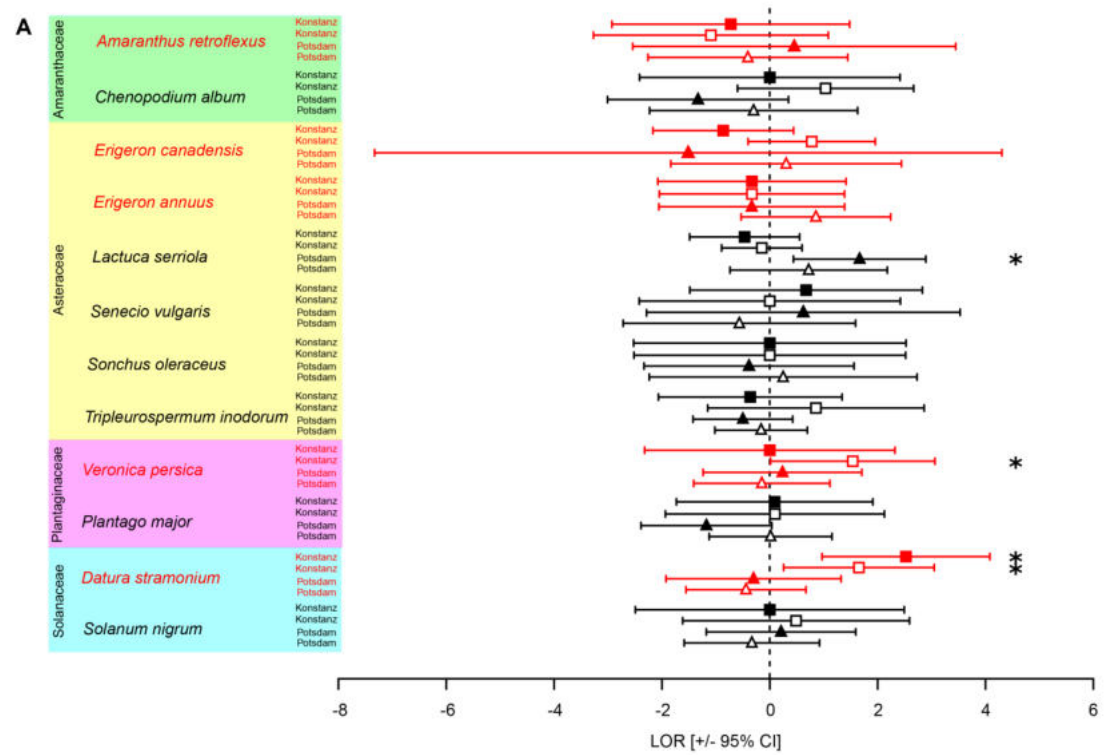
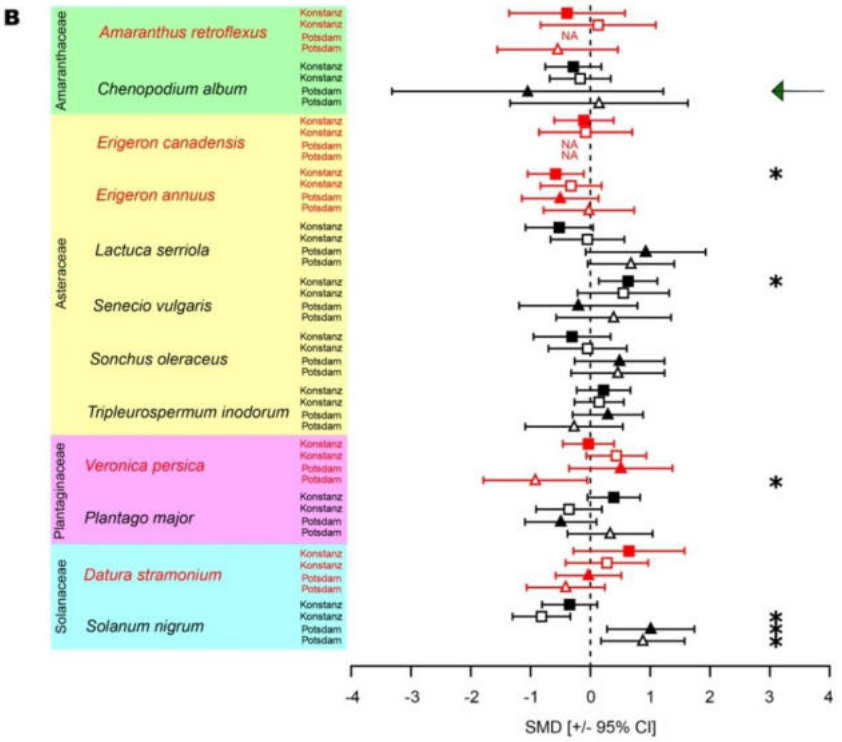


Figure A3.3: Prediction plots of the meta-regression models in Table 4.3. Plotted predictions for native and non-native plant species in the control and zebularine treatment in Konstanz and Potsdam, based on meta-regression models in Table 4.3. Squares illustrate Konstanz data and triangles illustrate Potsdam data. Closed and open symbols stand for control and zebularine treatments, respectively. Natives are marked in black and non-natives are marked in red. Stars denote effect sizes significantly different from 0 (i.e. 95% confidence intervals non-overlapping with 0). Survival model with 0.5 continuity correction (A), survival model with continuity correction based on the ratio of sample sizes between the compared groups (B), aboveground biomass model (C), flowering probability model with 0.5 continuity correction (D), flowering probability model with continuity correction based on the ratio of sample sizes between the compared groups (E), and reproductive biomass model (F).


A
Amaranthaceae
Amaranthus retroflexus
Chenopodium album
Asteraceae
Erigeron canadensis
Erigeron annuus
Lactuca serriola
Senecio vulgaris
Sonchus oleraceus
Tripleurospermum inodorum
Plantaginaceae
Veronica persica
Plantago major
Solanaceae
Datura stramonium
Solanum nigrum
Konstanz
Potsdam
-8
-6
-4
-2
0
2
4
6
LOR [+/- 95% CI]


B
Amaranthaceae
Amaranthus retroflexus
Chenopodium album
Asteraceae
Erigeron canadensis
Erigeron annuus
Lactuca serriola
Senecio vulgaris
Sonchus oleraceus
Tripleurospermum inodorum
Plantaginaceae
Veronica persica
Plantago major
Solanaceae
Datura stramonium
Solanum nigrum
Konstanz
Potsdam
NA
-4
-3
-2
-1
0
1
2
3
4
SMD [+/- 95% CI]

(**Figure A3.4** continued from previous page)

