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Evolutionary adaptive responses to rapid climate change in plants
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A case study of the widely distributed species *Silene vulgaris*

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It is the intertwined and interacting mechanism of evolution and ecology, each of which is at the same time a product and a process, that are responsible for life as we see it, and as it has been.

James W. Valentine (1973)

List of Papers

This dissertation is a cumulative work that is based on the following papers:

Kahl, S. M., Lenhard, M., & Joshi, J. (2019). Compensatory mechanisms to climate change in the widely distributed species *Silene vulgaris*. *Journal of Ecology*, *107*, 1918–1930. https://doi.org/10.1111/1365-2745.13133

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Chapter 1

General Introduction

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General Introduction

Today anthropogenic climate change represents the major threat to individual species but also to whole ecosystems on this planet (Parmesan and Hanley 2015, Malhi et al. 2020). However, as climate predictions vary, and other abiotic and biotic interactions come into play it is currently one of the greatest challenges in biological research to examine and assess the impact of climate change on the species living on Earth (Parmesan and Hanley 2015). This study contributes to this research by focusing on the widely distributed plant species *Silene vulgaris* and examining its' response to a simulated climate change scenario.

The anthropogenic climate change

Since approximately 1750 atmospheric greenhouse gas concentrations have been increasing constantly and can be traced back to human activities unequivocally (IPCC 2021). With these elevated concentrations of atmospheric greenhouse gases shifts in temperature and precipitation pattern are associated. For example, each of the last four decades has been warmer than any decade before since 1850 and the global surface temperature from 2001-2020 was on average 0.99°C warmer than during the period between 1850-1900 (IPCC 2021). At the same time global precipitation over land has on average increased since 1950 (IPCC 2021). Furthermore, glaciers and the Arctic sea ice area are retreating due to human contributions that resulted in a raise of the global mean sea level by 0.20 m between 1901 and 2018 (IPCC 2021). The climatic changes also lead to more extreme weather events like heat waves or floods across the whole globe (Easterling et al. 2000, Christensen et al. 2007, Vázquez et al. 2017, Thurman et al. 2020). As a results of the changing climatic conditions biological processes like species' phenologies, interactions, or distributions are starting to be disrupted with a land biosphere that has shifted poleward or has prolonged its' growing season by up to two days (Root et al. 2003, Parmesan and Yohe 2003, Tylianakis et al. 2008, Traill et al. 2010, Parmesan and Hanley 2015, Beever et al. 2017, Renner and Zohner 2018, Giménez-Benavides et al. 2018, Wang et al. 2020, Thurman et al. 2020, IPCC 2021). All these results show that the species living on this planet are currently facing the challenge of a rapidly changing environment. Moreover, these changes are happening at a pace unprecedented in the last 2000 years (IPCC 2021). Hence, it is an important subject of current research to investigate how species can adapt to these rapidly changing environmental conditions and if these mechanisms will help the species to survive the environmental changes induced through the anthropogenic climate change.

Species' response mechanisms to climate change

In principle, there are three major ways how species can behave under changing environmental conditions: They can persist, escape, or if the conditions are highly unfavourable species can go extinct (Sala et al. 2000, Chen et al. 2011, Thurman et al. 2020). However, it depends strongly on the species and the velocity of the change which of the three scenarios becomes true. The rapid changes the Earth's recent climate is experiencing represent a challenge for most organisms (Sala et al. 2000, Urban 2015). For the ongoing climate change several studies have already found persisting and escape strategies in many species (Parmesan and Yohe 2003, Parmesan 2006, Urban 2015). Among the escape strategies shifting distributions of species are commonly found (Root et al. 2003, Parmesan and Yohe 2003, Chen et al. 2011). They have been reported in several organism groups e.g., neotropical migratory birds, small mammals, marine fishes, montane insects, or New World plant communities (Perry 2005, Moritz et al. 2008, Rushing et al. 2020, Feeley et al. 2020, McCain and Garfinkel 2021). These reports describe that species respond to 'too hot conditions' by moving their original distribution pattern towards higher elevations, or latitudes, deeper waters, or deeper underground. However, organisms can also persist under changing environmental conditions which requires a certain amount of adaptability. In this context, phenotypic plasticity represents an important mechanism for organism persistence under climate change. Especially in various plant taxa those plastic responses were already found: different deciduous trees show a plastic response in photosynthesis (Gunderson et al. 2009) and various perennial steppe species exhibit plastically responding leaf traits (Liancourt et al. 2015) towards climate change. However, phenotypic plasticity needs to be adaptive, i.e. produce equivalent or higher fitness in the novel environment, to be an effective mechanism against a rapidly changing climate (Becklin et al. 2016). Thus, it depends strongly on its link to plant performance (Nicotra et al. 2010, Liancourt et al. 2015) if phenotypic plasticity can facilitate plant adaptation to climate change.

The current climate change will affect the ecology and evolution of every species on this planet (Sala et al. 2000). As different species responses will not only impact the species themselves but will also affect whole biodiversity patterns (Siepielski et al. 2017) it is crucial to understand in what way different species are responding towards climate change to comprehensively assess how climate change will alter the ecosystems on our planet. In this context the examination of widely distributed plant species can play a key role in understanding the effects of climate change on a wider range of ecosystems as they are often a part of many different plant communities.

Research objectives

This thesis aims at studying possible trait responses of the widely distributed species *Silene vulgaris* to a simulated climate change scenario examining putative response differences based on the geographic origin of the sampled populations (Chapter 2), their population genetics (Chapter 3) and the sex of the individual (Chapter 4).

The study organism Silene vulgaris

Silene vulgaris (MOENCH) GARCKE (Caryophyllaceae) is an herbaceous perennial plant (Figure 1) which covers a wide distributional range from North Africa (Morocco and Egypt) expanding over the entire European continent, including islands, towards Russia, the Middle East and parts of temperate Asia (Bushneva 2002, Taylor and Keller 2007, Pearl et al. 2009, Registry-Migration.Gbif.Org 2021). In North America, Australia, South Africa, Ethiopia and Japan *S. vulgaris* was introduced as well (Registry-Migration.Gbif.Org 2021, WFO 2021) and has become an invasive species in certain parts of Canada and the USA (McCauley et al. 2003, Taylor and Keller 2007). The species naturally grows under nutrient-poor to moderately nutrient-rich conditions (Sandner and Matthies 2017, Koźmińska et al. 2019). Most commonly it is found along roadsides, in meadows, cultivated fields or on abandoned lots in patches of 10-100+ individuals (Bailey and McCauley 2006, Taylor and Keller 2007). However, ecotypes of *S. vulgaris* are also known to be adapted to more extreme environmental conditions like serpentine, calamine or other heavy-metal polluted soil (Bratteler et al. 2006b, 2006a, Pacwa-Płociniczak et al. 2018, Muszyńska et al. 2019b, 2019a). Hence, they are known as facultative metallophytes (Muszyńska et al. 2020,

Figure 1. *Silene vulgaris* (MOENCH) GARCKE (Caryophyllaceae). Photo by Sandra Kahl.

Wiszniewska et al. 2020) displaying the immense adaptation potential for *S. vulgaris*. Further studies also deal with tolerance mechanisms towards drought and salt stress within the species (Koźmińska et al. 2019). In fact, its' strong adaptive behaviour may be the reason why the species has become a very successful neophyte in some parts of North America (Bailey and McCauley 2006, Olson et al. 2019, USDA and NRCS 2021). Particularly because of its wide distributional range *S. vulgaris* has also been subject to many studies on population biology. Several studies examined the species' genetic diversity and genetic structure and used it as a model organism to gain knowledge on the colonisation dynamics and invasion history of invasive species (McCauley et al. 2003, Bailey and McCauley 2006, Taylor and Keller 2007, Bernasconi et al. 2009) or to shed light on the re-colonisation of Europe after the last glacial maximum (LGM) (Prentice et al. 2011).

An important role in the population biology research of *S. vulgaris* plays the breeding system. Populations of *S. vulgaris* consist of female and hermaphrodite individuals therefore, it is known as a gynodioecious species. Hermaphrodite individuals of *S. vulgaris* are self-compatible and are likely to self at moderate rates (Jürgens et al. 1996, Bailey and McCauley 2006). However, selfing results into lower seed set, a lower weight of seeds and a lower germination rate (Jürgens et al. 1996). Hence, outcrossing is still an important process in *S. vulgaris* which is achieved through insect pollination. Originally it was thought that the species is solely pollinated by nocturnal moths or hawkmoths as other closely related species (e.g. *Silene alba*) (Pettersson 1991a, 1991b, Jürgens et al. 1996). However, it has been shown that bumblebees represent very effective pollinators as well, especially in the early morning hours, but even until dawn (Jürgens et al. 1996, Hintermeier and Hintermeier 1997). Although *Silene vulgaris* is a gynodioecious plant closely related species in the same genus possess different breeding systems. They reach from hermaphroditism (e.g. *S. paradoxa*), over gynodioecy (e.g. *S. vulgaris*, *S. nutans*) to dioecy (e.g. *S. latifolia*, *S. dioica*) (Muyle et al. 2021). Little wonder several species within the genus *Silene* have been the subject of studies on the evolution of breeding systems in plants (Sansome 1938, Desfeux et al. 1996, Lebel-Hardenack et al. 2002, Mrackova et al. 2008, Bernasconi et al. 2009, Cegan et al. 2012, Balounova et al. 2019, Bačovský et al. 2020, Muyle et al. 2021). Some *Silene* species possess sex chromosomes that have evolved at least twice independently within the genus (Mrackova et al. 2008, Cegan et al. 2012). In the species *S. vulgaris* however, the sex determination is more complex and is based on an interaction between multiple cytoplasmic male sterility (CMS) factors and autosomal male fertility restorer genes (McCauley and Taylor 1997, Olson and McCauley 2002). While CMS factors repress the production of fertile anthers in 'male-sterile' plants of *S. vulgaris*, the corresponding restorer genes can restore this trait, as their name suggests. CMS factors also show strong population structures which explains why the sex ratio can differ strongly among populations (Olson and McCauley 2002, Bailey and McCauley 2005). In a study by (Delph and Carroll 2001) it was further shown that ecological factors can influence the breeding system where hermaphrodites produced seeds of higher relative seed fitness under a high quality environment compared to female plants.

In summary, *S. vulgaris* has proven to be a well-established study organism (Bernasconi et al. 2009) that is capable of dealing with a high variety of environmental conditions on different levels. Hence, this species provides an excellent model species to study the influences of climate change on a widely distributed plant.

The experimental setup

To examine response differences to a climate change scenario in the chosen species *S. vulgaris* I conducted a greenhouse experiment in Potsdam, Germany from November 2015 to April 2016. I was using seeds from 25 different populations across a latitudinal gradient in Europe (site examples see Figure 2a and b). Each of these seeds were grown individually in pots with thirteen individuals per populations. 25 individuals (one per population) were then arranged in trays for better handling and an open top chamber was constructed around them with a heating device underneath (Figure 2c). These open top chambers included a thermostat as well and were used to heat up the temperature around the plants in a controlled manner. For the different climate change treatments, I was employing projections of potential climate change scenarios of central Europe. These scenarios predicted an increased temperature by 2.5-3.5°C and decreased precipitation by 15 mm or 25 mm (UBA 2006, IPCC 2007, Schwarz et al. 2007, Ahlström et al. 2012) which were referring to the average annual summer temperature and precipitation in East Germany (UBA 2006).

Figure 2. Collection sites of *S. vulgaris* seeds. a) Spanish site E1 near Alcanó. b) German site D2 in Berlin, Tempelhof. c) Experimental greenhouse setup in Potsdam.

To examine response differences in the different populations towards the climate change treatment various fitness-related traits were measured. I measured days until germination and days until flowering, dry mass, plant height, number of leaves, number of branches, number of flowers, leaf area, specific leaf area (SLA), and number of flowers. These traits have been proven to respond reliably to drought and temperature stress and have been used in several other studies (Rucker et al. 1995, Khan et al. 2015, Zeiter et al. 2016, Wellstein et al. 2017, Eziz et al. 2017, Meineri et al. 2020). Furthermore, the sex of each individual was determined to look for response differences between the sexes (described in Chapter 4). Leaf material was further collected from every plant within the experiment for the genetic population analysis via ddRAD sequencing (see Chapter 3).

Outline of the thesis

The following three chapters (Chapter 2-4) show published or drafted studies on the responses of *S. vulgaris* to a potential climate change scenario. All these chapters can be read independently as they represent separate research entities.

Chapter 2 deals with the general response of *S. vulgaris* to a simulated climate-change scenario studied in the greenhouse experiment over six months. To determine the response pattern different plant traits were measured and generalised mixed models used for the analysis. Furthermore, it was the aim of this study to investigate if different European populations (along a latitudinal gradient) respond differently to the employed climate-change scenario. This approach provides further insights into the adaptation potential of widely distributed species like *S. vulgaris* and facilitates our understanding of the role of phenotypic plasticity in plant adaptation to climate change.

In Chapter 3 the results from the greenhouse experiment were used and supplemented with a population genetic analysis of *S. vulgaris*. Here, a genome-wide approach was used to identify the underlying phylogeny of the sampled *S. vulgaris* populations across Europe. Afterwards the results from the greenhouse experiment were re-analysed to identify potential response differences among distinct genetic lineages of *S. vulgaris* towards the climate change scenario employed. Hence, this chapter provides a comprehensive approach to find a potential underlying phylogenetic influence on the adaptation of *S. vulgaris* to climate change.

Chapter 4 then deals with sexual dimorphism in *S. vulgaris*. It was first analysed if sexual dimorphism can be detected in the species. The second aspect dealt with the question if the sexes are influenced differently by the climate change treatment. The results of the analyses are then further discussed with regards to the evolution of the gynodioecious breeding system in *S. vulgaris* under the expected climate change. This analysis adds up to the underrepresented field of sexual dimorphism in gynodioecious species and provides further insights into sexually dimorphic traits under climate change.

Chapter 5 provides a general discussion of the previous chapters summarizing the overall results and conclusions regarding the research objectives. Furthermore, the chapter explains the constraints of the different studies and provides ideas and suggestions for additional research on the topic of adaptational responses to climate change in plants, i.e., in the widely distributed species *S. vulgaris*.

Chapter 2

Compensatory mechanisms to climate change in the widely distributed species *Silene vulgaris*

Chapter 2

Compensatory mechanisms to climate change in the widely distributed species *Silene vulgaris*

By Sandra Kahl, Michael Lenhard and Jasmin Joshi

Abstract

1. The adaptation of plants to future climatic conditions is crucial for their survival. Not surprisingly, phenotypic responses to climate change have already been observed in many plant populations. These responses may be due to evolutionary adaptive changes or phenotypic plasticity. Especially plant species with a wide geographic range are either expected to show genetic differentiation in response to differing climate conditions or to have a high phenotypic plasticity. 2. We investigated phenotypic responses and plasticity as an estimate of the adaptive potential in the widespread species *Silene vulgaris*. In a greenhouse experiment, 25 European populations covering a geographic range from the Canary Islands to Sweden were exposed to three experimental precipitation and two temperature regimes mimicking a possible climate-change scenario for central Europe. We hypothesized that southern populations have a better performance under high temperature and drought conditions, as they are already adapted to a comparable environment. 3. We found that our treatments significantly influenced the plants but did not reveal a latitudinal difference in response to climate treatments for most plant traits. Only flower number showed a stronger plasticity in northern European populations (e.g., Swedish populations) where numbers decreased more drastically with increased temperature and decreased precipitation treatment. 4. Synthesis. The significant treatment response in *Silene vulgaris*, independent of population origin – except for the number of flowers produced – suggests a high degree of universal phenotypic plasticity in this widely distributed species. This reflects the likely adaptation strategy of the species and forms the basis for a successful survival strategy during upcoming climatic changes. However, as flower number, a strongly fitness-related trait, decreased more strongly in northern populations under a climate-change scenario, there might be limits to adaptation even in this widespread, plastic species.

Introduction

Currently, many plant species must adapt to rapid climate changes. Global temperatures are predicted to rise by 2°C or more by the end of the century without intense measures to reduce greenhouse gas emissions (IPCC 2018) and precipitation patterns will change at a regional scale resulting in greater extremes of dry versus wet conditions (Easterling et al. 2000, Medvigy and Beaulieu 2012). Plants already respond to their changing environments via migration to more suitable habitats (Chen et al. 2011, Corlett and Westcott 2013, Pecl et al. 2017). However, not all plants are capable of long-distance migration via seed dispersal and fragmented landscapes further hinder their range expansion (Ellis 2015). An alternative strategy to cope with current climatic changes is thus adaptation while persisting in the changing habitat. Various changes of phenotypic traits as a result of a changing environment have already been reported in plant species including an alteration in leaf phenology (Estiarte and Peñuelas 2015), earlier flowering events (Cook et al. 2012, Ovaskainen et al. 2013), and changing growth rates or overall productivity (Norby and Zak 2011, Medeiros and Ward 2013). Adjusting the phenotype according to changing environmental conditions either requires phenotypic plasticity or rapid evolutionary adaptation mechanisms. Phenotypic plasticity describes the ability of a given genotype to produce different phenotypes in different environments and enables plant species to survive highly variable environmental conditions without permanently changing their phenotype through selection (Nicotra et al. 2010). In contrast, rapid evolutionary adaptation depends on heritable variation in fitness-related traits under different environmental conditions (Moritz and Agudo 2013). The relative importance of these two mechanisms under climate change is still debated. In their review, Nicotra et al. (2010) discuss the underlying theories as well as molecular and genetic mechanisms causing phenotypic plasticity in response to a changing climate. High levels of genetic variation within a population improve the adaptive potential of plants. Genetic variation in environmental signalling pathways, for example, play a crucial role in plastic phenotypic responses of plants by sensing abiotic and biotic cues of the environment (Nilson and Assmann 2010, Kvitek and Sherlock 2013). Chevin, Lande, & Mace (2010) proposed a model that incorporates phenotypic plasticity as well as heritable adaptation for a more accurate prediction of population persistence in a rapidly changing climate. However, the extent to which rapid evolution matters varies greatly between species and ecosystem properties (Ellner et al. 2011). When testing relationships between initial plasticity and subsequent adaptive evolution in natural populations, Ghalambor et al. (2015) revealed that adaptive plasticity might even constrain evolution in certain cases. In contrast, in a Mustard species it was found that phenotypic plasticity and adaptive evolution both contribute to an advanced flowering phenology in response to a warming climate (Anderson et al. 2012).

As the relative contributions of phenotypic plasticity and adaptive evolution to phenotype changes are still poorly understood (Van Kleunen and Fischer 2005, Valladares et al. 2007), we approached this question by conducting a greenhouse experiment. For this experiment, we sampled the widespread species *Silene vulgaris* (MOENCH) GARCKE (bladder campion) along a European latitudinal gradient. As a locally abundant species over a large geographic range (Rabinowitz et al. 1986) in meadows and ruderal places, *S. vulgaris* represents an ideal organism to address the topic of phenotypic adaptation as it may exhibit phenotypic plasticity as well as large genetic variation (Joshi et al. 2001). We examined the individual adaptive potential of ecologically important traits by exposing plants from 25 populations to experimental warming conditions (an increase by 3°C) and altered watering regimes (25 mm less precipitation) that reflect a possible climate change scenario for central Europe (IPCC 2007, Ahlström et al. 2012). In addition, we analysed whether the plant traits measured were correlated with local climatic conditions at sampling sites to account for possible trait adaptations to different environmental conditions. We measured biomass, plant height, number of flowers, number of branches, number of leaves, leaf area and specific leaf area (SLA) as fitness-related plant traits. These traits have been shown to negatively respond to drought and temperature stress (Rucker et al. 1995, Khan et al. 2015, Zeiter et al. 2016, Wellstein et al. 2017, Eziz et al. 2017). Thus, we hypothesized that biomass-related traits will have lower values under drought and increased temperature treatments. Biomass is also considered a reliable fitness-related trait that is positively associated with fecundity (Younginger et al. 2017). As a direct measure of fecundity, we used number of flowers as a proxy for number of seeds produced by an individual plant. As days to flowering is known to show considerable shifts towards earlier flowering with increasing temperature (Parmesan and Yohe 2003), we included this trait in our measurements. The right timing of flowering is essential for plant-pollinator interactions thus also influencing the reproductive success of a plant (Memmott et al. 2007).

It was our aim 1) to study putative trait responses in *Silene vulgaris* to a potential climate-change scenario of central Europe and 2) to investigate if latitudinal origin of different European *Silene vulgaris* populations influences the response to the climate-change scenarios applied. We hypothesized that southern European populations of *Silene vulgaris* will show a lower decrease in biomass-related traits and higher values in fecundity-related traits under higher temperatures and less precipitation as a result of pre-adaptation to these conditions.

Material and Methods

Study species and sampling sites

Figure 3. Sampling sites of *Silene vulgaris* from 25 populations.

Silene vulgaris (MOENCH) GARCKE (Caryophyllaceae) is an herbaceous perennial plant native to Eurasia. It is commonly found along roadsides, in meadows, cultivated fields or on abandoned lots (Taylor and Keller 2007). Local populations of *S. vulgaris* consist of female and hermaphrodite individuals (gynodioecy). Species in the genus *Silene* have served as model organisms for answering ecological and evolutionary questions for a long time. They have been used when examining adaptation to extreme soil environments (Bratteler et al. 2006b) or researching the evolution of plant sex chromosomes (Cegan et al. 2012, Papadopulos et al. 2015). Furthermore, *S. vulgaris* is a very popular study species for examining host-pathogen interactions with male-sterilizing fungi (Microbotryacea) (Hood et al. 2010, McArt et al. 2014). As a well-established study organism (Bernasconi et al. 2009), *S. vulgaris* is thus a suitable model species to investigate whether plant phenotypic changes responding to climate change are a result of rapid evolution or plasticity.

25 different populations were chosen at sites in Austria (A), Switzerland (CH), Germany (D), Spain (E) — including a population from the Canary Islands, France (F) and Sweden (S) (Table 1) spanning the natural latitudinal range of *S. vulgaris* in Europe (Figure 3). At each site, seeds were collected from 13 open pollinated plants in 2015 for the greenhouse experiment. The location of sampling sites was recorded using a GPS device (‘GARMIN GPS 72H’; accuracy <15 m; Garmin, Switzerland).

Greenhouse experiment

The experiment was conducted in a greenhouse in Potsdam, Germany from November 2015 – April 2016. From each population, 78 seeds were sown: six seeds from 13 mother plants per population to account for the six treatments. Thus, each mother plant was represented by six half-sib progenies across the six treatments. Plastic pots (Ray Leach 'Cone-tainers'^{TM}; 5 cm diameter; manufacturer: Stuewe and Sons, USA) were filled with standard potting soil (Einheitserde Classic, Profi Substrat, Pikiererde CL P; manufacturer: Einheitserde Werkverband e.V., Deutschland) combined with sand (3:1 v/v). Collected seeds were sown on the substrate described, one seed per pot and the pots were distributed to 78 trays (30 cm x 60 cm). This resulted in a total number of 1950 seeds. Treatments were randomly arranged to

Table 1. Sampling sites of 25 European *Silene vulgaris* populations. Populations are arranged according to their latitudinal origin.

Population	Country	Site	Elevation [m]	Latitude	Longitude
E4	Spain	La Palma	1100	28° 46' 58.0" N	17° 56' 22.0" W
E1	Spain	Alcanó	211	41° 29' 29.4" N	00° 26' 27.3" E
E3	Spain	La Sentiu de Sió	316	41° 49' 44.1" N	00° 54' 19.9" E
E2	Spain	Fontdepou	798	41° 57' 44.6" N	00° 45' 51.4" E
F2	France	Pommeraie	78	43° 56' 42.6" N	01° 22' 21.5" E
F4	France	Menton	177	45° 39' 57.7" N	05° 17' 30.9" E
CH2	Switzerland	Cadenazzo	202	46° 09' 09.0 "N	08° 56' 33.0" E
F3	France	La Noue du Bourg	73	46° 39' 28.0" N	01° 23' 13.6" W
F1	France	Les Rochette	65	46° 41' 00.8" N	01° 23' 58.0" W
CH1	Switzerland	Flims	2102	46° 52' 03.8" N	09° 14' 23.4" E
A1	Austria	Gschnitz	1234	47° 02' 51.0" N	11° 21' 29.0" E
D12	Germany	Langenargen	402	47° 36' 33.2" N	09° 31' 43.7" E
D10	Germany	Konstanz	400	47° 40' 23.0" N	09° 09' 10.6" E
F5	France	Normandie	83	49° 16' 06.0" N	01° 37' 39.0" E
D6	Germany	Heilsbronn	400	49° 20' 44.6" N	10° 47' 26.5" E
D11	Germany	Hamm	90	49° 44' 26.2" N	08° 26' 58.6" E
D5	Germany	Mainz	89	49° 59' 53.4" N	08° 13' 15.7" E
D9	Germany	Potsdam	90	52° 21' 44.6" N	13° 04' 34.9" E
D3	Germany	Berlin	46	52° 28' 14.4" N	13° 23' 44.4" E
D1	Germany	Berlin	30	52° 31' 58.7" N	13° 23' 10.2" E
D7	Germany	Gerswalde	70	53° 11' 06.3" N	13° 45' 28.0" E
D8	Germany	Geesower Hügel	30	53° 14' 32.9" N	14° 23' 06.9" E
D2	Germany	Hamburg	25	53° 40' 21.0" N	10° 05' 04.8" E
S2	Sweden	Vickleby	51	56° 34' 37.1" N	16° 27' 39.5" E
S1	Sweden	Södra Bäck	10	56° 40' 12.7" N	16° 40' 54.2" E

account for possible temperature or light gradients in the greenhouse and reflected conditions derived from climate-change prediction models (IPCC 2007, Ahlström et al. 2012). Referring to these predictions two temperature treatments were established in the greenhouse using open-top chambers with additional heating devices. Open-top chambers were built using polystyrene and transparent, unplasticised PVC foils (400 µm thickness; Pütz GmbH + Co. Folien KG, Germany) around the trays. Heating mats ('BioGreen WP 030-060'; Bio Green OHG, Germany) were placed under the pots to increase air temperature. A thermostat ('Universal UT 200-2'; manufacturer: ELV Elektronik AG, Germany) was used to measure and keep the temperature on a constant level. The control temperature treatment was set to 18°C and the warming treatment to 21°C. The control temperature was based on the average summer temperature for Eastern Germany (UBA 2006). The simulated climate change temperature was chosen based on predictions of a temperature increase of 2.5–3.5°C for central Europe (using datasets for Germany as a proxy; Schwarz, Harmeling, & Bals, 2007; UBA, 2006). For the precipitation treatment, the values were set to 90 mm, 75 mm, and 65 mm. We used these watering regimes based on precipitation values for Eastern Germany and climate models predicting a -15 mm to -25 mm decrease in precipitation for central Europe using datasets for Germany as a proxy (UBA 2006, IPCC 2007, Schwarz et al. 2007). Temperature and precipitation treatments were combined in a fully factorial design resulting in overall six treatment combinations.

The following plant-performance traits were measured for each plant individually: days until germination and flowering, biomass in g, plant height (at the day of first flowering; in cm), number of leaves (at the day of first flowering), number of branches (at the day of first flowering), number of flowers (after harvesting), leaf area in cm² and specific leaf area (SLA; in cm²/mg). As the number of seeds could not be determined reliably (ripening capsules lost seeds before sampling), the number of flowers was counted as a measure of fecundity and an estimate of reproductive plant fitness. Aboveground biomass of all plants was harvested after the withering of the majority of the inflorescences. To determine biomass, plants were dried at 80°C for 48 h after harvesting and weighed immediately. SLA was measured by removing one leaf per plant (4th leaf counted from the bottom) at the time of flowering. Leaves were air-dried for four weeks and weighed afterwards. To determine leaf area, leaves were glued on barcoded paper sheets with a size key. Paper sheets with leaves were digitized using either a flatbed scanner or a digital photo camera. The obtained images were further processed using the Python module scikit-image (van der Walt et al. 2014). Individual leaves were segmented using an intensity thresholding approach. Leaf area was extracted using the regionprops function in scikit-image.

Climate data

Historical climate data from the sampling sites were obtained from WorldClim from 1970–2000 (Fick and Hijmans 2017). These data included 19 bioclimatic variables in addition to solar radiation, wind speed and water-vapour pressure (Table A1, Table A2). Population sampling sites were analyzed for climatic patterns in a principal component analysis (PCA). PCA was performed using the function prcomp() in R (R Core Team 2017). To check if climate at a population' site was correlated to latitude of population a linear regression of principal component loadings and latitude was performed.

