Eco-evolutionary responses of plants to spatial and temporal climatic variation

Dissertation

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Eco-evolutionary responses of plants to spatial and temporal climatic variation

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“... for the times they are a-changin'.”

Bob Dylan, The Times They Are a-Changin' (1964)

Cover page picture: The island of La Gomera as viewed from the Teno mountains on Tenerife in autumn 2010.

Content

Background and motivation

Climate-related resources (water and energy) and their distribution and availability in space and time are crucial determinants of biogeographic and evolutionary histories of organisms. Organismal association and adaptation (see Box 1) to climatic parameters and their variation emerge over various spatial scales and organisational levels, from single genes and individuals, over populations, species and ecosystems to global biodiversity patterns, as well as from ecological (*e.g.* within single seasons) to long-term macro-evolutionary time scales (geological ages).

Current organisms and their traits, as well as their spatial distributions and assemblages are the result of environmental pressures and opportunities in dynamic biogeographical settings from the past to present. Within the limits of specific phylogenetic constraints, populations evolve by numerous eco-evolutionary steps that potentially sum up to the formation of new species and phylogenetic lineages. While the entirety of processes like speciation is hardly discernible within a researcher's lifetime, one can very well observe parts of the eco-evolutionary continuum. The study of species' and populations' ecological, evolutionary and biogeographical responses to changing environments is one of the most exciting topics in biology as it deals with an essential sign of life itself. Such snapshots of eco-evolutionary shifts thus provide invaluable insight into general processes in evolution and ecology.

It is also crucial for the needed forecasts of global climate change outcomes. The current climatic change is increasingly affecting ecological and evolutionary developments of species and ecosystems (Parmesan 2006; Lavergne *et al.* 2010). Consequences for biodiversity patterns and for human wellbeing are already observable and will intensify in the future considering climate projections for the coming decades (Pereira *et al.* 2010; IPCC 2014a; Selwood *et al.* 2015). Currently, this has generated an unprecedented societal interest in modes, capacities and magnitudes of organismal responses to environmental change.

In general, knowledge on adaptive variation to climatic parameters among populations and species on different spatial and temporal scales can bring valuable insights into their response capacities. Furthermore, drastic climatic changes are not exceptional but rather a reoccurring phenomenon in earth history. Taking references to past climate change outcomes can therefore be helpful in order to understand current processes when keeping in mind that the current anthropogenic climate change may not entirely be comparable to past climate shifts. The very rapid current pace of change demands for very fast responses, and many species may not be able to react with sufficient rapidity and efficacy (*e.g.* Jump & Peñuelas 2005; Urban *et al.* 2012; **Manuscript 1**).

Possible responses to climatic alterations include adaptive phenotypic reactions and evolutionary change, or dispersal (see Box 1) and range shifts following clines of suitable habitat. Global climate change is thus not only a challenge, but can be seen as an open-ended natural experiment, making it feasible to study different types of biotic reactions to environmental alterations. Nevertheless, the understanding of actual response capacities and underlying mechanisms, as well as of interactions with other factors of global change (*e.g.* land-use change, eutrophication, species invasions, biotic interactions) is still on an early stage.

This thesis aims to improve knowledge of relevant ecological and evolutionary processes related to climatic change and variation. Several types of organismal response to climatic changes are examined, and links between biological and geographical sciences are made. The thesis therefore takes special reference to the current (anthropogenic) climate change but additionally includes perspectives and studies from past climatic shifts and their biogeographic consequences. The work is focused on plants, as their individual immobility implements a special need for direct *in-situ* responses to environmental variation, making them very interesting and valuable objects for research on eco-evolutionary processes in the course of climatic change.

Structure of this thesis

My thesis starts with shortly introducing eco-evolutionary response types to environmental variation that are subject to this work. After that, I give an overview of contemporary climate change patterns and their relevance for plant life to depict the underlying pressures that lead to responses in plants and to show how such pressures may develop in the future. Subsequently, the different response types of plants to climatic variation are discussed by summarising recent concepts and findings in the respective research fields. The response types are discussed in separate chapters; however, links and possible feedbacks between them are shown, respectively, to provide an integrative picture. In this chapter, the findings from the included six manuscripts are integrated and brought in context to the current scientific progress. I finish the introduction with an outlook, depicting how the made research adds to the scientific knowledge and how future research might close left uncertainties and gaps. The manuscripts are presented afterwards after a short overview of all of them and a statement of my own contributions. As the thesis addresses readers from different research fields, a box of explanations and definitions of specific terms which are not explained in the text, is provided separately (Box 1).

Box 1: Definitions and explanations of terms as used in this dissertation.

Adaptation: Any internal process that increases or maintains fitness of organisms in their environment by bringing phenotypes closer to their (shifted) ecological fitness optima. These processes include evolutionary (genetic) changes due to selective pressures, but also plastic changes such as (reversible) modulation and acclimatisation or (irreversible) modification of overall phenotypes and single traits. This process-based and combined definition puts emphasis on the integrative understanding of ecological stress response and evolution that is basic for this thesis.

Alleles: Alternate variants of the same gene (or same genetic locus). This variation basically accounts for phenotypic heterogeneity within and among populations.

Allele frequency: The proportion of a particular allele among all considered alleles of a given locus within a gene pool, *e.g.* a population.

Dispersal: Successful (net gene flow) movements of propagules (diaspores and pollen) or individuals to a new area (leading to range shifts and expansions of species or populations) or between populations (leading to an exchange of genetic material, also termed migration).

Effective population size: The number of individuals in a real population that exhibits the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding (heterozygosity loss over time) as an idealised (randomly mating and with an equal sex ratio) population would show. This can be interpreted as the 'genetic size' of a given population, and is highly influenced by the number of individuals actually contributing to reproduction and by sex ratios within the population.

Epigenetic variation: Variation in gene expression that is not based on the DNA (nucleotide) sequences of genes but on various mechanisms that modify the configuration and local readability of DNA like methylation of nucleotides, chromatin re-modelling (*e.g.* regulation of DNA region compaction and gene accessibility by its wrapping around histone proteins), or attachments of repressor proteins to the DNA strand (*e.g.* to silencer or promotor regions of genes). These modifications can regulate genes, leading to functional trait variation. They can be passed on in cell lines and may or may not be heritable over generations. In a wider usage of the term, epigenetic modification also includes other factors and processes that influence the development of an organism without DNA sequence change, *e.g.* RNA transcript processing and translational controls by various mechanisms.

Genetic drift: The change in allele frequencies in a population over time due to the effect of random sampling of individuals. These result from stochasticity in reproductive mating, but also during dispersal processes (founder effects), or from non-selective random environmental events and resulting bottleneck effects (*e.g.* population declines due to a volcanic outbreak). Genetic drift may lead to complete loss or fixation (dominance) of alleles and depends strongly on effective population sizes.

Genotype: The genetic constitution of an individual organism, resulting from the set of genes and/or alleles in its genome.

Box 1 (continued).

Indirect effects of climate change: Secondary (derivative) effects resulting from changes of climatic conditions to organisms or other systems under consideration. For example, facilitation of parasites or pests by climatic alterations might cause even higher pressures on plant populations than direct physiological effects from climatic habitat change. Co-extinction by interruptions of mutualistic species interactions *e.g.* due to differential phenology shifts are also an illustrative example of indirect effects.

Oceanic islands: Islands that originated on oceanic crust, mainly by volcanism, but occasionally also by tectonic uplift. In warm regions, coral accretion in island peripheries and on seamounts additionally contributes to the build-up and maintenance of oceanic islands. Oceanic islands are thus characterised by their permanent isolation from other landmasses that have shaped peculiar biogeographic, ecological and evolutionary settings.

Phenotype: The composite of an organism's morphological, physiological, biochemical, developmental and phenological traits and characteristics. These result from the genotype of the organism (*i.e.* from expression of available genes), from environmental factors and from interactions between the two.

Phylogeography: The study of principles and processes for evolutionary relationships of genetic lineages within or among closely related species to their geographical locations and biogeographical histories. In this thesis the term is used only according to intraspecific lineage differentiation processes and patterns.

Population: A group of organisms of the same species that form a reproductive and thus evolutionary entity. That is, such organisms occupy a more or less well defined and continuous geographic area and have the capability of interbreeding. Ecological and reproductive interactions are thus more frequent among individuals of this group than with members of other populations. Populations are the biological units on which evolutionary processes take place.

Synergistic interactions of climate change effects: Interactions of direct or indirect climate change effects with effects of other (disconnected) factors and processes of relevance causing overall effects that are greater than the simple additive effects of the single factors. Besides numerous other examples, such interactions might occur if climatic stress on a plant population is amplified by anthropogenic habitat destruction. Resultant population size decrease facilitates the risks of environmental (*e.g.* climatic) and demographic stochasticity and lowers the capability to adaptively respond, which causes amplifying feedbacks and, potentially, extinction vortices.

Overview of eco-evolutionary responses to environmental variation

A population (see Box 1) can only persist when environmental conditions lie within the range of ecological tolerance of its individuals, so that population growth is equal to or greater than zero. This interplay of environmental factors (dimensions) and the ability to make use of or tolerate these factors is defined by the ecological niche of a given organism (Hutchinson 1957; Holt 2009). Ecological niches of species have been assumed to remain relatively unchanged over time, as described in the hypothesis of niche conservatism (Wiens & Graham 2005; Pearman *et al.* 2008). This conception, however, has been more and more challenged by numerous examples of past and contemporary rapid niche shifts of species or single populations (see Lavergne *et al.* 2010 and references therein). In the research field of climate change biology, such niche lability has particular relevance. If environmental conditions change to, or even beyond, the limits of an organism's actual niche dimensions (either by colonisation of a new habitat, or by environmental change), several responses are possible to re-establish or maintain niche realisation (*i.e.* accordance between ecological tolerance and environmental conditions).

A very extensive response is genetic adaptation to the novel conditions, *i.e.* the change of phenotypes in a population due to, or accompanied by, allele frequency shifts of relevant genes. However, genetic adaptation requires selective pressures staying within the ecological tolerance of at least some individuals for several generations as well as population sizes large enough to cover the demographic costs of selection (Jump & Peñuelas 2005; Kinnison & Hairston 2007). If environmental change proceeds faster than this or generally exceeds ecological tolerance and capacities of a population, genetic adaptation processes would not suffice to permit ongoing niche realisation and persistence (Jump & Peñuelas 2005; Hoffmann & Sgrò 2011).

A faster and more direct mechanism of adaptation to environmental fluctuation and to modulate ecological tolerance is (adaptive) phenotypic plasticity (Chevin *et al.* 2010; Nicotra *et al.* 2010), *i.e.* the capacity of a given organism (genotype) to express different phenotypes under different environmental conditions (Sultan 2000). Induced by environmental triggers, a variety of cellular and molecular mechanisms control for plasticity in phenotypic traits or in developmental pathways in organisms, with epigenetic modifications (see Box 1) probably being among the most important regulators (Nicotra *et al.* 2010). By changing the substrate for selection (the phenotypes), phenotypic plasticity may induce or precede genetic adaptations (Lande 2009; Chevin *et al.* 2010; Schlichting & Wund 2014). Also, phenotypic plasticity is itself under genetic control (Scheiner 1993; Nicotra *et al.* 2010; Des Marais *et al.* 2013); and plastic phenotype modification as well as epigenetic variation can be transmitted to following generations (Rossiter 1996; Youngson & Whitelaw 2008; Herman & Sultan 2011; **Manuscript 2**). Therefore, phenotypic plasticity is not only a short-term factor for adaptive responses to environmental change, but plays a key role in evolutionary processes (West-Eberhard 2005; Pfennig *et al.* 2010).

If changes in environmental conditions are beyond ecological and physiological tolerances and cannot be encountered by adaptive responses, populations will decline, eventually leading to local extirpation or even global extinction of the species (Selwood *et al.* 2015). Some taxa and gene pools manage to escape deteriorating conditions by dispersal to new habitats or by retreating into (micro-)refugia (Parmesan 2006; Corlett & Westcott 2013). However, environmental change can also foster the expansion of a species' distribution if suitable habitats increase in number or area, including the decline of formerly exclusive biotic interactions. Likewise, adaptations to new (beneficial) resources or spontaneous niche expansions to include formerly unexploited resources can take place, altering a species' ecology without detrimental environmental change (Holt 2003; Sexton *et al.* 2009).

In general, present species and their distributions integrate past and contemporary ecological and evolutionary responses to environmental variation (including anthropogenic factors) in space and time (*e.g.* Webb III & Bartlein 1992; Holt 2003). A main driver of such response processes is climate and climate change, influencing resource availability and eco-evolutionary processes on various scales, by several mechanisms and in numerous interactions with other factors (*e.g.* local site conditions and species pools).

Contemporary climatic changes and their impacts on plants

Temperature increase

Global climate warming is now unequivocal and is clearly associated to atmospheric concentrations of greenhouse gases that increasingly have been emitted since the industrial revolution in the 19th century (Diffenbaugh & Field 2013; IPCC 2013). Especially from 1950 to 2012, global temperatures increased by 0.12 °C per decade, with higher rates (0.15 – 0.16 °C per decade) after 1979, indicating an acceleration of the trend (IPCC 2013). Climate warming has been more pronounced on the Northern hemisphere and over land areas (IPCC 2013).

Increasing mean annual temperatures can have several implications for plants. Warmer growth conditions increase evapotranspiration and may exceed temperature optima for diverse physiological processes, including photosynthesis and respiration (Sage *et al.* 2008; Gunderson *et al.* 2010; Way & Yamori 2014). This can lead to modified habitat suitability and altered competitiveness for many plant species (Walther 2003; Gunderson *et al.* 2010). Also, climatic changes can enable range shifts of herbivores, microbial diseases, and parasites to formerly unaffected regions, thus creating new selective pressures on plants. This can result in extensive pest outbreaks with deep ecological changes, as can be seen in the current forest dieback in Western North-America due to a continuing outbreak and spread of the mountain pine beetle (*e.g.* Kurz *et al.* 2008; Bentz *et al.* 2010).