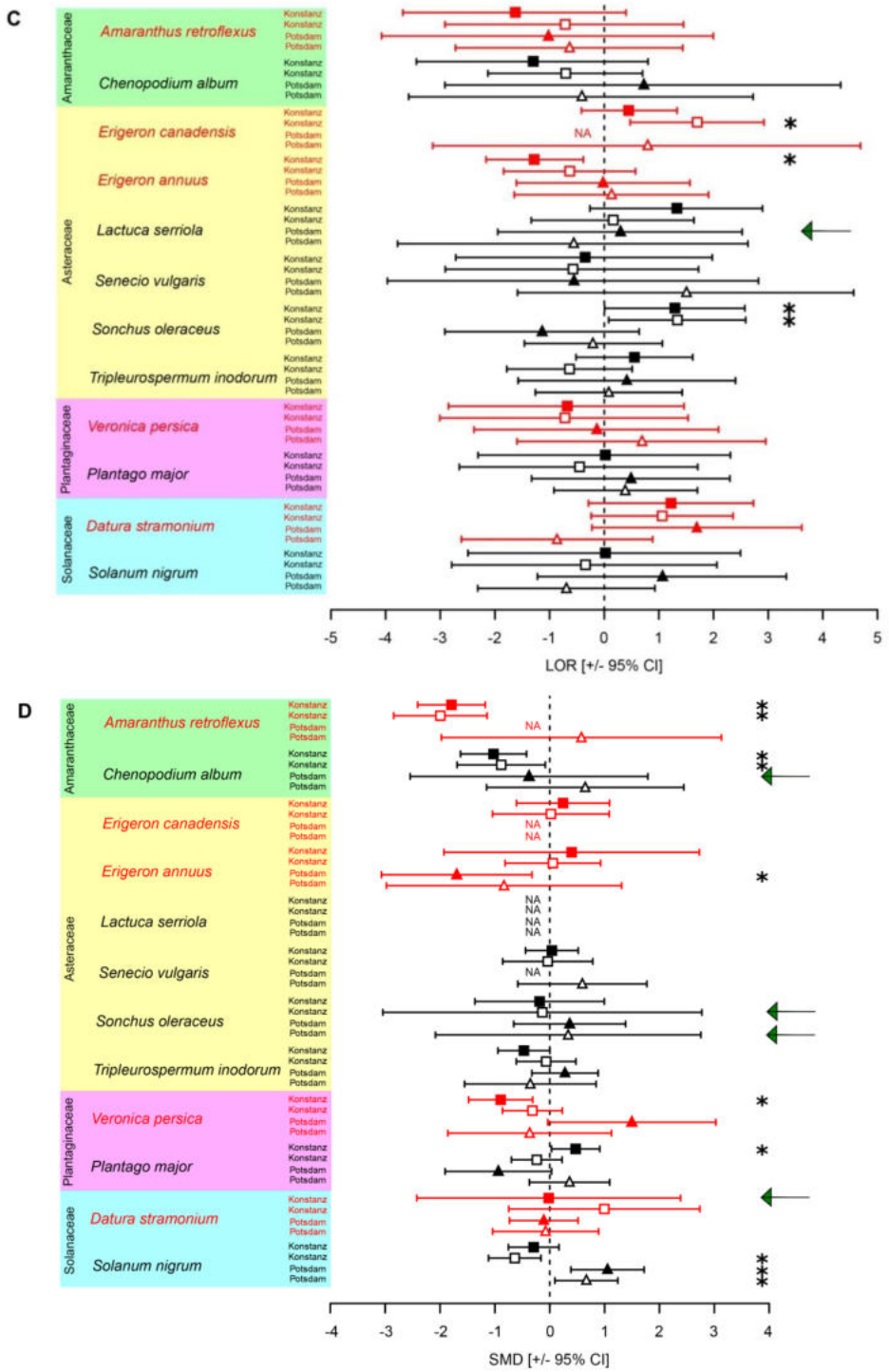


Figure A3.4: Forest plots with effect sizes of survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass summarized at the region-level (D). Squares illustrate Konstanz data and triangles illustrate Potsdam data. Closed and open symbols stand for control and zebularine treatment, respectively. Natives are marked in black and non-natives are marked in red. Stars denote effect sizes significantly different from 0 (i.e. 95% confidence intervals non-overlapping with 0). Green arrows denote cases with a single effect size, without summarization. NAs denote cases with insufficient data for effect size calculation. Survival and flowering probability effect sizes were calculated with continuity correction based on the ratio of sample sizes between the compared groups. LOR – log-transformed odds ratio, SMD – standardized mean difference.

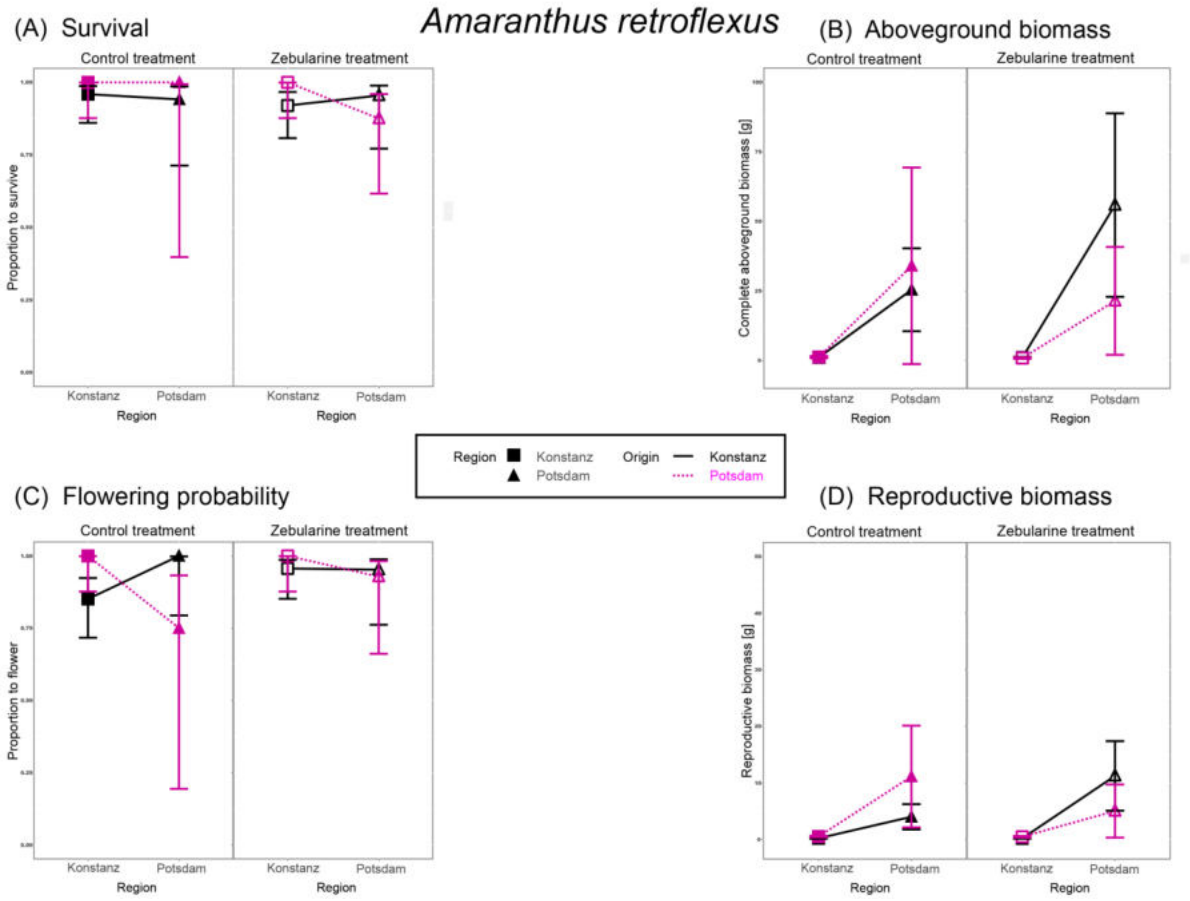


Figure A3.5: Reaction norms of the non-native species *Amaranthus retroflexus* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz site data and triangles indicate Potsdam site data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