Based on the WorldClim data (Fick and Hijmans 2017) the 25 populations covered a certain amount of climatic variation (Figure A1a). The first axis of the PCA on climatic variables (46.6% of the variation) was strongly correlated with 'annual mean temperature' (bio_1), 'mean temperature of warmest quarter' (bio_10), 'precipitation of driest month' (bio_14) or 'precipitation of warmest quarter' (bio_18) (Table A3). The variables 'temperature seasonality', 'mean temperature of wettest quarter', and 'precipitation of coldest quarter' were largely associated with principal component 2 (23.4% of the variation) (Table A3). The populations' positions on PC 1 and PC 2 reflect their latitudinal distribution (Figure A1a). Linear regressions of principal component scores and latitude were significantly correlated. With increasing latitude, the populations had significantly lower PC 1 loadings (R^2= 0.19, p= 0.03, n =25) but increasing PC 2 loadings (R^2= 0.51, p <0.001, n= 25) (Figure A1b, c). Therefore, populations from lower latitudes can be characterised by higher mean annual temperature, higher temperatures during the warmest quarter or lower precipitation during the driest month and warmest quarter of the year. With increasing latitude, the populations also experienced a stronger temperature seasonality (bio_4) and lower solar radiation (Table A3). The projections of the populations on the two axes did not reveal distinct groups, but one Swiss (CH1) and one Spanish population (E4) were located as climatic outliers on PC 1 and PC 2, respectively. The high alpine population CH1 is characterized by the highest precipitation values (Table A1, Table A2) whereas E4 exhibits the strongest precipitation seasonality and smallest annual temperature range (Table A1, Table A2) leading to its outlier position in the PCA.

Trait differentiation analysis

For statistical analyses of plant traits, we employed linear mixed models for traits fitting a normal distribution. For binary data (germination, survival, and flowering) and count data (number of flowers, number of leaves, number of branches) we used generalized mixed models with a binomial and Poisson distribution, respectively. Models were performed using the function lmer() and glmer() in R (R Core Team 2017). As fixed factors, we included: Treatment of plants (divided in temperature and precipitation), latitude of population origin (linear), temperature x precipitation, temperature x latitude, precipitation x latitude. Random factors were population identity and mother plant.

Plasticity index

To get an estimation of plasticity we calculated the Plasticity Index (PI) for the temperature and precipitation treatment separately. We used the approach introduced by Valladares, Wright, Lasso, Kitajima, & Pearcy (2000): PI=(mean(treatment1))-(mean(treatment2)) / max(mean(treatment1)),(mean(treatment2)). This index can be easily applied, is widely used in different studies and can be compared among plant traits (Valladares et al. 2006). A linear model was performed analysing the influence of latitude on the PI of each trait.

Results

Of a total of 1950 seeds, 86.5% germinated. The lowest number of seeds germinated in a Swiss (CH1) and a Spanish (E2) population (32.1% and 43.6%, respectively; Figure A2a). As treatments started two weeks after germination (to ensure seedling establishment), treatment effects could not be tested on speed of germination.

74.2% of surviving plants flowered. The days to flowering ranged from 81–171 days with a peak at 117 days after sowing. The percentage of flowering individuals per population differed strongly. By the end of the experiment, the German population D9 had the highest proportion of individuals flowering (98%) whereas the lowest proportion of flowering individuals was found in the two Swedish populations with both around 44% (Figure A2b).

The survival of plants during the experiment was high (97.4%). Most plants died in population CH1 (24%) whereas all individuals survived in the populations D1, D5, D6, D8, D10, D11, D12, E3 and E4 (Figure A2c).

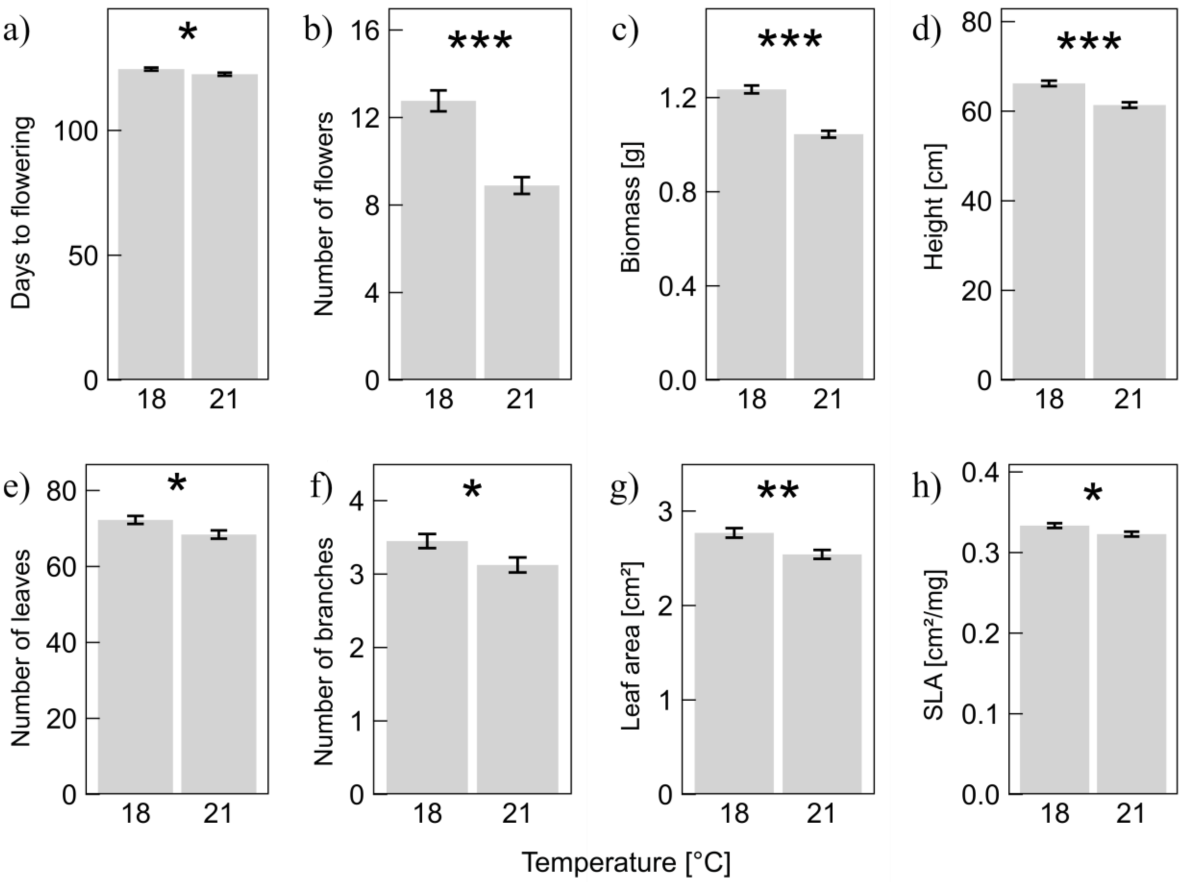

Figure 4. Responses of phenotypic traits (means ± standard errors) in *Silene vulgaris* plants with regard to the two experimentally applied temperature regimes (18°C and 21°C). a) days to flowering, b) number of flowers, c) dried biomass, d) height, e) number of leaves, f) number of branches, g) leaf area and h) specific leaf area (SLA). Asterisks indicate significant differences (* P <0.05, ** P <0.01, *** P <0.001).

Effects of simulated climate change on plant traits

Temperature significantly influenced all plant traits in our experiment leading to lower trait values with increasing temperature (Table 2, Figure 4). The strongest differences were found in the number of flowers with on average 12.8 flowers at 18°C compared with 8.9 flowers at 21°C (Figure 4b). Plant biomass reached 1.2 g versus 1.0 g and plant height reached on average 66.2 cm versus 61.4 cm at 18°C compared to the 21°C-treatment, respectively (Figure 4c, d). The number of leaves and branches per plant, leaf area and SLA were decreased under the 21°C-treatment, however to a less significant extent than the overall biomass (Figure 4e, f, g, h). Days to flowering advanced with increasing temperature from 124.6 days to 122.5 days on average (Figure 4a). However, fewer plants, 73% compared to 83%, flowered under the increased temperature treatment in the greenhouse.
Precipitation influenced half of all traits measured except for survival, flowering probability, days to flowering, leaf area and SLA (Table 2). The strongest differences were visible

Table 2. ANOVA results of plant phenotypic traits in *Silene vulgaris* grown under two different temperature and three different precipitation conditions. Latitude refers to the latitude at origin site of different *S. vulgaris* populations. Significant effects are indicated in bold. For binary data (germination, survival, flowering) a binomial distribution and for count data (number of flowers, leaves, branches) a Poisson distribution was assumed. Degrees of freedom for the population effect were 22 for all traits, except biomass and leaf area (df=23). SLA: Specific leaf area, df: degrees of freedom.

Trait		Germination		Survival		Flowering		Biomass		Height		Days to flowering	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temperature	1	-	-	0.10	0.75	**25.02**	**<0.001**	**94.7**	**<0.001**	**46.32**	**<0.001**	**5.96**	**0.01**
Precipitation	2	-	-	1.01	0.60	1.42	0.49	**173.1**	**<0.001**	**43.25**	**<0.001**	0.35	0.84
Latitude (linear)	1	0.24	0.62	0.13	0.72	**5.25**	**0.02**	1.9	0.17	3.31	0.07	1.69	0.19
Temp x Prec	2	-	-	1.67	0.43	1.30	0.52	0.4	0.84	1.74	0.42	1.65	0.44
Temp x Lat	1	-	-	0.20	0.65	1.94	0.16	0.2	0.67	0.73	0.39	0.13	0.71
Prec x Lat	2	-	-	0.76	0.68	2.59	0.27	0.3	0.85	3.62	0.16	0.27	0.88
R² marginal (conditional)		0.003 (0.31)		0.003 (0.05)		0.07 (0.20)		0.13 (0.31)		0.09 (0.46)		0.03 (0.30)	

Trait		Flower number		Leaf number		Branch number		Leaf area		SLA	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temperature	1	**536.55**	**<0.001**	**56.05**	**<0.001**	**9.34**	**<0.01**	**15.14**	**<0.001**	**6.47**	**0.01**
Precipitation	2	**147.44**	**<0.001**	**131.49**	**<0.001**	**18.96**	**<0.001**	4.54	0.10	5.49	0.06
Latitude (linear)	1	2.29	0.13	0.43	0.51	**6.54**	**0.01**	0.00	0.96	0.44	0.51
Temp x Prec	2	**10.02**	**<0.01**	**24.25**	**<0.001**	1.24	0.54	0.04	0.98	0.03	0.98
Temp x Lat	1	**9.00**	**<0.01**	2.87	0.09	0.02	0.88	2.51	0.11	0.48	0.49
Prec x Lat	2	**14.45**	**<0.001**	**12.48**	**<0.01**	0.35	0.84	0.14	0.93	0.28	0.87
R² marginal (conditional)		0.13 (0.85)		0.08 (0.63)		0.05 (0.18)		0.01 (0.33)		0.01 (0.12)	

between the lowest and highest precipitation treatment, i.e. between 65 mm and 90 mm precipitation: with lower precipitation, most trait values, decreased significantly (Figure 5). Plants reached a height of 66.2 cm and a biomass of 1.3 g under 90 mm of precipitation but only 60.7 cm and 1.0 g under the 65-mm precipitation treatment (Figure 5d, c). Number of leaves and branches decreased from 74 to 68 and from 3.5 to 3.0, respectively (Figure 5e, f). Of the leaf traits only SLA decreased significantly, with 0.32 cm²/mg under 65 mm of precipitation compared to 0.34 cm²/mg under the 90 mm treatment (Figure 5h).

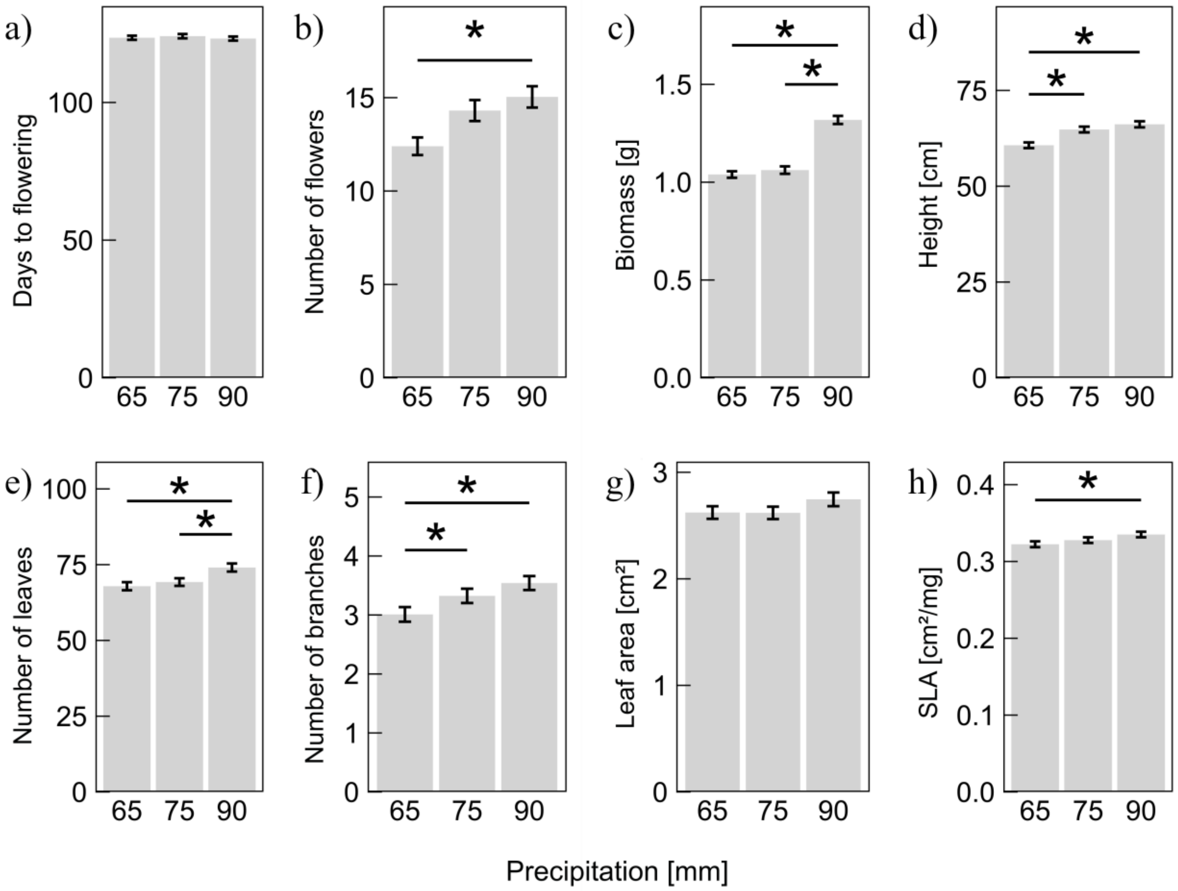

Figure 5. Responses of phenotypic traits (means ± standard errors) in *Silene vulgaris* plants regarding the three experimentally applied precipitation regimes (65 mm, 75 mm and 90 mm). a) days to flowering, b) number of flowers, c) dried biomass, d) height, e) number of leaves, f) number of branches, g) leaf area and h) specific leaf area (SLA). Asterisks indicate significant differences (* P <0.05, ** P <0.01, *** P <0.001).

Influence of population origin on plant responses to climate change

Except for the number of flowers and leaves produced, none of the trait responses to reduced precipitation differed along the latitudinal gradient (Table 2).

These non-significant treatment (temperature or precipitation) x latitude interactions indicated that the treatment effects were similar in all populations along the latitudinal gradient (Table

2). This is also reflected in the mainly parallel reaction norms, e.g. in biomass response to increased temperature and decreased precipitation, respectively (Figure A3c, Figure A4c). Of the two traits that differed in their response along a latitudinal gradient, the number of flowers showed strong temperature and precipitation responses (steeper slopes) with increasing latitude (Figure A5a, b) whereas the number of leaves (Figure A5c) only differed in their response to the precipitation treatment along the latitudinal gradient.

Plasticity index

The significant influence of the climate treatments on plant traits (Table 2) indicates a considerable degree of plasticity in phenotypic traits of *S. vulgaris*. Plasticity of biomass-related traits was higher than plasticity in days to flowering or SLA and was comparable between the temperature and precipitation treatment. (Figure A6, Figure A7). The plasticity index (PI) of flower number was significantly influenced by latitude for the temperature and precipitation treatment (Table 3). The PI of days to flowering was influenced by latitude only for the precipitation treatment. Number of flowers and days to flowering were more plastic at sites of higher latitude (Figure 6, Figure A7). For the remaining traits, we were not able to find a significant influence of latitude on PI, neither for the temperature nor for the precipitation treatment (Table 3, Figure A6, Figure A7). The standard errors in Figure 6 and Figure A6 show that the PI varied strongly within populations indicating that some maternal genotypes were more plastic than others within populations.

Table 3. The effects of latitude on plasticity index (PI) based on individual traits. PI was calculated separately for precipitation and temperature treatments. Tests were using linear regressions with *F*- and *p*-values given for 24 degrees of freedom. Plasticity of flower number significantly increased with latitude for temperature and precipitation treatment, plasticity of days to flowering significantly increased with latitude for precipitation treatment (stated in bold).

	Temperature		Precipitation	
	F	*p*	*F*	*p*
Biomass	0.0	0.91	0.0	0.94
Height	0.2	0.67	1.1	0.29
Days to flowering	2.4	0.12	**5.9**	**<0.05**
Flower number	**9.9**	**<0.01**	**6.6**	**<0.05**
Leaf number	0.1	0.80	0.1	0.80
Branch number	0.2	0.65	0.5	0.46
Leaf area	0.3	0.61	0.1	0.75
SLA	0.7	0.39	0.6	0.43

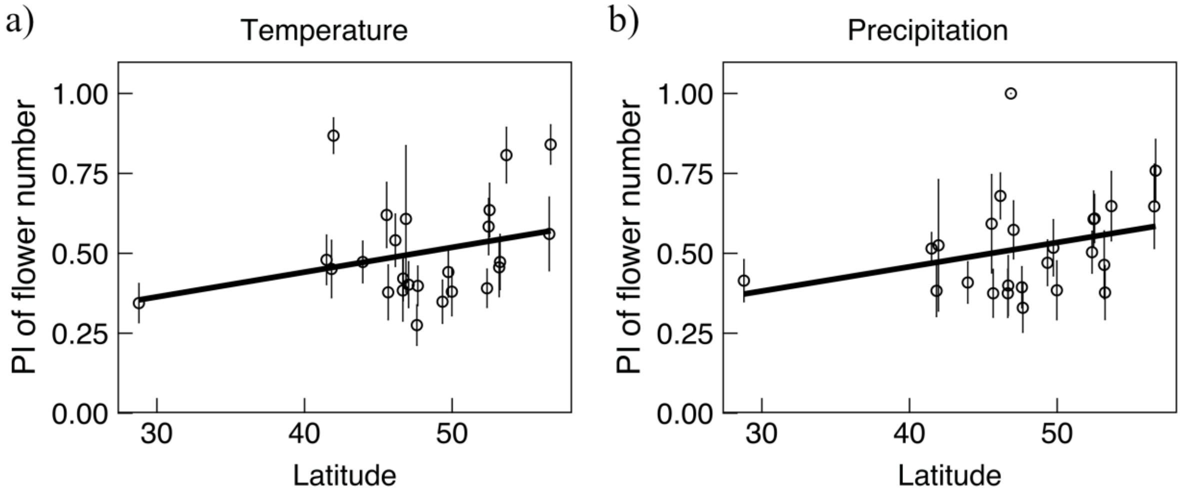

Figure 6. Plasticity index (PI; means ± standard errors) of flower number of *S. vulgaris* populations across their latitudinal origin. a) PI for temperature treatment, b) PI for precipitation treatment.

Discussion

Climate change is altering environmental conditions such as the availability of resources that are of great importance for the survival of plant species (Nicotra et al. 2010). Thus, it is crucial to understand how plants will behave under the climatic conditions forecasted for the near future. In our experiment, the applied increased temperature treatment had a significantly negative effect on the majority of observed plant traits in *Silene vulgaris* independently of plant origin. Earlier studies have shown that temperature is a major factor altering plant phenotypic traits as it influences growth as well as the timing of plant development (Cleland et al. 2007, Hatfield and Prueger 2015). A negative correlation between plant biomass or plant height and temperature, as found in our study, has already been shown in *Silene noctiflora* (Qaderi and Reid 2008) and *Erigeron speciosus* (de Valpine and Harte 2001) and was also observed for vegetation cover in geothermal areas (Nishar et al. 2017). Reduced plant height and smaller leaves result in a smaller transpiration surface and thus may help the plant to withstand the increased drought stress at higher temperatures. Increasing temperature also influenced phenology by decreasing the days to flowering by approximately 2 days in our experiment. This is in agreement with other studies that observed earlier flowering with increasing temperatures (Alatalo and Totland 1997, Park and Schwartz 2015) suggesting that climate change is a driving force in the shift of flowering times towards the beginning of the year (Parmesan and Yohe 2003, Franks et al. 2007, Bertin 2015). The correct timing of flowering is an essential ability to maximize reproductive output as plants additionally depend on optimal environmental conditions and out-crossing species on synchronous flowering and pollinator availability. Our results revealed an advanced flowering only by two days, this may not necessarily impair plant fitness with regard to pollinator interactions, but long-term effects over several generations cannot be excluded. In general, it has to be kept in mind that results of warming experiments are highly species- and ecosystem-specific and they are also strongly interconnected to the availability of water.

The experimental precipitation regimes influenced the plant traits to a lesser extent (Table 2) although a meta-analysis by Siepielski et al. (2017) showed that precipitation is a strong selective force often exceeding the influence of temperature on plant adaptation. Most significant differences in morphology were observed between the extreme precipitation regimes of 65 and 90 mm where biomass-related traits decreased significantly with less precipitation. We detected a 23% decrease in biomass, an 8% decrease in plant height and number of leaves as well as a 14% decrease in number of flowers under the lowest precipitation treatment. These results are in line with other studies where drought stress decreased stem and leaf biomass (Prieto et al. 2009, Dubois et al. 2017, Eziz et al. 2017). Decreased overall biomass under increasing drought was also found in alpine *S. vulgaris* in a study by Hamann, Kesselring, & Stöcklin (2018). Similar to heat response, drought response in plants focuses on minimising plant transpiration to lower water consumption. Therefore, the plant growth theory predicts, that this lack of resources leads to a decline in biomass production and lower SLA (Poorter and Remkes 1990). Our biomass-related traits were able to confirm this theory but leaf traits, however, were not significantly influenced by the precipitation treatment in our overall model. This contradicts the results of several other studies, where leaf area and SLA decreased under drought (Maes et al. 2009, Jung et al. 2014, Wellstein et al. 2017). Under dry conditions, plants are expected to decrease their leaf surface and SLA to prevent the evaporation of water and thus enhance the water-use efficiency. However, these leaf traits have also been shown to be highly species-specific (Griffin-Nolan et al. 2018) which impedes the prediction of general drought responses. Flower number decreased significantly with lower precipitation in our experiment. This result is in accordance with findings describing a lower number of flowers and seeds under increased drought conditions in ornamental and agricultural plants (Mwanamwenge et al. 1999, Flagella et al. 2002, Çakir 2004, Álvarez et al. 2009). A study by Levine, McEachern, & Cowan (2008), however, could not find a correlation between flower number and precipitation in natural populations of annual plants.

The PCA revealed a considerable degree of climatic variability among our populations that was also correlated to latitude. Latitude was therefore used as a proxy for climatic differences in the analysis of trait responses and plasticity. If trait-climate associations were adaptive, southern European populations (e.g. E4, E1, E3) should exhibit decreased leaf and biomass-associated traits that are favoured under warmer and drier conditions. In our mixed models traits did not change along the latitudinal gradient except for the number of branches. Hence, trait adaptation in southern populations could not be detected. This is not in accordance with common garden experiments where it has been shown that plant height decreases and SLA increases with latitude representing an adaptation strategy to colder, wetter environments (De Frenne et al. 2013). However, the sensitivity of trait response (i.e., the interaction of latitude and treatment) in our mixed model showed a significant latitudinal difference for number of flowers and number of leaves. These traits responded more drastically in populations from northern latitudes where significantly less flowers were produced under the higher temperature or lower precipitation treatment and where a lower number of leaves also correlated with lower precipitation. Especially, the number of flowers is a strong fitness related trait determining the number of offspring. With regard to our initial hypothesis, we can assume that northern populations of *Silene vulgaris* may have more difficulties in producing a feasible number of offspring under climate change. This might even be aggravated through herbivory on developing seeds through the lychnis moth, *Hadena bicruris*, or the anther sterilizing fungus *Microbotryum*. Both organisms, insects and pathogenic fungi, are likely to thrive through climate change and are predicted to have extensive impacts on ecosystems (Chakraborty and Newton 2011, Fisher et al. 2012, Meineke et al. 2018). To test if the decreased flower number could threaten the survival of individual *S. vulgaris* populations, a long-term experiment with several generations under climate-change conditions would be needed. However, it has to be recognized that the interaction effect of treatment and latitude in this trait is strongly influenced by the outlier position of the southernmost population (E4) leading to the significant increase in response sensitivity.

Phenotypic plasticity is assumed to be among the main mechanism for species to persist under changing climatic conditions as it allows for rapid phenotypic changes. In summary, the majority of plant traits showed a significant response towards the treatments thus indicating a considerable phenotypic plasticity for *S. vulgaris*. The calculated PIs support this result for the two treatments and the different populations. We found a markedly differing PI among populations and plant traits. Higher PI values found in biomass-related traits and leaf area indicate that *S. vulgaris* has the ability to respond to increased temperature and decreased precipitation through restricted growth. In contrast, days to flowering and SLA were more fixed traits under changing climatic conditions showing a lower PI. The fact that days to flowering is a less plastic trait may indicate stabilizing selection. In the gynodioecious *S.*

vulgaris the presence of pollinators during flowering time is crucial to ensure seed set also in exclusively female plants and increase the number of offspring. In this respect, climatic factors like precipitation or temperature represent less important selective forces leading to a change in flowering time. As phenotypic plasticity itself can be seen as a plant trait, it also possesses a genetic basis (Bradshaw 1965). The missing interaction of latitude and treatment (i.e. temperature or precipitation) in most traits suggests a low genetic differentiation in phenotypic plasticity among populations which is in line with other studies examining plasticity under different moisture and temperature conditions (Gugger et al. 2015, Münzbergová et al. 2017). Mainly the phenotypic plasticity of flower number showed a significant genetic differentiation along a latitudinal gradient. In this case, plasticity itself may represent a trait under selection which is exposed to differing climatic conditions and climate variability. According to the climatic variability hypothesis (CVH), higher latitudes possess a higher thermal variability and thus should also show greater phenotypic plasticity obtained through selective pressure (Janzen 1967, Molina-Montenegro and Naya 2012). In our study, latitude was used as a proxy for climate at population origin and it was positively correlated with annual temperature range or temperature seasonality, two indicators of thermal variability. The linear regression of latitudinal influence on plasticity index did not lead to a significant effect for most plant traits but we found a significant influence on the PI of flower numbers. This may indicate that northern populations are more likely to adjust their number of flowers to changing climatic conditions (i.e., temperature and precipitation). However, it is unlikely that the increased plasticity in flower number is adaptive. Through our greenhouse experiment we showed that northern populations are also more likely to reduce their number of flowers under climate change conditions. The higher plasticity in these populations may thus actually lead to fewer offspring during climate change and rather represents a maladaptation of this trait.

Conclusion

Climate-change scenarios expect an increase in temperature by up to 3.5°C and a decrease in precipitation by up to 25 mm in the summer months for central Europe. In our experiment, we mimicked these conditions for 25 populations of *Silene vulgaris* to study population differences in phenotypic responses. In summary, our results paint a complex picture of the adaptation of the widely distributed species *Silene vulgaris* to climate change. All populations survived the treatments and showed considerable variation in their plant traits and phenotypic response to treatments. When adapting to changing environmental conditions the combination of genetic and plastic response mechanisms was shown to be a common pattern. As our sampling approach covered a long latitudinal gradient from southern to northern Europe with high climatic variation, we expected that southern European populations would show higher biomass-related traits and higher fecundity-related values under the climate-change conditions as they already experience warmer and drier conditions compared to central Europe. However, our results did not confirm this theory and neither showed differences in phenotype nor in phenotypic plasticity for most traits along a latitudinal gradient. Therefore, *S. vulgaris* populations of central Europe are likely to deal with a future climate change scenario of 3°C temperature increase, and 25 mm precipitation decrease in a similar way as southern European populations. Flower number was the only trait that responded differently in southern compared to northern populations when exposed to the experimental temperature increase and precipitation decrease. In accordance with our initial hypothesis, flower number decreased more drastically in northern populations than in southern populations. An increase of phenotypic plasticity with latitude is also in accordance with the climate variability hypothesis, but the adaptive character of flower-number plasticity in our species is debatable. As flower number is directly related to number of offspring, a strong decrease in this trait might actually turn out to be maladaptive leading to an insufficient offspring number that is further threatened by pathogenic fungi or seed predators. For a complete assessment of *S. vulgaris* response to climate change, extreme weather events have to be taken into account as well. Extreme drought, for example, has the potential to reduce offspring by as much as 80−90% and also to decrease biomass production of different grassland species significantly (Beierkuhnlein et al. 2011, Hatfield and Prueger 2015). Further experiments with *Silene vulgaris* should therefore also mimic extreme droughts or flooding events. Although not along a latitudinal gradient, populations showed differences in their traits and trait responses. A population genetic approach or QTL mapping could be used to assess trait adaptation to climatic factors and to identify traits that are likely to adapt under ongoing climate change.