Additionally, longer vegetation periods (earlier onset and/or later cessation) cause phenological changes. This alters ontogenetic development, productivity and fitness patterns (Walther 2003; Yang & Rudolf 2010; **Manuscript 3**), but may also result in modifications or disintegration of community interactions due to mismatches of differently affected temporal ecological processes in or among species (Walther 2003; Memmott *et al.* 2007; Walther 2010; Urban *et al.* 2012).

For mid to high latitude ecosystems, higher winter temperatures can decrease shelter from long-lasting snow cover and may change soil freeze-thaw patterns (Kreyling 2010; Kreyling *et al.* 2010). Increased frost injuries of roots and shoots, altered nutrient cycling, plant productivity and phenology, or increased insect pest risks are possible effects (Kreyling 2010).

For the 21st century, temperature increases are projected to proceed (Karl *et al.* 2015), with varying strength and velocity depending on the presupposed anthropogenic greenhouse gas emission scenario. Recent climate change projections (*e.g.* IPCC 2013) include four scenarios

(Representative Concentration Pathways - RCPs; Vuuren *et al.* 2011): One stringent mitigation scenario (RCP2.6), two intermediate scenarios (RCP4.5 and RCP6.0) and one very high emission scenario (RCP8.5). For the period 2016—2035, global mean temperature increase will reach 0.3 to 0.7 °C relative to the period 1986—2005, without strong differences among the four RCPs (IPCC 2013). In later periods of the century, climate change projections substantially diverge for the different RCPs: In the period 2046—2065, projected mean global changes range from 1.0 (RCP2.6) to 2.0 °C (RCP8.5), and for the period 2081—2100 from 1.0 (RCP2.6) to 3.7 °C (RCP8.5). A strong regional variation is indicated with the highest increase in Arctic regions (up to > 11 °C) and large areas of the northern hemispheric continental regions (5 to 7 °C), whereas the Southern Hemisphere and most ocean regions receive lower temperature increases (0.5 to 7 °C, for 2081—2100 under RCP8.5, respectively; IPCC 2013).

Changes in precipitation patterns

Precipitation changes are less significant and far more heterogeneous in their direction, magnitude and regional pattern (Trenberth 2011; IPCC 2013, 2014b). There is a slight overall trend of precipitation increase over land for the mid-latitudes of the Northern hemisphere since 1901, while for other latitudinal zones no significant long-term overall trend could be quantified (IPCC 2013). However, some regional patterns emerge: Western Africa and the Sahel zone, the Mediterranean region, eastern Asia and eastern Australia have undergone precipitation decreases between 1951 and 2010 with up to −100 mm/a per decade, while other regions have received increasing precipitation up to 100 mm/a per decade, *e.g.* northern and eastern parts of Europe, some regions of South America, central parts of North America and north-western Australia (IPCC 2013).

Projections of mean precipitation change show strong regional differences as well. In general, an intensification of the global hydrological cycle is indicated. This results in trends of increasing mean annual precipitation in tropical and mid- to high latitudes (up to 60% in 2081—2100 compared to 1986—2005 for RCP8.5) while in subtropical regions precipitation tends to decrease (up to −40%; IPCC 2013).

Observations and projections also indicate changes in seasonal patterns of precipitation for many regions of the world (IPCC 2012, 2013; Li *et al.* 2013; Lee & Wang 2014; Zeppel *et al.* 2014). Altered seasonality can result from temporal redistributions of constant annual amounts but also from disproportional change, *e.g.* in one season's precipitation amount compared to other seasons in a region. Both would alter soil water contents, which may pose strong ecological change for plant species and communities, depending on local soil properties, microclimate, plant functional types and the nature of the change in timing (Zeppel *et al.* 2014 and references therein).

Changes in the amount and/or temporal distribution of precipitation (rain, snow, fog, hail) can lead to short-term but also to enduring situations of water stress for plants. Water deficiency drives non-adapted plants to decreased productivity and growth and can generally diminish their reproductive fitness and competitiveness (Goldstein & Suding 2014; Zeppel *et al.* 2014), as well as their resistance to antagonist species, *i.e.* herbivores (Fox *et al.* 1999; Franzke & Reinhold 2011), parasites and pathogens (Pautasso *et al.* 2010). Different responsiveness and induction of species-specific asynchronous phenological responses may disrupt interspecific interactions with effects on the community level (Walther 2010; Yang & Rudolf 2010). Similarly, for non-aquatic plant species, an excess of water (*e.g.* flooding, waterlogging) can cause oxidative stress or can generate conditions that facilitate antagonistic microbial activity (*e.g.* root rot or pathogen attacks) that decrease plant fitness (*e.g.* Jung *et al.* 2009; Muneepeerakul *et al.* 2011). In general, water regime shifts alter competitive processes and patterns in ecosystems, for example extraordinary water availability might favor mesic invaders over dry-adapted specialist species (*e.g.* Trueman & d'Ozouville 2010; Farrer *et al.* 2014). In the long run, the structure, chemistry and biota of soils may change (Young *et al.* 1998; Nielsen & Ball 2015).

Extreme events

Ecosystem transitions due to long-term climatic shifts are very likely to be promoted or even be dominated by increasing frequencies and magnitudes of extreme weather and climate events, *i.e.* droughts, heat waves, severe late frosts, hurricanes, or heavy rainfalls and flooding (IPCC 2012, 2014b). Such pulse events are projected to intensify in many regions of the world (Trenberth 2011; IPCC 2012; Fischer & Knutti 2015), and there is increasing evidence for extensive and long-lasting effects on plant species and ecosystems (Jentsch & Beierkuhnlein 2008; Smith 2011; Reyer *et al.* 2013; Zeppel *et al.* 2014).

While the affecting factors (*e.g.* increased temperature, drought) are generally the same as in gradual climatic changes, extreme events are temporally restricted but much more abrupt. This strongly limits the time for species to acclimatise or respond, and sometimes leads to exceedance of equilibria and survival thresholds of plants and ecosystems (Smith 2011; Reyer *et al.* 2013). Also, physical damages (*e.g.* from increased occurrence of storms, flooding or wildfire) might decrease fitness or even wipe out local populations if occurring too frequent, being too strong in magnitude or being accompanied by other detrimental disturbance factors (Thompson *et al.* 2007; Littell *et al.* 2009; Littell *et al.* 2010). If plant populations are not able to recover from, tolerate or adapt to such extreme event impacts, local species turnover and ecosystem changes will be a consequence (*e.g.* Jump & Peñuelas 2005; Thompson *et al.* 2007; Kreyling *et al.* 2008; Johnstone *et al.* 2010).

Interactions of climate change impacts with abiotic and biotic factors

Global change drivers often act simultaneously and may have very effective amplifying feedbacks and synergistic interactions (Brook *et al.* 2008; Bellard *et al.* 2014). This makes climate change outcomes very hard to predict for species and ecosystems and calls for more integrated research.

A known phenomenon of global change relevant for plant life is the increased atmospheric concentration of CO_2 (Leakey *et al.* 2009; Loehle 2011). Compared to 278 ppm in 1750, the atmospheric abundance of CO_2 in 2011 has increased by 40% to 390.5 ppm (IPCC 2013), and might reach levels of 490 ppm (RCP2.6) to 1370 ppm (RCP8.5) in 2100 (Vuuren *et al.* 2011). CO_2 enrichment has been shown to increase growth and water-use-efficiency in plants (Leakey *et al.* 2009) and to mitigate drought stress (*e.g.* de Graaff *et al.* 2006; Wertin *et al.* 2010). This can significantly reduce climate change effects on plants coming from temperature increase and altered precipitation patterns (Loehle 2011). However, increases in atmospheric CO_2 are also known to change plant species compositions in ecosystems (*e.g.* Owensby *et al.* 1999; Polley *et al.* 2012) and to facilitate invasions of neophyte species (*e.g.* Bradley *et al.* 2010 and references therein). Nevertheless, the exact effects of elevated CO_2 on plants and its interactions with other drivers of global change are not fully understood yet (Leuzinger *et al.* 2011).

Habitat destructions by land use change, overexploitation, pollution, soil erosion and the effects of invasive species set up environmental alterations that interact with and can amplify climate change effects on natural systems (Millenium Ecosystem Assessment 2005; Brook *et al.* 2008; Mantyka-Pringle *et al.* 2012). Declining habitats and census sizes decrease the evolutionary adaptability of populations (Jump & Peñuelas 2005) and increase risks of demographic and environmental stochasticity (Gilpin & Soulé 1986; Parmesan 2006; Brook *et al.* 2008) as well as the genetic risks of diminishing population sizes (Ellstrand & Elam 1993; Schaal & Leverich 2005; Aguilar *et al.* 2008). Fragmentation of habitats and possible dispersal routes due to anthropogenic land conversion aggravates these problems by hampering gene flow (Honnay & Jacquemyn 2007; Aguilar *et al.* 2008) and necessary range shifts to track climatic niches (Honnay *et al.* 2002).

Habitat loss can also come from sea level rise due to thermal extension of oceanic water volumes and melting of glaciers and polar ice caps. Between 1901 and 2010, a global mean sea level rise of 0.19 m has been observed (IPCC 2013). Until the end of the 21st century, sea level rises up to 0.82 m compared to the period 1980—1999 are projected (IPCC 2013), and other estimates and new data suggest significantly higher values (reviewed and discussed in **Manuscript 1**). Temporal or permanent inundations of coastal plains and marshland and of entire islands like atolls and low reef islands are very likely in the coming decades (IPCC 2014b). Habitat loss and deterioration might not only result from inundation, but also from groundwater salinisation (Ross *et al.* 1994; Ross *et al.* 2009) or indirectly from forced translocation of affected human settlements, agriculture and infrastructure to formerly unimpaired terrain (Wetzel *et al.* 2012; **Manuscript 1**). This can make sea level rise a very potent interaction partner of climatic alterations not only in low-lying coastal ecosystems.

Besides changes in the spatial setting and availability of habitats, alterations in species pools contribute to new pressures on biodiversity and to evolutionary mechanisms as well. Increasingly, invasive species are affecting native plant species by acting as competitors, herbivores, predators, parasites, diseases, disease vectors, hybridising congeners, or indirectly due to habitat transformations (see *e.g.* Mack *et al.* 2000; Millenium Ecosystem Assessment 2005; Chown *et al.* 2015). Invasions can be favoured by climatic alterations and in turn can amplify climate change impacts on native species and ecosystems (Walther *et al.* 2009; Mainka & Howard 2010).

Biotic interactions in general are important factors for the response and response capacity of plants to climate change (*e.g.* Lavergne *et al.* 2010; Walther 2010). Stable species communities and mutualism may buffer disturbance impacts like deteriorating climatic conditions, *e.g.* by facilitation and ecological redundancy effects (Chapin, III *et al.* 1997; Yachi & Loreau 1999; Beierkuhnlein & Jentsch 2005; Hooper *et al.* 2005). However, changing and disrupting ecological interactions between species and asynchronous responses to climatic alterations are also among the major threat factors for many species (Parmesan 2006; Memmott *et al.* 2007). Trophic and competitive balances in ecosystems can be changed by climatic alterations, potentially leading to increased vulnerability of some species, but also to facilitation of others that cope better with the new conditions (*e.g.* Lavergne *et al.* 2010).

Climate change-driven phenological mismatch between plants and their pollinators, diaspore dispersers and other ecological services may cause serious threats to species (Memmott *et al.* 2007; Yang & Rudolf 2010). Especially highly specialised taxa might suffer, *e.g.* from declining seed set and dispersal, or from missing herbivore predation (Memmott *et al.* 2007; Lavergne *et al.* 2010; Aslan *et al.* 2013).

Different responses of interaction partners also reduce the capacity of species to track their climatic niche by dispersal and range shifts, *i.e.* when interaction partners differ in their dispersal capacities or rates (Lavergne *et al.* 2010; Hsu *et al.* 2012; Gellesch *et al.* 2013). Mutualistic interactions may then be interrupted, with drastic consequences for the respective species if the ecological service cannot be substituted by another species. Further on, biotic interactions can efficiently restrict range shift attempts by competition and exclusion in the receiving communities along dispersal routes (Caplat *et al.* 2008; Lavergne *et al.* 2010; Norberg *et al.* 2012; Urban *et al.* 2012; Corlett & Westcott 2013).

Plants responding to climatic variation and change

Climatic changes alter environmental conditions for plant populations and may cause various ecological pressures. Many changes will lie within the environmental tolerance of individuals. However, persistence of individuals, populations and species will also require direct biotic responses, *i.e.* by phenotypic plasticity of individuals, by evolutionary adaptation within and among populations, or by dispersal to more suitable habitats. In this chapter, I will summarise concepts and recent findings concerning the different response types of plants to climatic variation.

General aspects of environmental tolerance

If a population is forced to adapt (by changing its genotypic and/or phenotypic composition) to climatic changes or to abandon a deteriorating habitat (*i.e.* local extirpation or relocation to more suitable habitat) depends on the exposure to local change and on the specific sensitivity, *i.e.* if the new conditions lie within the boundaries of the ecological niche and are tolerated by the affected organisms or not (Williams *et al.* 2008). Ecological optima and the ranges of tolerance to climatic habitat changes of plants are shaped by their genetically determined physiological, anatomical and morphological traits and life history (*e.g.* Williams *et al.* 2008; Munson 2013).