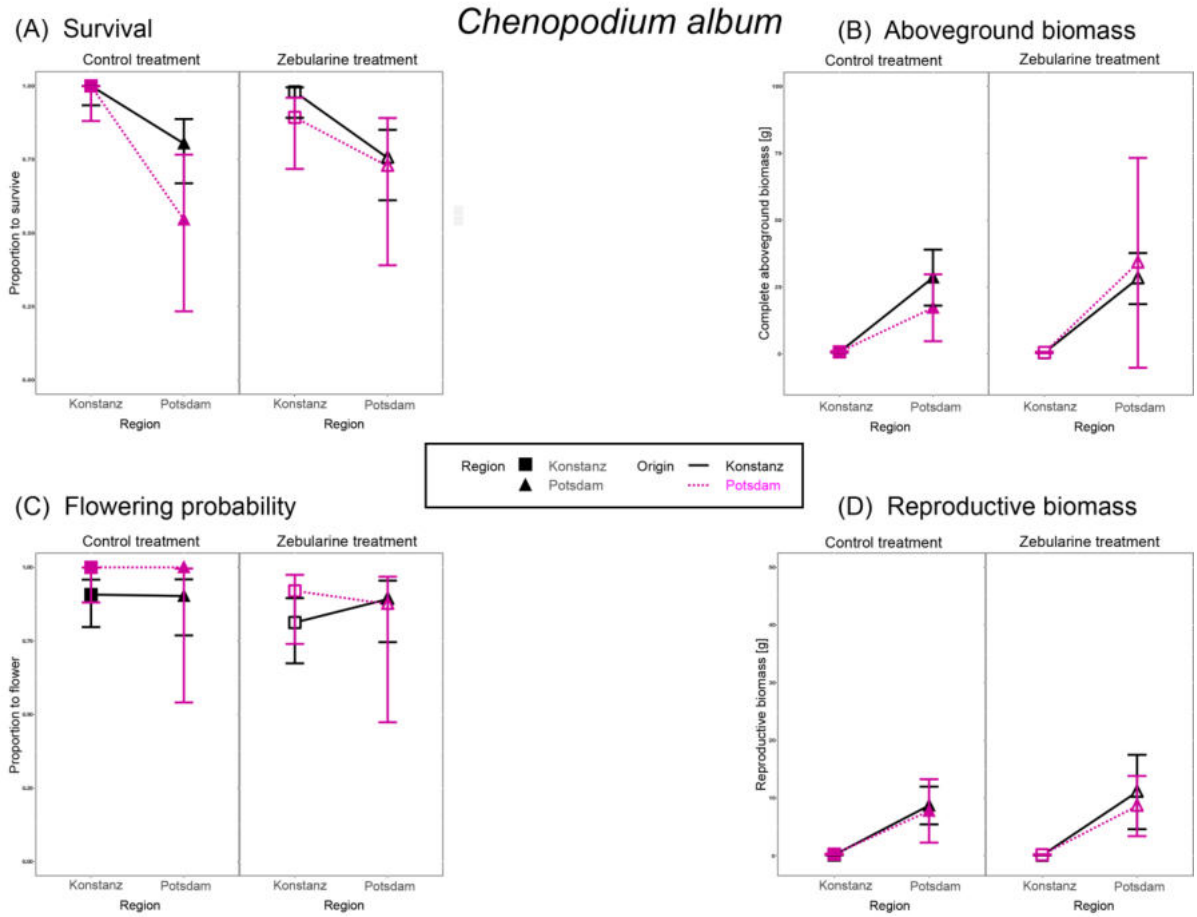


Figure A3.6: Reaction norms of the native species *Chenopodium album* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

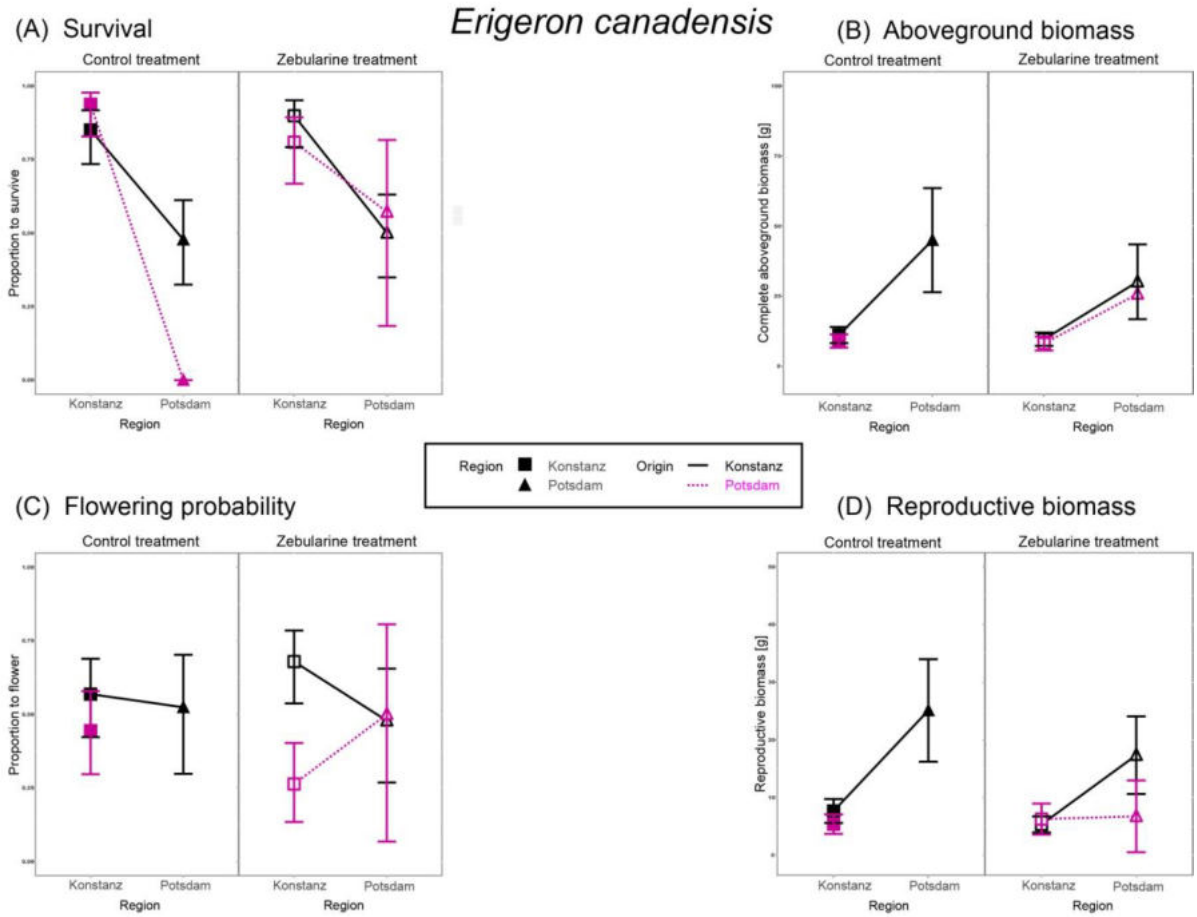


Figure A3.7: Reaction norms of the non-native species *Erigeron canadensis* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