Chapter 3

Phylogeography of a widely distributed plant species reveals cryptic genetic lineages with parallel phenotypic responses to warming and drought conditions

Chapter 3

Phylogeography of a widely distributed plant species reveals cryptic genetic lineages with parallel phenotypic responses to warming and drought conditions

By Sandra Kahl, Christian Kappel, Jasmin Joshi and Michael Lenhard

Abstract

To predict how widely distributed species will perform under future climate change it is crucial to understand and reveal their underlying phylogenetics. However, detailed information about plant adaptation and its genetic basis and history remains scarce and especially widely distributed species receive little attention despite their putatively high adaptability.

To examine the adaptation potential of a widely distributed species, we sampled the model plant *Silene vulgaris* across Europe. In a greenhouse experiment, we exposed the offspring of these populations to a climate-change scenario for central Europe and revealed the population structure through whole genome sequencing. Plants were grown under two temperature (18°C, 21°C) and three precipitation regimes (65 mm, 75 mm, 90 mm) to measure their response in biomass and fecundity related traits. To reveal the population genetic structure, ddRAD sequencing was employed for a whole genome approach. We found three major genetic clusters in *S. vulgaris* from Europe: one cluster comprising Southern European populations, one cluster of Western European populations and another cluster containing Central European populations. Population genetic diversity decreased with increasing latitude and a Mantel test revealed significant correlations between F_{ST} and geographic distances as well as between genetic and environmental distances. Our trait analysis showed that the genetic clusters significantly differed in biomass-related traits and in the days to flowering. However, half of the traits showed parallel response patterns to the experimental climate change scenario. Due to the differentiated but parallel response patterns, we assume that phenotypic plasticity plays an important role for the adaptation of the widely distributed species *S. vulgaris* and its intraspecific genetic lineages.

Introduction

Throughout their evolutionary history, organisms have had to cope with changing climates or other environmental changes. Species are able to deal with environmental changes through migration, phenotypic plasticity, and/ or genetic adaptation (Exposito-Alonso et al. 2018, Hämälä et al. 2018, de Villemereuil et al. 2018, Radchuk et al. 2019). However, as all species are limited by trade-offs and only possess a certain range of tolerable environmental conditions, rapid climate change may represent an intensive threat that affects their survival (Barnosky et al. 2011, Pacifici et al. 2015, Radchuk et al. 2019, Trisos et al. 2020).

To reliably predict biodiversity changes under climate change, it is also important to take a closer look at the phylogeography of species as genetic lineages on an intraspecific level may differ in their adaptive genetic responses (Prunier et al. 2012, Schwarzer and Joshi 2017). Past distribution patterns are often reflected in current species phylogenies and can be associated with divergent environmental conditions. This ghost of selection past (Samani and Bell 2016) may be a strong selective force leading to differing genetic adaptations (Prunier et al. 2012, García-Fernández et al. 2013). Especially widely-distributed species consist of a variety of populations that can show morphological differences or exhibit local adaptation (Joshi et al. 2001, Wright et al. 2006, Pearman et al. 2010). So far, only few studies have dealt with the adaptation potential or response differences on an intraspecific level; as a result little is known about the implications of ignoring phylogeographic structures when studying climate change responses (Pfenninger et al. 2007, Pearman et al. 2010). Studies on *Pinus* and other widely distributed species show that differences in intraspecific response to various climatic factors can be found and should be of importance when studying the impacts of climate change (Rehfeldt et al. 2002, Zhang et al. 2004, Oney et al. 2013, Brabec et al. 2017).

The herbaceous plant *Silene vulgaris* is such a widely distributed species that covers a South-North gradient from North Africa up to the far North of Europe and a West-East gradient from Iceland to the middle East and temperate Asia; the species was also introduced to North America, Australia, South Africa, Ethiopia and Japan (Registry-Migration.Gbif.Org 2021, WFO 2021). *S. vulgaris* possesses a variety of known ecotypes especially adapted to extreme environmental conditions (i.e., heavy-metal soil pollution) (Pacwa-Płociniczak et al. 2018, Muszyńska et al. 2019b). Furthermore, we reported in an earlier study that *S. vulgaris* responds considerably towards climatic changes through phenotypic plasticity (Kahl et al. 2019). These characteristics make *S. vulgaris* a suitable species to investigate the response differences of genetic lineages to climate change. To examine these response differences, we sampled 25 European populations of *S. vulgaris* spanning a latitudinal gradient and tested the response of the different genetic lineages to a simulated climate change scenario for central Europe. Population structure was phylogeographically evaluated using ddRAD sequencing and populations were exposed to a potential climate change scenario (with a temperature increase by 3°C and a reduced precipitation by 15 and 25 mm per summer month, respectively) to examine their phenotypic response. For the evaluation of phenotypic responses nine different plant traits were measured. We chose those plant traits that are known to strongly react to temperature and precipitation changes and are proxies for plant fitness (Memmott et al. 2007, Hatfield and Prueger 2015, Wellstein et al. 2017, Eziz et al. 2017).

It was the aim of the study (1) to reveal the genetic population structure of the sampled *Silene vulgaris* populations across Europe and (2) to test, if putatively different genetic lineages of *S. vulgaris* showed a different response to a simulated climate change scenario for central Europe.

Material and Methods

Model plant species, sample collection and greenhouse experiment

Silene vulgaris (Moench) Garcke is a diploid (2n=24), perennial plant of the family of Caryophyllaceae with a self-compatible reproductive system. Its native range covers the entire European continent including islands and expands towards Russia, North Africa (Morocco, Egypt), to the Middle East and parts of Asia (Bushneva 2002, Taylor and Keller 2007, Pearl et al. 2009, Registry-Migration.Gbif.Org 2021). Apart from its native ranges in Eurasia *S. vulgaris* has also colonized North America where it has become an invasive species in some locations (McCauley et al., 2003; Taylor & Keller, 2007). Populations of *S. vulgaris* consist of female and hermaphrodite individuals making it a gynodioecious plant. It typically occurs in open grasslands or cultivated fields as well as on abandoned lots and can exhibit a high heavy metal tolerance (Bringezu et al. 1999, Taylor and Keller 2007). The pollination of *Silene vulgaris* is primarily done by moths, bumble bees and hover flies (Pettersson 1991b, Jürgens et al. 1996). For the present study, a total of 325 plants were sampled in 2015 along a latitudinal gradient in Europe (Table 4). The plants belonged to 25 different populations of which seed samples were collected from 13 open pollinated plants per population. One of the populations (F3) was collected at three different locations at the southern coast of France (Figure 9). The sampling of each of the locations did not result in sufficient seed material for a balanced experimental design. Hence, we combined the seeds from the different locations into one single population (F3) as we considered the coastal area of France an important addition to the experiment based on the different climate conditions. Six seeds per plant were grown in a greenhouse and exposed to two different constant temperatures (18°C and 21°C) and three different precipitation conditions (90 mm, 75 mm, and 65 mm per month) as described in Kahl et al. (2019). The temperatures were held constant using a setup of heating mats (‘BioGreen WP 030-060’; Bio Green OHG, Germany) and thermostats (‘Universal UT 200-2’; manufacturer: ELV Elektronik AG, Germany), see Kahl et al. (2019). For a precise watering, a commercial bottle-top dispenser was used, and each pot was watered individually. The conditions resembled a possible climate change scenario for central Europe with increased average annual temperatures and decreased rainfall (Ahlström et al. 2012, IPCC 2013).

Table 4. Summary of locations and genetic analyses of the *Silene vulgaris* populations studied (altitude in m a.s.l.).

Population ID	Site	Altitude [m]	Latitude	Longitude	F_{IS}	He	Ho	π
A1	Gschnitz	1234	47° 02' 51.0" N	11° 21' 29.0" E	0.002	0.039	0.058	0.060
CH1	Flims	2102	46° 52' 03.8" N	09° 14' 23.4" E	0.026	0.051	0.069	0.084
CH2	Cadenazzo	202	46° 09' 09.0 "N	08° 56' 33.0" E	0.014	0.054	0.071	0.079
D1	Berlin	30	52° 31' 58.7" N	13° 23' 10.2" E	0.053	0.069	0.067	0.096
D2	Hamburg	25	53° 40' 21.0" N	10° 05' 04.8" E	0.025	0.056	0.065	0.079
D3	Berlin	46	52° 28' 14.4" N	13° 23' 44.4" E	0.046	0.064	0.063	0.088
D5	Mainz	89	49° 59' 53.4" N	08° 13' 15.7" E	0.027	0.048	0.053	0.068
D6	Heilsbronn	400	49° 20' 44.6" N	10° 47' 26.5" E	0.004	0.038	0.056	0.059
D7	Gerswalde	70	53° 11' 06.3" N	13° 45' 28.0" E	0.031	0.054	0.059	0.076
D8	Geesower Hügel	30	53° 14' 32.9" N	14° 23' 06.9" E	0.037	0.048	0.047	0.068
D9	Potsdam	90	52° 21' 44.6" N	13° 04' 34.9" E	0.022	0.044	0.051	0.064
D10	Konstanz	400	47° 40' 23.0" N	09° 09' 10.6" E	0.002	0.043	0.068	0.069
D11	Hamm	90	49° 44' 26.2" N	08° 26' 58.6" E	0.029	0.060	0.070	0.087
D12	Langenargen	402	47° 36' 33.2" N	09° 31' 43.7" E	0.016	0.051	0.063	0.072
E1	Alcanó	211	41° 29' 29.4" N	00° 26' 27.3" E	-0.006	0.075	0.119	0.117
E2	Fontdepou	798	41° 57' 44.6" N	00° 45' 51.4" E	-0.009	0.064	0.107	0.102
E3	La Sentiu de Sió	316	41° 49' 44.1" N	00° 54' 19.9" E	-0.022	0.072	0.118	0.106
E4	La Palma	1100	28° 46' 58.0" N	17° 56' 22.0" W	-0.009	0.067	0.103	0.099
F1	Les Rochette	65	46° 41' 00.8" N	01° 23' 58.0" W	0.011	0.042	0.057	0.064
F2	Pommeraie	78	43° 56' 42.6" N	01° 22' 21.5" E	0.027	0.052	0.055	0.070
F3	Menton	73	43° 47' 13.7" N	07° 29' 58.2" E	-0.008	0.059	0.092	0.087
	La Ciotat	44	43° 11' 55.2" N	05° 37' 53.3" E				
	Forcalquier	554	43° 56' 42.6" N	05° 48' 34.9" E				
F4	La Noue du Bourg	177	46° 39' 28.0" N	01° 23' 13.6" W	0.023	0.051	0.063	0.077
F5	Normandie	83	49° 16' 06.0" N	01° 37' 39.0" E	0.020	0.049	0.058	0.069
S1	Södra Bäck	10	56° 40' 12.7" N	16° 40' 54.2" E	0.044	0.066	0.069	0.094
S2	Vickleby	51	56° 34' 37.1" N	16° 27' 39.5" E	0.021	0.049	0.060	0.073

The following fitness related plant traits were measured to assess the performance under the experimental conditions: germination, survival, flowering, biomass, plant height, days to flowering, number of flowers, number of branches, number of leaves, leaf area and specific leaf area (SLA). This plant trait data has been already used in an earlier analysis in Kahl et al. (2019), but the current publication extends these findings by including a population genomic analysis via ddRAD sequencing.

ddRAD library preparation and sequencing

For the extraction of genomic DNA, leaf-tissue samples were taken from 13 randomly chosen individuals from each population in the greenhouse. Therefore, each mother plant was represented by six half-sib progenies. The tissue samples were dried in silica gel until further processing. Genomic DNA was extracted from the samples using Qiagen DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The library preparation was performed as in Peterson et al. (2012a) with some modifications. Double-digest reactions were carried out in a volume of 100 µl containing ~500 ng of genomic DNA, 20 U of MspI and EcoRI and 10x CutSmart® buffer (NEB, Frankfurt am Main, Germany). Individual adapters were ligated on to 50 ng of digested DNA for a final pooling of 48 individuals (Table A4, Table A5). Size selection was carried out using Pippin Prep targeting fragments between 276 - 476 bp. Multiplexing indices (6 bp) and Illumina sequencing primers were added to ~20 ng of size-selected sample and CloneAmp™ HiFi PCR Premix (Takara, Saint-Germain-en-Laye, France) in a final PCR amplification. Each sample was amplified in a 20 µl reaction volume with 14 cycles following the manufacturer’s protocol. Sample libraries were pooled in equal amounts and quantified using Agilent 2200 TapeStation System (Agilent Technologies, Waldbronn, Germany). Paired-end sequencing was performed using Illumina NextSeq 500 System (Illumina, München, Germany).

RAD-seq data analysis and SNP identification

Raw Illumina reads were de-multiplexed by their unique barcode and adapter sequences into unique reads for each individual using the process_radtags command in STACKS (v1.47) (Catchen et al. 2013). Reads were shortened to 140 bp to obtain equal length. ddRAD-seq loci were assembled using the de novo pipeline ustacks, cstacks, sstacks, tsv2bam, and gstacks in STACKS (v2.4) (Rochette et al. 2019) due to the lack of a reference genome. Programs were run with the following parameters: ustacks -t gzfastq -f [sample].1.fq.gz -i [number] --name [sample] -o stacks/ -p 22 (for each sample separately); cstacks -P stacks/ -M population-map.txt -n 4 -p 22, and sstacks -P stacks/ -M population-map.txt -p 22; tsv2bam -P stacks/ -M population-map.txt --pe-reads-dir fastq/ -t 22; gstacks -P stacks/ -M population-map.txt -t 22. SNP calling took place at the gstacks step.

Population genetic analyses and genetic structure

The populations program in STACKS (v.2.4) was used to calculate observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient of populations (F_{IS}), nucleotide diversity (π), and the inter-population component (F_{ST}). Population structure was estimated using fastSTRUCTURE (Raj et al. 2014) with simple prior. Best model complexity from 2 to 7 was chosen using its chooseK.py program.

We calculated a population level phylogenetic tree based on nucleotides fixed within populations and variant among them obtained using the --phylip parameter of the populations program in STACKS (v2.4). The phylogenetic analysis was done using the maximum likelihood approach in RAxML (v8.2.9) (Stamatakis 2014). We employed a GTR + gamma model of sequence evolution for single full ML tree searches. Nodal support of the phylogenetic tree was evaluated by 500 replicates of RAxML's rapid bootstrap algorithm. The robustness of the obtained phylogenetic tree is indicated by the 100% bootstrap support for the three major branches.

Genetic and environmental differentiation

To examine possible genetic and environmental correlations amongst sampled populations mantel tests were performed. Climate variables of population sampling sites were acquired from the WorldClim database from 1970–2000 (Fick and Hijmans 2017) and are listed in the appendix (Table A1, Table A2). The climate data included annual mean temperature, mean diurnal temperature range, isothermality, temperature seasonality, maximum temperature in warmest month, minimum temperature in coldest month, temperature’ annual range, mean temperature of wettest, driest, warmest and coldest quarter, annual mean precipitation, precipitation of wettest and driest month, precipitation seasonality, precipitation of wettest, driest, warmest and coldest quarter, solar radiation, wind speed and water-vapor pressure. The data represent average values from the years 1970-2000. With this comprehensive approach we aimed to account for the large distribution of samples across Europe and potential differences in climatic zones. R (version 3.4.3; R Core Team, 2017) was used to calculate dissimilarity matrices between population sampling sites of *Silene vulgaris* based on the

Table 5. Genetic differentiation (F_{ST}) of 25 *Silene vulgaris* populations.

	A1	CH1	CH2	D1	D2	D3	D5	D6	D7	D8	D9	D10	D11	D12	E1	E2	E3	E4	F1	F2	F3	F4	F5	S1	S2
A1	-																								
CH1	0.32	-																							
CH2	0.26	0.25	-																						
D1	0.24	0.25	0.21	-																					
D2	0.28	0.29	0.24	0.22	-																				
D3	0.25	0.26	0.21	0.18	0.22	-																			
D5	0.31	0.30	0.23	0.21	0.26	0.22	-																		
D6	0.28	0.28	0.22	0.19	0.24	0.20	0.23	-																	
D7	0.26	0.27	0.22	0.18	0.23	0.19	0.21	0.20	-																
D8	0.29	0.30	0.23	0.21	0.25	0.22	0.24	0.23	0.22	-															
D9	0.29	0.30	0.23	0.19	0.25	0.21	0.25	0.22	0.21	0.24	-														
D10	0.29	0.29	0.22	0.19	0.24	0.20	0.24	0.22	0.20	0.23	0.23	-													
D11	0.25	0.25	0.21	0.19	0.22	0.20	0.20	0.20	0.20	0.21	0.21	0.20	-												
D12	0.27	0.28	0.22	0.20	0.24	0.21	0.24	0.22	0.21	0.24	0.23	0.22	0.20	-											
E1	0.29	0.29	0.25	0.25	0.26	0.25	0.26	0.25	0.26	0.27	0.27	0.26	0.23	0.26	-										
E2	0.31	0.29	0.25	0.24	0.26	0.23	0.27	0.24	0.25	0.27	0.27	0.27	0.22	0.26	0.18	-									
E3	0.32	0.31	0.27	0.26	0.28	0.27	0.28	0.27	0.28	0.29	0.29	0.29	0.23	0.28	0.19	0.22	-								
E4	0.31	0.31	0.26	0.26	0.27	0.27	0.28	0.26	0.27	0.28	0.29	0.27	0.24	0.28	0.19	0.22	0.22	-							
F1	0.33	0.28	0.27	0.26	0.30	0.27	0.31	0.29	0.29	0.31	0.30	0.31	0.26	0.29	0.29	0.29	0.31	0.31	-						
F2	0.32	0.27	0.28	0.27	0.30	0.27	0.31	0.29	0.29	0.32	0.30	0.31	0.27	0.29	0.29	0.29	0.31	0.31	0.21	-					
F3	0.29	0.28	0.24	0.25	0.24	0.25	0.26	0.24	0.26	0.27	0.27	0.26	0.23	0.26	0.20	0.21	0.22	0.22	0.28	0.29	-				
F4	0.32	0.24	0.27	0.26	0.28	0.27	0.30	0.29	0.28	0.30	0.30	0.30	0.26	0.29	0.26	0.27	0.29	0.29	0.22	0.22	0.24	-			
F5	0.30	0.27	0.26	0.24	0.27	0.25	0.28	0.27	0.27	0.29	0.28	0.28	0.24	0.27	0.27	0.27	0.29	0.29	0.25	0.24	0.26	0.25	-		
S1	0.24	0.26	0.21	0.19	0.21	0.19	0.23	0.20	0.19	0.21	0.21	0.20	0.20	0.21	0.25	0.24	0.27	0.27	0.27	0.28	0.25	0.27	0.26	-	
S2	0.26	0.27	0.22	0.18	0.22	0.19	0.23	0.21	0.19	0.22	0.21	0.20	0.20	0.21	0.25	0.25	0.28	0.27	0.28	0.28	0.25	0.28	0.26	0.17	-

climate data listed above and pairwise geographic distances. Geographic distances between sampling sites were calculated with the R package geosphere and transformed into Euclidean distances. Environmental variables were normalized due to varying units and scales using the scale function in R and Euclidean distances were calculated between populations. Mantel tests were performed between environmental and genetic distances (expressed as F_{ST}; Table 5), geographic and genetic distances and environmental and geographic distances with 100,000 repetitions.

Table 6. ANOVA results of plant phenotypic traits in *Silene vulgaris* grown under two different temperature and three different precipitation conditions.

		Height		Flower number		Leaf number		Branch number		Leaf area	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temp	1	**46.3**	**<0.001**	**539.0**	**<0.001**	**56.3**	**<0.001**	**9.5**	**<0.01**	**15.1**	**<0.001**
Prec	2	**43.8**	**<0.001**	**147.5**	**<0.001**	**131.8**	**<0.001**	**18.5**	**<0.001**	4.2	0.12
Cluster	2	**26.5**	**<0.001**	**11.6**	**<0.01**	**8.3**	**<0.05**	**20.9**	**<0.001**	**9.1**	**<0.05**
Temp x Prec	2	1.7	0.42	**9.6**	**<0.01**	**23.4**	**<0.001**	1.2	0.56	0.0	0.98
Temp x Cluster	2	**7.8**	**0.01**	4.0	0.14	**9.9**	**<0.01**	**7.5**	**<0.05**	**6.3**	**<0.05**
Prec x Cluster	4	8.7	0.07	**11.8**	**<0.05**	**33.3**	**<0.001**	**11.1**	**<0.05**	1.1	0.89
R^2 marginal (conditional)		0.27 (0.46)		0.29 (0.85)		0.20 (0.63)		0.12 (0.21)		0.10 (0.33)	

		Germination		Survival		Flowering		Biomass		Days to flowering		SLA	
	df	*t*	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temp	1	-	-	0.1	0.75	**25.3**	**<0.001**	**94.7**	**<0.001**	**6.0**	**0.01**	**6.4**	**0.01**
Prec	2	-	-	1.3	0.53	1.4	0.50	**172.9**	**<0.001**	0.3	0.85	5.5	0.06
Cluster	2	**13.0**	**<0.01**	2.1	0.34	2.4	0.30	**4.7**	**<0.05**	**7.1**	**<0.05**	0.6	0.73
Temp x Prec	2	-	-	1.5	0.48	1.4	0.50	0.4	0.83	1.6	0.46	0.0	0.99
Temp x Cluster	2	-	-	0.6	0.73	0.9	0.64	0.2	0.95	1.0	0.60	1.9	0.38
Prec x Cluster	4	-	-	2.6	0.62	4.6	0.33	0.4	0.95	0.9	0.92	2.1	0.71
R^2 marginal (conditional)		0.11 (0.29)		0.01 (0.06)		0.04 (0.20)		0.14 (0.31)		0.07 (0.30)		0.01 (0.12)	

Note: Cluster refers to the genetic cluster affiliation revealed for the *S. vulgaris* populations in the genetic analysis through STRUCTURE (Figure 9). Significant effects are indicated in bold. For binary data (germination, flowering, survival) a binomial distribution and for count data (number of flowers, leaves, branches) a Poisson error distribution was assumed. SLA: Specific leaf area, df: degree of freedom.

Trait differentiation analysis towards climate change

The three main groups revealed by the population genetic analysis through STRUCTURE were included in the analysis of phenotypic changes related to a possible climate change scenario. Using different mixed models, we tested for phenotypic differences in *S. vulgaris* between our experimental climate change conditions and between the three genetic clusters revealed through the population genetic analysis (see above). Linear mixed models were employed for the statistical analyses of plant traits that fitted a normal distribution (biomass, plant height, days to flowering, leaf area and specific leaf area). For binary data (plant survival, and flowering) and count data (number of flowers, number of leaves, number of branches) we used generalized mixed models with a binomial and Poisson distribution, respectively. Furthermore, we also tested if the genetic clusters responded differently to the climate-change conditions by including the interaction term (Temp x Cluster; Prec x Cluster, Table 6) in our analysis. Models were performed using the function lmer() and glmer() in R (version 3.4.3, R Core Team 2017). We included experimental treatment (divided in temperature and precipitation), cluster affiliation, temperature x precipitation, temperature x cluster affiliation, and precipitation x cluster affiliation as fixed factors (Table 6). Random factors were population identity and mother plant. Differences in germinating seed numbers were evaluated through Welch´s two sample t-test due to unequal sample sizes in the three genetic clusters. The influences of experimental conditions on germination could not be tested as treatments started two weeks after germination to ensure a maximum germination and seedling survival rate. The analysis via mixed-effect models revealed significant effects of the precipitation and temperature treatments on *S. vulgaris* traits (Table 6). Details of this influence were not within the scope of the present study and have been discussed further in Kahl et al. (2019). The aim of the present study is to analyse trait differences in relation to the phylogenetic relationship between *Silene* populations.

Results

Summary statistics

Illumina sequencing of the ddRAD libraries provided 286 million fragments. Sample average and median were 971,650 and 767,040 respectively. The inbreeding coefficient (F_{IS}) was close to zero for all populations ranging from 0.024 (A1) to 0.087 (D1; Table 4), hence not providing evidence for inbreeding. Expected heterozygosity ranged from 0.038 (A1) to 0.070

(E1; Table 4). Observed heterozygosity of populations ranged from 0.037 (CH1, D11) to 0.060 (E3; Table 4). Values for nucleotide diversity of the different populations ranged from 0.049 (A1) to 0.087 (E1). The spatial distribution of population heterozygosity revealed a significant latitudinal pattern only for observed heterozygosity (Figure 7). Observed heterozygosity declined significantly with higher latitudes (Figure 7b, *p* <0.001). We also found a trend towards a decline of expected heterozygosity and nucleotide diversity with increasing latitude; however, this was not significant (Figure 7a, c).

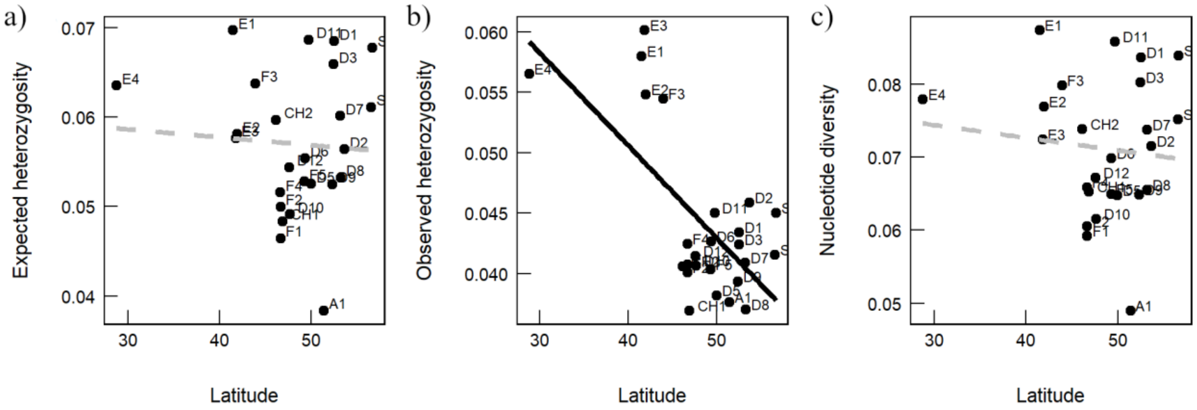

Figure 7. Relationship between a) expected heterozygosity and latitude ($p > .05$), b) observed heterozygosity and latitude ($p < .001$), c) nucleotide diversity (π) and latitude ($p > .05$) of 25 European *Silene vulgaris* populations.