Depending on the environmental and phylogenetic context, some plant characteristics have been shown to correlate with high tolerance to climatic change. For example, Soudzilovskaia *et al.* (2013) found that traits promoting conservative leaf water economy (higher leaf mass per area, thicker leaves) and large investments in belowground reserves (root carbon content) were good predictors of species' abundance increase during 28 years of temperature increase among Caucasian alpine plants. Likewise, summer dormancy, water status maintenance in basal tissues during drought, and root biomass were shown to be correlated with drought survival (Volaire 2008). Especially for woody plants resistance to cavitation (air emboli in the xylem under water stress) is also an important physiological trait shaping drought tolerance (Maherali *et al.* 2004; Anderegg 2015). In contrast, differences between plants of C_3 and C_4 photosynthetic pathways, concerning their responsiveness and tolerance to the interacting effects of increased CO_2, increased temperature and water stress, have mixed and ambiguous findings in the literature (see *e.g.* Wand *et al.* 1999; White *et al.* 2000; Drake 2014).

On the population level, biotic interactions and local abiotic conditions (Liancourt *et al.* 2013; Munson 2013) as well as prior local genetic adaptation (*i.e.* ecotypic variation, see *e.g.* Liancourt *et al.* 2013) shape and modify the ranges of ecological tolerance. Further on, earlier expositions to stress within a plant's lifetime (and hence conditioning and hardening, see *e.g.* Walter *et al.* 2013; Backhaus *et al.* 2014) are very important for tolerance to climatic change. In general, it must be noted that intraspecific variation in tolerances to climatic changes can be as high as tolerance differences among species (Anderegg 2015).

Phenotypic plasticity

Phenotypic plasticity is defined as environmentally induced shifts in phenotypes and is among the primary means by which plants react to climate change impacts (*e.g.* Nicotra *et al.* 2010). It is thus an active mechanism of ecological tolerance to environmental variation and can dilate the ecological niche width of populations and species beyond the limits of genetically inherited trait values (van Valen 1965; Richards *et al.* 2006). Adaptive plasticity can efficiently maintain (or increase) fitness parameters under new conditions and shields genotypes from selective pressures or might even generate new substrate for selection to act on (Ghalambor *et al.* 2007). It comprises changes in the morphology, phenology and physiology, as well as in life histories and reproductive patterns in plants (*e.g.* Parmesan 2006; Matesanz *et al.* 2010; Nicotra *et al.* 2010).

Phenotypic plasticity is controlled by differential expression of alternative genes, gene activation and inhibition, or by up- or downregulation of transcripts and other relevant cellular components. This is realised by physiological, genetic and/or epigenetic control mechanisms on single genes associated to the ecologically relevant trait(s), but more often on multiple independent or interacting genes. The following cellular processes are known to control gene expression changes subsequent to the reception of the external stimulus (an environmental signal) and initiation of a signalling cascade (see Nicotra *et al.* 2010): i. Post-translational modification of the components of signalling pathways, *e.g.* by protein phosphorylation; ii. Regulatory gene transcription by changes in transcription factor provision or activity; iii. Changes in chromatin, *e.g.* chromatin remodelling, histone modification or DNA methylation; iv. Activation and jumping of transposable elements; v. Demethylation of transposable elements and upregulation of adjacent genes; vi. Expansion of short repeat sequences, affecting gene expression; vii. Changes in the population of small RNAs with effects on post-transcriptional control of gene transcripts (RNA interference) as well as on chromatin modifications. However, although these mechanisms were shown to be involved in plastic responses to environmental variability, many open questions are still not solved (*e.g.* on the contributions of the different mechanisms and on how they evolve). Also, only few non-model species were part of the underlying studies (Chinnusamy & Zhu 2009; Nicotra *et al.* 2010).

For plant population persistence during climatic changes, adaptive phenotypic plasticity may provide the possibility to rapidly respond to earlier springs and prolonged vegetation periods, increased temperature and changed precipitation regimes, extreme weather events (heat spells, heavy rain events, droughts) and other direct or indirect impacts of global change. Further on, it may help plant populations at the leading front of range shifts to colonise and establish in new regions when habitats within their former range limits deteriorate or when formerly unsuitable regions become suitable by climatic changes (for overviews and examples of plastic responses of plants to climate change see Parmesan 2006; Matesanz *et al.* 2010; Nicotra *et al.* 2010).

The fact that the responsiveness and magnitude of phenotypic plasticity can vary among populations within a species is subject of a dynamic research field (*e.g.* Ghalambor *et al.* 2007; Vitasse *et al.* 2013; **Manuscript 3**). Such variation has strong implications for specific response capacities of populations under different environmental alterations, for the development of species ranges, and for evolutionary impacts of plasticity (see below). One assumption for the origin of such variation are different evolutionary histories of populations, *i.e.* different selection patterns that integrate past advantages of plasticity relative to the costs (see *e.g.* Scheiner 1993; Ghalambor *et al.* 2007). However, the fact that a large fraction of observed plastic responses is either non-adaptive (neutral in terms of fitness parameters or even maladaptive) and/or non-heritable limits the generality of past selection differences as a reason for varying plasticity among populations (Ghalambor *et al.* 2007).

Nicotra *et al.* (2010) also suggest an influence of standing (*i.e.* pre-existing) genetic variation within a population for its capability of reacting to environmental variability by phenotypic plasticity. Variation in genes encoding for compartments of the cellular machinery that enables plants to sense changes in the environment and to process these stimuli to a cellular and organismal response could help plant populations to rapidly adapt to changing conditions (Nicotra *et al.* 2010). This makes sense as the maintenance of environmental sensitivity and regulatory machineries is posing the highest ecological and evolutionary costs of phenotypic plasticity in organisms (Scheiner 1993; DeWitt *et al.* 1998).

In fact, in **Manuscript 3**, we found a relationship between mean plastic responses of *Fagus sylvatica* populations under different experimental climatic conditions and genetic diversity values, which, in turn, reflected phylogeographic patterns in this species. Accordingly, Pleistocene species range contractions to glacial refugia (including the mixing of genotypes from different biogeographical origins and evolutionary histories) and subsequent re-colonisations of northern regions in the Holocene (accompanied by genetic drift) shaped genetic compositions and diversity parameters of populations. Indeed, experimental groups from past refugial regions in Southeast Europe with high allelic diversity showed a higher mean plasticity of height increments under different treatments than proveniences of re-colonised Central European regions (**Manuscript 3**).

By adapting phenotypes of individuals to shifting ecological optima, phenotypic plasticity enables populations to evade or attenuate losses of genetic variation due to strong selection in altered environmental conditions and extreme events (Ghalambor *et al.* 2007). It therefore helps to maintain genetic variation, which, in turn, is known to be necessary for evolutionary adaptation processes (*e.g.* Jump *et al.* 2009) and thus has a strong conservative aspect. However, if adaptive plasticity produces phenotypes that completely match the new ecological optimum (favoured by a new and enduring selection regime), the population is likely to persist but adaptive evolution is probably slowed down. Selection would then act not directional but stabilising on the phenotype and its actual induction (Ghalambor *et al.* 2007).

In contrast, phenotypic plasticity is also increasingly understood as a driver of evolution. If the adjusted phenotypes are close enough to what is favoured by the new selection regimes to persist but not completely matching this ecological optimum, selection will act directional and is likely to result in evolutionary progress (Ghalambor *et al.* 2007). Although plasticity is environmentally triggered, it relies on underlying genetic variation in the inducibility and expression of phenotypic variants (West-Eberhard 2005; Ghalambor *et al.* 2007). If natural selection continuously favours certain plastically generated phenotypes within a population, it can improve their form, regulation and phenotypic integration until the new phenotype finally becomes genetically encoded and constitutively expressed, a process known as genetic accommodation (West-Eberhard 2005). An extreme form of this, genetic assimilation, leads to reduction and even final loss of plasticity if selection favours reduced responsiveness of the new (accommodated) phenotype (*i.e.* canalisation; Pigliucci & Murren 2003; Schlichting & Wund 2014).

Both mechanisms are described for natural populations and are increasingly supposed to be major factors for evolutionary change within and differentiation among populations (see reviews in Pfennig *et al.* 2010; Schlichting & Wund 2014). Environmentally initiated novelties may thus have greater evolutionary potential than those derived from (much slower) mutational processes, and genetic change seems to be more often a follower than an initiator of phenotypic change (West-Eberhard 2005; Pfennig *et al.* 2010). The role of phenotypic plasticity for plant responses to climate change impacts is thus not limited to short-term persistence effects but must be strongly considered for long-term evolutionary adaptation as well.

Transgenerational plasticity

Environmental variation can also lead to direct phenotypic responses in the (affected or unaffected) offspring generation(s) of plants, *i.e.* without the process of prior genetic accommodation, and without the necessity for cross-generational or re-occurring environmental stimuli. Such transgenerational environmental effects have been known for a long time; however, only in the last years has their adaptive potential gained interest (Herman & Sultan 2011; Salinas & Munch 2012, **Manuscript 2**).

The most obvious mechanisms behind transgenerational plasticity are modifications in nutrient provisioning to seeds by affected mother plants, which changes the initial resource availability for seedlings (Bloedner *et al.* 2007; Donohue 2009; Germain & Gilbert 2014). More recent findings identified changes in the quantity and composition of maternally derived mRNAs and/or microRNAs, primary and secondary metabolites or plant hormones provided to the seeds, as well as inheritable epigenetic marking as mechanisms behind phenotypic variation in offspring plants (Chinnusamy & Zhu 2009; Herman & Sultan 2011).

Many observed transgenerational effects are non-adaptive or even mal-adaptive (Rossiter 1996; Youngson & Whitelaw 2008), *e.g.* smaller seeds of stressed mother plants leading to decreased germination success or seedling growth. However, there is increasing evidence for specific phenotypic adjustments in offspring that are functionally adaptive to the parental conditions that induced them (see Herman & Sultan 2011 for a literature overview).

Yet, most investigations on adaptive transgenerational plasticity in plants have been concentrating on herbaceous species and not many studies exist that investigate such effects in connection with climatic variation (Herman & Sultan 2011). However, perennial species play an important role for the structure and dynamics in ecosystems and might be even more reliable on non-genetic adaptation to climatic alterations than short-lived species, as long generation times could hamper rapid evolutionary adaptation and fast range shifts.

In **Manuscript 2**, long-term transgenerational effects of precipitation extreme events (drought, heavy rain) on two woody heathland species were shown. Drought stress on mother plants of *Genista tinctoria* advanced germination timing in their offspring and heavy rain decreased germination success. Further on, a reduced leaf carbon / nitrogen ratio (C/N) and increased relative growth rates were detected for offspring plants of heavy rain-treated mothers. The second species, *Calluna vulgaris*, showed no significant transgenerational plasticity in response to drought or heavy rain treatments. However, transgenerational effects of different experimental plant communities of the mother plants were obvious for this species. Mothers that grew in communities consisting of two species (*i.e.* with higher intraspecific competition) produced more rapidly germinating seeds and lower seedling leaf C/N compared to mothers that grew in communities consisting of four species. These results may indicate an adaptive response, that is, stress-exposure of mother plants leads to earlier germination as an avoidance strategy of possibly recurrent stress. Lower C/N in offspring of heavy rain-treated mothers could indicate an adaptive increase of metabolic *vs.* structural tissue to take use of “expected” high resource availability. The same can be assumed for lower competition for resources as a result of lower intraspecific neighbourhood (see **Manuscript 2**).

Transgenerational environmental effects can directly influence traits, phenological patterns and demographic compositions in offspring generations (Rossiter 1996; Donohue 2009). By increasing the generation of new phenotypes and their exposition to selection, transgenerational plasticity might thus amplify the evolutionary and ecological potential of phenotypic plasticity during global climate change. **Manuscript 2** provides insight in species differences and an integrated perspective on transgenerational plasticity patterns by considering community effects.

Evolutionary adaptation

Evolutionary change is the shift of allele frequencies in functionally relevant genes driven by the forces of mutation, recombination, genetic drift and natural selection. Only selection has a purely non-random and directing effect and thus can cause adaptive evolutionary change. According to this, climate is a very important selective force for plants, which is reflected in the pervasiveness of climatic adaptations throughout the plant kingdom (see *e.g.* Donoghue & Sanderson 2015). Evolutionary differentiation among plant populations and species, functional trait evolution, the size and shape of species ranges, as well as patterns of biodiversity on various spatial scales are often influenced or driven by climate-related evolutionary processes and adaptive patterns.

Processes of biodiversity pattern generation due to adaptive mechanisms in plants are particularly well observable on high elevation oceanic islands. Peculiar environments in climatically heterogeneous landscapes, geographical isolation and non-saturated niche space enhance adaptive processes, which has led to high proportions of endemic species in island floras (Whittaker & Fernández-Palacios 2007; Kier *et al.* 2009). In **Manuscript 4**, biodiversity and endemism richness on La Palma, Canary Islands, was shown to be highly influenced by climatic patterns. This island provides remarkable climatic heterogeneity, mainly due to two factors. First, strong precipitation differences exist between the humid north-eastern sectors that receive high amounts of rain and cloud drip due to their trade wind exposure, and the dry south-western sectors where a discernible rain shadow effect is present. Second, the geology of La Palma comprises steep elevational gradients. Besides temperature change with elevation, the highest zones of La Palma are also beyond the trade wind inversion line, meaning not only a cooler, but also a significantly drier and sunnier environment for their species than in lower zones. Reflecting these patterns, especially the percentage of endemics in local species pools could be well predicted by climatic factors like elevation, rainfall seasonality, solar radiation and mean annual temperature. Particularly in places where climatic conditions are harsh or uncommon (*i.e.* peculiar combinations of climatic conditions), the presence of specialised endemics was increased. As most endemics on oceanic islands have evolved *in situ*, such clear associations to climatic variables can indicate focal species' origins and suggest climatic variation as a determining driver of evolution on the island (see **Manuscript 4**).