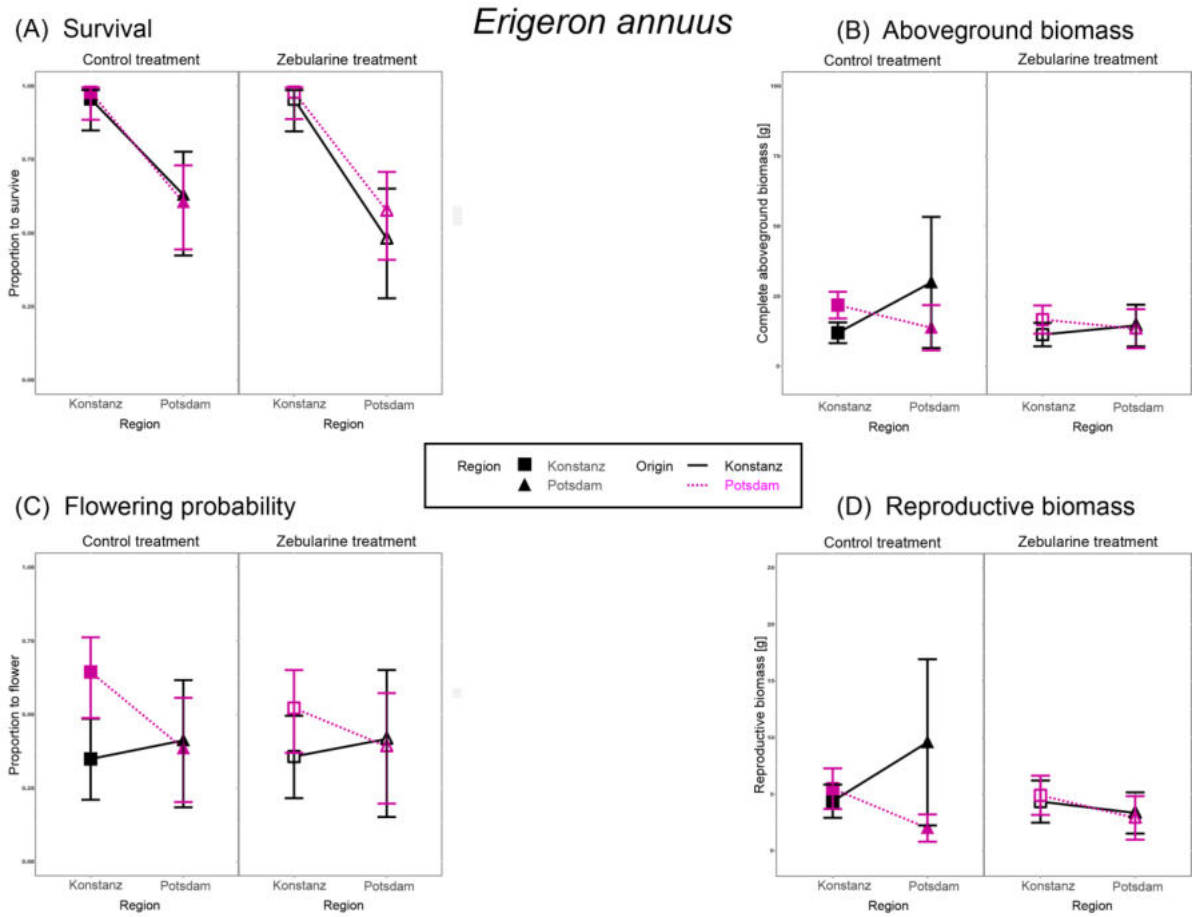


Figure A3.8: Reaction norms of the non-native species *Erigeron annuus* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

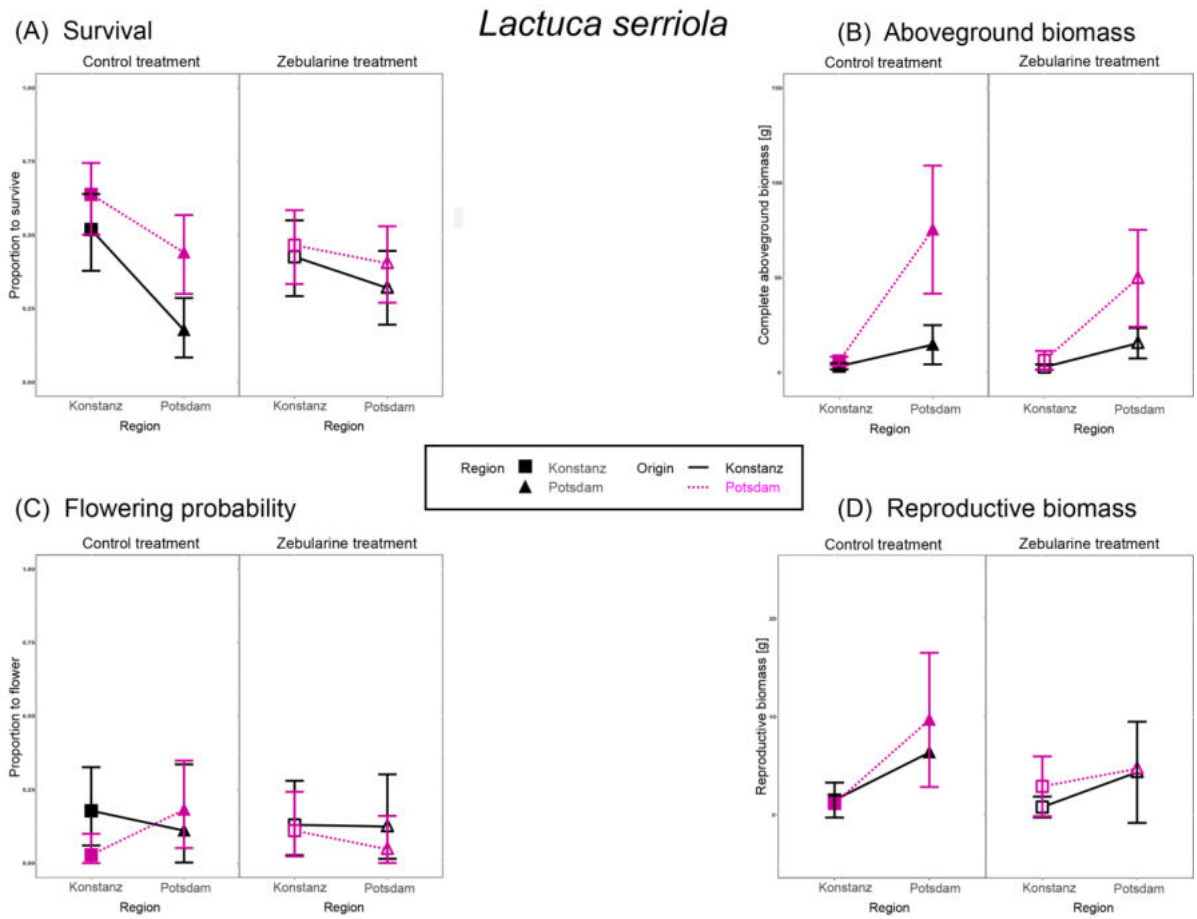


Figure A3.9: Reaction norms of the native species *Lactuca serriola* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