Population genetic structure and analysis

The phylogenetic analysis revealed three major clusters among the European *S. vulgaris* populations sampled. All Spanish (E1, E2, E3, E4) and one southern French population (F3) formed one major monophyletic cluster of South-Western Europe (hereafter referred to as cluster 'South') whereas the second monophyletic branch comprised the German (D1, D2, D3, D5, D6, D7, D8, D9, D10, D11, D12), Austrian (A1), Swedish (S1, S2) and one Southern Swiss population (CH2) of Central-Europe (hereafter referred to as cluster 'Central'). The third cluster will be referred to as cluster 'West' comprising most French populations (F1, F2, F4, F5) and the remaining Swiss alpine population (CH1, Figure 8). Within the South cluster the Spanish populations formed a monophyletic group with one French population as a sister group with 100% bootstrap support. The Central European populations of *Silene vulgaris* formed one distinct cluster with comparably low resolution. Within this cluster no further geographic differentiation was possible with the data available, and the position of each population showed low bootstrap support. We found two Swedish populations (S1, S2) that formed sister groups (S1 and S2) as well as many of the German populations (e.g., D1 and D9

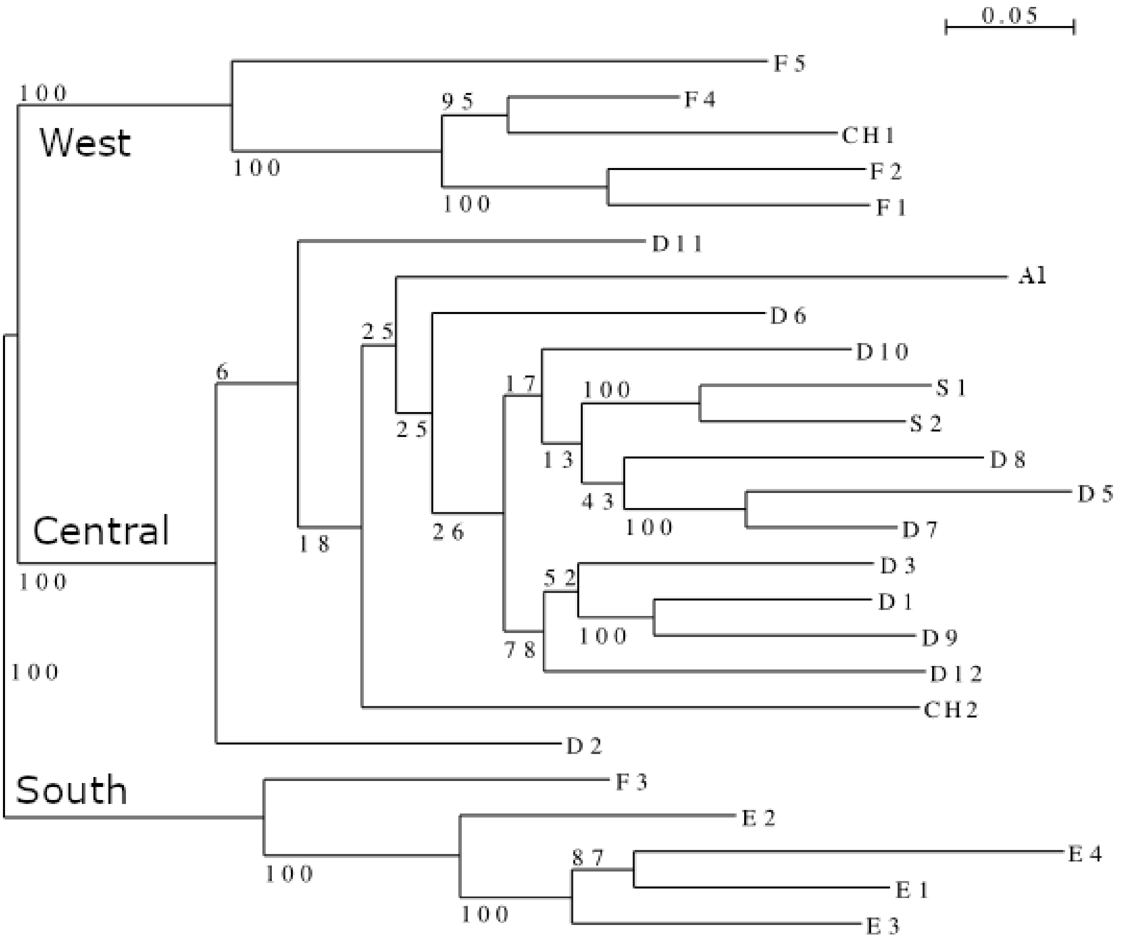

Figure 8. Maximum likelihood tree for the 25 populations of *S. vulgaris* generated by RAxML. Numbers represent bootstrap values (in %) from 500 replicates. Three major clusters were identified and used for further analysis of plant traits: “Central” = central-north European cluster, “West” = West European cluster and “South” = South European cluster.

from Potsdam and Berlin). However, the remaining genetic relationships did not correspond to a geographic pattern within this central European cluster (Figure 8). In the 'West' cluster the Swiss alpine population CH1 was nested amongst the French populations.

The multivariate analysis of SNPs using STRUCTURE largely agrees with the phylogenetic structure (Figure 9). At K = 3 cluster ‘South’ is identical with populations E1, E2, E3, E4, and F3. The remaining clusters from the STRUCTURE analysis only differ slightly by including the Swiss alpine population CH1 in the ‘Central’ cluster instead of the ‘West’ cluster. As this clustering based on STRUCTURE more closely reflects the geographical proximity of populations, we used these three clusters for the further analyses below.

In our additional analysis through the pairwise F_{ST} value comparison a very similar pattern to the phylogenetic analysis was found (Figure A8): here, the ‘South’ cluster is identical comprising populations E1, E2, E3, E4, and F3 (see Figure 8). However, it shows that populations CH1 and A1 both cluster in the ‘West’ cluster together with the remaining French populations. Apart from these differences, the third cluster in the pairwise F_{ST} value comparison included the same populations as in the phylogenetic analysis (D1, D2, D3, D5, D6, D7, D8, D9, D10, D11, D12 and CH2; Figure A8). The three groups had a significantly higher genetic similarity within than amongst each other.

Mantel tests and population differentiation

The Mantel tests revealed a strong genetic pattern for the correlation between environmental, geographic, and genetic distances (Figure A9). Based on the climate data analysed from the populations' sites (Table A2) we found a significant positive correlation of geographic and environmental distances with r=0.89 and *p* <0.001 (Figure A9a). Furthermore, Mantel tests resulted in significant correlations between F_{ST} and geographic distances (in r=0.29, *p* <0.05; Figure A9b) as well as in significantly positive correlations between genetic and environmental distances (r=0.34, *p* <0.01; Figure A9c).

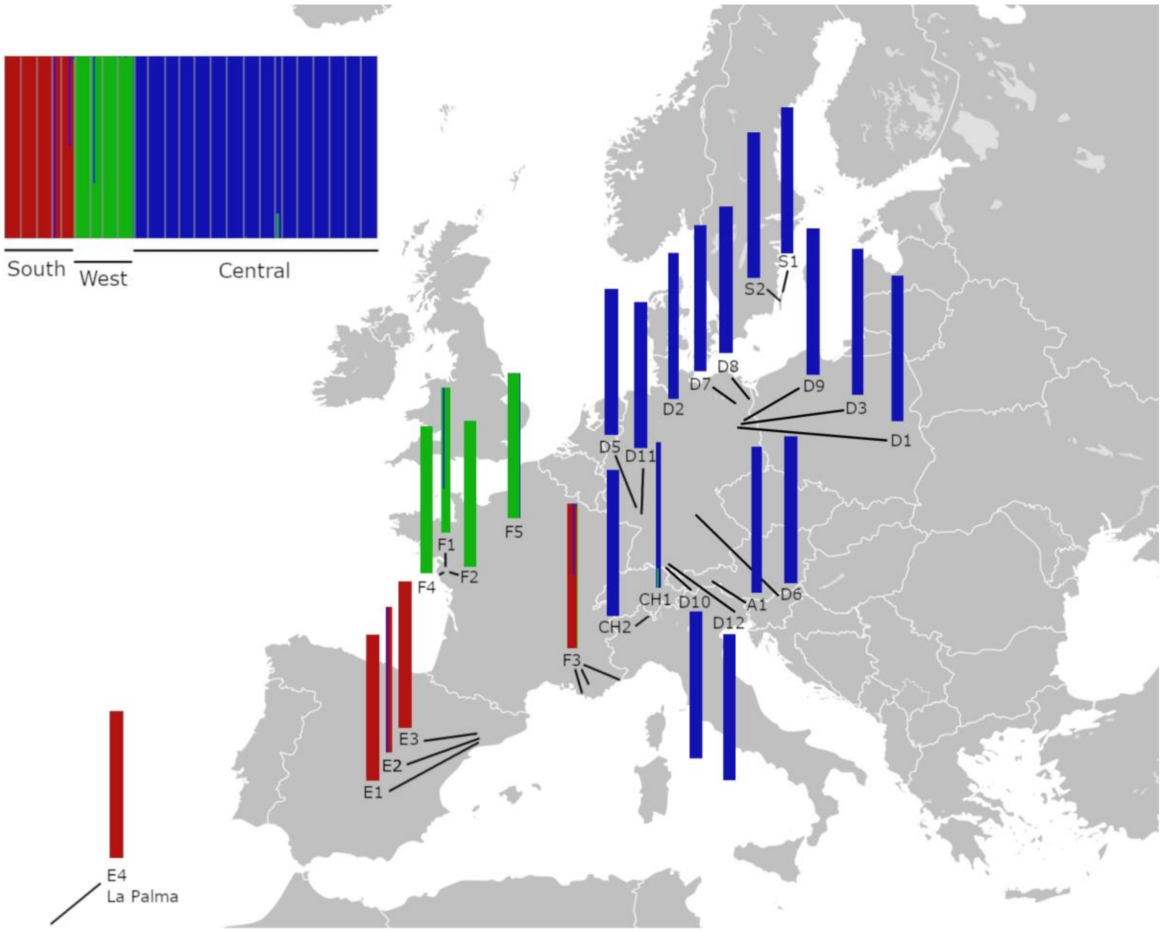

Figure 9. Multivariate analysis via ddRAD sequencing of 25 *Silene vulgaris* populations. The map shows the locations of the sampled populations including their population genetic structure. The different populations cluster in three major groups referred to as “South”, “Central”, and “West”.

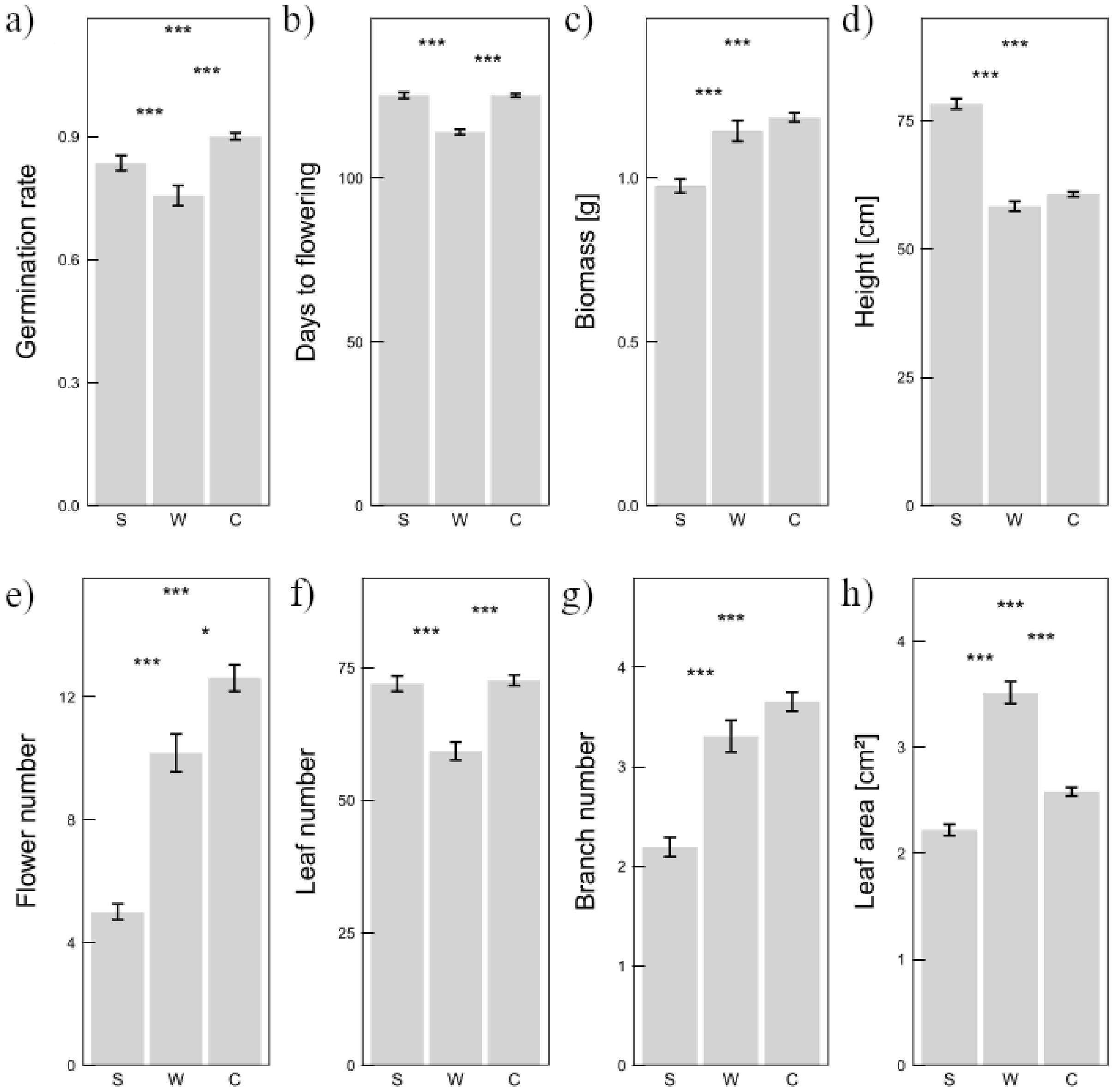

Figure 10. Phenotypic differences between genetic clusters (South (S), West (W), and central (C) European clusters as defined in Figure 9.

Responses of genetic clusters towards climate change

Analyses with mixed-effect models revealed that genetic clusters - as defined by STRUCTURE - significantly differed in the number of germinating plants, in days to flowering, plant biomass, plant height, flower, leaf, and branch number as well as in leaf area (Table 6, Figure 10). For germination rate and flower number the average values for the plants in the Central cluster were significantly higher than in the other clusters (Figure 10a, e). Time to flowering was shortest in the West cluster and plant biomass was lowest in the South cluster (Figure 10b, c). Plant height and leaf area showed a different pattern, with the tallest plants being found in the South cluster, and plants from the West cluster having the biggest

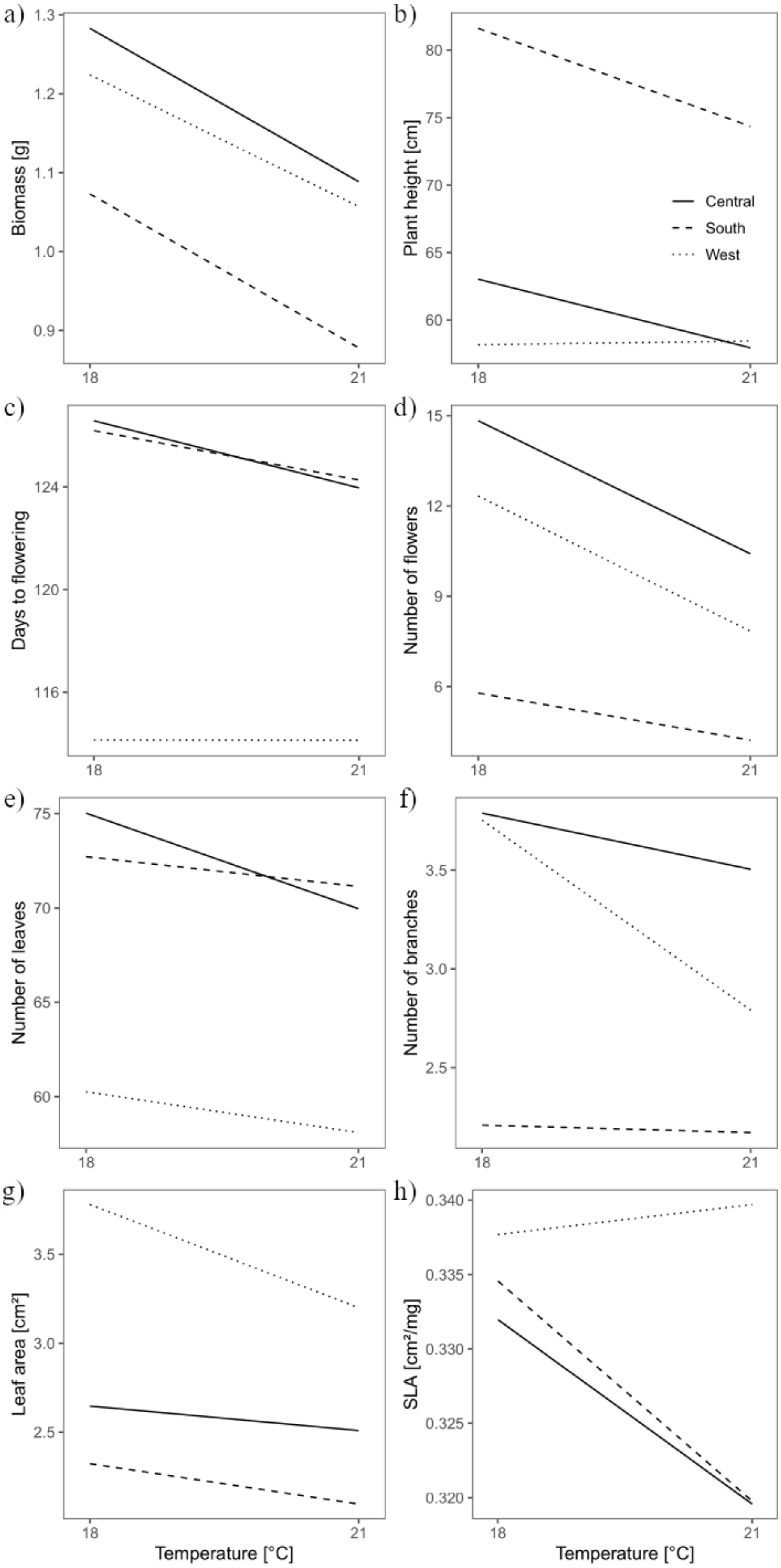

Figure 11. Reaction norms for temperature treatments of the three genetic clusters of *Silene vulgaris* (South, West, and central European clusters as defined in Figure 9). SLA: Specific leaf area.

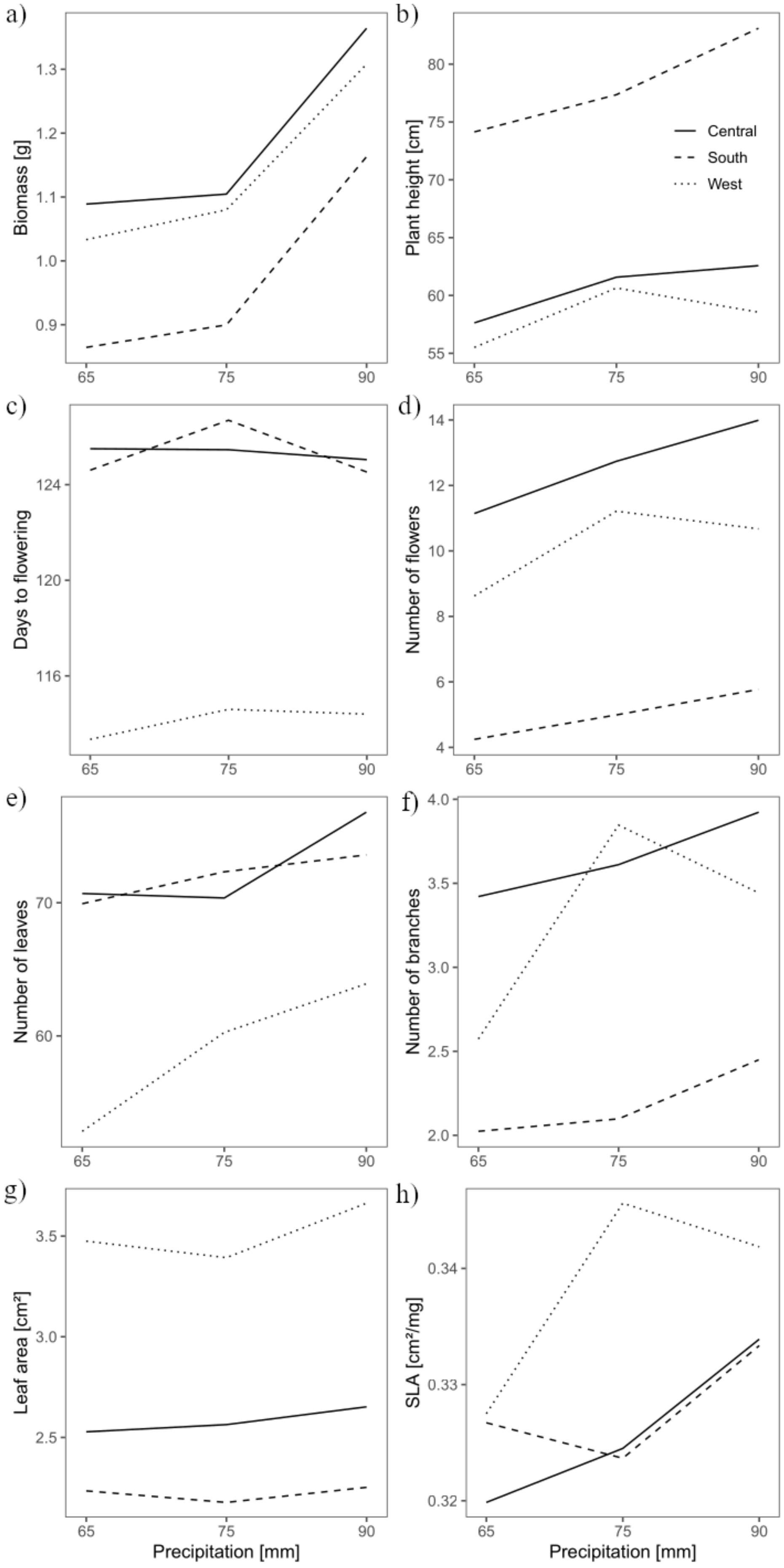

Figure 12. Reaction norms for precipitation treatments of the three genetic clusters of *Silene vulgaris* (South, West, and central European clusters as defined in Figure 9). SLA: Specific leaf area.

leaves (Figure 10d, h). However, the number of leaves was lowest in the West cluster and plants from the South cluster possessed fewer branches (Figure 10f, g).

There was no significant interaction between the genetic clusters and either temperature or precipitation conditions for six of the traits (germination, survival, flowering, biomass, days to flowering, specific leaf area; Table 6). For the remaining five traits (leaf number, branch number, flower number, leaf area, plant height) the three clusters differed in their responses to the temperature and/or precipitation treatments (Table 6). In the South and Central cluster plant height strongly decreased with increasing temperature, whereas it slightly increased in the West cluster (Figure 11b). Number of leaves and branches and the leaf area decreased in all clusters with an increased temperature but showed a stronger response in the Central and West Cluster respectively (Figure 11e, f, g). The reaction norms of flower, leaf and branch number differed in the three genetic clusters in response to the different precipitation treatments (Figure 12d, e, f): With increased precipitation the number of flowers in the South and Central cluster was increased whereas the West cluster showed a peak in flowers at 75 mm precipitation (Figure 12d). The number of leaves showed a decrease from 90 mm to 75 mm precipitation in all three clusters that further declined in the South and West cluster from 75 mm to 65 mm. The Central cluster showed a similar number of leaves under the 75 mm and 65 mm treatment (Figure 12e). For the number of branches the genetic clusters revealed a similar reaction norm for the Central and South cluster, whereas the West cluster exhibited a strongly increased number of branches under the 75 mm treatment (Figure 12f). For plant biomass, however, the three genetic clusters mainly showed a parallel pattern in reaction norms (Figure 11a, Figure 12a).

Discussion

How different plant species can adapt to a changing climate represents a crucial ecological and evolutionary question. As widely distributed species often possess many genetic lineages or subspecies (Van Rossum et al. 2018), revealing their population structure may help to predict their response to different environmental conditions at present and under future conditions (Collart et al. 2021). In many European plant species a phylogeographic pattern can be found that resulted from the survival in different refugia during the last glacial maximum (LGM) (Beatty and Provan 2011, Taberlet et al. 2012, Bagnoli et al. 2016, Sebasky et al. 2016, Listl et al. 2017, Roces-Díaz et al. 2018, Krebs et al. 2019, Schwarzer and Joshi 2019). During the LGM Northern Europe was covered with glaciers that were also scattered in the mountainous regions of central Europe (Heyman et al. 2013). The climatic change associated with the LGM affected many plant species and drove their distribution to defined refugia forming biodiversity hotspots that also served as post-glacial sources for re-colonization (Petit, 2003; Hewitt, 2004; Morelli et al., 2016). The recent genetic lineages that were formed from these past events often show differential adaptation to environmental conditions (Prunier et al. 2012, Yan et al. 2019, Walden et al. 2020). Uncovering these population structures may thus facilitate the understanding of plant responses to a changing climate in the future.

In the present study we used the model plant species *Silene vulgaris* to uncover its genetic population structures within its European distributional range. Secondly, we analysed whether the response to experimentally induced climate change differed between the genetic clusters detected.

The phylogeographic pattern and genetic diversity of Silene vulgaris

The genome-wide analysis of SNPs identified three major clusters originating from Southern, Western and Central Europe possibly representing a disruption pattern of the pan-European species *Silene vulgaris* during the last glacial maximum. Spanish (E1, E2, E3, E4) and French (F1, F2, F4, F5) *S. vulgaris* populations analysed in this study appear in separate clusters. A similar pattern was also found in the population analysis by Sebasky et al. (2016) who also proposed a separate cluster for France in contrast to a cluster found on the Iberian Peninsula for *S. vulgaris*. Comparable population structures were also found for the closely related *Silene nutans* with a separate historic refugium on the Iberian Peninsula expanding towards south-western France (Van Rossum et al. 2018). In the present phylogenetic analysis, the Central European populations of *Silene vulgaris* formed a third clade with comparably low resolution. Within this clade no further geographic differentiation was possible with the data available. Interestingly, *S. vulgaris* populations from Sweden (S1, S2) did not stand out from the remaining Central European populations. The two Swedish populations (S1, S2) clustered together in a well-supported branch within the third cluster. The phylogenetic analysis has shown that *S. vulgaris*, a species widely distributed in Europe, shows a considerable range of phylogenetic diversity but can be structured in three major clusters. This phylogenetic pattern can be of different origin. One possibility is that this pattern is a result of the recolonisation of Europe from distinct refugia after the LGM. In the sampled *S. vulgaris* populations indicators for genetic diversity (He, Ho, π) declined with increasing latitude. The most southern populations of *S. vulgaris* showed the highest values of observed heterozygosity. Similar observations were found for nucleotide diversity in the present study: Nucleotide diversity was lower in populations from higher latitudes compared to populations from lower latitudes. These findings may indicate that southern European populations have been functioning as refugia during the LGM. During that time populations in the South of Europe remained larger as they were not disrupted by snow covered areas or glaciers. When temperatures were increasing again a subsequent recolonization of central and northern Europe was likely to start from these southern refugia. The discovered pattern in *S. vulgaris* of decreased genetic diversity with increasing latitude is a commonly found pattern in different species after the last ice age (Hewitt 1999, Breen et al. 2009, Beatty and Provan 2011, Chung et al. 2018) and can be traced to the subdivision of populations in southern refugia and small population sizes during the recolonization. Overall, we detected three major genetic clusters in South, West and central Europe for the sampled *Silene vulgaris* populations whose genetic diversity decreased with increasing latitude. However, both, the genetic clusters and decreasing genetic diversity with latitude are only unspecific indicators of the populations' evolutionary past. The recent genetic lineages may be the result of past disruptive population genetic events during the last ice age and *S. vulgaris* may share the same glacial refugia with other *Silene* species (Taylor and Keller 2007, García-Fernández et al. 2013, Meindl et al. 2016, Van Rossum et al. 2018, Tausch 2019). However, the hypotheses about *S. vulgaris*' evolutionary past need further investigation and for an unambiguous identification of the refugia during the LGM, further population genetic analyses are needed. Therefore, we suggest undertaking further distribution modelling to identify possible refugia and use coalescent-based methods to reliably analyse the genetic variation presently found in *S. vulgaris* (Rosenberg and Nordborg 2002, Sebasky et al. 2016).

Interestingly, we found for *S. vulgaris* that only observed heterozygosity significantly decreased with higher latitudes whereas for He and π we only found a decreasing trend. A possible explanation for this result is an increased inbreeding in northern populations. This could consequently lead to an increase in the number of homozygotes and thus a lower Ho. However, at the same time this extent of inbreeding did not lead to a loss of overall genetic diversity at the population level and could be the reason why He and π do not show this significant decrease. A similar case has been described by Bemmels and Dick (2018) where Ho was significantly decreased in southern populations of North American hickory tree species. In general, we found relatively low values of F_{IS} suggesting a strong outbreeding behaviour of the populations. Although hermaphrodite individuals of *S. vulgaris* are self-compatible outcrossing is preferred as it leads to a higher fitness of offspring (Bailey and

McCauley 2006). Also, female individuals in *S. vulgaris* are clearly dependent on the pollination by hermaphrodites. This fact makes outbreeding a necessity at least for the female individuals of this species.