The prevalence of evolutionary adaptations on oceanic islands is also apparent in the commonness of adaptive species radiations. These are defined as diversifications of an ancestor organism into new forms within relatively short time by the means of evolutionary adaptation and concomitant speciation processes, particularly when a change in the environment makes new resources available or creates new challenges (Schluter 2000; Nosil 2012). The special biogeographical settings and ecological peculiarity of oceanic island environments have enhanced numerous of such radiations (Whittaker & Fernández-Palacios 2007).

An iconic example for adaptive plant species radiations is the genus *Aeonium* WEBB & BERTHEL. (Crassulaceae). It has evolved on the Canaries archipelago (Mes *et al.* 1996; Mort *et al.* 2002), where it comprises about 28 species plus a series of distinct subspecies (Liu 1989; Arechavaleta Hernández *et al.* 2010). Strong differences in their ecological niches, their morphology and ecophysiology (Liu 1989; Lösch 1990; Mort *et al.* 2007) suggest that speciation events were driven or at least supported by adaptation to different (micro-) climatic environments (Lems 1960; Lösch 1990; Jorgensen & Frydenberg 1999; Jorgensen 2002; Thiv *et al.* 2010). However, intraspecific variation as a possible response to differential environmental conditions, and thus processes of adaptive evolution in species of this genus, has not been studied yet.

To test the hypothesised importance of climatic variation for island evolution and to check if environmental heterogeneity on the small scale of an oceanic island can drive evolutionary divergence in plants, we tested two island-endemic *Aeonium* species of La Palma for genetic population differentiation related to potential spatial and climatic determinants (**Manuscript 5**). Although differentiation was generally low within both species, clear topographic effects could be shown in the population genetic patterns, as well as signatures of adaptive variation related to temperature and precipitation variables in one of the tested species. Although it is not possible to make definite statements about the future evolutionary outcome of such variation (*e.g.* if it depicts incipient speciation), the study showed the potential of climatic heterogeneity to drive evolutionary processes, even on the small scales of La Palma (see **Manuscript 5**).

The mentioned patterns, nevertheless, are the results of evolutionary and biogeographical long-term processes over time scales from centuries to millions of years. The actual presence of these organisms tells us that they coped with past climatic pressures and changes by different means of ecological and evolutionary responses. However, in contrast to past environments, the current anthropogenic global change is setting up conditions and dynamics that raise questions about the adaptability of many species and local populations. Climatic conditions are globally changing with paces higher than those of many past climatic changes (Diffenbaugh & Field 2013; IPCC 2013). Additionally, climatic alterations are accompanied by other (new) drivers of environmental change, *e.g.* anthropogenic habitat destruction and fragmentation, pollution, overexploitation and introduced invasives (Millenium Ecosystem Assessment 2005; Brook *et al.* 2008; Mantyka-Pringle *et al.* 2012). These factors result in demanding novel environmental conditions and selective pressures on many organisms. Most likely, the new conditions initiate adaptive processes within and among populations "in attempt" to maintain or enhance fitness and survival. However, there is a vivid debate going on about the capacity of evolutionary adaptations to ensure local persistence of species and populations under current and future climatic changes (*e.g.* Jump & Peñuelas 2005; Parmesan 2006; Kinnison & Hairston 2007; Bell & Collins 2008; Chevin *et al.* 2010; Lavergne *et al.* 2010; Hoffmann & Sgrò 2011).

With respect to oceanic island floras, **Manuscript 1** provides the first global overview of expectable climatic alterations on oceanic islands for the 21st century and potential impacts on their floras. Here, the need for evolutionary adaptations of plants to changing environments is particularly high because island ecosystems often do not provide the necessary space for range shifts. On the other hand, many oceanic island species are characterised by small overall population sizes, limited genetic variation, long generation and reproduction times, and high vulnerability to novel disturbance types (*e.g.* invasive species) due to their evolution in oceanic isolation, factors that are likely to hamper rapid evolutionary adaptations (see **Manuscript 1** and references therein).

The uncertainty in evolutionary adaptation being able to rescue threatened plant species from recent climate change is, however, not limited to oceanic island floras. In general, evolutionary adaptability of a given population or species is influenced by a number of biotic and abiotic factors. These factors differ between species, populations, individuals and traits, which in turn suggests strong differences in specific capacities for evolutionary change and adaptation.

Basic to all evolutionary responses within a population is the underlying strength and continuity of the local selective pressure (*e.g.* a sufficient rate and intensity of climatic changes) in combination with some trait maladaptation(s) to the new environmental conditions (*i.e.* the difference between the initial mean trait value and the new optimum value of the trait). Given this, one of the most important biotic factors for evolutionary adaptation is the genetic variation in functionally relevant traits that is present in the affected population (Hoffmann & Sgrò 2011). It is the basic variation on which the new selection can act on (Jump *et al.* 2009; Hoffmann & Sgrò 2011). However, heritability (*i.e.* the contribution of genetic variation to trait variation in contrast to environmental/plastic effects and random chance) needs to be high to enable selection responses (Chevin *et al.* 2010; Franks & Hoffmann 2012). Further on, genetic interactions between traits and different directions of selection across multiple traits can strongly constrain their evolutionary adaptation to changing environments (Walsh & Blows 2009; Hoffmann & Sgrò 2011). That is, if genetically determined trait correlations are antagonistic to the direction of selection on these traits, such interdependencies can significantly slow down climate change adaptation (*e.g.* Etterson & Shaw 2001; Walsh & Blows 2009). Additionally, pleiotropy (the common case of one gene influencing multiple traits) can hinder evolutionary adaptation. Allele shifts in pleiotropic genes that are beneficial for one trait's function under a new environmental condition may simultaneously decrease fitness due to their effects on other functional traits, which might receive different selective pressures (Walsh & Blows 2009; Hoffmann & Sgrò 2011).

Selection does not only act on standing genetic variation, but also on newly introduced alleles. These might come from gene flow from other populations or from mutation. High mutation rates and the evolution of new (adaptive) genetic variants can therefore be a beneficial factor for rapid climate change adaptation (Bell & Collins 2008). Both, standing genetic variation and mutational variation are proportional to (effective) population size (see Box 1), making large populations more likely to undergo evolutionary adaptation (Lynch & Lande 1993; Hoffmann & Sgrò 2011). In turn, population size itself has tremendous effects on persistence under environmental change. Large population sizes can buffer impacts of environmental stochasticity and the demographic costs of adaptation (Jump & Peñuelas 2005; Kinnison & Hairston 2007). However, common species with large population sizes (especially obligate outcrossers) can be even more vulnerable to sudden population declines. Often, their mating systems and life histories are not adapted to small population sizes, decreased connectivity and/or low individual density (see *e.g.* Honnay & Jacquemyn 2007; Aguilar *et al.* 2008), which might decrease their capacity to overcome demographic depressions during phases of strong selection (Kinnison & Hairston 2007).

Gene flow from other populations into a population under strong selective pressure can maintain the necessary genetic variation or may even introduce new beneficial genetic variants (Kinnison & Hairston 2007). Habitat fragmentation and anthropogenic gene flow barriers are thus assumed to decrease persistence potentials and adaptability of natural populations (*e.g.* Jump & Peñuelas 2005). On the other hand, gene flow can introduce maladapted genotypes and alleles, which might result in fitness costs and prevent adaptive responses to local selection (Kirkpatrick & Barton 1997; Hoffmann & Sgrò 2011). Such trade-offs are particularly important in range periphery populations, where selection pressures often are stronger than in the range centre of a species and which thus often represent sinks for migrants (Kirkpatrick & Barton 1997). Further on, it is a widespread pattern that populations in range peripheries bear lower genetic variation (Eckert *et al.* 2008; **Manuscript** 6).

Another characteristic of plants relevant for their evolutionary adaptability is their generation time and life history. Short life cycles are advantageous for evolutionary responses to changing environments, making annuals and other short-lived plant species more likely to cope with the evolutionary challenges of climate change (Chevin *et al.* 2010; Hoffmann & Sgrò 2011).

Many traits are genetically variable and evolutionary change has been shown for many species and populations. It is therefore likely that many populations will undergo adaptive processes during global climate change and that some threatened populations will be rescued if the initial effects of strong selective pressures (like decreases of population sizes and of genetic variation) can be overcome (see *e.g.* Kinnison & Hairston 2007; Lavergne *et al.* 2010; Hoffmann & Sgrò 2011).

Dispersal and range shifts

Successful dispersal (see Box 1) depends on the accessibility of suitable target areas, specific dispersal capacity and migrant establishment. If co-occurring with local extirpation at former range areas where conditions deteriorate, dispersal would result in range shifts and might rescue some populations and species from total extinction if toleration or adaptation to the changing conditions fails. However, species can also expand their ranges in response to environmental change if increases in the ecological suitability of formerly unoccupied but accessible regions or changing biogeographic dispersal routes or barriers would be the result.

Dispersal is the most important response type of plants to climatic changes in the paleontological records for the Quaternary (*e.g.* Huntley 1991). Likewise, numerous phylogeographical studies suggest that many plant species showed high and recurrent capacities of shifting their ranges by dispersing or contracting into refugia and re-expanding when conditions ameliorated (*e.g.* Taberlet *et al.* 1998; Hewitt 2000; Petit *et al.* 2003; Hewitt 2004; see also **Manuscript 6**). However, this prevalence of dispersal responses is probably, at least in parts, due to the fact that past plastic and small scale-evolutionary responses are far more difficult to detect and/or to relate to specific climatic changes.

Range shift processes interact with the above-mentioned eco-evolutionary responses (Holt 2003; Lavergne *et al.* 2010), and the influences of such interactions in past climate change responses remain unclear. For example, it has been shown that if environmental conditions favour high dispersal capacities, traits relevant for dispersal, colonisation and establishment (*e.g.* propagule production, propagule traits, and germination timing control) can be rapidly modulated by plastic changes (*e.g.* Martínez-Berdeja *et al.* 2015; Ronce *et al.* 2005) or evolutionary shifts (*e.g.* Huang *et al.* 2015; Shine *et al.* 2011). It is likely that such plastic and evolutionary processes have influenced the efficiency and rates of dispersal for many plant species during past climate changes and that they modify current and future dispersal responses to climatic variation. Additionally, prior ecotype variation and population-specific tolerances to expected climatic change should not be neglected in assessments and models of future range shift capacities and range developments of species (Pearman *et al.* 2010; Benito Garzón *et al.* 2011; Oney *et al.* 2013; Valladares *et al.* 2014).

The process of dispersal itself is strongly influenced by several other factors. First, general dispersal capacities and potential dispersal rates strongly differ between species (Malanson & Cairns 1997; Urban *et al.* 2012). Second, biotic interactions like competition or loss of positive species interactions (Norberg *et al.* 2012; Urban *et al.* 2012) and edaphic factors (Bertrand *et al.* 2012; Beauregard *et al.* 2014) can cause strong constraints for range shift success of plants, even though climatic and other factors might be suitable in a potential new habitat or along dispersal routes. Third, (accessible) space for range shifts is not given everywhere and for all species. Especially species living solely in Arctic or high elevation ecosystems or in areas that are limited by strong dispersal barriers towards the main directions of expectable range shifts are likely to become threatened by impossible dispersal responses to climate change (*e.g.* Parmesan 2006, **Manuscript 1**). Fourth, availability and quality of potential dispersal routes and stepping stone habitats is a crucial factor. Anthropogenic land use, habitat destruction and landscape fragmentation is new compared to past climate-induced range shift situations, probably causing difficulties for many species to track their climatic niche (Mantyka-Pringle *et al.* 2012; Corlett & Westcott 2013). Particularly species being specialised to rare natural or semi-natural habitats, having only small total areas of occurrence, and/or showing limited dispersal abilities might not be able to overcome unsuitable landscape compartments and regions in between their former and potential new habitats. However, some authors generally question if the potential dispersal velocities of plant species will be sufficient at all to track the predicted broad-scale climatic shifts (see Corlett & Westcott 2013).

Beside Arctic and mountaintop species, a special vulnerability to the impacts and challenges of rapid climate change can be assumed for many species of oceanic islands. Here, a large fraction of global plant biodiversity can be found in the form of local endemics (Kreft *et al.* 2008; Kier *et al.* 2009). The small and restricted land surfaces of oceanic islands strongly limits range shift potentials for island biota during climatic changes, forcing negatively affected species to adapt to the new conditions or to retract to refugia in order to avoid extinction. Being highly heterogeneous in their physical and ecological nature (geology, topography, landscape structuring, microclimatic patterns, ecosystem configurations, anthropogenic impairments, *etc.*), oceanic islands can be expected to vary strongly in their provisioning of micro-climatic refugia, landscape permeability and general range shift potentials for their species. These differences and general patterns of climate change influences on oceanic islands are extensively discussed in **Manuscript 1**, a review paper which for the first time specifically targets the effects of climate change on oceanic island floras.

Dispersal can also be a driver of evolutionary differentiation. Colonisation of new areas, subsequent limitation or loss of gene flow between source and founder populations and differing selective pressures can initiate evolutionary divergence. Indeed, phylogeographical studies show that intraspecific isolation after range shifts or retraction to disjunct refugia have often resulted in genetic lineage differentiation (Hewitt 1999, 2000, see also **Manuscript 6**). Such past refugium disjunctions can have effects on present regional variation in (adaptive) phenotypic population characteristics, as shown *e.g.* for *Populus nigra* (DeWoody *et al.* 2015), *Geum montanum* (Scheepens *et al.* 2015), or *Campanula thyrsoides* (Scheepens *et al.* 2013). Further on, post-glacial re-colonisation histories (*i.e.* the successional spread of species from their refugia to de-glaciated areas) have also shaped intraspecific genetic patterns (Hewitt 1999; Petit *et al.* 2003). Genetic variation within species has mostly diminished along with re-colonisation advances by the processes of genetic drift, but also by possible selection pressures and primer effects (competitive exclusion of succeeding genotype establishment after colonisation of new terrain by founder individuals) in front edge populations.