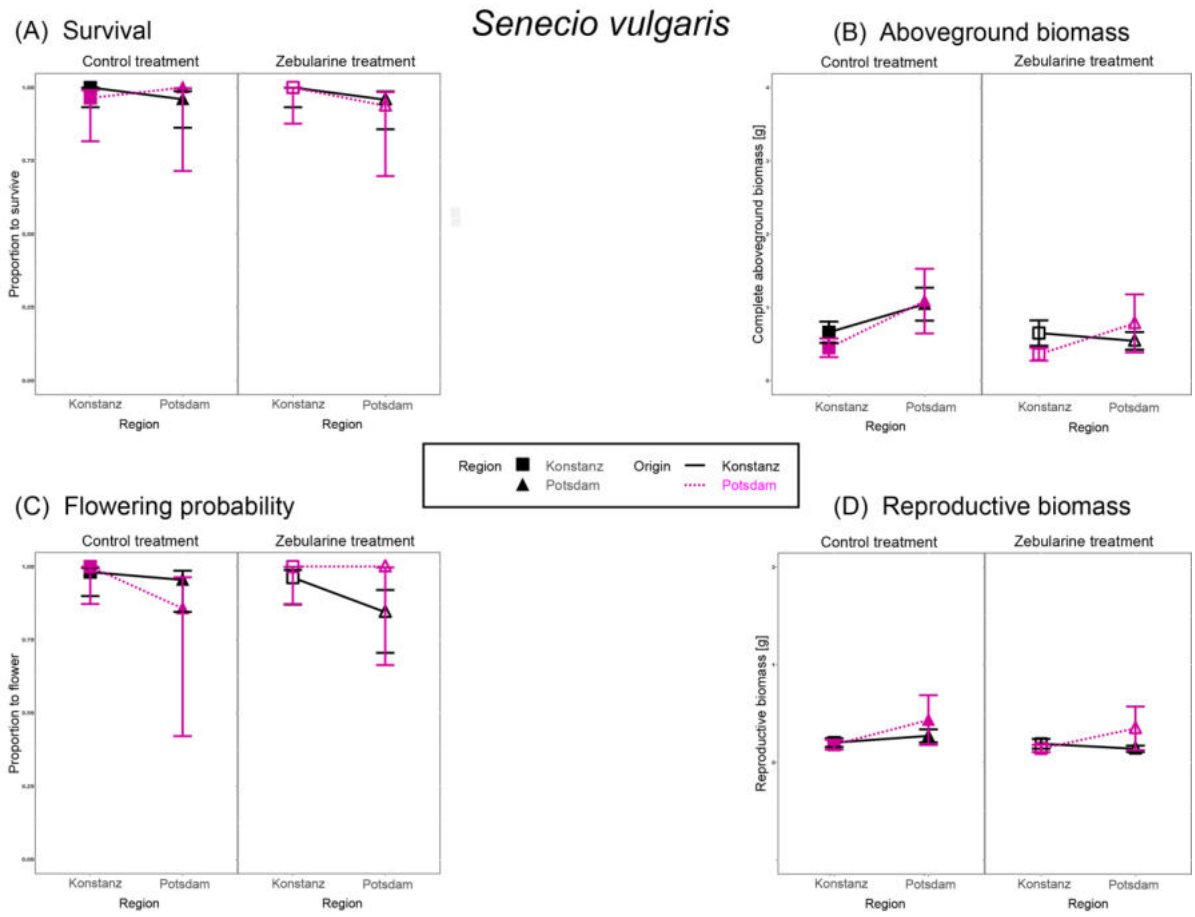


Figure A3.10: Reaction norms of the native species *Senecio vulgaris* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

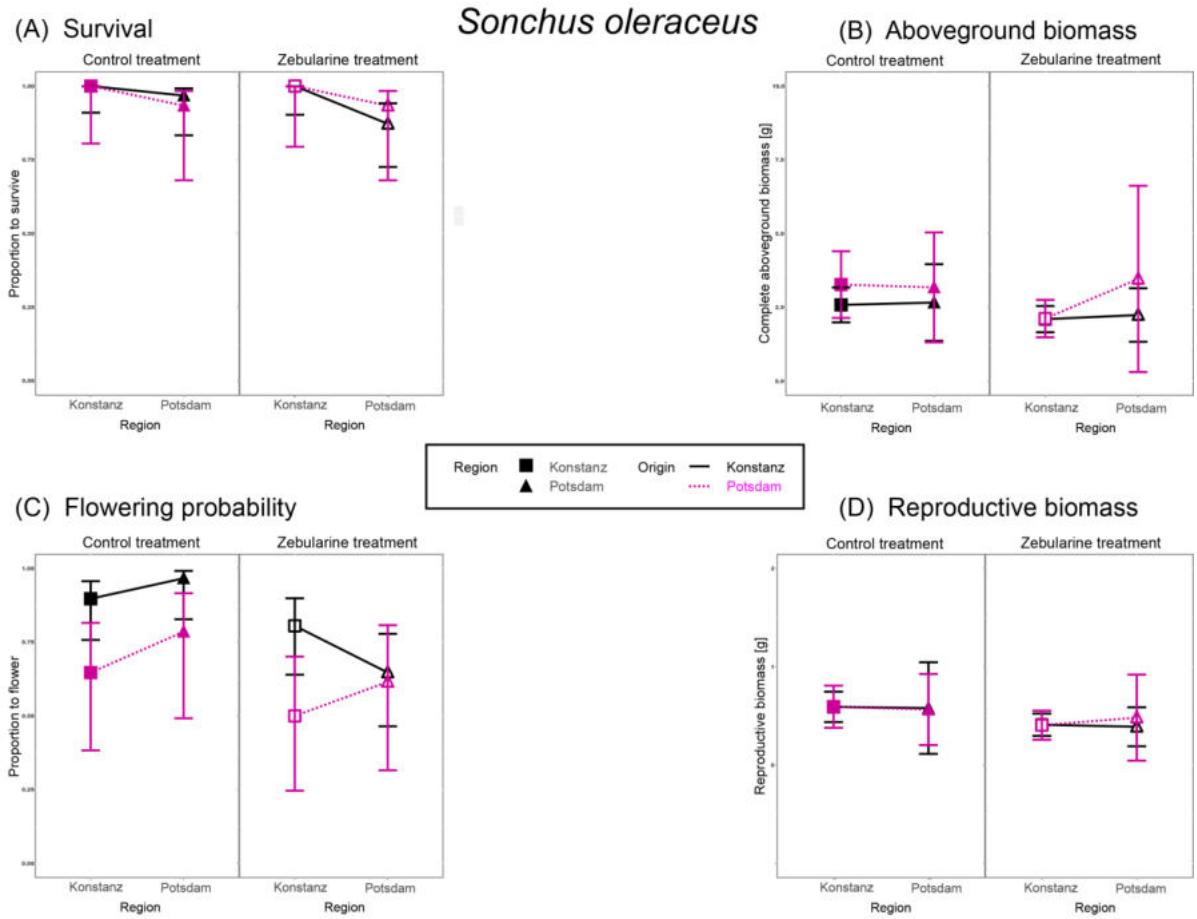


Figure A3.11: Reaction norms of the native species *Sonchus oleraceus* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