Environmental drivers of population structure

In our Mantel test analysis, the populations examined showed increased genetic differences with increasing geographic distance. This is a typical pattern for isolation by distance where stronger genetic differences are expected with an increasing distance between populations (Meirmans 2012). More importantly we found a significant positive correlation between geographic and environmental distance at the populations' sites of origin. As we included climatic factors in the analysis, we can conclude that those populations situated further apart from each other also experience stronger differences in climatic conditions (e.g., temperature or precipitation). These climatic conditions represent strong selective forces (Blackman et al. 2017, Moore et al. 2020, Santana et al. 2020). This is supported by the significant correlation between genetic and environmental distances in our study and underlines the likely importance of climatic factors as selection factors for *S. vulgaris*. In the present study we were able to identify several fitness related traits (e.g., plant height, biomass, number of flowers etc.) and time to flowering that differed significantly among the three genetic lineages. Possibly these trait differences are an adaptation to different environmental habitat conditions of the genetic lineages. It is known from *S. vulgaris* that it possesses several different ecotypes with strong genetic differentiation that are adapted to extremely unfavourable habitat conditions (i.e. heavy-metal pollution) (Bratteler et al. 2006b, Muszyńska et al. 2019b). The exceptional adaptation of *S. vulgaris* to these extreme environments suggests that the species also shows adaptation to putatively less strong selective forces i.e., the different climate zones in continental Europe. In conclusion we can state that populations of *S. vulgaris* show stronger between-population genetic differences when their habitats are less similar.

Cluster differences in response to temperature and precipitation treatment

At the species level, *S. vulgaris* possesses a considerable phenotypic plasticity with regard to temperature and precipitation changes that likely helps this species to adapt (Kahl et al. 2019). To investigate differences in the response towards a possible climate change scenario between the genetic clusters we measured several fitness-related traits that are known to respond to drought and temperature stress (Rucker et al. 1995, Khan et al. 2015, Zeiter et al. 2016, Wellstein et al. 2017, Eziz et al. 2017, Meineri et al. 2020).

The general response pattern on a population level has been described before (Kahl et al. 2019). In the present analysis, we focused on putative differences in response to experimental climate change conditions amongst the genetic lineages. As an overall result we found that the genetic clusters differed significantly in most of the plant traits examined. Furthermore, we showed that the populations' environmental habitat conditions were correlated with the genetic diversity of the specimens we sampled. The differences in plant traits between the genetic clusters lead to the conclusion that *S. vulgaris* already shows adaptation towards different climatic conditions in Europe where the clusters are situated (South, West and central Europe). With the currently ongoing climatic changes mean temperatures are rising in Europe and precipitation patterns are changing drastically (Bindi and Olesen 2011, Trnka et al. 2011, Marx et al. 2018). If we are interested in how species can adapt to these habitat changes, their genetic background has to be considered (Bowles and Whelan 1996, Vandergast et al. 2008, Anderson et al. 2011, McMahon et al. 2014, Corlett 2017). The inclusion of intraspecifc lineages is of strong importance here as it has been proven to substantially impact analyses on ecological niche modelling (Collart et al. 2021). Therefore, we included the phylogenetic data to investigate possible response differences in *S. vulgaris* to temperature increase and precipitation decrease from a possible climate change scenario. Our analysis revealed differences in the phenotypic response of the genetic clusters. The response of flower, leaf, and branch numbers, as well as leaf area and plant height to the precipitation and temperature treatments differed significantly between the three genetic clusters. The illustration of phenotypic plasticity shows that in these traits one of the genetic clusters showed an opposing trait response (e.g., cluster West between 75 and 90 mm of precipitation). Because we used one climate change scenario for all *S. vulgaris* plants it is possible that the highest precipitation treatment is out of the optimum range for the West cluster. Hence the number of flowers or branches decreased with an increased precipitation of 90 mm. With the knowledge we gained from the phylogeographic analysis it would be of interest to expose plants from the different clusters to differing climate change scenarios specified for the region they originate from. This approach could further facilitate the understanding of climate adaptation in the genetic lineages of *S. vulgaris*. In general, we found that under most treatments the clusters' responses point in the same direction (see reaction norms; Figure 11, Figure 12). Thus, the genetic clusters we found in European *S. vulgaris* populations mostly did not show contradicting differences when responding to changing temperature and precipitation conditions. Climate change may thus not favor one or the other phylogenetic cluster as all three seem to have a comparable basis of adaptability. Hence, even though we found potential differences in the genetic diversity and habitat adaptations at a population level, overall *S. vulgaris* does not show striking differences in its response to an experimental climate change scenario. An earlier study suggests that *S. vulgaris* possesses a considerable amount of phenotypic plasticity towards temperature and precipitation regimes (Kahl et al. 2019). Hence, in the case of this widely distributed species phenotypic plasticity may play an important role in adaptation processes. In other species it has also been shown that phenotypic plasticity provides a strong mechanism to mitigate negative effects of climate change (Frank et al. 2017, Richardson et al. 2017, Kingsolver and Buckley 2017, Peterson et al. 2018). In this context knowledge on genetic markers of phenotypic plasticity for *Silene vulgaris* would help to verify this hypothesis.

In the future, climate change will either lead to a shift in distribution patterns in plants or will force them to adapt locally (He et al. 2019, Ahrens et al. 2020, Anderson and Wadgymar 2020, Metz et al. 2020). The present study used a comprehensive approach including a pan-European sampling and a greenhouse experiment on climate change adaptation paired with a population genetic analysis to understand the interaction of population genetics and current trait responses. The results revealed three genetic clusters for *S. vulgaris* showing distinct trait differences. However, the three clusters did not show major differences in their response to experimental climate-change conditions. Hence, for the widely distributed *Silene vulgaris*, phenotypic plasticity seems to represent an important aspect when facing the obstacles of rapid climate change.

Chapter 4

Does sex matter? - Sex differences of biomass allocation in a gynodioecious species under environmental stress

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By Sandra Kahl, Michael Lenhard and Jasmin Joshi

Abstract

Sexual dimorphism describes the differences in vegetative or reproductive traits between the different sexes in a species. In plant breeding systems sexual dimorphism can occur in dioecious as well as in gynodioecious species where gynodioecy is often seen as an evolutionary linkage between hermaphroditism and dioecy. As climate change is becoming an important factor influencing plant morphology it remains unclear if and how different sexes may respond in sexually dimorphic species. To examine this question, we employed the gynodioecious species *Silene vulgaris* looking at sexual differences of several plant traits. We examined number of flowers, leaves and branches, specific leaf area (SLA), leaf area, biomass, plant height and time to flowering. Furthermore, we exposed the plants to a climate change scenario with two different temperature (18°C, 21°C) and three different precipitation regimes (90 mm, 75mm, 65mm) to examine response differences between female and hermaphrodite individuals. In general, we detected, that hermaphrodites had a higher number of flowers but a lower number of leaves. With regards to the climate change treatment, we found that hermaphrodites showed a milder negative response to higher temperatures in the number of flowers and SLA compared to females. Hence, as we expect temperatures to rise under climate change female plants will be affected more strongly. A potential outcome could be a shift towards hermaphroditism if females cannot compensate for this discrepancy through other reproductive traits. Our results show that some traits of *S. vulgaris* exhibit sexual dimorphism and that both sexes are affected differently by a potential climate-change scenario. These findings provide a solid basis for further analyses on how environmental factors could alter the dynamic of the gynodioecious breeding system in *S. vulgaris*.

Introduction

Sexual dimorphism describes the differences in vegetative and reproductive traits between the sexes in a species. These may result from natural selection or sexual selection. In animals the evidence for sexual dimorphism has been recognized widely and studied extensively by early luminaries of science like Darwin (Darwin 1859). In plants however, sexual dimorphism is not that obvious. As most plants are hermaphrodites, separate female and male specimen where differences could be studied do not occur (Christopher et al. 2019). That said, there is a wide variation of reproductive systems in plants, ranging from hermaphroditic species over monoecious (with flowers from different sexes on the same specimen) and dioecious species (where individuals are of either one or the other sex) to gynodioecious species (where populations are formed by hermaphrodite and female specimen). As a result, in most of these reproductive systems male and female attributes are carried by the same individual or even the same flower and sexual dimorphism is much harder to examine. Nonetheless sexual dimorphism has been studied in several plant species. In *Rumex nivalis* and *Spinacia oleracea*, for example, male seeds are on average heavier than female seeds. Apart from seed size other reproductive trait differences between males and females have been observed as well: They include flower phenology (Thomas and LaFrankie 1993), flower size (Delph et al. 1996), flower number per plant (Delph et al. 2005) or flower longevity (Primack 1985). For example, the multistemmed understory shrub *Lindera triloba* exhibits a larger number of flowering ramets in males and males also show a larger extent of inter-annual variation in inflorescence numbers (Matsushita et al. 2011). Further differences were also reported in the floral fragrances (Ashman 2009) or nectar production (Bawa and Opler 1975) between male and female individuals. Sexual dimorphism was furthermore reported in vegetative plant traits. In the genus *Leucadendron* male and female individuals show a distinct difference in plant architecture (Midgley 2010). In the clonal aquatic *Sagittaria latifolia* the clonal reproduction was significantly lower in females than in males but females showed a higher nitrogen content of clonal corms compared to those of males (Van Drunen and Dorken 2012). The review by (Geber et al. 1999) also shows that females of *Silene latifolia* grow larger and live longer than male individuals. These examples show that plants can exhibit sexually dimorphic traits. However, every morphological trait comes with a cost. Especially if we put these plants with their trait differences into an environmental context, they will experience additional selective pressure. Hence, a changing environment may enhance or reduce sexual differences that were detected before.

Sexual dimorphism in a changing environment

Within the last decades a large number of studies have suggested that male and female plants are differently affected by various environmental factors like climate, competition, herbivory or parasitism (Ågren et al. 1999, Baldoni et al. 2004, Díaz-Barradas et al. 2018, Seyedi et al. 2019, Zhang et al. 2019). In the study by Xu et al. (2008) female specimen of *Populus cathayana* showed lower values for different biomass allocation measures under elevated temperatures and induced drought stress compared to male individuals. *Corema album* (Díaz-Barradas et al. 2018) exhibits a sexual dimorphism based on leaf water potential and water use efficiency that is only manifested during the dry period in more arid regions. Eppley (2001) showed that in the saltmarsh grass *Distichlis spicata* significantly more female seedlings survive an extreme high tide compared to male seedlings. Furthermore, a review by Ågren et al. (1999) showed that in dioecious plant species males usually allocate more resources in flowers during flowering time while female plants have greater total reproductive allocation because of seed production. A fundamental difference in allocation of reproductive resources can be one reason why also vegetative traits might be affected when environmental conditions change, and plants experience more stressful conditions. Especially the influence of climate change will intensify the environmental stress for plants in the future. It is expected that droughts will become more frequent and more severe (Swann 2018). Furthermore, climate change predictions also include hotter temperatures or changing rainfall patterns (IPCC 2013). In present studies sexual differences are usually addressed in dioecious species often providing more distinct trait features. The employment of gynodioecious species has been widely neglected although this breeding system represents an interesting linkage between hermaphroditism and dioecy.

The genus Silene - a model to study the evolution of breeding systems

The genus *Silene* contains several hermaphrodite, gynodioecious and dioecious species; therefore it has been the subject of many studies on the evolution of reproductive systems in plants (Sansome 1938, Desfeux et al. 1996, Lebel-Hardenack et al. 2002, Mrackova et al. 2008, Bernasconi et al. 2009, Balounova et al. 2019, Bačovský et al. 2020). Within the genus dioecy and hermaphroditism (including the evolution of selfing) have evolved at least twice independently (Desfeux et al. 1996). Furthermore, it was shown that in several dioecious *Silene* species (e.g. *S. latifolia*, *S. colpophylla*, *S. dioica*) the sexes are determined by sex chromosomes (Lebel-Hardenack et al. 2002, Cegan et al. 2012, Slancarova et al. 2013), with the sex-determination system reflecting that of mammals (Bačovský et al. 2020). The sex-determining systems in dioecious plants are generally assumed to have gradually evolved from a hermaphroditic or monoecious ancestor via gynodioecy (Balounova et al. 2019). As a gynodioecious plant the species *S. vulgaris* is therefore often used to examine different theories on the evolution of sex-determination. In contrast to other *Silene* species *S. vulgaris* does not possess sex chromosomes that are responsible for the expression of the different sexes. In *S. vulgaris* sexes are determined by an epistatic interaction of nuclear and cytoplasmic genes (Charlesworth 1981). Female plants carry cytoplasmic male sterility genes (CMS), while hermaphrodite individuals may or may not have a male sterile cytoplasm in addition to nuclear restorer genes which restore the male function (Bailey and McCauley 2005). Therefore, female plants with this sexual determination system are often also referred to as ‘male-sterile’ plants. Furthermore, several CMS and nuclear restorer alleles can often be found in gynodioecious populations and specific nuclear restorers only rescue male function in conjunction with specific CMS genes. Hence, both females and hermaphrodites can carry nuclear restorer alleles that lead to an expression of the male function in dependence of their CMS background (Bailey and McCauley 2005). As a result of this complex sex determination system an evolutionary conflict in CMS fitness can arise between females and hermaphrodites: whereas CMS factors are inherited maternally and may influence seed production, restorer genes are inherited biparentally and may influence seed and pollen production (Bailey and McCauley 2005). This conflict has been examined in several studies which reported advantages of females over hermaphrodites in a higher number of flowers per plant, more ovules per flower, more seed capsules per plant, more seeds per capsule or greater seed weight (Pettersson 1992). Presumably these differences in sexes cannot only be observed in traits directly related to their reproduction (e.g. seed and offspring related traits), but also in vegetative traits (Dykstra et al. 2009).

For the current analysis we used *Silene vulgaris* as a representative of a gynodioecious breeding system and examined trait differences between female and hermaphrodite individuals. We also measured sexual response differences under a potential climate change scenario to find out if females will be affected differently by climate change than hermaphrodites.

Material and Methods

Study species and sampling sites

Silene vulgaris (MOENCH) GARCKE (Caryophyllaceae) is known in the literature as a well-established study organism (Bernasconi et al. 2009). It is an herbaceous perennial plant that is native to Eurasia. The species is highly adaptable and can be found along roadsides, in meadows or cultivated fields (Taylor and Keller 2007), but also in highly polluted soil of former mining regions (Bratteler et al. 2006b). *S. vulgaris* is a gynodioecious species and its populations consist of female and hermaphrodite individuals. Within the genus *Silene* several different sexual systems exist – from hermaphrodite over gynodioecious to dioecious plants. This is why the genus has been employed extensively for studies on the evolution of sexes in plants (Cegan et al. 2012, Papadopulos et al. 2015). With its strong adaptive potential and its solid background as an established model organism for evolutionary studies we found *S. vulgaris* to be a suitable species to examine potential effects of climate change on female and hermaphrodite individuals. We collected 25 different populations at sites in Austria (A), Switzerland (CH), Germany (D), Spain (E) — including a population from the Canary Islands, France (F) and Sweden (S) (Table 1). From each population seeds were collected from 13 open pollinated plants in 2015 for the following greenhouse experiment. The location of sampling sites was recorded using a GPS device (‘GARMIN GPS 72H’; accuracy <15 m; Garmin, Switzerland).

Greenhouse experiment

The experiment was conducted in a greenhouse in Potsdam, Germany from November 2015 – April 2016. For a detailed description of the experiment please refer to Kahl et al. (2019). 1950 seeds from 25 different European populations were sown individually into pots that were distributed onto 78 trays. Trays were then exposed to two different temperature treatments that resulted in an average temperature of 18°C (control temperature) and the warming treatment of 21°C. Three different precipitation treatments were applied on top: plants were exposed to 90 mm, 75 mm, and 65 mm of precipitation. Treatments were randomly arranged to account for possible temperature or light gradients in the greenhouse and reflected conditions derived from climate-change prediction models (UBA 2006, IPCC 2007, Schwarz et al. 2007, Ahlström et al. 2012). Temperature and precipitation treatments were combined in a fully factorial design resulting in six treatment combinations.

The following plant biomass traits were measured for each plant individually: biomass in g, plant height (at the day of first flowering; in cm), number of leaves (at the day of first flowering), number of branches (at the day of first flowering), number of flowers (after harvesting), leaf area in cm² and specific leaf area (SLA; in cm²/mg). Aboveground biomass was harvested after the majority of the inflorescences withered. To determine biomass, plants were dried after harvesting for 48 h at 80°C and weighed afterwards. SLA was measured by removing the 4th leaf counted from the bottom of the plant at the time of flowering. Leaves were air-dried for four weeks and weighed afterwards. To determine leaf area, leaves were glued on barcoded paper sheets with a size key. Paper sheets with leaves were digitized using either a flatbed scanner or a digital photo camera. The obtained images were processed using the Python module scikit-image (van der Walt et al. 2014). Individual leaves were segmented using an intensity thresholding approach. Leaf area was extracted using the regionprops function in scikit-image. Plant sex was determined visually after the plants opened their flower. Plants that did not flower were excluded from the analysis.

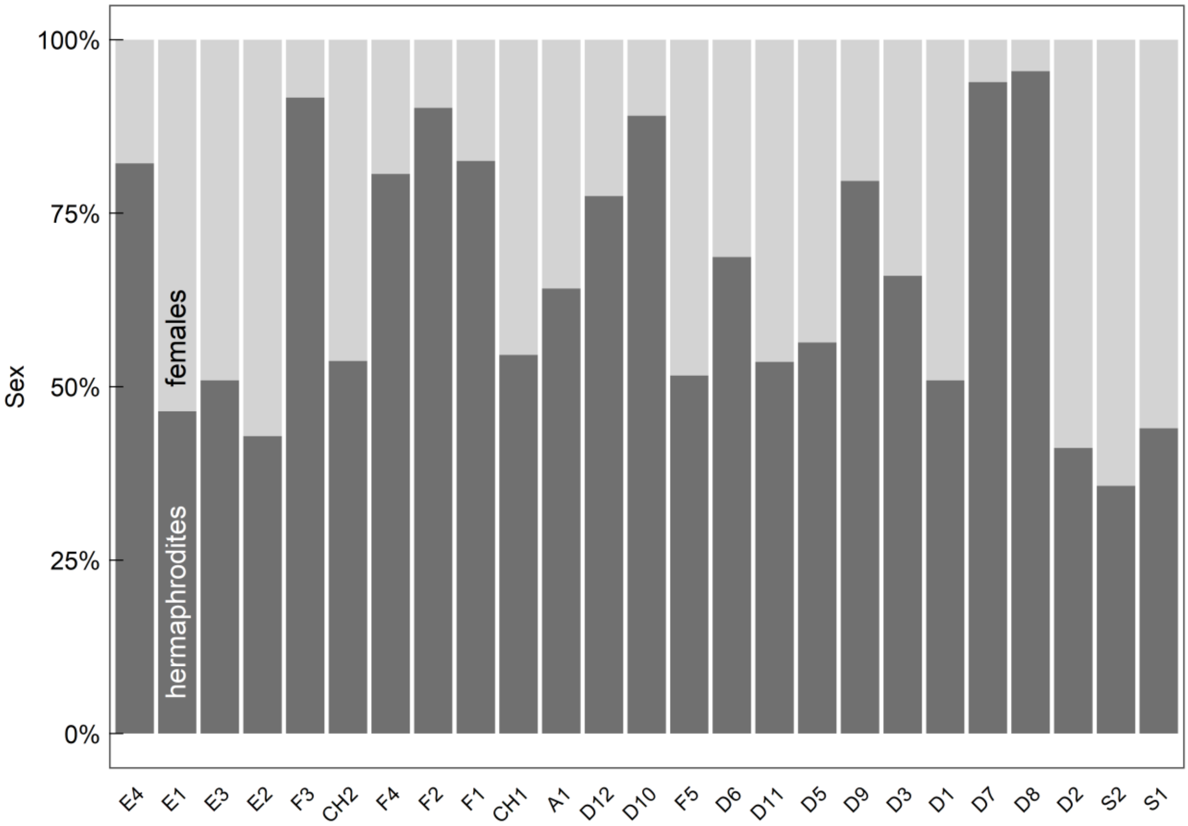

Figure 13. Percentage of female and hermaphrodite plants per population of *Silene vulgaris*. Populations are shown in latitudinal order from South (left) to North (right).

Trait response analysis

To determine if sexes responded differently towards the climate change treatment we set up (generalized) linear mixed models. Models used the function lmer() and glmer() in R (version 3.4.3, R Core Team 2017). As fixed factors, we included: Treatment of plants (divided in temperature and precipitation), plant sex, temperature x precipitation, temperature x sex, precipitation x sex. Random factors were population identity, latitude of population origin and mother plant.

Table 7. ANOVA results of plant phenotypic traits in *Silene vulgaris* grown under two different temperature and three different precipitation conditions. Significant effects are indicated in bold. For count data (number of flowers, leaves, branches) a Poisson distribution was assumed. SLA: Specific leaf area, df: degrees of freedom.

		Days to flowering		Biomass		Height		Leaf area	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temp	1	**5.68**	**0.02**	**79.62**	**<0.001**	**45.46**	**<0.001**	**15.70**	**<0.001**
Prec	2	0.38	0.83	**180.73**	**<0.001**	**42.78**	**<0.001**	4.78	0.09
Sex	1	0.26	0.61	3.37	0.07	1.35	0.25	0.67	0.41
Temp:Prec	2	1.55	0.46	0.72	0.70	1.55	0.46	0.03	0.99
Temp:Sex	1	0.90	0.34	1.29	0.26	0.15	0.70	1.11	0.29
Prec:Sex	2	1.44	0.49	0.27	0.87	0.65	0.72	0.06	0.97
R^2 marginal (conditional)		0.01 (0.29)		0.14 (0.33)		0.04 (0.44)		0.01 (0.32)	

		SLA		Number of flowers		Number of branches		Number of leaves	
	df	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Temp	1	**6.61**	**0.01**	**299.03**	**<0.001**	**9.27**	**<0.01**	**51.43**	**<0.001**
Prec	2	5.13	0.08	**121.30**	**<0.001**	**18.45**	**<0.001**	**129.50**	**<0.001**
Sex	1	0.00	1.00	**7.24**	**<0.01**	**4.07**	**0.04**	**18.34**	**<0.001**
Temp:Prec	2	0.07	0.97	**15.98**	**<0.001**	1.31	0.52	**22.39**	**<0.001**
Temp:Sex	1	**6.34**	**0.01**	**33.05**	**<0.001**	1.85	0.17	3.25	0.07
Prec:Sex	2	1.04	0.60	**7.70**	**0.02**	1.01	0.60	**12.93**	**<0.01**
R^2 marginal (conditional)		0.01 (0.12)		0.06 (0.82)		0.02 (0.20)		0.07 (0.64)	

Results

Of 1950 plants 677 did not flower or their sex was not unambiguously to be identified and thus had to be excluded from the analysis. Overall, we recorded 390 female and 883 hermaphrodite plants. Populations differed strongly in the percentage of sexes. While two German populations had the lowest percentage of female individuals (D7: 6.1%; D8: 4.5%; Figure 13), one Swedish population had the lowest percentage in hermaphrodite individuals (S2: 35.7%; Figure 13). We also aligned the populations along their latitudinal gradient to look for a geographic pattern of sex distribution. However, we could not observe a latitudinal pattern across the European populations as the percentage of female/ hermaphrodite individuals differed independent of latitudinal origin.

Morphological trait differences between the sexes

Not all the examined traits showed differences between the two sexes. Female and hermaphrodite individuals in *S. vulgaris* did not differ significantly in height, leaf area and SLA, nor in time to flowering (Table 7). However, we were able to observe differences in the number of flowers, branches and leaves that were grown. Numbers of flowers and branches were significantly lower in female plants, whereas the number of leaves was significantly lower in hermaphrodites (Table 7, Figure 14). The overall biomass of plants differed significantly on a 10% level between females and hermaphrodites (Table 7) with females having a slightly higher biomass (Figure 14).

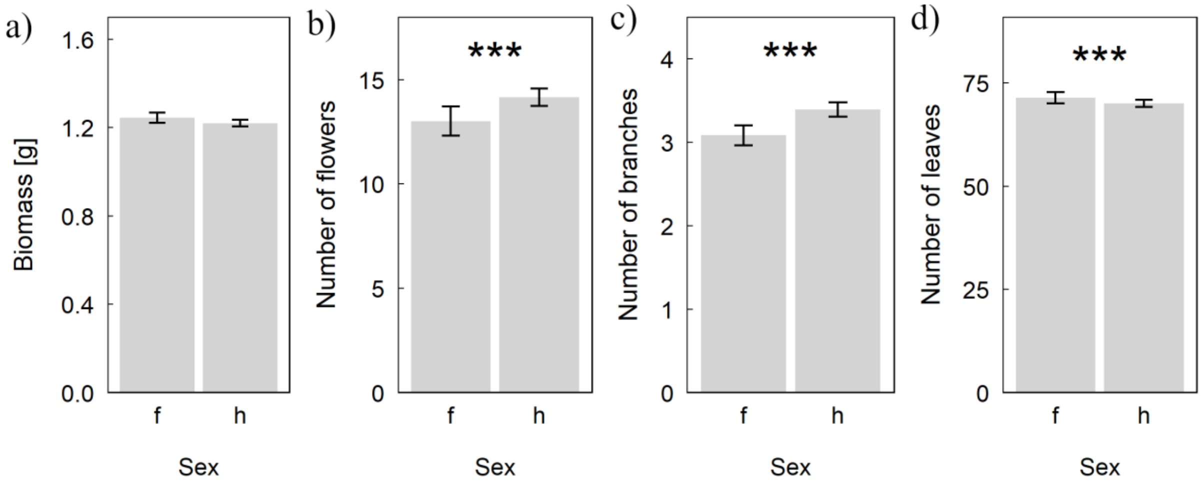

Figure 14. Trait differences in hermaphrodite (h) and female plants (f) of *Silene vulgaris*. (* p <0.05, ** p <0.01, *** p <0.001).