Both, adaptive variation among phylogeographic lineages and differences in genetic diversity among populations due to different glacial and post-glacial histories are likely to affect population responses to current and future climatic changes. Persisting (and potentially cryptic) adaptations to different refugial conditions may include climatic adaptations or adaptiveness relevant for future conditions (see *e.g.* DeWoody *et al.* 2015). And although phylogeographic studies mostly rely on (presumably) neutral genetic markers (intergenic regions not subject to selection), differences in genetic diversity among populations of different Quaternary histories are likely to at least partially correlate with functional genetic variation (Merilä & Crnokrak 2001; Corre & Kremer 2003; Nosil *et al.* 2009; Orsini *et al.* 2013; see also **Manuscript 3**). During species range contractions in cooling periods in the Pleistocene diverse lineages probably have converged in southern refuge regions. Refugial populations persisted in relatively stable climatic conditions during glacial periods, which enabled them to maintain large portions of their genetic variation or even evolve new variants. The resulting variation within populations is beneficial for future climate change responses, not only by provisioning an 'option value' against environmental stressors and for evolutionary adaptation (*sensu* Jump *et al.* 2009), but also as facilitator of (adaptive) phenotypic plasticity, as indicated and discussed in **Manuscript 3** (see also Nicotra *et al.* 2010 and discussions in previous chapters).

Genetic diversity patterns due to phylogeographic histories often coincide with diversity patterns of range centre-periphery gradients (Guo 2012; **Manuscript 6**). In range peripheries, several possible factors can lead to decreased genetic diversity in and higher genetic differentiation among populations. These include lower environmental suitability and higher selection pressures, smaller effective population sizes, higher genetic drift due to increased environmental and demographic stochasticity, as well as greater geographical isolation and lower gene flow among peripheral populations compared to populations in more central regions of a given species' range (Holt *et al.* 2005; Eckert *et al.* 2008; Sexton *et al.* 2009; Gerst *et al.* 2011; Lira-Noriega & Manthey 2014). Additionally, lower genetic diversity in peripheral populations might also be due to late colonisation from more central regions, *i.e.* more or less recent founder effects (Hampe & Petit 2005).

However, combined considerations of range centre-periphery patterns and past large-scale dispersal processes are still uncommon, although shown as meaningful in models of genetic diversity differences among populations (see Guo 2012). **Manuscript 6** introduces novel metrics to quantify marginality in complex and discontinuous range shapes using the example of *Corynephorus canescens*, a widespread but highly specialised European grass species. We found that genetic diversity was significantly better explained when range marginality of populations was combined with directions of post-glacial re-colonisation (the longitudinal and latitudinal gradients) as predictor variables, compared to models including only re-colonisation directions or only marginality. The shown intraspecific variation reflected a past climate-associated re-colonisation process but additionally hints towards possible differences in adaptive capacities among populations. In many species, populations in past glacial refugia in southern regions now represent the potential rear edge of northward range shifts in response to climate warming. For example, Mediterranean regions are projected to receive considerable environmental pressures from increasing summer temperatures, decreasing precipitation and more frequent extreme weather events like droughts or heavy rain (IPCC 2013, 2014b). However, Mediterranean populations often also possess high standing genetic variation, probably beneficial for eco-evolutionary response capacity and their persistence in changing environments (Hampe & Petit 2005; see also **Manuscript 3 and 6**).

Outlook

If ecological tolerance to changing climatic conditions is insufficient, plastic and evolutionary adaptation is missing or incomplete and/or dispersal to regions of environmental suitability is impossible or fails so that ecological niche cannot be maintained extinction will follow as a result. Projections indicate that biodiversity will significantly decline towards the end of the century if species will not be able to cope with climate change impacts and if it will not be possible to mitigate the effects of global change (*e.g.* Pereira *et al.* 2010; Bellard *et al.* 2012; Selwood *et al.* 2015). However, although predicted to be a major driver of species extinctions in the future, the proximate causes of recent extinctions and threats from climate change are known only for a handful of species (Cahill *et al.* 2013). In the paleontological record, extinctions are also hard to relate to specific (proximate) causes, even when they occurred in periods of strong climatic change. Therefore, knowledge on the processes of species extinctions due to climatic changes are limited and mostly based on deductive reasoning. More research in this direction is necessary to infer process-based models to assess extinction risks and to develop possible mitigation measures.

The probability of different taxa and populations to persist during climatic habitat alterations is mainly depending on i. the magnitudes, rates and variability (*i.e.* fluctuations of magnitudes and rates, see *e.g.* Bell & Collins 2008) of climatic changes and their direct and indirect impacts, and ii. the capacities of affected organisms to tolerate or respond to those changes by the means of the above discussed mechanisms. Both issues still show large knowledge gaps. For many regions of the world, only coarse climate data are available (low spatial and temporal resolution). This makes it hard to model sufficiently resolved regional climate change projections and to infer potential future conditions and developments for regional and local species and ecosystems. Particularly in regions of high biodiversity, biological uniqueness (*e.g.* endemism) and thus high threat potential from global change impacts, data on past and current climatic trends, as well as reliable projections are often missing (*e.g.* in many tropical and subtropical developing countries with coarse networks of climate stations and on small islands, see **Manuscript 1**). Often, the topographical complexity of a region and thus micro-climatic conditions and potential micro-refugia are underrepresented in spatial climate projections.

Specific response capacities of most species and populations are also still largely unclear, and possible ecotype differences within species, phenotypic plasticity and evolutionary adaptation are only in the beginning of being integrated into models of organismal responses to climate change. Existing frameworks of potential climate change impacts on the persistence of organisms rely on observed trends (*e.g.* demographic and distributional data) or projected changes (*e.g.* niche distribution models) (Thomas *et al.* 2011; Selwood *et al.* 2015) or are purely conceptual with rather qualitative considerations on relevant factor interactions (*e.g.* Williams *et al.* 2008; **Manuscript 1**). More empirical data on organismal response capacities and integrated assessment tools to forecast risks (and benefits) of climate change on species and ecosystems are needed.

In this thesis, several advancements in the understanding of biological responses to climatic variation are made and new research options are opened.

- First, an initial global and integrative framework for scientific and conservational treatments of climate change impacts on oceanic islands is provided (**Manuscript 1**). This fills a research gap on these very peculiar ecosystems, considering their evolutionary and ecological significance and their importance for global biodiversity. The suitability of oceanic islands for research in climate change ecology is depicted and options for needed scientific efforts are identified.
- Second, lasting transgenerational effects of extreme weather events have been shown for perennial species, including germination and offspring performance (**Manuscript 2**). This emphasises the relevance of extreme events in shaping sustainable changes in population processes and phenotypic variation that may contribute to eco-evolutionary processes. Further research is needed to test later developmental steps in offspring individuals or even in the following generations. Transgenerational plasticity should be more considered as a medium-term response of organisms to climate change impact, additional to direct phenotypic plasticity and long-term evolutionary adaptation.
- Third, a relation between phenotypic plasticity patterns and genetic diversity in populations were suggested, indirectly including phylogeographic gradients into considerations of phenotypic plasticity (**Manuscript 3**). More studies with additional species and greater geographical sampling are needed to elucidate the found effects and to investigate their relevance for intraspecific variation in response capacity towards climatic change.
- Fourth, the importance of climatic and topographical landscape heterogeneity on eco-evolutionary processes was shown by analyses of biodiversity patterns (**Manuscript 4**) and intraspecific genetic structuring (**Manuscript 5**) of endemic species on an oceanic island. Although ecological heterogeneity is known to drive evolution and species diversity patterns on islands this has rarely been quantified on the intra-island scale and within species. Additional research should further focus on the underlying processes of niche divergence, speciation and biogeographic differentiation within and among species on the scale of single oceanic islands. For example, in-depth phylogeographic analyses and genomic studies could unravel evolutionary trajectories and traits and genes involved in species evolution of island endemics in order to test island biogeographical and species radiation theory.

- Fifth, an enhancement in the analyses of large-scale population genetic patterns was made by integrating the effects of range centre-periphery gradients into phylogeographical patterns in a widespread European species due to Quaternary climate fluctuation (**Manuscript 6**). To do so, new metrics of range marginality were invented and tested, which may have general relevance for studies on range centre-periphery gradients.

The presented findings contribute to the knowledge on plant responses to climate change and their eco-evolutionary capabilities in various spatial and temporal scales of climate variation. In a broader perspective, the challenge for future studies of species responses to climatic variation will be to integrate the interacting processes of phenotypic plasticity and evolution into models and projections (*e.g.* in species distribution models). Intraspecific variation in response capabilities must find better consideration and we should refrain from looking at ecological response and evolution as discrete processes. Likewise, climate change impacts cannot be seen isolated from other pressures of global change. The interactive nature of and feedbacks between different drivers of change must be considered when studying risks and response capabilities of species and ecosystems. Although this is not a trivial task, it will help to understand the constant interplay of evolution and ecology and to assess the future of biodiversity and organismal distributions under changing climates.

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Manuscripts of this thesis

Overview

In the following, I will provide short summaries of the manuscripts presented in this thesis, highlighting their relevance for the scientific landscape, contributions to answering open questions, or achieved methodological or conceptual advances. Manuscripts are ordered according to their appearance in the introduction. Table 1 provides an overview on conference contributions during my PhD period related to this thesis. After a declaration of my personal contributions to them, the manuscripts are presented in their latest form. A list of publications and manuscripts to which I contributed but which are not included in this thesis is given in Appendix 1.

Manuscript 1 compiles and analyses climate data and literature knowledge to a global overview and conceptual vulnerability analysis for climate change patterns and impacts on oceanic islands and their floras. The role of anthropogenic climate change for oceanic island biodiversity has been largely neglected yet, probably due to presumed climatic buffering from surrounding water masses, supposed dominating effects of habitat destruction, and a previous focus of climate change research on continental systems. We show by projecting most recent global climate projections to oceanic islands and by extensively reviewing existing literature from climatology, biology and other research fields that there is a great potential of risks resulting from current and future climatic changes and from specific attributes of oceanic islands and their species. Limited space for dispersal responses, small population sizes and high ecological specialisation of many island endemics, plus low functional redundancy in oceanic island ecosystems are factors for high vulnerability against climatic alterations. We further argue that interactions of local impairments (*e.g.* from habitat destruction and neobiota invasions) with climatic habitat changes and potential indirect effects of forced human activity shifts (*e.g.* translocation of settlements due to sea level rise) might cause the most severe problems for island biota. Differences in impact potentials and possible responses among island types and species are discussed. Although the paper compiles a growing body of evidence for climate change risks on oceanic island floras, great uncertainties still exist, largely resulting from missing high resolution climate models for most oceanic islands und from insufficient knowledge on species' response potentials. We identify knowledge gaps, provide ideas for potential research and depict the general suitability of oceanic islands for research in global change ecology.

Manuscript 2 is an experimental study of transgenerational effects of extreme weather events (drought and heavy rain) on two perennial plant species (*Genista tinctoria* and *Calluna vulgaris*), recurrently experienced by mother plants. Additionally, effects of different community compositions (resulting in different levels of intraspecific competition) of *C. vulgaris* mother plants were tested. We can show that drought stress on *G. tinctoria* and high competition for *C. vulgaris* mother plants cause earlier germination of seeds. Additionally, offspring of heavy rain-treated *G. tinctoria* mothers showed reduced overall germinability and delayed germination, higher growth rates in their second year and a lower leaf C:N ratio, indicating less structural, C-rich tissue and more metabolic, N-rich tissue. The found transgenerational plasticity is discussed regarding potential response strategies of mother plants to avoid stress and high competition for their offspring. Germination and growth are crucial factors for offspring establishment and competitiveness. The observed maternal effects can thus shape long-term population dynamics and community interactions in natural ecosystems. Our study is the first to show that maternal effects after extreme events or changes in community composition cannot be neglected when estimating ecological and evolutionary responses of species and populations to environmental change. It is also a rare case study of transgenerational plasticity in perennial species, revealing persistent maternal effects.

Manuscript 3 investigates the influence of genetic diversity within populations of European Beech (*Fagus sylvatica*) on variation in phenotypic plasticity among populations to experimental extreme weather events (drought and/or warming). The manuscript thus directly tests an assumption made in recent synthesis papers on phenotypic plasticity. We show that genetic diversity differences related to the known phylogeographical history of the analysed populations from Bulgaria (glacial refuge region) and Germany (post-glacial re-colonisation region) were partially correlated to phenotypic plasticity variation. Potential adaptive values of the measured plasticity variation and phylogeographic implications on plasticity on the population level are discussed. Thereby, the manuscript hints towards a further value of genetic diversity besides its evolutionary importance. As a parameter influencing plasticity in populations, genetic diversity might have relevance for assessments of response capacities of plant populations to climate change and in conservation planning.