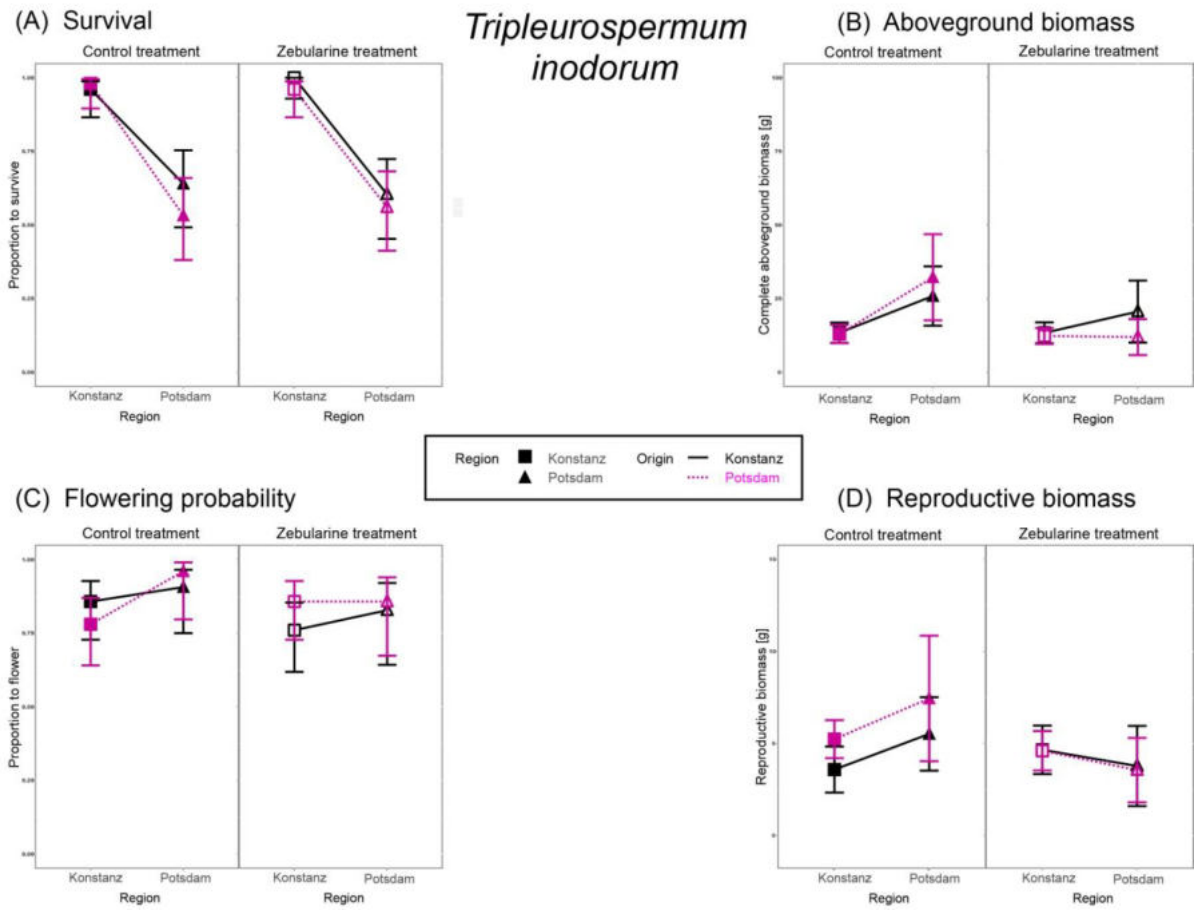


Figure A3.12: Reaction norms of the native species *Tripleurospermum inodorum* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

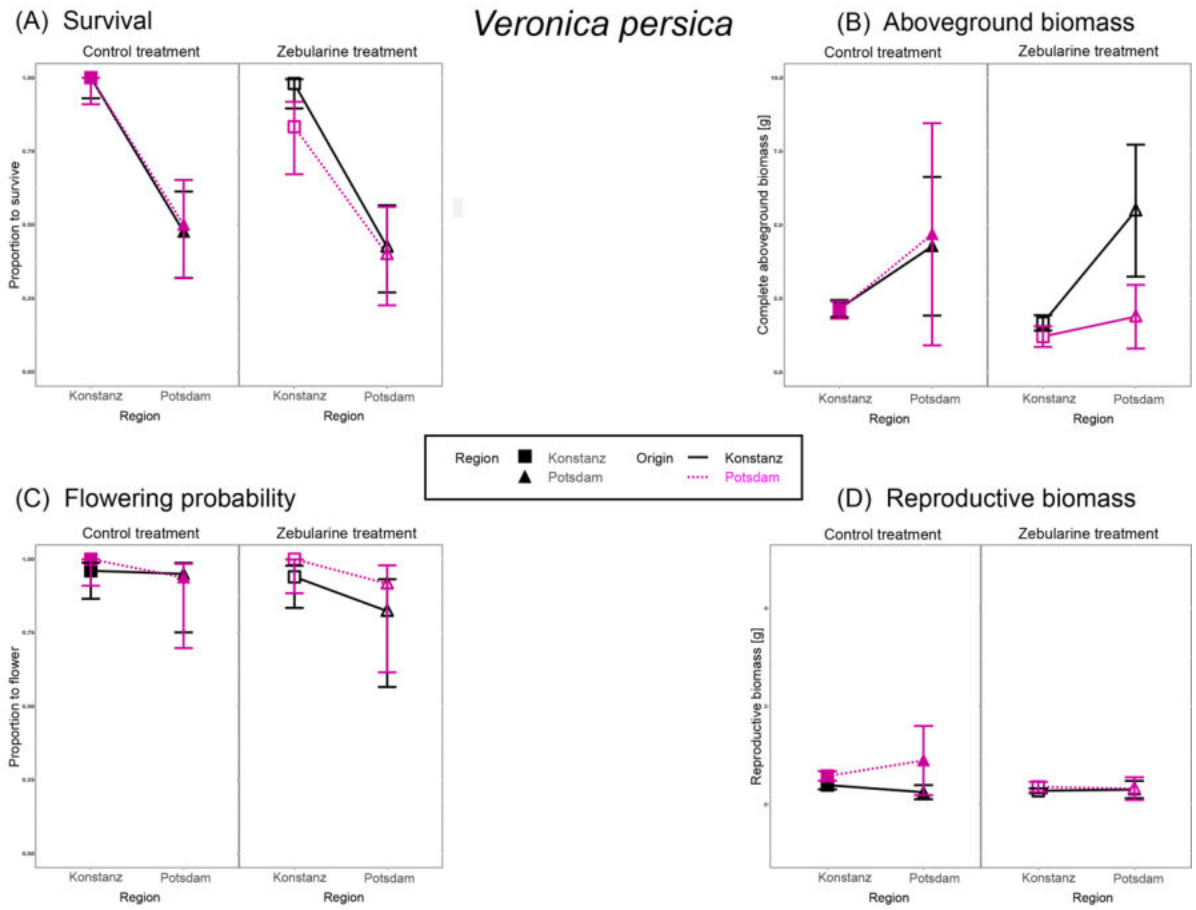


Figure A3.13: Reaction norms of the non-native species *Veronica persica* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