Sex differences in trait response

We found significantly different responses between the sexes in SLA, number of flowers and numbers of leaves to the experimental climate change conditions (Table 7). The effect of reduced precipitation and/ or increased temperature led to significant differences between the sexes in the number of flowers that were produced (Table 7). Females showed a stronger decrease in the number of flowers that were produced under increased temperature conditions (Figure 15a). While female plants showed a 36% decrease in number of flowers in the 21°C treatment compared to the 18°C treatment, hermaphrodites also showed a decrease in flowers

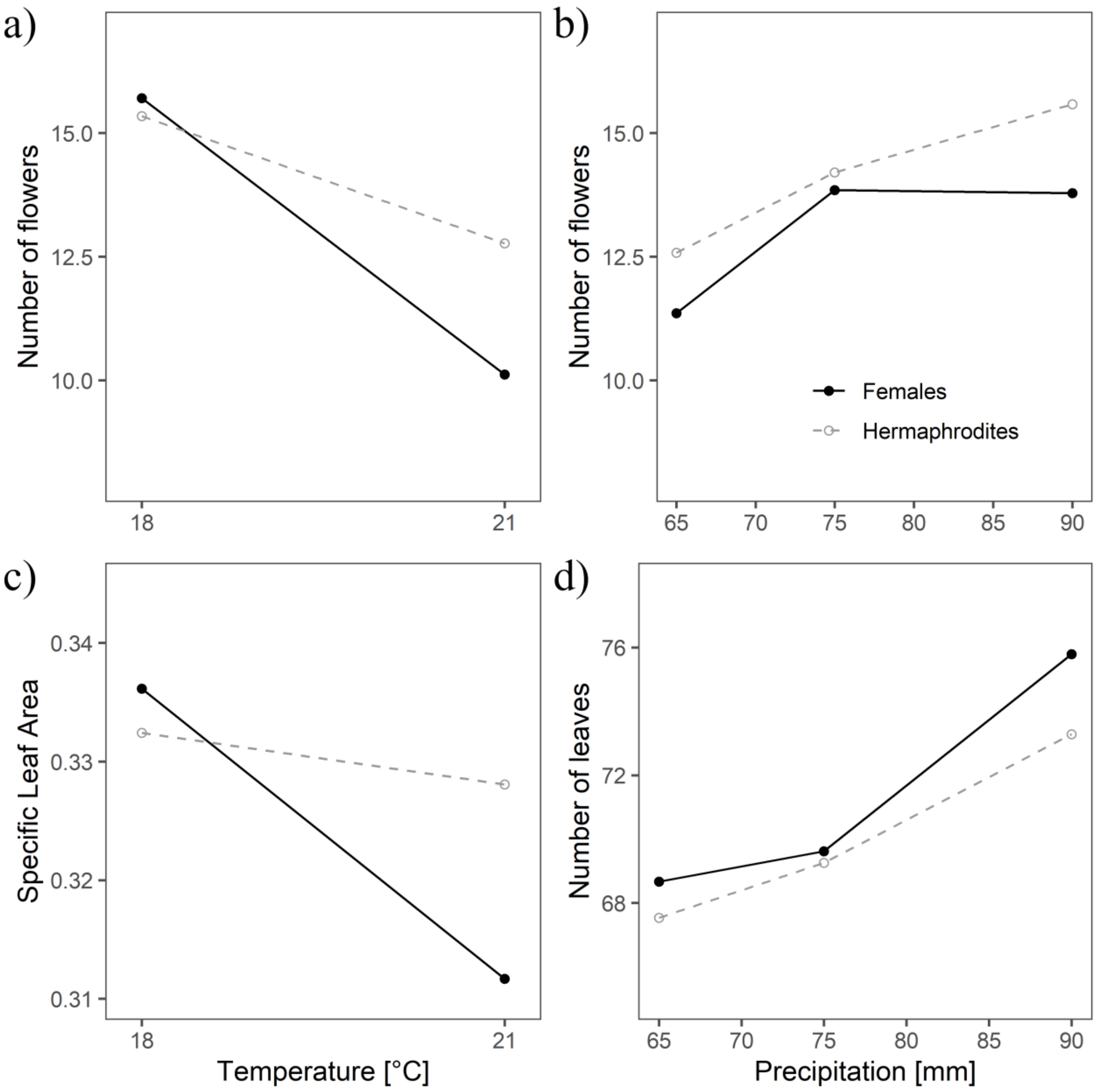

Figure 15. Significant trait response differences in hermaphrodite and female plants of *Silene vulgaris* under reduced precipitation and increased temperature conditions. Specific leaf area (SLA) in cm^2/mg.

but to a lesser extent. Hermaphrodites produced 16% fewer flowers under the higher temperature treatment (Figure 15a). With regard to precipitation, female plants hardly showed any response differences between the 90 mm and 75 mm precipitation treatment, but flower number was strongly reduced at the 65 mm treatment dropping by 17% (Figure 15b). Hermaphrodites on the other hand, showed a steady decrease amongst the temperature treatments: 8% fewer flowers were produced at 75 mm and 19% fewer flowers were produced at 65 mm of precipitation compared to a precipitation of 90 mm (Figure 15b). Overall hermaphrodites showed a higher performance in the number of produced flowers cross all precipitation treatments (Figure 15b). For SLA we found that females were affected more strongly by a temperature increase compared to hermaphrodites: while SLA decreased from 0.336 cm²/mg to 0.312 cm²/mg in female plants, hermaphrodites showed a comparably mild decrease from 0.332 cm²/mg to 0.328 cm²/mg (Figure 15c). Lastly, we were also able to detect a significant response difference in the number of leaves that were produced. In this trait we detected a steadily decreased number of leaves with decreasing precipitation in hermaphrodites: between the 90 mm and 75 mm treatment the number of leaves decreased from an average 73.3 to 69 and further to 68 leaves per plant under 65 mm precipitation. Female plants showed a more drastic decrease from 76 to 70 leaves at the 90 mm and 75 mm precipitation level, respectively. Between the 75 mm and 65 mm level, however, the curve flattens to an average number of 69 leaves per female plant. In general females show more leaves per individual than hermaphrodites, regardless of the precipitation level.

Discussion

The current study aims at investigating the differences between female and hermaphrodite individuals of *S. vulgaris*. Furthermore, it is investigated how vegetative and reproductive traits respond to a climate-change scenario in the two sexes. The diverse effects climate change may have on the two sexes of the species have not been examined previously. Below, we discuss the obtained results from this study demonstrating a potential trade-off between vegetative and reproductive traits in females compared to hermaphrodites in *S. vulgaris*. We also display differences in trait responses between the sexes and review the impact climate change may have on the evolution of the gynodioecious breeding system in *S. vulgaris*.

Sexual dimorphism in Silene vulgaris

In the plants investigated, females produced on average a larger vegetative biomass and number of leaves compared to hermaphrodites. As all plants were harvested at the same time it is possible that the increased vegetative biomass and number of leaves in females is related to a stronger growth in females after the onset of flowering. Similar observations were made in different biomass traits in *Phacelia linearis* (Eckhart and Chapin 1997). However, the measured differences for *S. vulgaris* should be taken with care. In our case biomass differed only with a significance level of 10% and earlier studies in other species failed to demonstrate sexual dimorphism in similar vegetative traits (Kohn 1989, Ashman 2005). Therefore, the differing vegetative features between *S. vulgaris* females and hermaphrodites need to be studied further. Our analysis also revealed that hermaphrodites and females of *Silene vulgaris* differed significantly in the number of flowers they produced during this experiment. Similar results have also been found in other gynodioecious species (Eckhart 1999, Shykoff et al. 2003, Ashman 2005). In our experiment, hermaphrodites produced a significantly higher number of flowers compared to female individuals. As we have not measured other flower traits, we may only hypothesize that females could have a higher performance in other reproductive traits that account for the trade-off of fewer flowers. If gynodioecy is maintained within the populations, females, in theory, have to show a higher performance in other reproductive traits (Lewis 1941). In other studies, for example, females produced bigger flowers, heavier seeds, had a higher seed production, or seeds germinated better (Shykoff et al. 2003, Chang 2006). To better understand how *S. vulgaris* compensates for fewer flowers in females, more reproductive traits would need to be included in analyses of sex differences in this species. With the present results we were able to demonstrate that hermaphrodites and females of *S. vulgaris* differ in reproductive and vegetative traits. The question that remains is if the sexes in *S. vulgaris* also show differing trait responses under changed environmental conditions.

Trait responses to climate change in females and hermaphrodites

Due to climate change temperatures will rise and precipitation is likely to decrease in central Europe (IPCC 2007, Ahlström et al. 2012). As a result, plants are likely to face more stressful conditions and thus their allocation of resources might change as it has already been reported for several species (Eziz et al. 2017). In a gynodioecious species like *S. vulgaris* this could impact females and hermaphrodites differently. For example, while hermaphrodite individuals must invest more in their flowers to create ovaries and stamens, a potential trade-off arises, and they might not be able to invest as much into further growth after the onset of flowering as females may under the same conditions. Under stressful conditions like drought or heat these trait differences of hermaphrodites and females might even increase as it has been seen in other sexually dimorphic species (Sakai and Harada 1998, Galen 2000, Xu et al. 2008, Li et al. 2019, Xia et al. 2020, Liu et al. 2020). Hence, climate change might influence the strength of sexual dimorphism we currently see in *S. vulgaris*. In the present analysis we found that both sexes were negatively affected by the treatments in their number of flowers, number of leaves, and specific leaf area. In general females were affected more strongly in these traits under drier and warmer conditions. Similar sex-specific response differences were reported under drought, differing nutrient levels or foliar shade for other species (Galen 2000, Dykstra et al. 2009, Putney et al. 2020). Especially the number of flowers was reduced drastically in female individuals of *S. vulgaris*. Taking into consideration that females of *S. vulgaris* already produce fewer flowers than hermaphrodites this trait difference might be even enhanced under a warmed climate. It is currently known from other gynodioecious species that females compensate for the lower number of flowers by producing seeds of higher fitness compared to hermaphrodites (Lewis 1941). With increasing temperatures and the hence decreasing number of flowers in females, it is possible that they will no longer be able to coexist alongside hermaphrodite individuals if seed fitness is not increased at the same time. For further studies additional flower and reproductive traits should thus be included in the analyses of this species under environmental stress. Other studies have shown that flower size, biomass allocation in female flower parts, flower scent or nectar production can also differ strongly between the two sexes in gynodioecious species (Klinkhamer et al. 1990, Kendziorski 2018, Stone and Olson 2018, Canelles et al. 2018) and thus might influence the fitness of the female and hermaphrodite sex differently. With regards to pollen and nectar production it has been shown, that female and hermaphrodite *S. vulgaris* plants are visited by different pollinators based on the pollinator’s preference for pollen or nectar. In this context females produce more seeds than hermaphrodites when pollinated by moths (Stone and Olson 2018). With a changing climate the abundance of pollinating moths can be strongly influenced and thus also impact the number of pollinated female *S. vulgaris* individuals in a population. Hence, climate change could also impact the breeding system in *S. vulgaris* shifting towards hermaphroditism through the availability of certain pollinators. Apart from the number of flowers we also found that females showed a more plastic response of SLA towards temperature with a much lower SLA at higher temperatures. In our experiment lower SLA probably arose from a low leaf water content. It has already been shown in several non-succulent species that SLA is positively correlated to leaf water content (Vendramini et al. 2002). With higher temperatures in our experiment evapotranspiration from the leaves probably increased and the leaf water content was lowered. However, as we did not measure the physiological parameter of leaf water content, we can only assume that this is the underlying process. As we found a decreased SLA in female plants under higher temperatures it is possible, that their leaf water content was more drastically lowered than in hermaphrodites and that they experienced stronger heat stress through increased evapotranspiration. For further conclusions however, more physiological parameters would need to be examined that are associated with drought and heat stress.

Sex ratio in different S. vulgaris populations

In our analysis we were not able to detect a latitudinal pattern of sex ratio amongst the different populations that were examined. However, in general we were able to find a strong variation in the sex ratios. It was also shown in the study by (Klaas and Olson 2006) on *Silene acaulis* that populations on a wider geographical range tend to differ strongly in their populations' sex ratios. Based on the complex genetic system that is underlying the sex determination in *S. vulgaris* it would be of further interest to analyse the various cytoplasmic male sterility factors and corresponding restorer genes and how they are distributed within the examined populations. It is possible that the genetic basis of sex determination reveals a similar geographic pattern for *S. vulgaris* as it was shown for other species already (Klaas and Olson 2006).

Conclusion

Climate change has major impacts on species morphology, distribution, and survival. However, the consequences this has on sexually dimorphic species have largely been neglected. In the present study we explored these consequences in the widely distributed species *Silene vulgaris*. We exposed this gynodioecious species to a potential climate change scenario measuring different morphological traits. From our results we were able to paint a complex picture that revealed sexually dimorphic traits and distinct response patterns to climate change. As for sexually dimorphic traits we found that females showed higher values in traits related to vegetative biomass (number of leaves and branches), whereas hermaphrodites produced larger numbers of flowers. We assume, as hermaphrodites have to invest energy into ovules and anthers at the same time, they are facing a trade-off with regards to vegetative biomass. However, it is possible that the larger number of flowers in hermaphrodites is a mechanism to compensate for other reproductive traits where female plants exceed (e.g., seed germination, seed weight). As other reproductive traits were not measured the reason behind the differing numbers of flowers in females and hermaphrodites of *S. vulgaris* remains open for further investigation. As for the sexually distinct response pattern to a potential climate-change scenario we found that the number of flowers and SLA in females were reduced more strongly under our climate change treatment, hence creating a potential disadvantage for females in comparison to hermaphrodite individuals.

In summary, female plants of *S. vulgaris* may possess a potential disadvantage through a smaller number of flowers that might currently be compensated for by other advantageous traits to maintain the gynodioecious breeding system within populations. With ongoing climate change the disadvantage present in females might even increase according to the results of our experiment (lower flower number and SLA) and further compensatory mechanisms in female flowers would be needed to avoid a possible shift towards hermaphroditism.

Chapter 5

General Discussion

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Climate change is one of the most important challenges of our times as it represent a major threat to species survival (Thomas et al. 2004, Knowlton and Graham 2010). Through the human-induced climate change that is ongoing temperatures have followed an ever-increasing trend (NOAA National Centers for Environmental Information 2021) leading to extreme heat waves in different regions (Muthers et al. 2017, Lhotka et al. 2018, Guo et al. 2018) and other extreme weather phenomena around the globe (Trenberth 2011, Peterson et al. 2012b). Furthermore, the current climate change also influences precipitation patterns drastically leading to a stronger variability in raining events (Trenberth 2011, Konapala et al. 2020) that also vary strongly on a local scale (Cook and Wolkovich 2016, Takkis et al. 2018). These examples show that the current climate change is altering environmental conditions that eventually impact the availability of resources for the species that live on our planet (Nicotra et al. 2010). Hence, it is crucial to understand how plants will behave in the future and if they could potentially adapt to the climate conditions they will have to face.

The present dissertation aimed at revealing how the widely distributed plant species *Silene vulgaris* responds to a possible climate change scenario with regard to its' morphological and phenological traits. Furthermore, it was the aim to unveil if these trait responses differ on a population level, among different European genetic clusters detected or between the sexes of the species.

Trait responses of S. vulgaris to climate change

Temperature and precipitation are important drivers for plant responses as they represent strong selective forces (Siepielski et al. 2017, Takkis et al. 2018). Hence, it was not surprising to see distinctive response patterns in the *S. vulgaris* populations in the experiment when they were exposed to a simulated climate-change scenario (Chapter 2). Regarding the temperature treatment, this dissertation is the first study to find that the increased temperature had a negative effect on most of the analysed plant traits in *S. vulgaris*. Similar results were also obtained in other *Silene* species, e.g., *S. noctiflora* or *S. latifolia* where higher temperatures led to fewer flowers, thicker leaves, lower biomass or smaller plant height (Qaderi and Reid 2008, Brothers et al. 2016). Increasing temperatures do not only change morphological traits in plants but can also significantly alter their phenology (Cleland et al. 2007, Rosbakh et al. 2021). In Chapter 2 the flowering of plants advanced by two days under the higher temperature treatment. This is in accordance with the findings for *S. latifolia* and *S. acaulis* (Alatalo and Totland 1997, Brothers et al. 2016). As similar observations were also made for many other plant species (Cleland et al. 2007, Anderson et al. 2012, Park and Schwartz 2015, Hatfield and Prueger 2015, Mo et al. 2017, Iler et al. 2019) this suggests that the current climate change will shift the flowering times in plants towards the beginning of the year (Parmesan and Yohe 2003, Lu et al. 2006, Franks et al. 2007, Bertin 2015, Iler et al. 2019). Especially with regards to pollinator abundance and availability the right timing of flowering is crucial for producing plant offspring. In the present greenhouse experiment the flowering only advanced by two days which is most likely not going to result into less pollinator interactions. However, it is possible that long-term effects over several generations may lead to a mismatch of pollinator availabilities and flowering times in *S. vulgaris* if pollinator availability is not changing to a comparable extend. With the observations made in this thesis this dissertation is the first to provide a basis for further research on flower phenology under climate change in *S. vulgaris*.

As climate change is also going to alter precipitation patterns on a global and local scale (Trenberth 2011, Cook and Wolkovich 2016, Takkis et al. 2018, Konapala et al. 2020) the present dissertation also implemented different precipitation treatments in the greenhouse experiment. The results showed that the experimental precipitation patterns influenced the plant traits to a lesser extent than temperature. However, biomass-related traits decreased significantly with decreasing precipitation which is confirmed the results of other studies (Prieto et al. 2009, Dubois et al. 2017, Eziz et al. 2017, Hamann et al. 2018, Tello-García et al. 2020, Lozano and Rillig 2020). Furthermore, the present greenhouse experiment in this dissertation also showed fewer flowers for the lower precipitation regime. Comparable findings were also described under drought conditions for several other ornamental and agricultural plant species (Mwanamwenge et al. 1999, Flagella et al. 2002, Çakir 2004, Álvarez et al. 2009).

In summary, Chapter 2 describes the phenotypical responses measured for 1925 individuals of *S. vulgaris* under six different temperature and precipitation combinations of a simulated climate change scenario. Hence, this experimental approach is the first study to allow for a comprehensive assessment of how morphological and phenological traits might change under future climatic changes in the species *S. vulgaris*.

Apart from the isolated trait responses, the present dissertation also aimed at revealing possible response differences between different European populations of *S. vulgaris* that might represent advantages or disadvantages during the ongoing climate change.

Population differentiated responses to climate change

In the greenhouse experiment described in Chapter 2 I was able to detect considerable climatic variability that correlated with the latitudinal origin of the different *S. vulgaris* populations. Hence, I hypothesised that southern European populations (e.g., originating from Spain) would exhibit morphological traits that are adapted to a warmer, drier climate. Hence, this may lead to lower values of leaf and biomass-related traits. However, the employed mixed models were not able to support this: in general, most traits did not change along the latitudinal gradient and thus e.g., a trait adaptation to a warmer climate in populations from southern Europe could not be detected. In other experiments this lack of latitudinal influence was not found. On the contrary: it was shown that morphological traits changed along a latitudinal gradient and the direction of the change depended on the species under investigation (De Frenne et al. 2011, 2013, Moles et al. 2014). Nonetheless, the mixed models in Chapter 2 showed that the sensitivity of the trait responses for number of flowers and number of leaves was significantly correlated with latitude. This means that populations from higher latitudes showed a more drastic response to the treatments producing less flowers under the higher temperature and low precipitation level and significantly less leaves also under the low precipitation level. These findings are of high importance as especially number of flowers is a fitness-related trait determining the offspring of a plant. Hence, it is possible that in northern populations of *S. vulgaris* the number of offspring plants might be reduced due to higher temperatures and less rain during climate change. From my knowledge this dissertation is the first study to find latitudinal differences in the sensitivity of trait responses towards climate change in *S. vulgaris*.

Widely distributed species are often underrepresented in the research on climate change impacts as they are usually not the target species of conservational efforts. However, as they are part of many different plant communities, they play a crucial role when assessing the impacts of climate change on a community level. The greenhouse experiment in this dissertation provides a comprehensive approach that included different European populations of different latitudinal origin of the widely distributed species *S. vulgaris*. The results from Chapter 2 showed that all populations of *S. vulgaris* had distinct response mechanisms to the simulated climate change scenario and differed in the response sensitivity. These results provide a solid basis to understand the responses of widely distributed plants and can thus help to gain a better understanding on how plant populations and communities might be affected by climate change.

In addition to the latitudinal origin, the genetic background of the plants might provide additional support to understand the adaptational mechanisms towards climatic factors in *S. vulgaris*. Therefore, the genetic population analysis described in Chapter 3 dealt with revealing the underlying genetic structure.

Population genetic structure and differential trait responses of genetic lineages

Widely distributed plants like *S. vulgaris* often possess many genetic lineages or subspecies with considerable local adaptation (Liepelt et al. 2010, Stölting et al. 2015, Van Rossum et al. 2018, Collart et al. 2021). By revealing the underlying genetic structure of the sampled *S. vulgaris* populations I assumed to find differences among the genetic lineages in their response towards the climate-change scenario that was employed in the greenhouse experiment in Chapter 2. In Chapter 3 I used a genome-wide approach via ddRAD sequencing and SNPs were analysed through three different approaches including phylogeny, multivariate analysis via STRUCTURE and pairwise F_{ST} comparison. All analyses revealed three major genetic clusters across the *S. vulgaris* populations sampled in Europe with only slight differences among the approaches. In principle, the clusters combined populations from Spain and southern France into a southern, from western France into a western, and from Germany, Switzerland, Austria, and Sweden, into a central European cluster. This pattern basically reflects those of earlier studies on *S. vulgaris* or closely related species within the same genus (Sebasky et al. 2016, Van Rossum et al. 2018). Through the analysis I was able to show that *S. vulgaris* possesses a considerable phylogenetic diversity that generally aligns into three major clusters. However, where these clusters derive from remains unrevealed. Many European plant species possess a phylogeographical pattern that resulted from historical expansion or disruption events, for example during the last glacial maximum (LGM) in Europe (Beatty and Provan 2011, Taberlet et al. 2012, Bagnoli et al. 2016, Sebasky et al. 2016, Listl et al. 2017, Roces-Díaz et al. 2018, Van Rossum et al. 2018, Krebs et al. 2019, Schwarzer and Joshi 2019). The analysis for *S. vulgaris* showed that indicators for genetic diversity (He, Ho, π) declined with increasing latitude meaning that it is possible that southern European populations were functioning as glacial refugia during the LGM. However, the indicators for genetic diversity can only be taken as one piece of evidence for this hypothesis. Although several other species, including closely related *Silene* species, show a comparable pattern (Hewitt 1999, 2000, Taylor and Keller 2007, Breen et al. 2009, Beatty and Provan 2011, García-Fernández et al. 2013, Meindl et al. 2016, Chung et al. 2018, Van Rossum et al. 2018, Tausch 2019) further analysis is needed to unambiguously proof these results.

Having revealed the phylogeography of the *S. vulgaris* populations used in the present experiment it was another aim of Chapter 3 in this dissertation to analyse whether the different genetic lineages exhibit different responses to a simulated climate-change scenario. As the anthropogenic climate change is leading to an increase of annual mean temperatures and drastic changes in the precipitation patterns (Bindi and Olesen 2011, Trnka et al. 2011, Marx et al. 2018, NOAA National Centers for Environmental Information 2021) it is crucial to take the genetic background of species into account as well. It has been shown already in several other studies especially in a conservational context that knowing the species genetics or genomics can help to facilitate species survival and understand their adaptation mechanisms (McMahon et al. 2014, Corlett 2017). This is why I included the genetic data gained from the multivariate approach with STRUCTURE (described in Chapter 3) into the analysis of morphological and phenological response patterns to the simulated climate-change scenario. The mixed models from Chapter 3 revealed that most of the analysed traits showed comparable responses under the different treatments and did not differ among the genetic clusters. In the light of ongoing climate change this could mean that none of the genetic lineages analysed in this study show an advantageous adaptation to climate change based on the employed climate-change scenario.

For plant species that are in the focus of conservational measures it is quite common to have their population genetics analysed or even to target specific genes responsible for the adaptation towards heat or drought. However, in more common species as *S. vulgaris* this is hardly done. According to my knowledge this work is he first to combine the population genetics of *S. vulgaris* with an analysis of the phenotypic trait responses to a climate change treatment. Hence, the results give us a first insight in how the genetic lineages of *S. vulgaris* might respond to anthropogenic climate change and can provide the fundamental knowledge to start working on other widely distributed species.

Sexual dimorphism under climate change

As an additional angle in the research of the climate change impact on *S. vulgaris*, the present dissertation also aimed at examining the response differences between female and hermaphrodite individuals. Under the changing climate many plants experience more stressful environments that might change their allocation of resources (Eziz et al. 2017). In *S. vulgaris* this may differ between the different sexes as it has been reported for other sexually dimorphic species (Xu et al. 2008, Brothers et al. 2016, Liu et al. 2020) and could even influence the breeding system dynamics of this species comparable to *Lobelia spicata* where high temperatures and low precipitation favoured the success of female plants (Ruffatto 2012). Hence, I determined in a first approach the morphological differences between the sexes in *S. vulgaris*. The results from Chapter 4 showed that females produced more vegetative biomass (dry biomass, number of leaves) compared to hermaphrodites as it has been shown in some studies (Eckhart and Chapin 1997) or failed to be proven in others (Kohn 1989, Alonso and Herrera 2001, Ashman 2005). Furthermore, I was able to show in Chapter 4 that hermaphrodites produced a significantly higher number of flowers than female plants which is in accordance to earlier studies in other species (Eckhart 1999, Shykoff et al. 2003, Ashman 2005, Wang et al. 2021). With examining a potential sexual dimorphism in *S. vulgaris* this dissertation is one of the few studies addressing this topic in this specific species and in a gynodioecious species in general. Thus, this dissertation helps to understand and shed light on the underrepresented topic of sexual dimorphism in gynodioecious plants.

As a second aspect Chapter 4 dealt with the actual response differences between the sexes towards the simulated climate-change scenario. I found that both sexes were negatively affected by the climate-change treatment in many of the examined traits. However, females showed a stronger negative response under the drier and warmer experimental conditions. It has been found in other species that sex-specific response differences occur under different stressful conditions: drought, shade, or regarding the nutrient levels (Galen 2000, Dykstra et al. 2009, Brothers et al. 2016, Putney et al. 2020). The results of Chapter 4 furthermore showed that specifically the number of flowers was drastically reduced in females under the climate-change scenario. As females of *S. vulgaris* have a lower number of flowers in general that decreased further under the climate-change scenario the sexual dimorphism detected might even increase in the long run. This could represent a disadvantage for the females of *S. vulgaris* compared to hermaphrodites. To ensure a co-existence alongside hermaphrodites female plants would need e.g., higher seed fitness to account for this disadvantage (Lewis 1941). Unfortunately, further reproductive traits regarding e.g., the seed fitness were not measured during the experiment and hence could not be analysed in Chapter 4. It is therefore highly speculative if female plants of *S. vulgaris* really have a strong disadvantage under environmental stress (induced through climate change) that eventually leads to fewer offspring. However, this work is one of the few that deals with sexual dimorphisms in a gynodioecious plant in general and in *S. vulgaris* specifically. Especially the trait analysis of both sexes in the light of climate change is rarely done in gynodioecious species and from my knowledge has never been done in *S. vulgaris* before. This work thus provides a fascinating new aspect to the research on the breeding system of *S. vulgaris* and how it might be affected through the climate change that the species is facing.

The role of phenotypic plasticity in the adaption to climate change

Phenotypic plasticity is considered an important mechanism for plants to persist in a changing environment as it often results in rapid phenotypic changes (Charmantier et al. 2008). In Chapter 2 a significant response was observed in most of the plants towards the climate change scenario. This indicates that phenotypic plasticity might play an important role in *S. vulgaris* when adapting to changing climatic conditions. Comparable results were also found for other species where phenotypic plasticity was able to account for negative effects of climate change (Frank et al. 2017, Richardson et al. 2017, Kingsolver and Buckley 2017, Peterson et al. 2018). The PIs that were calculated in Chapter 2 support this assumption differing among populations and plant traits examined suggesting that *S. vulgaris* can respond very plastically to the increased temperature and reduced precipitation treatments through its' growth. Apart from being an adaptational mechanism phenotypic plasticity can also be seen as a trait itself that possesses a genetic basis and is heritable (Bradshaw 1965, Thompson 1991). In the mixed models that were employed for the temperature and precipitation treatments for the different *S. vulgaris* populations no interaction between the latitude where the populations originated from, and the treatment was found (Chapter 2). This suggests a low genetic differentiation for the phenotypic plasticity among the populations examined (sensu (Pigliucci 2001)) and has been already shown in other studies that tested for the interaction of genotype (represented by latitude in Chapter 2) and environment (represented by treatment in Chapter 2) (Gugger et al. 2015, Münzbergová et al. 2017). For the number of flowers, however, a significant genetic differentiation with regards to latitudinal origin was found. In this case it is possible that plasticity may represent a trait under selection exposed to the impacts of climatic changes. Furthermore, the results from Chapter 2 show that *S. vulgaris* individuals from higher latitudes possessed greater values of PIs for flower number. This means that plants from higher latitudes might be more likely to adjust their number of flowers more plastically towards varying temperature conditions. Summing up the present thesis shows that *S. vulgaris* possesses considerable phenotypic plasticity at the species level regarding temperature and precipitation changes. This has never been found before in this species and represents an important scientific contribution to understand the adaptational mechanisms towards climate change in widely distributed species. Hence, examining the adaptational mechanisms in further widely distributed species could facilitate a realistic assessment of the impacts of climate change on a number of plant communities.

Limitations and suggestions for future research

In Chapter 2 the simulated climate change treatment resulted in a decrease of most biomass-related traits. However, results of warming experiments are highly species- and ecosystem dependent and they can vary strongly. Therefore, conclusions for other species cannot be drawn easily. Regarding the precipitation treatment it is important to mention that the performance of plants is highly interconnected to the availability of water. Hence, the results of the greenhouse experiment should be handled with care and cannot be applied to other species or ecosystems without effort. The greenhouse experiment revealed that northern populations of *S. vulgaris* produced less flowers under the climate-change treatment. This could imply that populations from higher latitudes could be threatened by climate change as they might produce fewer offspring. However, to test for this possibility a long-term experiment would need to be set up where the development of flower production across several generations in different populations under a climate-change treatment would need to be examined. In this approach the inclusion of further fitness related traits (e.g., number of produced seeds, germination rate in produced seeds etc.) could also support further statements on long-term effects. Furthermore, it is important to point out, that the same climate-change scenario was employed for all the different European populations. These conditions are not ideal to reflect a climate-change scenario. Hence, the conditions may have been more favorable for some populations than for others. Ideally, I propose to employ different climate change scenarios that reflect the regional climate-change projections for the local populations examined. A common-garden transplant experiment is yet another possibility to ensure that single populations face differing environments than where they originate from. Especially, the transplantation of individuals from any of the three genetic lineages (see Chapter 3) to any of population sites where the other lineages derive from should be considered for further experiments. In this context of local adaptation it would have been of further interest to analyse functionally important genetic variation in the three genetic lineages as it has been done in other species (Stölting et al. 2015). However, the genome of *S. vulgaris* is comparably big with regard to other plants species and has not been fully sequenced yet which makes a more in-depth analysis more complicated and time consuming. In Chapter 3 I hypothesised that the different genetic lineages of *S. vulgaris* derive from a potential isolation scenario of single populations in distinct refugia during the last glacial maximum (LGM). However, this hypothesis about *S. vulgaris*' evolutionary past needs further examination. The refugia would need to be identified unambiguously where a distribution modelling of the species could support. Furthermore, a coalescent-based method would be needed to reliably analyse the present genetic variation in *S. vulgaris* and trace it back to possible historical patterns in the species similar to the studies of Rosenberg and Nordborg (2002) and Sebasky et al. (2016). With regards to the differences between the sexes in *S. vulgaris* a long-term experiment would be favourable. In this experiment the proportion of females and hermaphrodites should be studied intensively, and further flower and reproductive traits should be measured. Through this a more comprehensive conclusion could be drawn on the development of the breeding system under climate change. An additional genetic analysis of the distribution of underlying cytoplasmic male sterility factors and corresponding restorer genes in the populations could further complete this approach (see Klaas and Olson, 2006). As mentioned before, phenotypic plasticity can be seen as a trait itself that possesses a genetic determination. Hence, to analyse the influence of phenotypic plasticity on the adaptation to climate change it could help to employ known genetic markers of phenotypic plasticity on *S. vulgaris* and look for differences in the distinct genetic lineages.