Manuscript 4 directly tests the (proposed) fundamental roles of climate and topography on distributions of plant diversity and its implications on eco-evolutionary processes. We take the island of La Palma as a model system for analysing distributions of plant biodiversity metrics (species richness, endemic richness and endemicity, *i.e.* percentage of endemics) on the landscape scale and relating those patterns to various climatic and topographic variables. By sampling and analysing data from 890 plots distributed over the entire island we were able to reliably interpolate patterns to the very high spatial resolution of 100 m × 100 m. Spatial patterns of biodiversity measures showed up to be partially decoupled and to be related to different explanatory variables. A combination of topographic and climatic variables accounted for the overall species richness pattern, whereas endemic richness was mainly driven by topographic variables (mainly topographic complexity, slope, solar radiation), and climatic drivers (mainly elevation, rainfall seasonality, temperature) were dominant in explaining endemicity patterns. Provisioning of habitat diversity and micro-refugia from past climatic variability and from human impairments (*e.g.* land use, introduced herbivores) are suggested as main explanations for the positive influence of topographic complexity and slope on endemic richness. In contrast, endemicity indicates floristic uniqueness and specialisation, and can thus be interpreted as a measure of *in situ* speciation in response to specific local selection pressures, supporting a strong relevance of climatic factors for adaptive evolutionary processes in plants. Besides these findings, the relevance of the study is the downscaling of (partially known) biogeographic patterns to the landscape level and depicting the geographical decoupling of the different biodiversity measures. Both aspects increase the general understanding of biodiversity patterns and have relevance for conservation management.

Manuscript 5 is a landscape genetic study targeted at the vivid debate about drivers of species radiations on oceanic islands and about evolutionary processes on small spatial scales in general. We analyse population genetic patterns within two island-endemic species of *Aeonium* on La Palma to identify drivers of evolutionary processes on the island scale. Although the genus represents an iconic textbook example for species radiations on oceanic archipelagos, this study is the first that comprehensively examines population genetic patterns within species of this interesting group. Moderate levels of genetic differentiation within the two species are revealed, which can be related to different landscape structures, emphasizing the importance of topography for evolutionary processes on small oceanic islands. Further on, indications of divergent selection due to climatic factors (summer precipitation, temperature) point to ecological population differentiation. Topographical and ecological heterogeneity within single islands can thus be stated probable drivers for speciation in the radiation of *Aeonium* on the Canary Islands. Additionally, by comparing intraspecific range-wide patterns within a widespread ecological generalist *vs.* a specialist species on the same island, possible effects of ecological niche width on the potential of evolutionary divergence within species could be discussed.

Manuscript 6 enhances the understanding of genetic diversity patterns within species ranges along the two gradients between glacial refugia to re-colonisation regions and between range centres to range margins. The study analyses large-scale population genetic patterns within the grass Corynephorus canescens, which is an uncommon species due to the facts that i) it has an Atlantic distribution ranging from the Iberian Peninsula to Eastern Europe but without the two classical glacial refuge regions on the Appenine and Balkan peninsulas and ii) it is highly dependent on disturbance and thus can be assumed to have a very dynamic population ecology. We thus test the hypotheses that Corynephorus canescens shows genetic patterns of i) uniform re-colonization from the Iberian peninsula towards the Eastern parts of Europe, ii) decreasing diversity from the centres of its distribution range to the range boundaries, and iii) interacting effects of high gene flow and disturbance-driven genetic drift. The found re-colonisation patterns are more complex than hypothesised; however, we can show that phylogeographic patterns interact with centre-margin patterns but also with the special ecology of this species. This integrative approach is innovative, as in most other studies these patterns are studied separately, despite their high biogeographical interdependence. We also invent and test new indices to quantify marginality, which has relevance for future studies that need to account for marginality on a continuous scale and/or in scattered or discontinuous range shapes.

Table 1: Presentation of research related to this thesis at scientific conferences. Only presentations where I was presenting author are listed.

Date	Organisation	Conference and location	Title	Type
01 / 2015	International Biogeography Society (IBS)	7th International Conference, University of Bayreuth, Germany	Holocene re-colonisation, central-marginal-distribution and habitat specialisation shape population genetic patterns within an Atlantic European grass species	Poster
01 / 2015	International Biogeography Society (IBS)	7th International Conference, University of Bayreuth, Germany	Island-scale spatial and ecological differentiation within two species of a radiating genus on the Canarian archipelago	Poster
07 / 2014	University of Hawaii at Manoa	Island Biology 2014, Honolulu, Oahu, Hawaii, USA	The impacts of global climate change on the floras of oceanic islands – a general framework	Oral
10 / 2012	Bayreuth Center of Ecology and Environmental Research (BayCEER)	BayCEER Workshop, University of Bayreuth, Germany	Spatial and ecological effects on population differentiations of two island endemic species of a radiating plant genus on the Canary Islands	Oral
09 / 2012	Ecological Society of Germany, Austria and Switzerland (GfÖ)	42nd Annual Meeting, Lüneburg, Germany	The biogeography of population differentiations in a radiating genus of the Canary Islands: Spatial or ecological drivers?	Oral
02 / 2012	Society for Tropical Ecology (gtö)	Annual Conference, Erlangen, Germany	Genetic differentiations within island endemics: A biogeographical evolution assay in selected *Aeonium* species	Oral
05 / 2011	AK Biogeographie im Verband der Geographen an Deutschen Hochschulen (VGDH)	Jahrestreffen, Hamburg, Germany	Genetic differentiations within selected *Aeonium* species – processes of adaptive radiation?	Oral

Declarations of own contributions to each manuscript

Manuscript 1:

Authors: Harter, D.E.V.; Irl, S.D.H.; Seo, B.; Steinbauer, M.J.; Gillespie, R.; Triantis, K.; Fernández-Palacios, J.M.; Beierkuhnlein, C.

Title: **Impacts of global climate change on the floras of oceanic islands – Projections, implications and current knowledge**

Journal and status: **Perspectives in Plant Ecology, Evolution and Systematics** 17(2), April 2015, Pages 160–183

Own contribution: writing: 75%, idea and concept: 70%, literature survey: 80%, data analysis and figures: 80%, corresponding author

Manuscript 2:

Authors: Walter, J.*; Harter, D.E.V.*; Beierkuhnlein, C.; Jentsch, A.

*: authors contributed equally to the manuscript

Title: **Transgenerational effects of extreme weather: Plant offspring shows modified germination, growth and stoichiometry**

Journal and status: **Journal of Ecology**, in press

Own contribution: writing: 20%, idea and concept: 50%, experimental work and data acquisition: 90%, data analysis and figures: 50%

Manuscript 3:

Authors: Harter, D.E.V.*; Nagy, L.*; Backhaus, S.; Beierkuhnlein, C.; Fussi, B.; Huber, G.; Jentsch, A.; Konnert, M.; Thiel, D.; Kreyling, J.

*: authors contributed equally to the manuscript

Title: **A comparison of genetic diversity and phenotypic plasticity among European beech (*Fagus sylvatica* L.) populations from Bulgaria and Germany under drought and temperature manipulation**

Journal and status: **International Journal of Plant Sciences** 176(3), March 2015, Pages 232–244

Own contribution: writing: 70%, idea and concept: 50%, experimental work and data acquisition: 0%, data analysis and figures: 70%, corresponding author

Manuscript 4:

Authors: Irl, S.D.H.; Harter, D.E.V.; Steinbauer, M.J.; Gallego Puyol, D.; Fernández-Palacios, J.M.; Jentsch, A.; Beierkuhnlein, C.

Title: **Climate *vs.* topography – spatial patterns of plant species diversity and endemism on a high-elevation island**

Journal and status: **Journal of Ecology** 103(6), November 2015, Pages 1621–1633

Own contribution: writing: 20%, idea and concept: 10%, field work and data acquisition: 15%, data analysis and figures: 5%

Manuscript 5:

Authors: Harter, D.E.V.; Thiv, M.; Weig, A.; Jentsch, A.; Beierkuhnlein, C.

Title: **Spatial and ecological population genetic structures within two island-endemic *Aeonium* species of different niche width**

Journal and status: **Ecology and Evolution** 5(19), October 2015, Pages 4327–4344

Own contribution: writing: 90%, idea and concept: 80%, field work and data acquisition: 70%, data analysis and figures: 90%, corresponding author

Manuscript 6:

Authors: Harter, D.E.V.; Jentsch, A.; Durka, W.

Title: **Holocene re-colonisation, central–marginal distribution and habitat specialisation shape population genetic patterns within an Atlantic European grass species**

Journal and status: **Plant Biology** 17(3), May 2015, Pages 684–693

Own contribution: writing: 80%, idea and concept: 60%, field work and data acquisition: 0%, data analysis and figures: 80%, corresponding author

Manuscript 1

Impacts of global climate change on the floras of oceanic islands – Projections, implications and current knowledge

Perspectives in Plant Ecology, Evolution and Systematics 17(2), April 2015, Pages 160–183

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Abstract

Recent climate projections indicate substantial environmental alterations in oceanic island regions during the 21st century, setting up profound threats to insular floras. Inherent characteristics of island species and ecosystems (*e.g.* small population sizes, low habitat availability, isolated evolution, low functional redundancy) cause a particular vulnerability. Strong local anthropogenic pressures interact with climate change impacts and increase threats. Owing to the high degree of endemism in their floras, a disproportionally high potential for global biodiversity loss originates from climate change impacts on oceanic islands. We reviewed a growing body of research, finding evidence of emerging climate change influences as well as high potentials of future impacts on insular species and ecosystems. Threats from global climate change are not evenly distributed among the world's oceanic islands but rather vary with intrinsic (*e.g.* island area, structure, age and ecological complexity) and extrinsic factors (regional character, magnitude and rate of climatic alterations, local human influences). The greatest flora vulnerabilities to climate change impacts can be expected on islands of small area, low elevation and homogeneous topography. Islands of low functional redundancies will particularly suffer from high rates of co-modifications and co-extinctions due to climate-change-driven disruptions of ecological interactions. High threat potentials come from synergistic interactions between different factors, especially between climatic changes and local anthropogenic encroachments on native species and ecosystems. In addition, human responses to climate change can cause strong indirect impacts on island floras, making highly populated islands very vulnerable to secondary (derivative) effects. We provide an integrated overview of climate change-driven processes affecting oceanic island plants and depict knowledge gaps and uncertainties. The suitability of oceanic islands and their ecosystems for potential research on the field of climate change ecology is highlighted and implications for adequate research approaches are given.

Key-words

Global warming, island biogeography, island endemics, oceanic buffering, sea-level rise, susceptibility

Contents

Introduction

Oceanic islands have always been stimulating to scientists as natural showcases of ecology, evolution and biogeographical processes (*e.g.* Darwin, 1859; Wallace, 1880; MacArthur and Wilson, 1967; Carlquist, 1974; Whittaker and Fernández-Palacios, 2007). Their origin from oceanic crust and final erosion beneath sea level has been persistent and characteristic traits during all times of earth history, defining special conditions and time scales for evolution and extinction. Isolation from continental landmasses sets up an efficient filter for the immigration of terrestrial organisms to oceanic islands. Successful colonisers are confronted with novel environments and evolutionary opportunities, leading to unique species and species assemblages (MacArthur and Wilson, 1967; Gillespie and Roderick, 2002; Whittaker and Fernández-Palacios, 2007). Thus, oceanic islands generally have lower overall species numbers per unit area (Whittaker and Fernández-Palacios, 2007) but show higher percentages of endemism than mainland areas (Kier *et al.*, 2009). As a consequence, the extremely limited insular areas host a disproportional high fraction of global biodiversity (Kreft *et al.*, 2008; Kier *et al.*, 2009).

Although most climate change research is focused on continental ecosystems, ocean regions, too, will be affected by global climate change. For many oceanic islands, changing temperatures and precipitation patterns, shifting frequencies and intensities of extreme weather events (*e.g.* droughts, storm surges, hurricanes), as well as altered patterns of seasonal and mid-term weather systems (El Niño Southern Oscillation, monsoon, etc.) and sea level rise can be expected (IPCC, 2012, 2013b). These changes can pose profound and challenging environmental alterations to terrestrial biota in general (*e.g.* Jump and Peñuelas, 2005; Parmesan, 2006; Cahill *et al.*, 2013), but have specific relevance for island biota (Loope, 1995; Mimura *et al.*, 2007; Caujapé-Castells *et al.*, 2010; Fordham and Brook, 2010; Bramwell, 2011).

This specific relevance is due to several aspects of oceanic islands: First, as a consequence of their isolated evolution, oceanic island species are often insufficiently prepared to changing environments (Cronk, 1997; Gillespie *et al.*, 2008; Fordham and Brook, 2010). For plants on oceanic islands, Carlquist (1974) introduced the “island syndrome”, meaning (i) a tendency to reduce their dispersability (see also Cody and Overton, 1996; Fresnillo and Ehlers, 2008; Gillespie *et al.*, 2012), (ii) a tendency to be poor competitors in the face of introduced species and (iii) a lack of defensive mechanisms against (non-native) herbivores (see also Bowen and van Vuren, 1997; Vourc'h *et al.*, 2001). Second, the restricted areas of oceanic islands and the surrounding ocean limit the options of island species to migrate and escape potentially deteriorating conditions via range shifts. This requires affected species to either retreat to potential refuge habitats within their island (if available and within reach), adapt rapidly to changing conditions or to become extinct (Gillespie *et al.*, 2008; Levine *et al.*, 2008). Third, due to the restricted size of oceanic islands, the total range of endemics is also comparably small. This is usually connected to a low overall population size, leading to higher vulnerability to stochastic (but also deterministic) threats (Kruckeberg and Rabinowitz, 1985; Gilpin and Soulé, 1986; Frankham, 1998; Gillespie *et al.*, 2008). Especially very narrow endemics, which represent a high percentage of oceanic islands’ biodiversity (*e.g.* Sakai *et al.*, 2002), are likely to exhibit low tolerances and a high vulnerability to extrinsic disturbances (Kruckeberg and Rabinowitz, 1985; Lavergne *et al.*, 2004; Hermant *et al.*, 2013). Also, oceanic isolation acts as a very efficient dispersal filter, drastically limiting the potential of species‘ responses to ecological shifts, such as climate change, by migration to other landmasses.