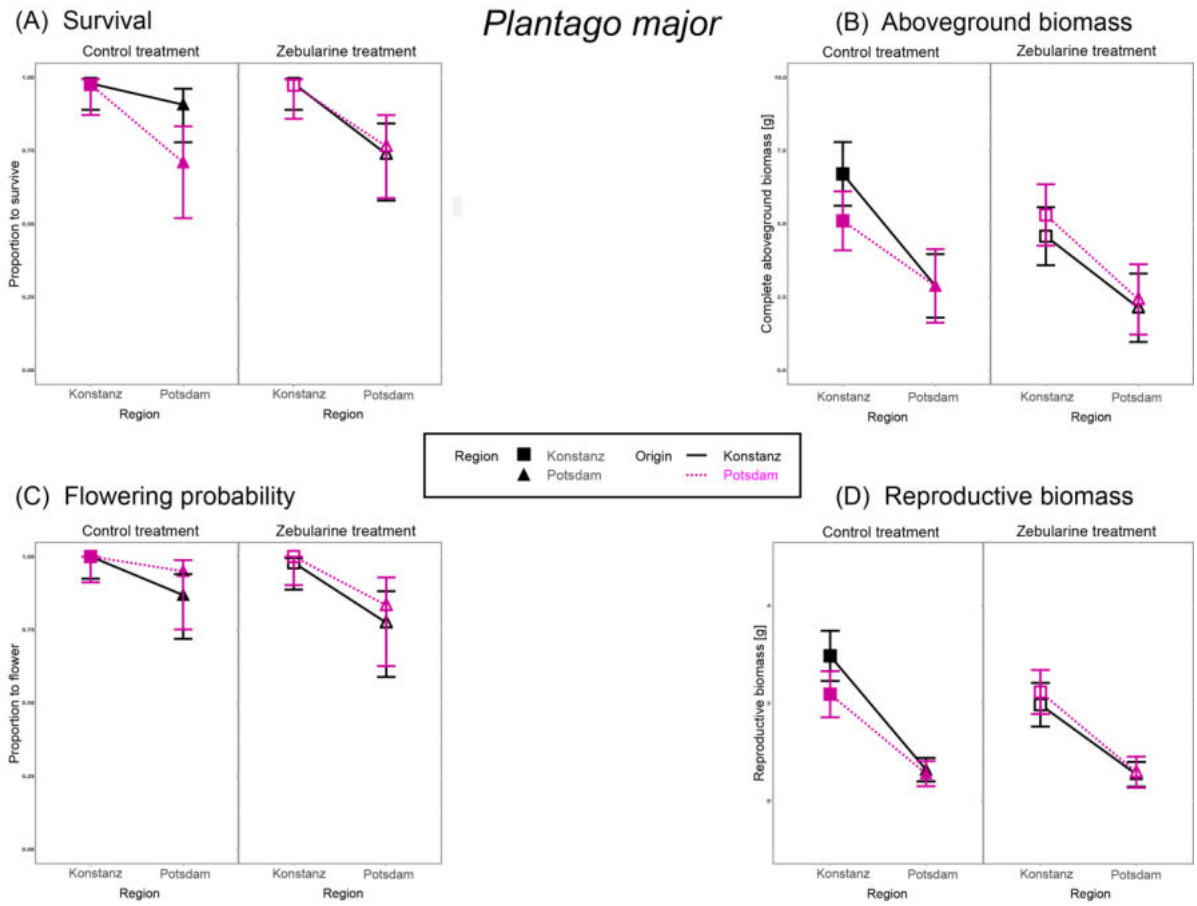


Figure A3.14: Reaction norms of the native species *Plantago major* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

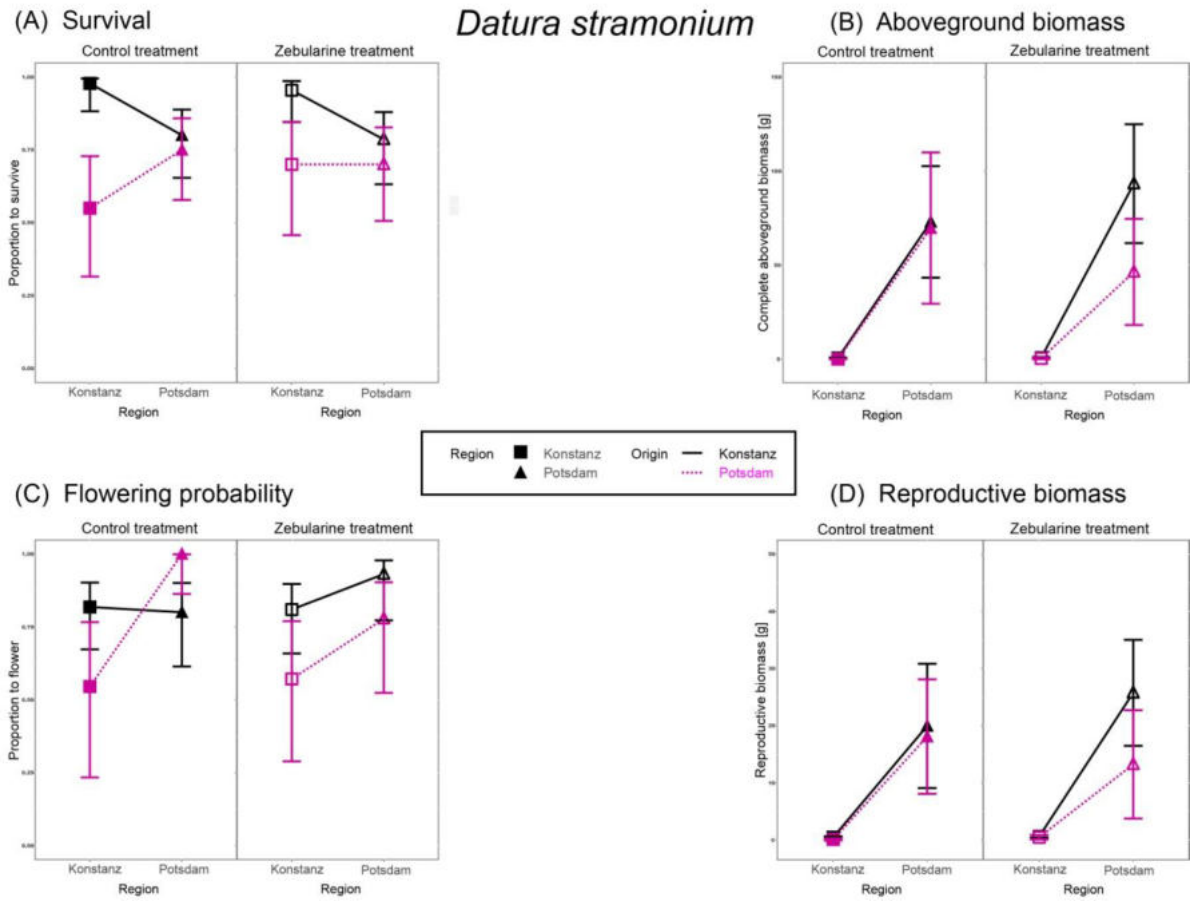


Figure A3.15: Reaction norms of the non-native species *Datura stramonium* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