Conclusion

It has already been reported in several previous studies that to adapt to current and future climate changes plants are shifting their distribution patterns or can adapt locally to the new conditions (He et al. 2019, Ahrens et al. 2020, Anderson and Wadgymar 2020, Metz et al. 2020). The present study builds on this knowledge to understand the responses to a climate-change scenario in the widely distributed species *S. vulgaris*. The study employed a comprehensive approach that is unmet in this species: An extensive pan-European sampling and results from an ecological greenhouse experiment were combined with a population genetic analysis as well as with a differential sex-analysis. The results showed distinct trait differences in the different European populations and genetic lineages but failed to reveal response differences to a potential climate-change scenario among them. Phenotypic plasticity may thus represent a possible coping strategy in a widely distributed plant like *Silene vulgaris* to deal with current and future environmental changes. Additionally, trait differences between the two sexes could only be detected in the number of flowers and these differences were strengthened by the climate-change scenario. Hence, it is possible that the gynodioecious breeding system of *S. vulgaris* could be influenced through a changing climate.

In conclusion, this work is the first to show that phenotypic plasticity is a possible adaptation strategy towards climate change in *S. vulgaris*. This provides a solid basis to examine phenotypic plasticity as an adaptational mechanism towards climate change in other widely distributed and gynodioecious plants. With climate change being one of the biggest threats of our times the present work exemplifies one possibility of how plant species can adapt and how they can be affected in various ways through rapidly changing climatic conditions.

Summary

The ongoing climate change is altering the living conditions for many organisms on this planet at an unprecedented pace. Hence, it is crucial for the survival of species to adapt to these changing conditions. In this dissertation *Silene vulgaris* is used as a model organism to understand the adaption strategies of widely distributed plant species to the current climate change. Especially plant species that possess a wide geographic range are expected to have a high phenotypic plasticity or to show genetic differentiation in response to the different climate conditions they grow in. However, they are often underrepresented in research.

In the greenhouse experiment presented in this thesis, I examined the phenotypic responses and plasticity in *S. vulgaris* to estimate its' adaptation potential. Seeds from 25 wild European populations were collected along a latitudinal gradient and grown in a greenhouse under three different precipitation (65 mm, 75 mm, 90 mm) and two different temperature regimes (18°C, 21°C) that resembled a possible climate change scenario for central Europe. Afterwards different biomass and fecundity-related plant traits were measured.

The treatments significantly influenced the plants but did not reveal a latitudinal difference in response to climate treatments for most plant traits. The number of flowers per individual however, showed a stronger plasticity in northern European populations (e.g., Swedish populations) where numbers decreased more drastically with increased temperature and decreased precipitation.

To gain an even deeper understanding of the adaptation of *S. vulgaris* to climate change it is also important to reveal the underlying phylogeny of the sampled populations. Therefore, I analysed their population genetic structure through whole genome sequencing via ddRAD. The sequencing revealed three major genetic clusters in the *S. vulgaris* populations sampled in Europe: one cluster comprised Southern European populations, one cluster Western European populations and another cluster contained central European populations. A following analysis of experimental trait responses among the clusters to the climate-change scenario showed that the genetic clusters significantly differed in biomass-related traits and in the days to flowering. However, half of the traits showed parallel response patterns to the experimental climate-change scenario.

In addition to the potential geographic and genetic adaptation differences to climate change this dissertation also deals with the response differences between the sexes in *S. vulgaris*. As a gynodioecious species populations of *S. vulgaris* consist of female and hermaphrodite

individuals and the sexes can differ in their morphological traits which is known as sexual dimorphism. As climate change is becoming an important factor influencing plant morphology it remains unclear if and how different sexes may respond in sexually dimorphic species. To examine this question the sex of each individual plant was determined during the greenhouse experiment and the measured plant traits were analysed accordingly. In general, hermaphrodites had a higher number of flowers but a lower number of leaves than females. With regards to the climate change treatment, I found that hermaphrodites showed a milder negative response to higher temperatures in the number of flowers produced and in specific leaf area (SLA) compared to females.
Synthesis – The significant treatment response in *Silene vulgaris*, independent of population origin in most traits suggests a high degree of universal phenotypic plasticity. Also, the three European intraspecific genetic lineages detected showed comparable parallel response patterns in half of the traits suggesting considerable phenotypic plasticity. Hence, plasticity might represent a possible adaptation strategy of this widely distributed species during ongoing and future climatic changes. The results on sexual dimorphism show that females and hermaphrodites are differing mainly in their number of flowers and females are affected more strongly by the experimental climate-change scenario. These results provide a solid knowledge basis on the sexual dimorphism in *S. vulgaris* under climate change, but further research is needed to determine the long-term impact on the breeding system for the species.

In summary this dissertation provides a comprehensive insight into the adaptation mechanisms and consequences of a widely distributed and gynodioecious plant species and leverages our understanding of the impact of anthropogenic climate change on plants.

Zusammenfassung

Der derzeitige Klimawandel verändert die Lebensbedingungen für viele Tiere und Pflanzen auf unserem Planeten in nie da gewesenem Maße. Damit Arten überleben, ist es von besonderer Wichtigkeit, dass sich diese an die sich ändernden Klimabedingungen anpassen können. Die vorliegende Dissertation befasst sich mit der Modellpflanze *Silene vulgaris* und versucht zu ergründen, wie sich solch weit verbreitete Pflanzenarten an den Klimawandel anpassen. Dabei ist zu erwarten, dass sie eine hohe phänotypische Plastizität besitzen, durch die sie sich gut anpassen können oder, dass sie sich durch eine genetische Differenzierung als Antwort auf die vorherrschenden Umweltbedingungen auszeichnen. Im experimentellen Ansatz dieser Dissertation untersuchte ich daher die phänotypischen Anpassungen und die phänotypische Plastizität von *S. vulgaris* an ein mögliches Klimawandelszenario für Zentraleuropa. Dabei wurden die Samen von 25 europäischen Populationen gesammelt und in einem Gewächshausexperiment unter drei verschiedenen Niederschlagsbedingungen (65 mm, 75 mm, 90 mm) und zwei verschiedenen Temperaturbedingungen (18°C, 21°C) herangezogen. Im Anschluss wurden verschiedene Biomasse- und Fertilitätsmerkmale gemessen. Für ein tiefergehendes Verständnis der Anpassungsmöglichkeiten von *S. vulgaris* an den Klimawandel ist es zudem wichtig, auch die zugrundeliegende Phylogenie der Populationen zu verstehen. In diesem Zusammenhang nutzte ich eine genomweite Sequenziermethode mittels ddRAD.

Die Bedingungen im Gewächshausexperiment beeinflussten die Pflanzen signifikant in ihren phänotypischen Merkmalen, jedoch ließ sich kein Unterschied zwischen Population unterschiedlicher Herkunft erkennen. Lediglich die Anzahl der Blüten zeigte eine größere Plastizität in nördlichen europäischen Populationen, wo sich die Blütenzahl stärker dezimierte unter höheren Temperaturen und stärkerer Trockenheit. Die populationsgenetische Analyse ergab drei distinkte phylogenetische Gruppen für die untersuchten europäischen Populationen von *S. vulgaris*: eine Gruppe beinhaltete südeuropäische Populationen aus Spanien und Südfrankreich, eine weitere Gruppe bestand aus den gesammelten Individuen der westfranzösischen Populationen, während die dritte Gruppe, die Populationen aus Mittel- und Nordeuropa enthielt. Diese genetischen Gruppen wurden anschließend ebenfalls der Merkmalsanalyse unter den Gewächshausbedingungen unterzogen. Dabei stellte sich heraus, dass sich die genetischen Gruppen in ihren phänotypischen Merkmalen unterschieden, jedoch

eine ähnliche Anpassung ihrer Merkmale an die experimentellen Klimawandelbedingungen zeigten.

Der dritte Aspekt dieser Dissertation befasste sich mit möglichen Anpassungsunterschieden zwischen den Geschlechtern in *S. vulgaris*. Als gynodiözische Art bestehen ihre Populationen sowohl aus weiblichen, also auch aus zwittrigen Individuen. Die phänotypischen Merkmale beider Geschlechter können sich dabei unterscheiden, was man als Sexualdimorphismus bezeichnet. Es ist bereits bekannt, dass sich Pflanzenmerkmale durch den anhaltenden Klimawandel bereits verändern, jedoch ist es nicht gut erforscht, ob und wie sich die unterschiedlichen Geschlechter bei einer sexuell dimorphen Art unter diesem Selektionsdruck verhalten. Während des Gewächshausexperiments wurden daher die Geschlechter der Individuen bestimmt und die phänotypischen Unterschiede zwischen weiblichen und zwittrigen Pflanzen analysiert. Allgemein lässt sich sagen, dass zwittrige Individuen mehr Blüten aber weniger Blätter hatten als weibliche. Im Hinblick auf die experimentellen Klimawandelbedingungen konnte ich zudem feststellen, dass Hermaphroditen in ihrer spezifischen Blattfläche und der Blütenanzahl weniger stark negativ auf höhere Temperaturen reagierten.

Synthese – Die signifikanten Merkmalsanpassungen an die Gewächshausbedingungen waren unabhängig von der geographischen Herkunft oder genetischen Gruppe der Individuen. Dies lässt ein hohes Maß an universeller, phänotypischer Plastizität vermuten. Dementsprechend kann davon ausgegangen werden, dass phänotypische Plastizität ein möglicher Anpassungsmechanismus für diese weit verbreitete Art an den Klimawandel sein könnte. Im Hinblick auf den Sexualdimorphismus in *S. vulgaris* lässt sich sagen, dass sich beide Geschlechter vornehmlich in der Anzahl der Blüten unterscheiden und dass weibliche Pflanzen stärker von den Bedingungen des Gewächshausexperiments beeinflusst wurden. Diese Dissertation konnte damit erstmals darüber Aufschluss geben, wie sich *S. vulgaris* im Hinblick auf ihren Sexualdimorphismus unter Klimawandelbedingungen verhält. Weitere Forschung wird nun benötigt, um auch den Langzeiteffekt des Klimawandels auf das Fortpflanzungssystem dieser Art abschätzen zu können.

Zusammenfassend lässt sich sagen, dass die vorliegende Arbeit einen umfassenden Einblick in die Anpassungsmechanismen einer weit verbreiteten Pflanzenart an den anthropogenen Klimawandel gibt. Zudem bestärkt sie unser Verständnis der Auswirkungen, die sich daraus für eine gynodiözische Art, wie *S. vulgaris* ergeben.

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Appendix

Supporting information for Chapter 2

Table A1. Climate data of *Silene vulgaris* sampling sites obtained from WorldCim (Fick & Hijmans, 2017). All temperature variables are in °C.

Pop	Site	bio_1	bio_2	bio_3	bio_4	bio_5	bio_6	bio_7	bio_8	bio_9	bio_10	bio_11
A1	Gschnitz	5.6	8.6	31.7	692.9	18.2	-8.9	27.1	14.0	-3.0	14.0	-3.0
CH1	Flims	1.0	7.1	32.9	547.7	12.2	-9.2	21.4	8.1	-4.8	8.1	-4.8
CH2	Cadenazzo	11.2	9.4	32.1	728.2	25.4	-3.9	29.3	15.0	2.3	20.3	2.3
D1	Berlin	9.9	7.1	27.6	696.8	22.0	-3.8	25.8	18.6	5.2	18.6	1.5
D2	Hamburg	8.8	7.3	30.4	613.3	20.2	-3.9	24.1	16.6	4.4	16.6	1.5
D3	Berlin	9.7	7.4	28.1	697.0	22.1	-4.3	26.4	18.4	5.1	18.4	1.3
D5	Mainz	10.2	7.8	30.3	649.3	22.3	-3.6	25.9	16.8	3.5	18.3	2.4
D6	Heilsbronn	8.6	8.3	30.4	687.5	21.4	-5.8	27.2	15.7	4.1	17.1	0.3
D7	Gerswalde	8.5	7.9	29.1	683.5	21.1	-5.9	27.0	17.0	3.8	17.0	0.2
D8	Geesower Hügel	8.7	7.5	28.0	696.2	21.2	-5.7	26.9	17.4	1.2	17.4	0.3
D9	Potsdam	9.2	7.6	28.5	687.7	21.6	-4.9	26.5	17.8	4.6	17.8	0.9
D10	Konstanz	9.6	8.2	30.2	701.3	22.4	-4.9	27.3	18.3	2.4	18.3	1.0
D11	Hamm	10.5	8.2	30.9	668.7	22.9	-3.6	26.5	17.3	3.7	18.9	2.5
D12	Langenargen	9.5	7.3	28.8	677.2	21.4	-4.1	25.5	17.9	2.5	17.9	1.3
E1	Alcanó	15.0	12.3	39.4	684.6	30.1	-1.2	31.3	15.5	23.7	23.7	6.9
E2	Fontdepou	12.1	11.3	37.3	667.5	27.2	-3.2	30.4	13.4	5.4	21.0	4.6
E3	La Sentiu de Sió	14.1	12.0	38.9	678.9	28.9	-2.0	30.9	14.7	7.4	22.8	6.3
E4	La Palma	14.5	6.2	41.3	323.7	22.5	7.5	15.0	11.2	18.3	18.9	11.0
F1	Les Rochette	12.1	8.8	38.9	519.6	22.8	0.2	22.6	6.7	18.6	18.6	5.9
F2	Pommeraie	12.4	11.4	38.9	661.2	27.1	-2.3	29.4	13.0	20.9	20.9	4.7
F3	La Noue du Bourg	11.6	9.0	36.8	572.7	23.2	-1.1	24.3	8.2	18.8	18.8	4.8
F4	Menton	11.5	9.3	33.7	665.6	24.7	-2.9	27.6	11.7	3.5	19.9	3.5
F5	Normandie	11.7	8.4	31.9	649.7	24.3	-2.0	26.3	11.9	4.0	20.0	4.0
S1	Södra Bäck	7.2	7.5	30.3	655.2	19.3	-5.5	24.8	15.1	1.8	15.7	-0.2
S2	Vickleby	7.3	7.8	29.9	678.0	20.1	-6.1	26.2	15.2	1.7	16.1	-0.4

Note: Pop: Population. bio_1: annual mean temperature, bio_2: mean diurnal range (mean of monthly (max temp - min temp)), bio_3: isothermality (bio_2/bio_7), bio_4: temperature seasonality (standard deviation), bio_5: maximum temperature warmest month, bio_6: minimum temperature coldest month, bio_7: temperature annual range (bio_5-bio_6), bio_8: mean temperature of wettest quarter, bio_9: mean temperature of driest quarter, bio_10: mean temperature of warmest quarter, bio_11: mean temperature of coldest quarter.

Table A2. Continued climate data of *Silene vulgaris* sampling sites obtained from WorldCim (Fick & Hijmans, 2017). All precipitation variables are in mm.

Pop	Site	bio_12	bio_13	bio_14	bio_15	bio_16	bio_17	bio_18	bio_19	s_rad	w_sp	w_v_pr
A1	Gschnitz	977	140	37	45	402	124	402	124	11861	1.9	0.7
CH1	Flims	1965	211	117	17	607	390	607	398	12866	4.1	0.5
CH2	Cadenazzo	1473	171	61	32	476	215	460	215	12618	1.4	1.1
D1	Berlin	575	65	36	19	176	117	176	137	9993	3.7	0.9
D2	Hamburg	792	81	45	17	223	159	223	193	9607	3.6	0.9
D3	Berlin	577	69	35	20	182	116	182	134	10061	3.7	0.9
D5	Mainz	574	64	36	18	181	116	167	125	10411	2.8	1.0
D6	Heilsbronn	743	83	48	18	234	154	227	165	10695	3.0	0.9
D7	Gerswalde	538	70	29	28	189	95	189	106	10098	3.2	0.9
D8	Geesower Hügel	510	62	27	26	176	94	176	101	10158	2.9	0.9
D9	Potsdam	558	65	36	19	172	115	172	133	10151	4.0	0.9
D10	Konstanz	879	110	47	27	304	151	304	163	11268	2.1	1.0
D11	Hamm	601	71	36	21	198	116	182	122	10569	2.7	1.0
D12	Langenargen	1094	132	57	29	386	183	386	191	11445	2.0	1.0
E1	Alcanó	366	49	10	37	121	62	62	72	15336	2.6	1.2
E2	Fontdepou	662	77	33	24	200	126	151	134	15263	3.1	0.9
E3	La Sentiu de Sió	490	58	21	28	153	93	106	94	15140	2.5	1.1
E4	La Palma	432	80	1	78	216	6	21	186	19890	6.2	1.0
F1	Les Rochette	773	88	39	28	259	121	121	243	12508	3.9	1.1
F2	Pommeraie	739	94	32	24	235	142	142	173	14923	3.3	1.0
F3	La Noue du Bourg	770	76	51	13	215	159	159	201	12112	3.4	1.1
F4	Menton	971	108	65	19	299	200	222	200	12677	2.9	1.0
F5	Normandie	838	95	50	21	259	159	205	159	12715	3.1	1.0
S1	Södra Bäck	524	56	32	19	156	101	147	123	10149	4.8	0.9
S2	Vickleby	509	55	29	20	157	96	146	117	10171	4.7	0.9

Note: bio_12: annual mean precipitation, bio_13: precipitation wettest month, bio_14: precipitation driest month, bio_15: precipitation seasonality (coefficient of variation), bio_16: precipitation wettest quarter, bio_17: precipitation driest quarter, bio_18: precipitation warmest quarter, bio_19: precipitation coldest quarter, s_rad: solar radiation in kJ/(m^2*d), w_sp: wind speed in m*s^{-1}, w_v_pr: water vapor pressure in kPa.

Table A3. Loadings of climatic variables on principal component 1 and 2. For abbreviations see captions of Table A1 and A2.

Climatic variable	PC 1	PC 2
bio_1	0.29	-0.05
bio_2	0.15	0.04
bio_3	0.17	-0.30
bio_4	-0.06	0.39
bio_5	0.26	0.06
bio_6	0.24	-0.25
bio_7	0.05	0.30
bio_8	0.01	0.35
bio_9	0.24	-0.17
bio_10	0.27	0.08
bio_11	0.27	-0.18
bio_12	-0.25	-0.17
bio_13	-0.23	-0.20
bio_14	-0.27	-0.10
bio_15	0.11	-0.23
bio_16	-0.24	-0.19
bio_17	-0.26	-0.10
bio_18	-0.28	-0.04
bio_19	-0.19	-0.31
s_rad	0.15	-0.31
w_speed	0.05	-0.19
w_v_pressure	0.26	0.01

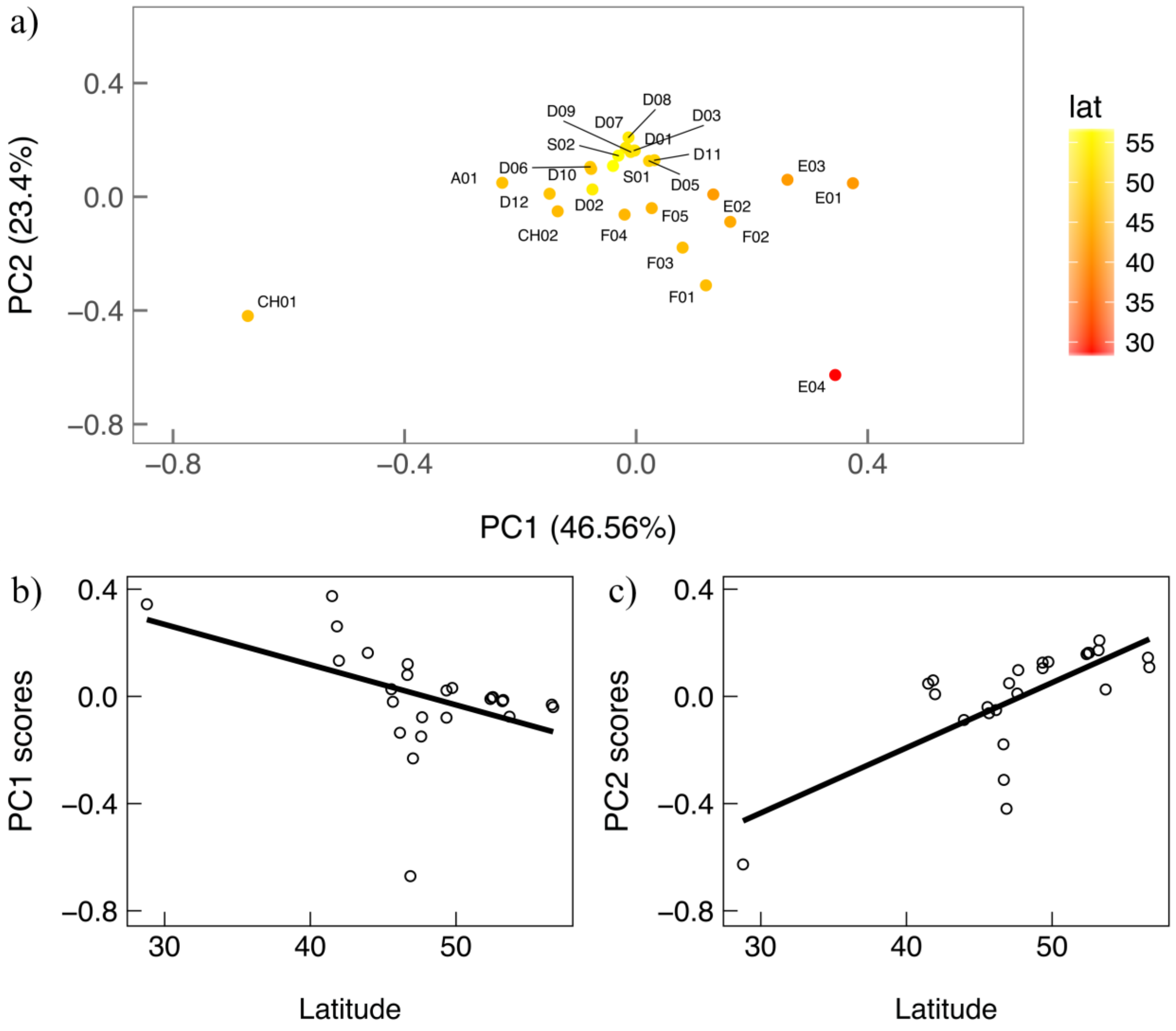

Figure A1. a) Principal component analysis of climate data for individual populations (for population abbreviation see Table 1). The contribution of each component to the total variation is denoted in each axis. b) Correlation between PC 1 scores and latitude and c) PC 2 scores and latitude for individual *Silene vulgaris* populations. lat: Latitude of *Silene vulgaris* populations.

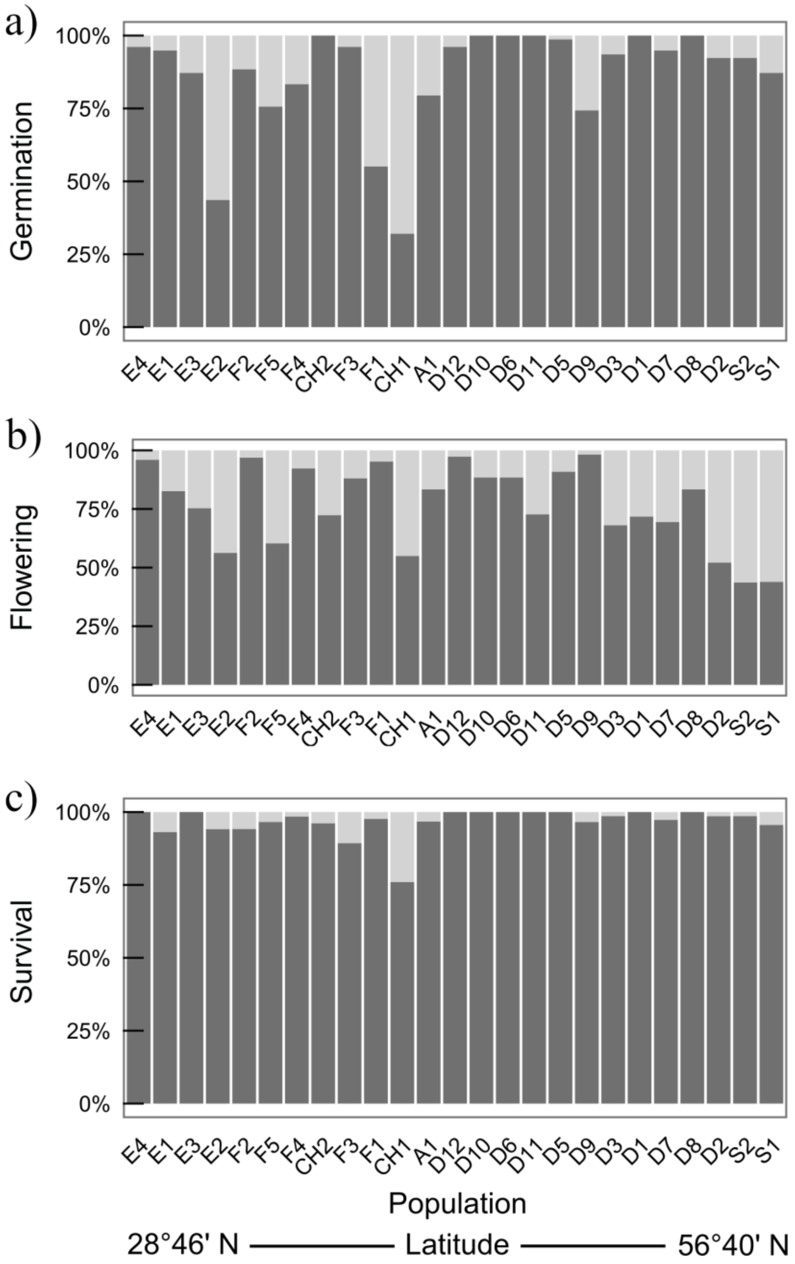

Figure A2. Proportion of individuals per population. a) germination (dark grey: germinated; light grey: did not germinate), b) flowering (dark grey: flowered; light grey: did not flower) and c) survival (dark grey: survived; light grey: did not survive). Populations are arranged according to their latitudinal origin from the southernmost population E4 (left; 28° 46’ 58’’ N) to the northernmost population S1 (right; 56° 40’ 12’’ N).