The imminent sensitivity of many island endemics to rapid environmental changes and extraordinary encroachments is reflected in the disproportionally high numbers of extinctions after human arrival on remote islands (Cronk, 1997; Sadler, 1999; Steadman, 2006; Caujapé-Castells *et al.*, 2010; Fordham and Brook, 2010). Although this pattern is more obvious for animals than for plant species (*e.g.* Sax *et al.*, 2002; Whittaker and Fernández-Palacios, 2007), numerous examples prove novel anthropogenic stressors (introduction of neobiota, selective use or exploitation, land use and habitat destruction) have caused past and current losses of island endemic plant species (*e.g.* Bouchet *et al.*, 1995; Jaffre *et al.*, 1998; Sadler, 1999; de la Luz *et al.*, 2003; Fall, 2005; Schäfer, 2005; Prebble and Dowe, 2008; Meyer and Butaud, 2009; Baider *et al.*, 2010; Connor *et al.*, 2012; Restrepo *et al.*, 2012).

Besides already irreversible losses, extinction debts and cases of imminent extinctions are also common in oceanic island floras (Sakai *et al.*, 2002; Sax and Gaines, 2008; Kaiser-Bunbury *et al.*, 2010; Heywood, 2011) and other island organisms (Triantis *et al.*, 2010; Halley *et al.*, 2014).

Compared to local human encroachments, global climate change alters environmental conditions beyond the island scale. Despite some climatic buffering effects by the surrounding oceans, climate change thus has the potential to significantly affect island biota. Evidence from Quaternary climatic alterations suggests substantial ecological shifts on oceanic islands, including losses of plant species (*e.g.* Loope and Giambelluca, 1998; Pau *et al.*, 2012; Boer *et al.*, 2013; Nogué *et al.*, 2013). Current observations of vegetation changes due to climate alterations suggest even stronger climate effects on plant life when climatic changes proceed into the future (*e.g.* Roux and McGeoch, 2008; Kapfer *et al.*, 2012; Krushelnycky *et al.*, 2013). Species vulnerability modelling according to 21st century climate change scenarios showed that considerable proportions of island plants are threatened by future conditions, *e.g.* by strong shifts or even complete losses of climatic niches of some species (*e.g.* Fortini *et al.*, 2013 for Hawaiian plants, or Marrero-Gómez *et al.*, 2007; Lloret and González-Mancebo, 2011; Patiño *et al.*, 2013 for the Canary Islands). Taken together, insights from past climatic changes, current trends and projections of future conditions show that oceanic islands and their floras are affected by climatic alterations and that oceanic buffering, although undoubtedly existent, cannot be generally expected to shelter those systems.

Extinctions of oceanic island plants by climate change would contribute disproportionally to global biodiversity decline (Mimura *et al.*, 2007; Fordham and Brook, 2010). This is particularly true considering that about one quarter of all vascular plant species are endemic to islands (sharing only 5 % of the Earth's land area, not considering Greenland), many to oceanic islands (Kreft *et al.*, 2008). However, specific literature on climate change impacts on oceanic island floras is scarce. Apart from case studies of potential local climatic alterations and resulting environmental consequences on selected species (*e.g.* Roux *et al.*, 2005; Marrero-Gómez *et al.*, 2007; Levine *et al.*, 2008; McEachern *et al.*, 2009; Shapcott *et al.*, 2012; Krushelnycky *et al.*, 2013) or on particular ecosystems, single islands or oceanic regions (*e.g.* Sperling *et al.*, 2004; Gillespie *et al.*, 2008; Wetzel *et al.*, 2012; Fortini *et al.*, 2013; Scott and Kirkpatrick, 2013; Bellard *et al.*, 2014), general examinations of impacts and potential influences of global climatic change on island plants are yet missing. Thus, the aims of this study are (i) to evaluate current climate change effects on oceanic island floras and discuss possible scenarios of climate-change driven impacts on island plant diversity, (ii) to assess how island- and species-specific characteristics may either intensify or attenuate impacts of climate change, and (iii) to identify knowledge gaps and research challenges.

Climate change patterns among island regions

Global temperature and precipitation change on oceanic islands

Oceanic islands are defined in this study as islands that have never had contact to other landmasses, including islands of volcanic or coralline origin, as well as raised coral or limestone islands and all intermediate forms. We compiled and analysed data on mean annual temperature and mean annual precipitation changes on such islands worldwide, based on median climate simulation outputs by recent CMIP5 model ensembles (Coupled Model Intercomparison Project Phase 5, Taylor *et al.*, 2012) for three future time periods and four greenhouse gas scenarios, respectively.

For this purpose, a dataset of globally distributed islands were obtained from the UNEP-based Island Directory Website (Dahl, 1991; http://islands.unep.ch/isldir.htm), which includes nearly 2000 islands with important island variables, including geological origin. Starting from this dataset we extracted all oceanic islands. Missing geological information on some islands was added if available in the literature and internet sources and all records were checked carefully. Coordinates were corrected (if necessary) and permanent isolation from other landmasses (surrounding sea depth of min. 160 m) was assured by visual assessment of bathymetry in GoogleEarth™ version 7.1.2.2041 (Google Inc. 2013), resulting in a final data set of 787 oceanic islands.

CMIP5-based, multi-model ensemble projections for global mean temperature above surface and precipitation were downloaded from the IPCC AR5 (Intergovernmental Panel on Climate Change fifth assessment report, IPCC, 2013b) publication and data download homepage (http://www.climatechange2013.org/report/full-report/). These data are presented and described in Annex I of the WGI report (IPCC, 2013a) and include 20-year average changes for the near term (2016–2035), mid-term (2046–2065) and the long term (2081–2100), relative to a reference period of 1986–2005. For each period, data for four Representative Concentration Pathway scenarios (RCP 2.6, RCP 4.5, RCP 6.0 and RCP 8.5; van Vuuren *et al.*, 2011) were obtained. From the 42 CMIP5 climate models in total, RCP 2.6 projections are based on an ensemble of 32, RCP 4.5 on the full 42, RCP 6.0 on 25, and RCP 8.5 on 39 model experiments, respectively (IPCC, 2013b). Spatial data was given on a 2.5° grid, consisting of regional median, 25%- and 75%-quantile responses of the respective model ensemble. The conversions of the original netCDF files to raster grids, the extraction of the raster values and their subsequent analyses were carried out with R 2.15.2 (R Core Team, 2012) using the packages 'raster' (Hijmans, 2013) and 'RNetCDF' (Michna, 2012).

To compare climate change projections for oceanic islands with the expected worldwide changes , we additionally analysed projection values from continental grid cells, and, to account for putatively differing coastal climates, from grid cells of coastal areas, including large continental islands. In obtaining continental climate change data, we identified cells that were entirely covering landmasses, *i.e.* purely inland pixels with no contact to seashores (hereafter termed 'continental'). For 'coastal' climate change expectations, we selected cells that contained shorelines of landmasses bigger than 100,000 km^2 (to illustrate, the North Island of New Zealand has an area of 113,729 km^2). The latitudinal range of oceanic islands in our data set ranged from –69° S to 71° N. To assure a reasonable comparison, we restricted the latitudinal range of our coastal and continental grid cell selection to the latitudinal range of –75° S to 75° N, yielding 1331 coastal and 1885 continental pixels in total. Values were extracted from all pixels, respectively, and analysed in the same way as the oceanic island values.

Mean annual temperature is projected to increase appreciably for our set of 787 islands, indicated by consistently positive directions in average change values among greenhouse gas scenarios (Fig. 1A). Model uncertainty for temperature (represented by the vertical extension of boxes in Fig 1A, showing the upper and lower average quartiles) is moderate. According to average median values, RCP 2.6 would likely result in average temperature increases below 1 °C even at the end of the century, whereas this is true in the other scenarios only for the near term future (period 2016 – 2035). For the mid-term (2046 – 2065) future, increases between ~1 °C (RCP 4.5 and RCP 6.0) and 1.5 °C (RCP 8.5) are possible, and for the long-term future (2081 – 2100) temperature increases range from 1.3 °C (RCP 4.5) to 1.6 °C (RCP 6.0) and even 2.8 °C (RCP 8.5).

However, there is a large geographic variation (indicated by the error bars in Fig. 1, showing the standard deviation among islands for the given median model response), which increases in long-term projections. Likewise, the overall uncertainty among climate models (the interquartile distance) is higher for the long-term simulation ensembles. For mean annual precipitation, the pattern is less distinctive (Fig. 1B). The average median model response indicates slightly more precipitation for the majority of islands in the future and an increasing trend towards the end of the century (from below 1% in all near-term projections to 1.8 – 3.2% for the period 2081 – 2100, the latter value being the average median for RCP 8.5). Nevertheless, there is a huge variation among islands (standard deviations ranging from 3.4% to 18.1%) and among climate model outputs within simulation ensembles. Both types of variability are indicated to increase from moderate to high greenhouse gas scenario and from near-term to long-term projections. This means strong uncertainties in projecting even the direction of precipitation change for many oceanic islands, considerably depreciating the robustness of assessments of future climatic conditions. Limited predictability of precipitation developments is a known source of uncertainty in climate projections (see below and discussion of precipitation projections and model variance in IPCC, 2013b and Lee and Wang, 2014). Lack of direct observations, methodological uncertainties and high geographical trend variation as well as high variability of major climate drivers like the El Niño-Southern Oscillation (ENSO) and climate oscillations of longer duration (*e.g.* Pacific Decadal Oscillation) may account for this uncertainty (Mote *et al.*, 2011; Keener *et al.*, 2012; IPCC, 2013b).

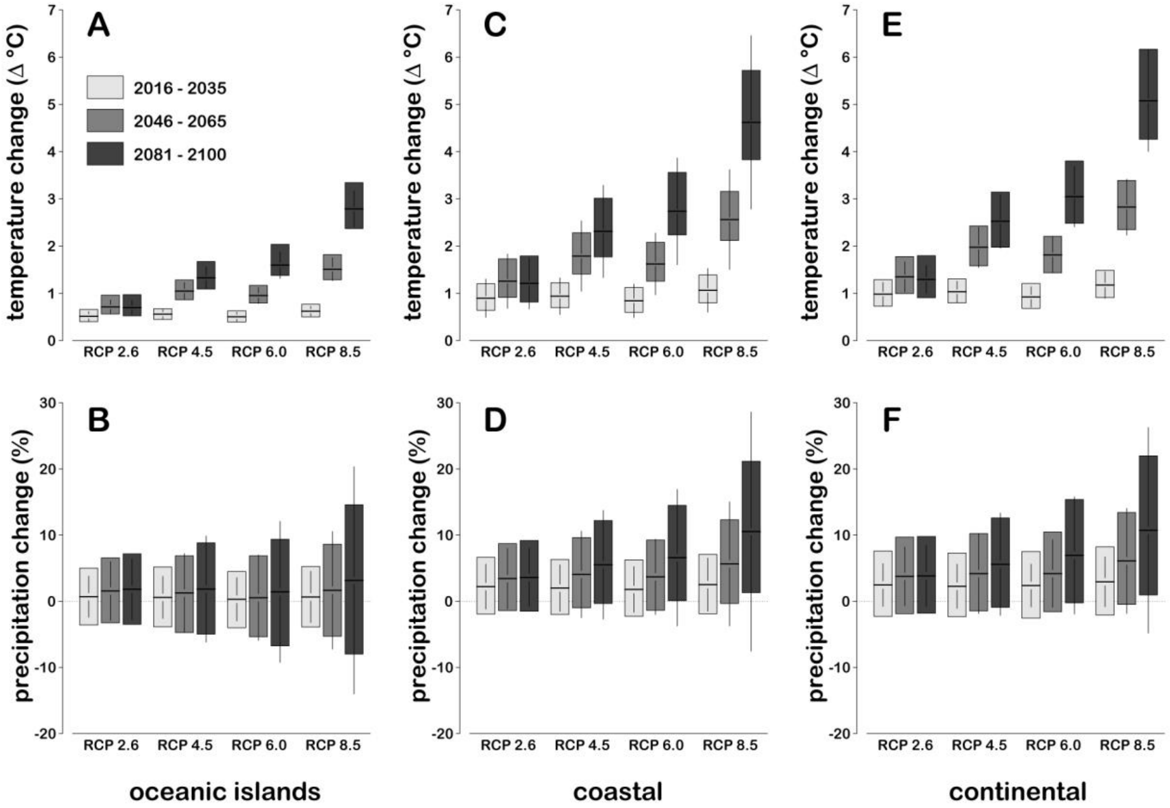

Fig. 1: Average absolute temperature change (A) and percentage precipitation change (B) projections over 787 oceanic islands in the course of the 21st century according to four different greenhouse gas concentration scenarios (RCPs). Single island values were based on regional median model outputs of CMIP5 simulation ensembles (32/42/25/39 out of 42 scenario experiments for RCP 2.6/4.5/6.0/8.5, respectively). Reference period is 1986 – 2005. Boxes show the averaged medians (central horizontal bar) and averaged upper and lower quartiles (upper and lower horizontal bars, respectively) of the climate model ensembles over the whole set of islands to indicate the model variability. The vertical lines depict the standard deviations of averaged medians to show the large differences among islands. Projections of temperature and precipitation changes for coastal (C, D) and continental regions (E, F) are depicted in the same way, respectively.