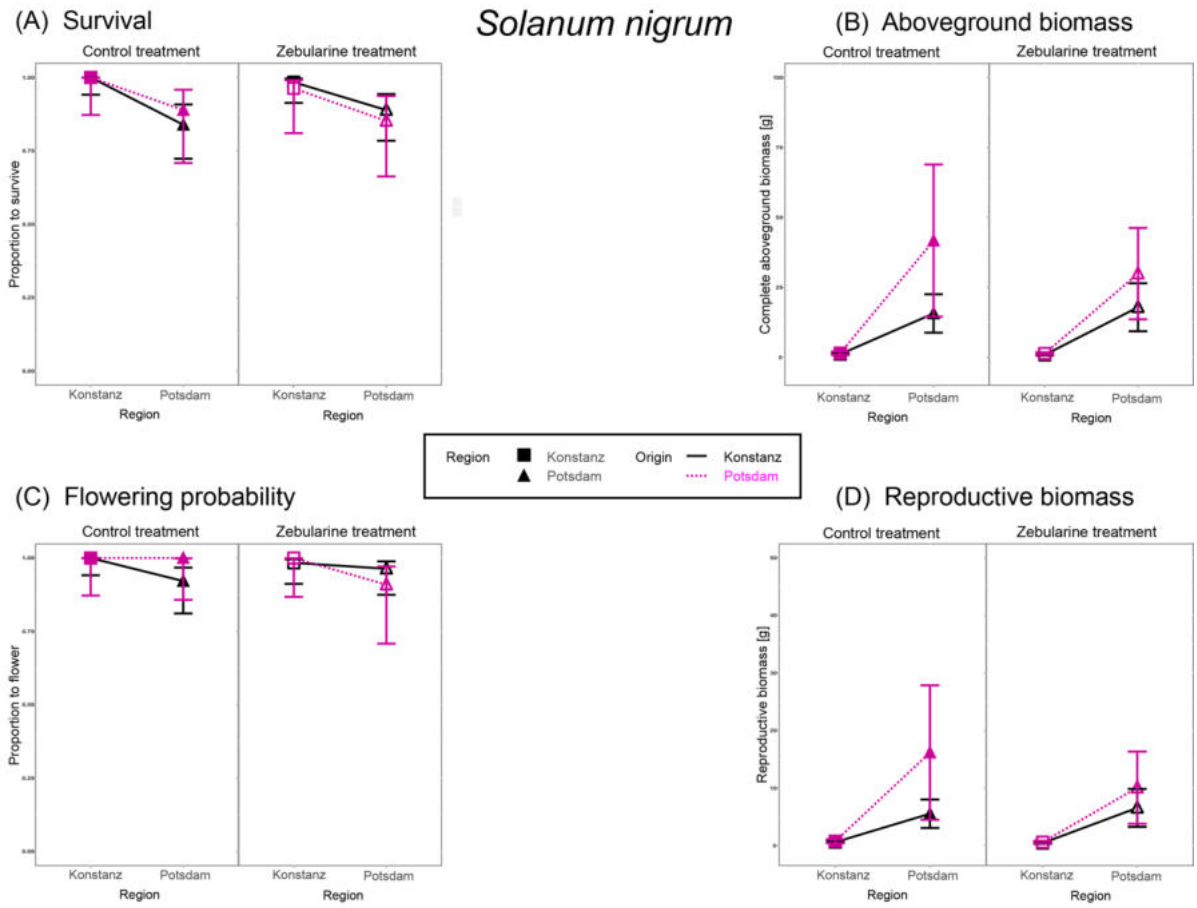


Figure A3.16: Reaction norms of the native species *Solanum nigrum* for survival (A), aboveground biomass (B), flowering probability (C) and reproductive biomass (D). Mean and 95% confidence intervals for untransformed data. Closed and open symbols denote control and zebularine treatment, respectively. Squares indicate Konstanz region data and triangles indicate Potsdam region data. Reaction norms for origins in the Konstanz transplant region are indicated in black, and origins in the Potsdam transplant region are indicated in purple.

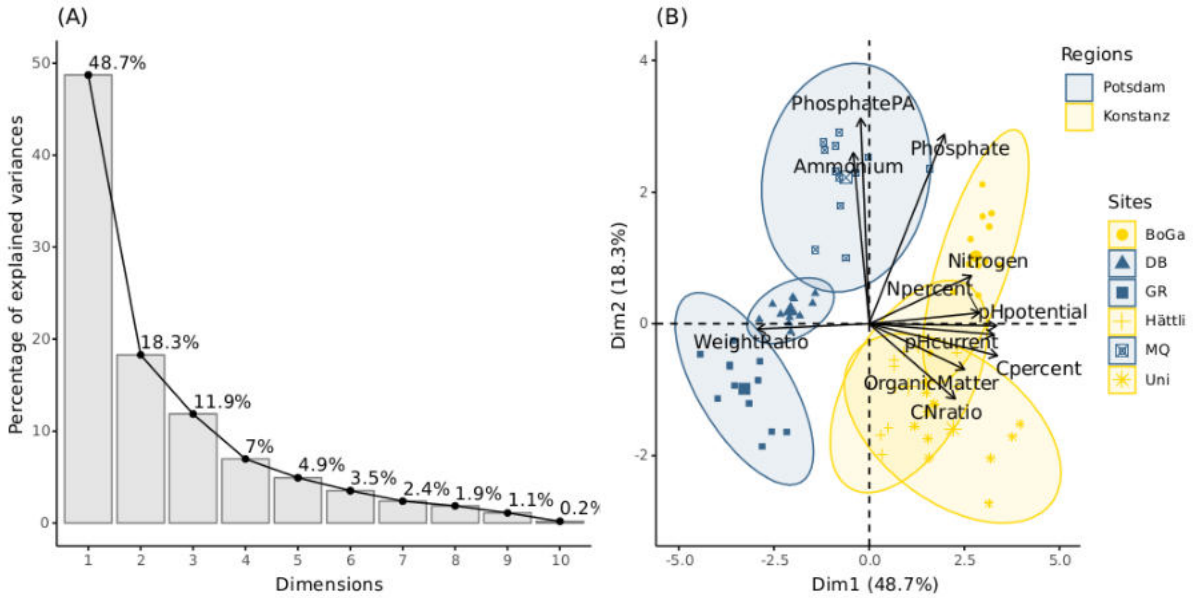


Figure A3.17: Soil parameters at field sites. Scree plot (A) and PCA and loadings for soil parameters at transplant field sites (B). BoGa – Botanical Garden of Konstanz; DB - Potsdam/Botanical Garden; GR - Ludwigsfelde/Gröben (close to Potsdam); Hättli - Konstanz/Hättelihof; MQ – Potsdam-Marquardt; Uni – University of Konstanz; Ammonium – plant-available ammonium [mg kg^{-1} dry mass]; Cpercent – relative [%] carbon content of dry mass; Npercent – relative [%] nitrogen content of dry mass; CNratio – carbon-to-nitrogen ratio of dry mass; Nitrogen – total nitrogen content [g kg^{-1} dry mass]; OrganicMatter – relative [%] organic matter in dry mass after loss-on-ignition; pHcurrent – pH value in ddH_2O; pHpotential – pH value in $CaCl_2^-$; Phosphate – total phosphate content [g kg^{-1} dry mass]; PhosphatePA – plant-available phosphate content [mg L^{-1} solved dry mass]; WeightRatio – dry-to-fresh mass ratio. (See Methods S4 for a detailed description of sampling and soil analysis.)

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Eidesstattliche Erklärung

Ich, Silvia Eckert, erkläre hiermit, dass die vorliegende Dissertation *Trait variation in changing environments: Assessing the role of DNA methylation in non-native plant species* von mir eigenständig sowie ausschließlich mit den angegebenen, zitierten Quellen und Hilfsmitteln angefertigt wurde. Des Weiteren versichere ich, dass die eingereichte elektronische Form der vorliegenden Dissertation mit der eingereichten schriftlichen, gedruckten Form vollständig übereinstimmt. Weiterhin erkläre ich, dass ich diese Arbeit noch keiner anderen Hochschule im In- oder Ausland zur Prüfung vorgelegt habe.

Potsdam, den 18. Mai 2022

Silvia Eckert