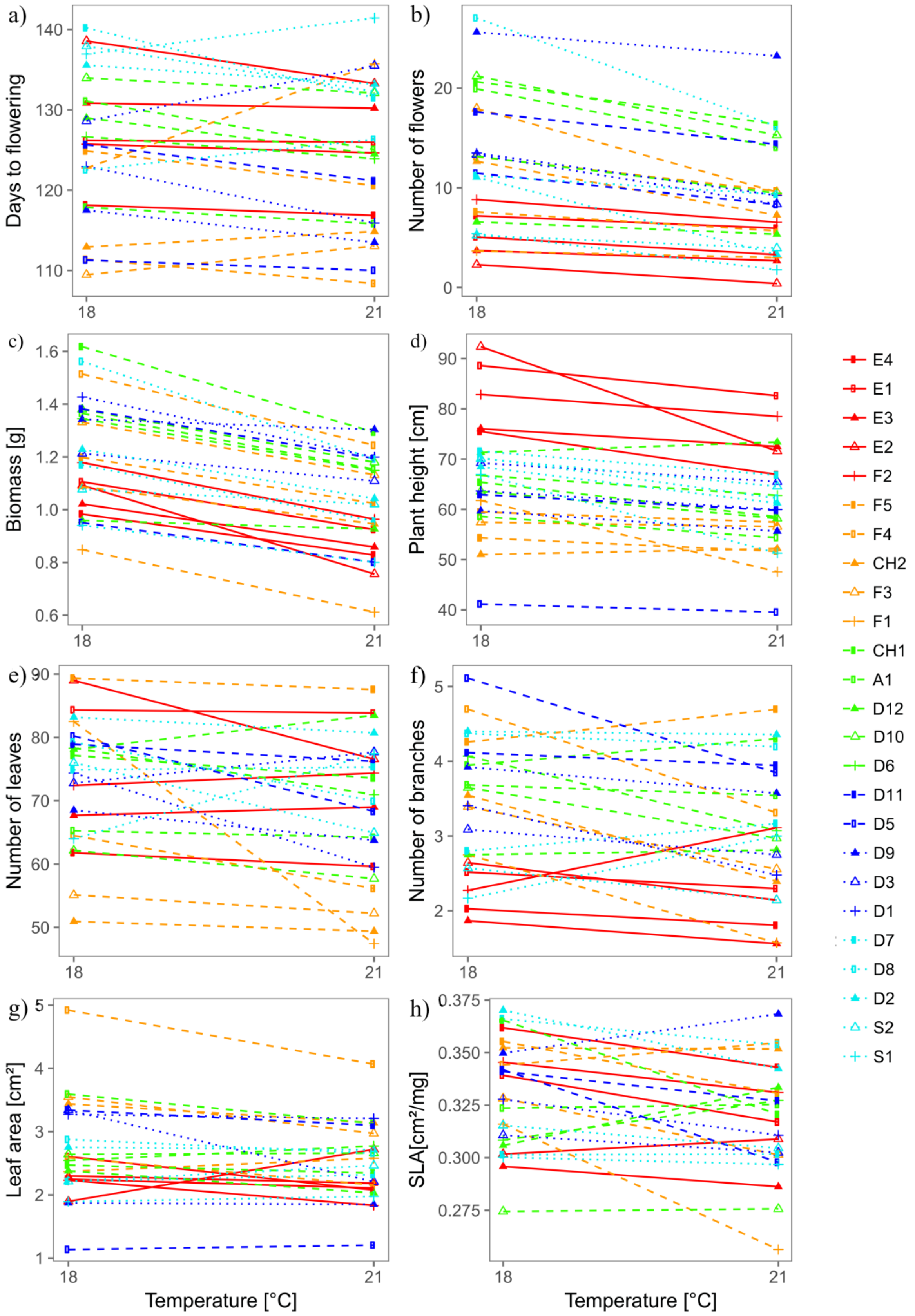

Figure A3. Reaction norms for temperature treatments of individual *Silene vulgaris* populations. Populations are ordered from South to North (top to bottom). SLA: Specific leaf area.

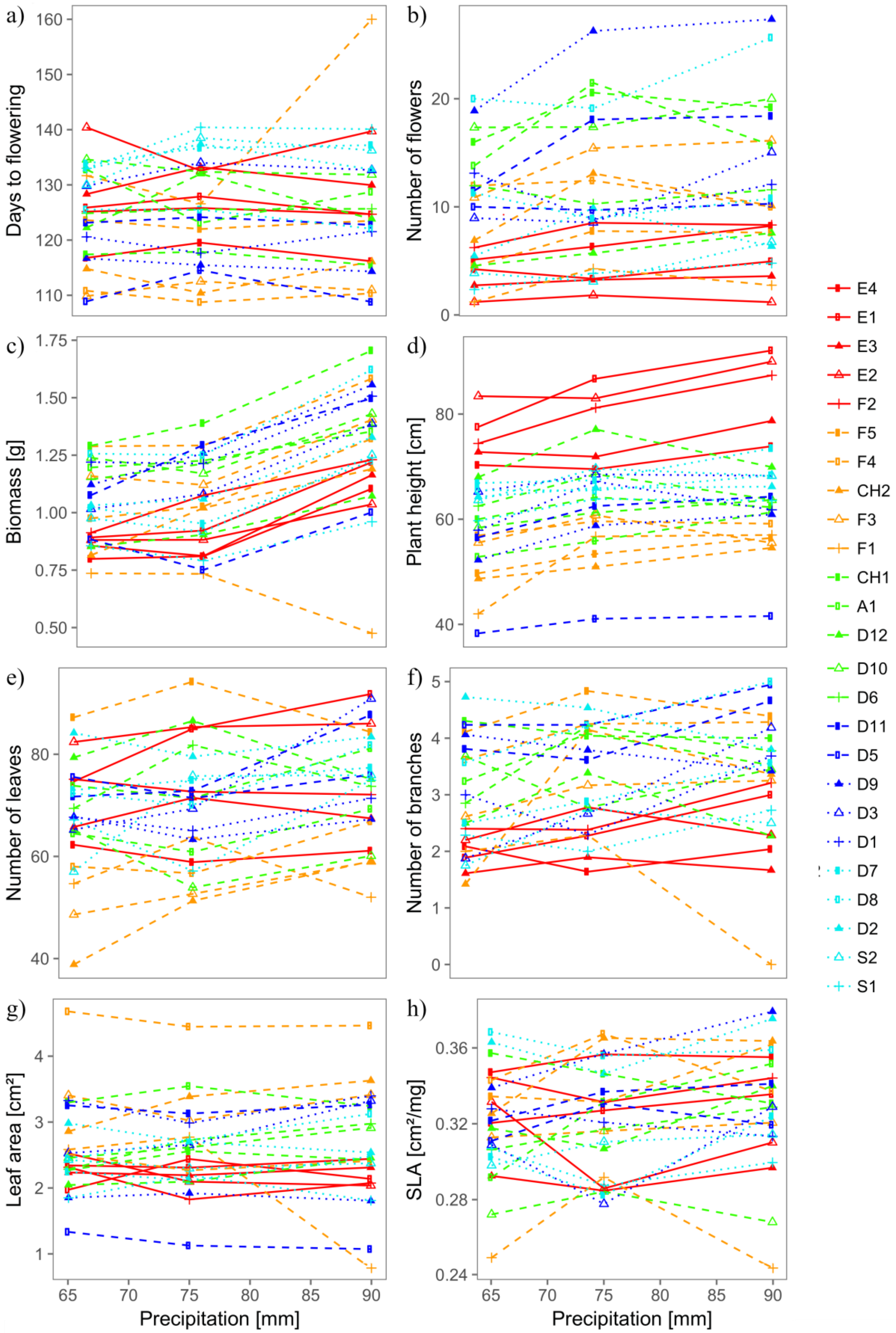

Figure A4. Reaction norms for precipitation treatments of individual *Silene vulgaris* populations. Populations are ordered from South to North (top to bottom). SLA: Specific leaf area.

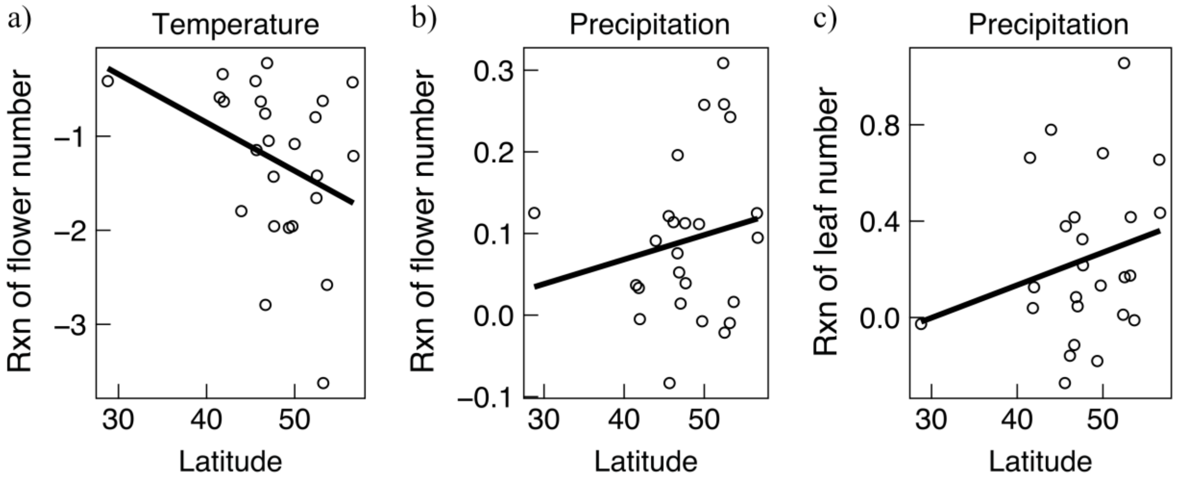

Figure A5. Reaction norm (Rxn) slopes for number of flowers regarding temperature a) and precipitation treatment b) and for number of leaves regarding precipitation treatment c). Reaction norm slopes of number of flowers and number of leaves became steeper along the latitudinal gradient indicating a stronger sensitivity towards the treatment in northern populations of *S. vulgaris*.

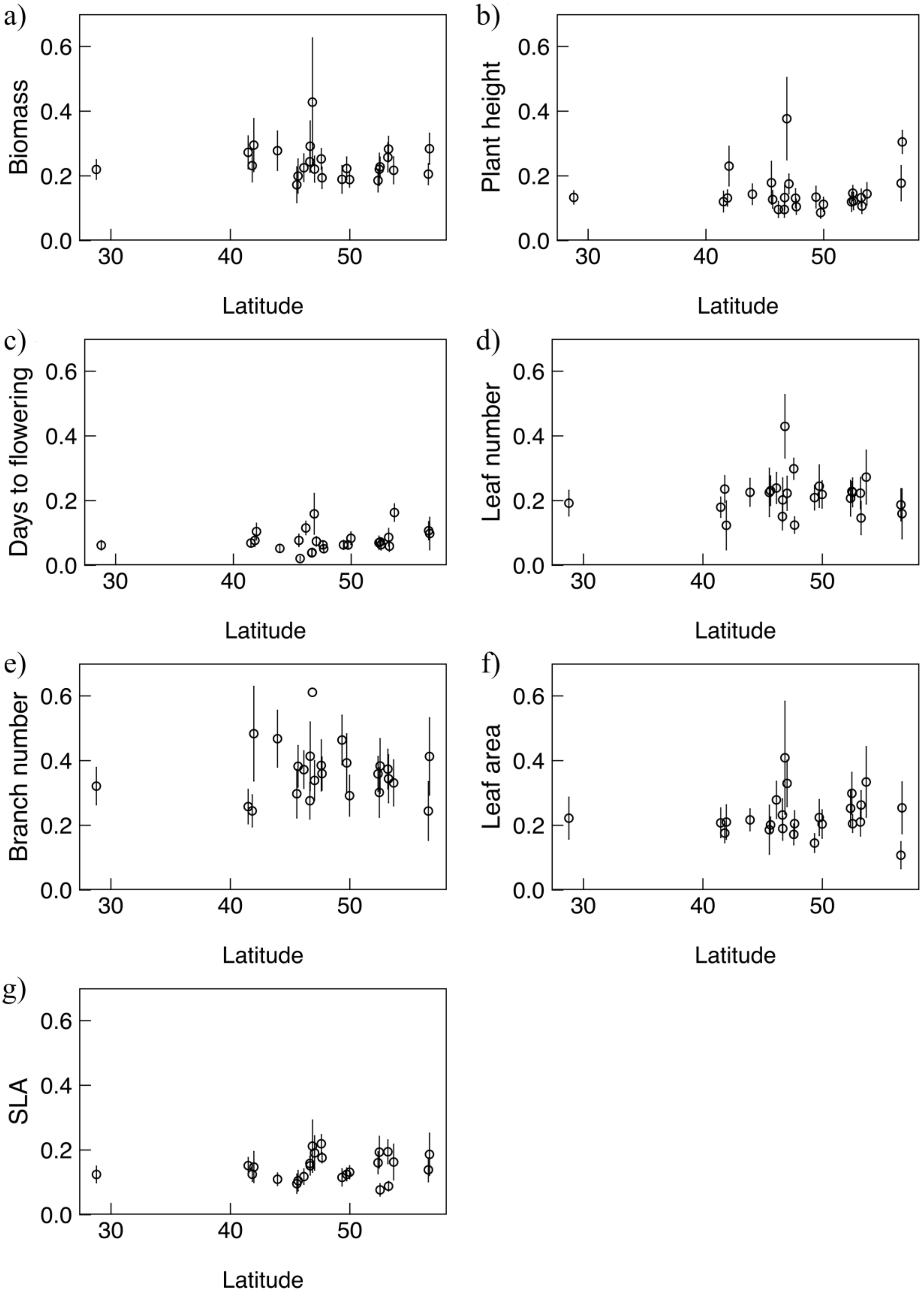

Figure A6. Plasticity index (PI; means ± standard errors) of plant traits for the temperature treatment. PI plotted for each *S. vulgaris* population at their latitudinal origin.

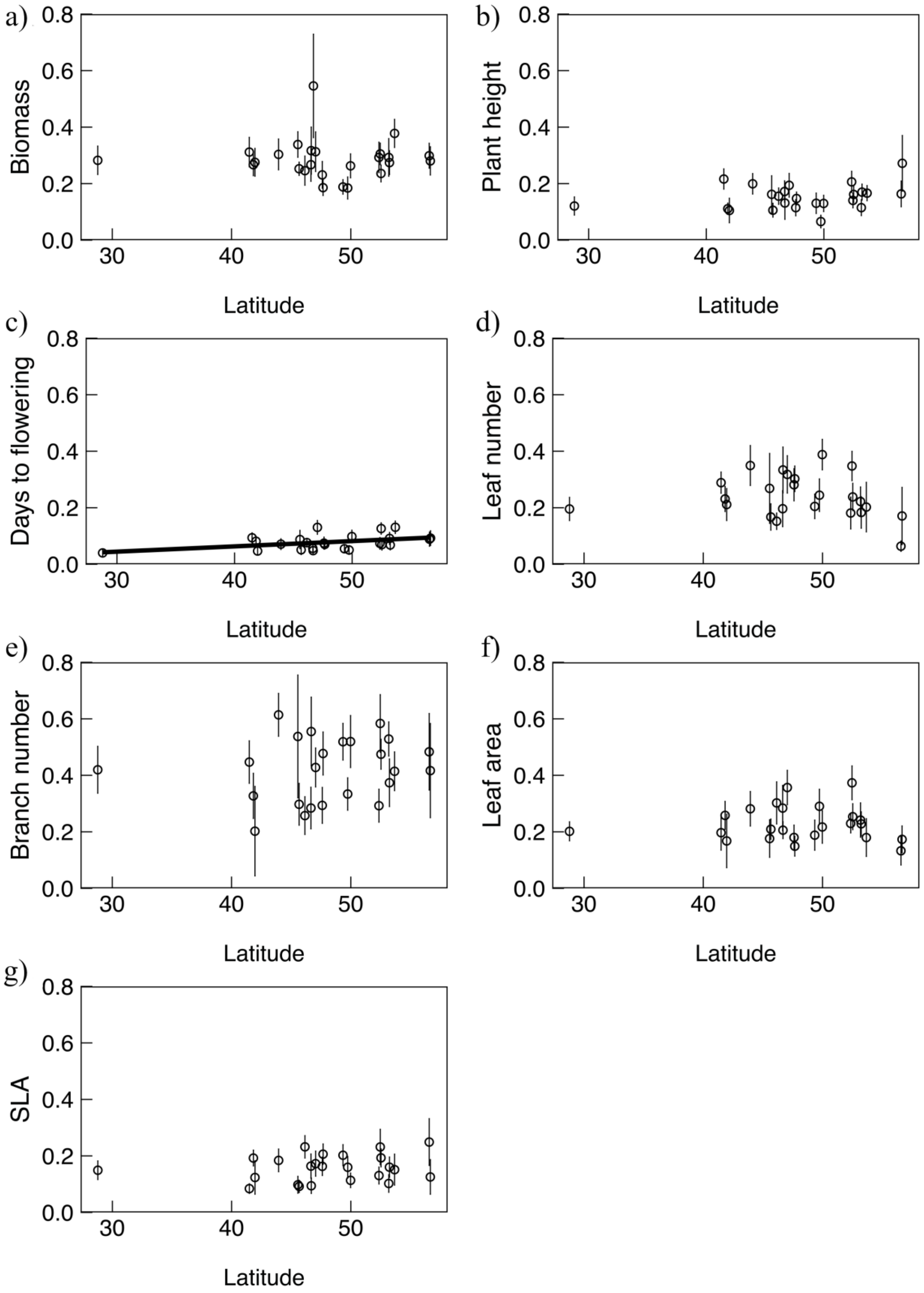

Figure A7. Plasticity index (PI; means ± standard errors) of plant traits for the precipitation treatment. PI plotted for each *S. vulgaris* population at their latitudinal origin.

Supporting information for Chapter 3

Table A4. The 48 EcoRI barcode adapters with unique 5 base pair sequences and the MspI and Illumina PCR oligo sequences used in this study.

Name	Sequence
EcoRI	
EcoRI_P1.1.1	GCATG
EcoRI_P1.1.2	AACCA
EcoRI_P1.1.3	CGATC
EcoRI_P1.1.4	TCGAT
EcoRI_P1.1.5	TGCAT
EcoRI_P1.1.6	CAACC
EcoRI_P1.1.7	GGTTG
EcoRI_P1.1.8	AAGGA
EcoRI_P1.1.9	AGCTA
EcoRI_P1.1.10	ACACA
EcoRI_P1.1.11	AATTA
EcoRI_P1.1.12	ACGGT
EcoRI_P1.1.13	ACTGG
EcoRI_P1.1.14	ACTTC
EcoRI_P1.1.15	ATACG
EcoRI_P1.1.16	ATGAG
EcoRI_P1.1.17	ATTAC
EcoRI_P1.1.18	CATAT
EcoRI_P1.1.19	CGAAT
EcoRI_P1.1.20	CGGCT
EcoRI_P1.1.21	CGGTA
EcoRI_P1.1.22	CGTAC
EcoRI_P1.1.23	CGTCG
EcoRI_P1.1.24	CTGAT
EcoRI_P1.1.25	CTGCG
EcoRI_P1.1.26	CTGTC
EcoRI_P1.1.27	CTTGG
EcoRI_P1.1.28	GACAC
EcoRI_P1.1.29	GAGAT
EcoRI_P1.1.30	GAGTC
EcoRI_P1.1.31	GCCGT
EcoRI_P1.1.32	GCTGA
EcoRI_P1.1.33	GGATA
EcoRI_P1.1.34	GGCCA
EcoRI_P1.1.35	GGCTC
EcoRI_P1.1.36	GTAGT
EcoRI_P1.1.37	GTCCG
EcoRI_P1.1.38	GTCGA

Table A5. Continued. The 48 EcoRI barcode adapters with unique 5 base pair sequences and the MspI and Illumina PCR oligo sequences used in this study.

Name	Sequence
EcoRI_P1.1.39	TACCG
EcoRI_P1.1.40	TACGT
EcoRI_P1.1.41	TAGTA
EcoRI_P1.1.42	TATAC
EcoRI_P1.1.43	TCACG
EcoRI_P1.1.44	TCAGT
EcoRI_P1.1.45	TCCGG
EcoRI_P1.1.46	TCTGC
EcoRI_P1.1.47	TGGAA
EcoRI_P1.1.48	TTACC
MspI	
MspI_P2.1	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
MspI_P2.2	/5Phos/CGAGATCGGAAGAGCGAGAACAA
Illumina	
PCR1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
PCR2_Idx_1_ATCACG	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_2_CGATGT	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_3_TTAGGC	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_4_TGACCA	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_5_ACAGTG	CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_6_GCCAAT	CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_7_CAGATC	CAAGCAGAAGACGGCATACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGC
PCR2_Idx_8_ACTTGA	CAAGCAGAAGACGGCATACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGC

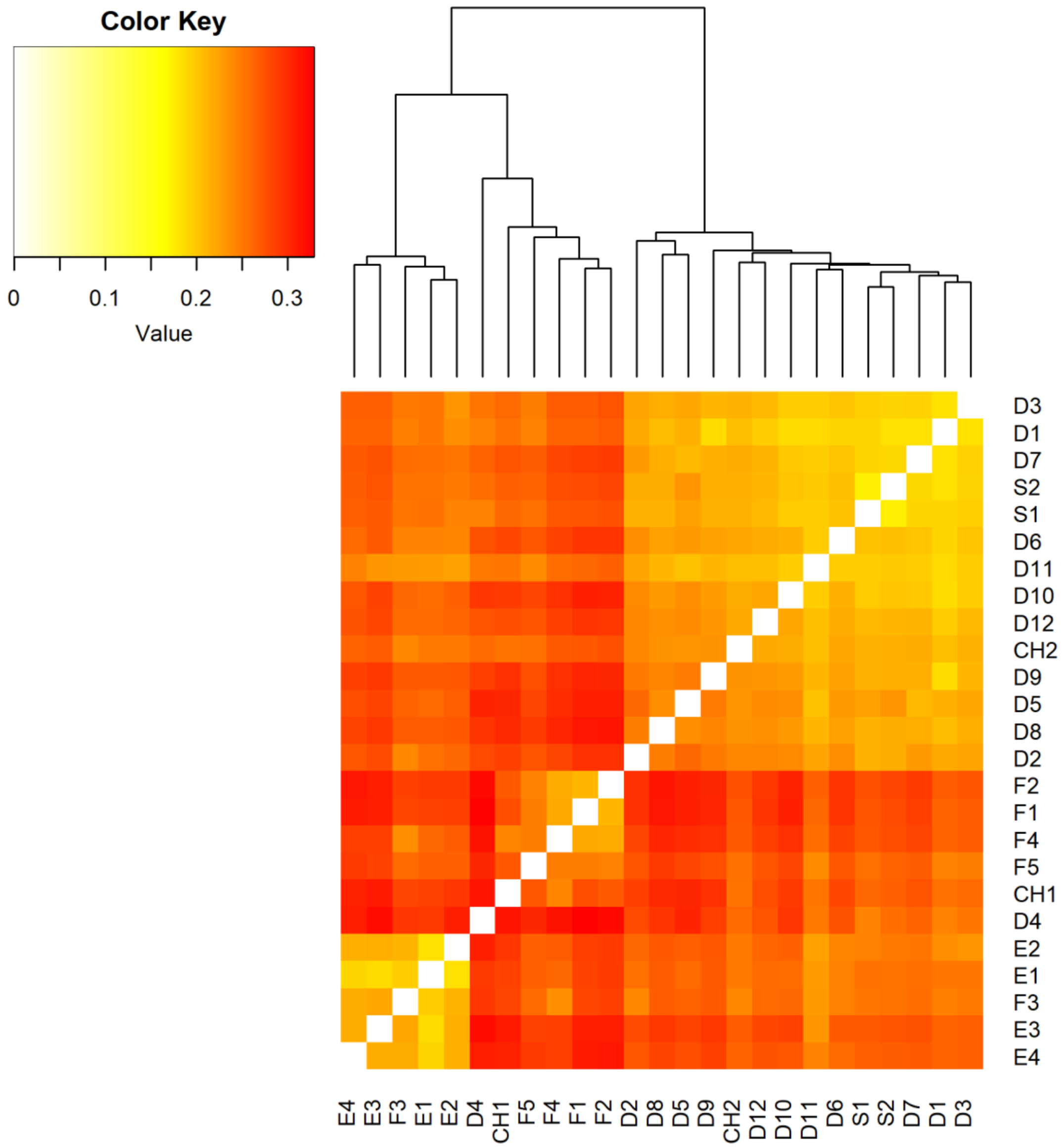

Figure A8. F_{ST} population dendrogram and heat map based on F_{ST} values among the 25 *Silene vulgaris* populations. The heat map colour illustrates the F_{ST} values.

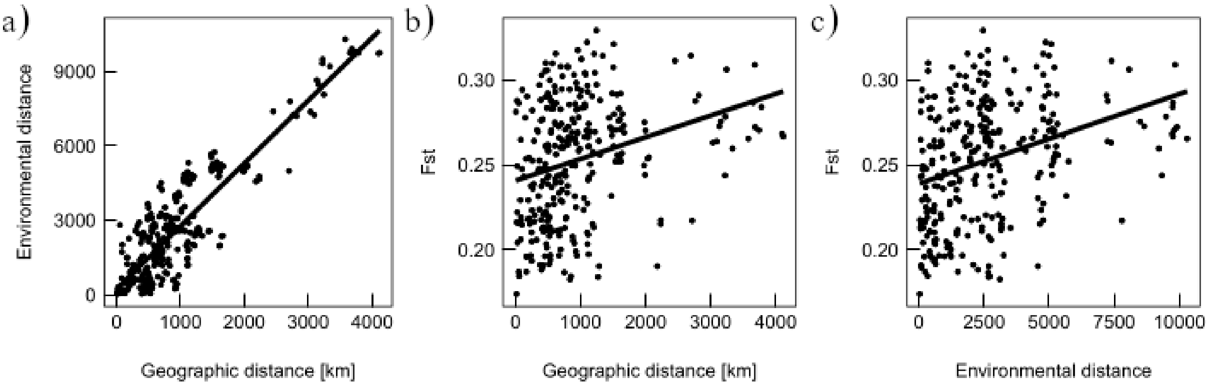

Figure A9. Relationship between a) environmental (Euclidean) and geographic distances (r=0.89, p <0.001), b) genetic (F_{ST}) and geographic distances (r=0.29, p <0.05) and c) genetic (F_{ST}) and environmental (Euclidean) distances (r=0.34, p <0.01) of the 25 *Silene vulgaris* populations.

Vollständige Publikationsliste

Kahl, S. M., Kappel, C., Joshi, J., & Lenhard, M. (2021). Phylogeography of a widely distributed plant species reveals cryptic genetic lineages with parallel phenotypic responses to warming and drought conditions. *Ecology and Evolution*, 11(20), 2045–7758. https://doi.org/10.1002/ece3.8103

Romero-Mujalli, D., Rochow, M., **Kahl, S.**, Paraskevopoulou, S., Folkertsma, R., Jeltsch, F., & Tiedemann, R. (2021). Adaptive and nonadaptive plasticity in changing environments: Implications for sexual species with different life history strategies. *Ecology and Evolution*, 11(11), 6341–6357. https://doi.org/10.1002/ece3.7485

Neiber, M. T., **Kahl, S. M.**, Wiggering, B., & Glaubrecht, M. (2019). Adding the West-African riverine component: Revision of the Recent freshwater snails belonging to *Pseudocleopatra* Thiele, 1928 (Caenogastropoda, Cerithioidea, Paludomidae). *Zootaxa*, 4674(3), 301–328. https://doi.org/10.11646/zootaxa.4674.3.1

Kahl, S. M., Lenhard, M., & Joshi, J. (2019). Compensatory mechanisms to climate change in the widely distributed species *Silene vulgaris*. *Journal of Ecology*, 107, 1918–1930. https://doi.org/10.1111/1365-2745.13133

Zwickel, T., **Kahl, S. M.**, Rychlik, M., & Müller, M. E. H. (2018). Chemotaxonomy of Mycotoxigenic Small-Spored *Alternaria* Fungi – Do Multitoxin Mixtures Act as an Indicator for Species Differentiation? *Frontiers in Microbiology*, 9, 1368. https://doi.org/10.3389/fmicb.2018.01368

Zwickel, T., **Kahl, S.**, Klaffke, H., Rychlik, M., & Müller, M. (2016). Spotlight on the Underdogs—An Analysis of Underrepresented *Alternaria* Mycotoxins Formed Depending on Varying Substrate, Time and Temperature Conditions. *Toxins*, 8(11), 344. https://doi.org/10.3390/toxins8110344

Kahl, S.M., Ulrich, A., Kirichenko, A. A., & Müller, M. E. H. (2015). Phenotypic and phylogenetic segregation of *Alternaria infectoria* from small-spored *Alternaria* species isolated from wheat in Germany and Russia. *Journal of Applied Microbiology*, 119(6), 1637–1650. https://doi.org/10.1111/jam.12951

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Eidesstattliche Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation „Evolutionary adaptive responses to rapid climate change in plants – A case study of the widely distributed species *Silene vulgaris*“ von mir eigenständig und ohne unerlaubte Hilfe verfasst wurde und ich keine anderen als die zitierten Quellen und Hilfsmittel verwendet habe. Weiterhin erkläre ich, dass diese Arbeit noch keiner anderen Hochschule im In- oder Ausland zur Prüfung vorgelegt wurde.

Berlin, den 10.11.2021

Sandra Kahl