It should also be noted that large-scale precipitation projections might disregard influences of island topographies on precipitation patterns on smaller scales. High-elevated islands often possess a diversity of mesoclimates due to orographic cloud formation, luv/lee-effects and other topographically determined climatic patterns resulting in heterogeneous distributions of precipitation patterns among island regions and/or elevational zones (Whittaker and Fernández-Palacios, 2007). Further on, many oceanic islands have highly structured landscapes of valleys and ridges with broad and steep slopes, which can generate numerous micro-climatic conditions. Both, regional differences within islands and small-scale landscape structures potentially constitute climatic micro-refugia for species during climate change (see *e.g.* Hannah *et al.*, 2014 for general considerations of micro-refugia). Additionally, it is possible that climate change impacts will vary among different areas within islands, *e.g.* increasing humidity in windward slopes but drying conditions in leeward island areas due to disparate changes in cloud formation or cloud layer elevation leading to changing rain shadow effects (Scholl *et al.*, 2007; Chu *et al.*, 2010). While large-scale projections can show regional trends important for general change perspectives, fine resolution island-scale climate models and downscaled climate projections will be necessary to account for geographical variation within high-elevated oceanic islands and to provide better resolved scenarios.

Likewise, large-scale temperature projections in general only refer to air temperatures close to the water surface and thus do not entirely represent the potential changes on small oceanic islands with extents much smaller than the climate model resolution (Mimura *et al.*, 2007; Heywood, 2011; Nurse and McLean, 2014). Air temperature increases above land surfaces on islands might exceed projections for the surrounding open sea surfaces (Mimura *et al.*, 2007) due to the lower specific thermal capacity of land surface. This could lead to a possible underestimation of temperature changes in current climate models.

The projections for oceanic islands differ from those for coastal and continental regions. Although of appreciable magnitude, temperature increases are projected to be lower on islands (Fig. 1A, C and E). This indicates an oceanic buffering effect that attenuates air temperature increases, probably by the relatively high heat capacity of large water masses surrounding the islands (Kutzbach and Webb III, 1993). While for the near future differences lie within a 1 °C range for all RCP scenarios, mid and long term projections show that average temperature increases in coastal and continental regions may exceed those of oceanic islands by more than 2 °C (RCP 8.5). Interestingly, temperature increase in continental regions is projected to become only very slightly higher than in coastal regions, showing a strong influence of large landmasses to climate conditions even in marginal regions, compared to the peculiar situation of oceanic climates. This difference between oceanic and non-oceanic regions is also true for precipitation change projections (Fig. 1B, D and F). While no qualitative difference is observable between coastal and continental regions, averaged projections for oceanic islands show lower average increases of precipitation and more drying trends in some climate models. Also, the increase of precipitation during the course of the 21st century seems to be smaller for oceanic islands than for other landmasses. This shows that climate change on oceanic islands may differ in some aspects compared to other terrestrial regions of the world.

Regional variation of change and drivers of variability

Observed and projected changes in climate are known to differ regionally (IPCC, 2013b). For our dataset of 787 islands, projected changes in temperature and precipitation for the period 2081 – 2100 under the medium to high greenhouse gas scenario RCP 6.0 are given in Fig. 2.

Temperature increase is indicated to be highest on arctic and sub-arctic islands (*e.g.* Jan Mayen, Aleutian Islands, Kuril Islands 2.5 – 4.9 °C) and in equatorial regions (*e.g.* Galápagos, Gulf of Guinea islands 2 – 2.5 °C; Fig. 2A). Also, the southernmost Antarctic oceanic islands show high increases (*e.g.* Peter-I-Island 2 – 2.5 °C). However, contrasting to northern-hemispheric regions it is observable that most islands in southern ocean regions seem to expect comparably moderate temperature increases (0.8 – 1.5 °C). According to this, an inversely hump-shaped relationship between the simple latitudinal gradient from the Antarctic oceans to Arctic oceans and projected temperature change explains considerable portions of the spatial variance ($R^2 = 0.49$; Fig. A.1A, see Online Appendix for a description of regression models).

As already indicated in Fig. 1, coastal and continental regions are projected to receive higher temperature increases in general (Fig. A.1A). However, especially in low (equatorial) latitudes temperature increase projections for islands and coastal regions overlap while continental regions seem to exceed oceanic values more continuously, confirming the oceanic buffering effect. Supporting this, the increase in temperature on islands is slightly negatively related with distance to the next continent ($R^2 = 0.18$; Fig. A.1B), which indicates an increasing influence of oceanic buffering and a decreasing influence of continents on climate conditions and climatic changes on islands.

Besides latitude, the position in ocean currents or major climate systems is crucial for the macro- and mesoclimatic conditions of an island or archipelago, which is especially important for precipitation patterns. In our analysis, strong increases of precipitation are indicated in equatorial islands (especially Pacific: southern Line Islands up to 55%, Gilbert Islands up to 50%, Galápagos 30 – 40%; but also Gulf of Guinea islands or Maldives up to 20%) and in polar islands (*e.g.* Jan Mayen, Aleutian Islands, Peter-I.-Island up to 20%; Fig. 2B). Projections vary strongly among regions in mid latitudes. Slight increases (up to 10%) are mainly projected for large parts of the western Pacific and for southern oceanic regions in general. In contrast, projections for central and eastern parts of the Pacific, central parts of the Indian Ocean and most Atlantic Ocean regions, including the Caribbean, indicate decreases in mean annual precipitation. Strongest decreases (–10 to –15.6%) are predicted for southeastern French Polynesia, Pitcairn Islands, Easter Island, Juan Fernández and Desventuradas Islands, some of the Lesser Antilles, Trindade and Martim Vaz, Ascension, Madeira and Aeolian Islands. A moderately distinct overall geographic pattern can thus again be described by an inversely hump-shaped relationship between precipitation change and absolute values of latitude (R^2 = 0.48; Fig. A.1C). This pattern is far more distinct for oceanic islands than for coastal and continental regions due to the exceedingly high precipitation increases in circum-equatorial ocean regions contrasting to declines or constancy in precipitation amounts in subtropical high pressure belt regions (Fig. A.1C), and seems to be a likely future phenomenon (see also Keener *et al.*, 2012; IPCC, 2014; Lee and Wang, 2014; Nurse and McLean, 2014). Additionally, for many regions, trends indicate above average precipitation in regions of originally high precipitation and a further decline in regions of low precipitation (IPCC, 2014), but this pattern is less prominent in the recent CMIP5 results compared to older projections (Lee and Wang, 2014). It has to be noted that increasing precipitation can also have negative effects, especially on originally dry ecosystems (*e.g.* by facilitation of invasive species and outcompeting of dry-adapted endemics), as stated for the Galápagos archipelago by Trueman and d'Ozouville (2010).

However, some differences between outputs of different climate models and their versions account for uncertainties and limited regional predictability, particularly for precipitation. Difficulties in simulating future alterations of precipitation in mid and low latitudes are due to their dependence on rather unpredictable large-scale climate systems. On tropical and subtropical islands, climate changes are often associated with dynamics of El Niño occurrences (*e.g.* Cao *et al.*, 2007. The El Niño-Southern Oscillation (ENSO) interacts with a variety of large-scale atmospheric and oceanic circulation systems and is thus related to inter-annual variability in temperature and precipitation. ENSO variability is naturally very high and its sensitivity to human-induced climate change is still not proven (Collins *et al.*, 2010). However, there is evidence for an increase in ENSO variability during the twentieth-century (Cobb *et al.*, 2013). Higher ENSO activity is associated with larger climate variability and fluctuations of the Inter-Tropical Convergence Zone (ITCZ) and thus trade wind regimes, which are major determinants of climatic conditions on low and mid latitude islands (Crausbay *et al.*, 2014b).

Alterations of seasonal patterns may have high impacts on ecosystems, especially when precipitation patterns in the seasonal cycle change (IPCC, 2013b). Global monsoon activity is expected to rise with an increase in global surface temperature. For global oceanic regions this means an 6.3 % spread of the overall monsoon domain, a longer monsoon period duration and increased monsoon rainfall, especially for the northern hemisphere (NH; Lee and Wang, 2014). In large parts of the southern hemispheric (SH) oceanic regions, significant decreases are projected, a pattern due to the higher temperature and thus higher humidity increase in the NH, compared to the SH (Lee and Wang, 2014).

Alterations in temperature seasonality will especially affect islands at higher latitudes, where projected temperature increases are higher and more pronounced for winter periods (IPCC, 2013b). This can extend vegetation periods (Parmesan, 2007) but also may increase the associated potential risks of late frost events (Kreyling, 2010).

Extreme weather events (*e.g.* droughts, heavy precipitation events, heat waves, storms with increased wind speeds, tropical cyclones) can cause very effective pulse disturbances to ecosystems and species, additional to impacts from changes in long-term average conditions (Jentsch and Beierkuhnlein, 2008; IPCC, 2012). This might have particular relevance for oceanic islands due to their small ecosystems and (endemic) species' ranges, allowing for more comprehensive impacts compared to larger continental systems. However, only little information is available on climate and weather extreme shifts in oceanic island regions, which is due to a lack of long-term observational data and high uncertainties in projecting the frequency or strength of such anomalies in oceanic regions (IPCC, 2012).

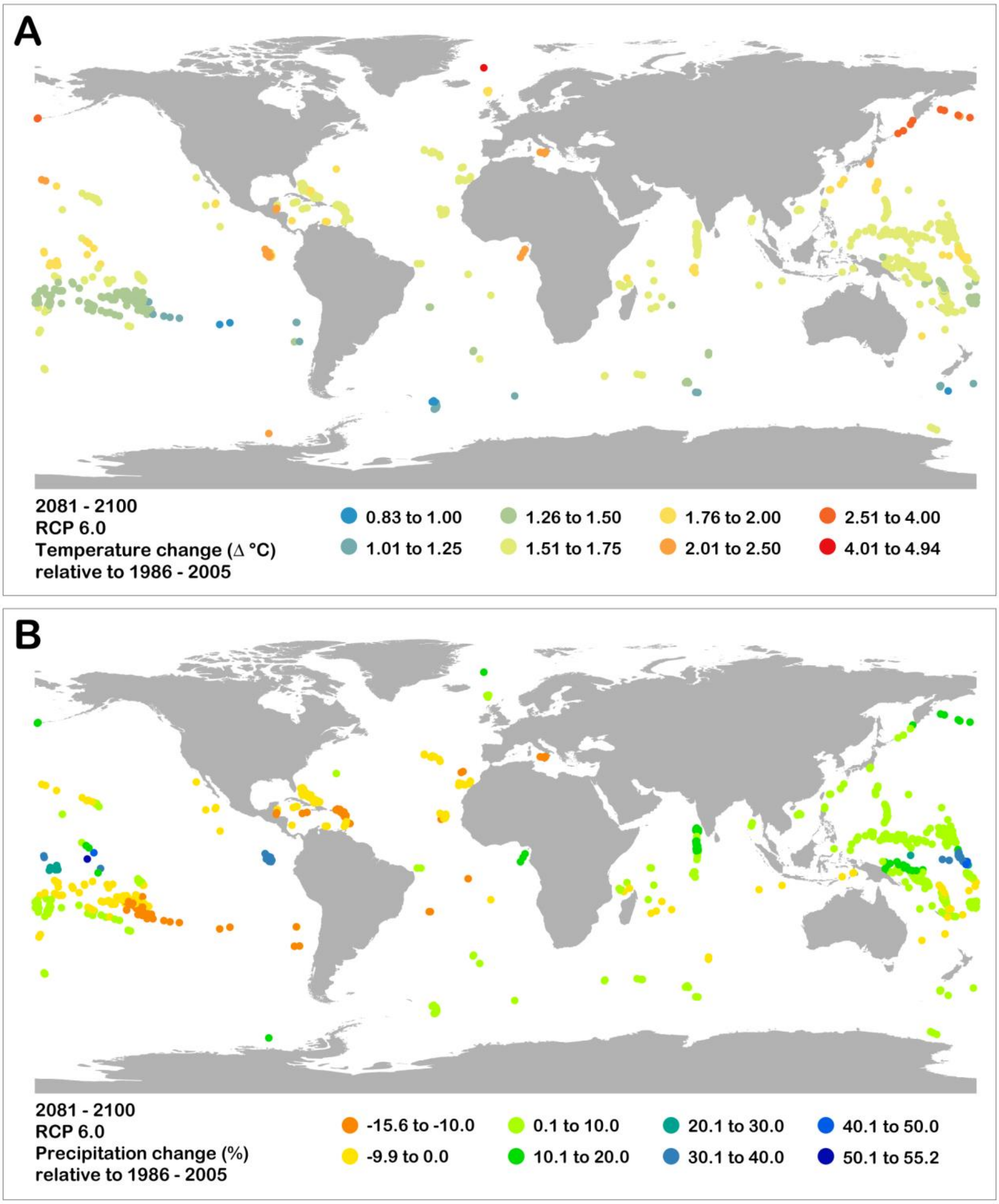

Fig. 2: Maps of (A) projected temperature change and (B) projected precipitation change on 787 oceanic islands for the end of this century (2081 – 2100) under the medium to high greenhouse gas concentration scenario RCP 6.0, based on regional median response projections over 25 climate models. Note that the scale subdivision for temperature change is not linear anymore beyond the 2 °C increase. Data were compiled from the IPCC AR5 publication and data download homepage (http://www.climatechange2013.org/report/full-report/).

Heavy precipitation events have showed a decrease in frequency since the 1950s in many Pacific regions, *e.g.* Hawaii (Chu *et al.*, 2010; Elison-Timm *et al.*, 2011), Guam (Lander and Guard, 2003) or Pohnpei Island, Micronesia (Lander and Khosrowpanah, 2004). Nevertheless, large scale projections for the 21st century indicate regionally highly variable trends for frequencies and intensities of heavy rain days as well as for drought events for large parts of the south-western Pacific (Australian Bureau of Meteorology and CSIRO, 2014). While there is high confidence for increasing frequencies of daily temperature extremes in almost any analysed region, considerable uncertainties remain in projections of precipitation extremes. Depending on the region and model, decreasing, stable and increasing extreme event frequencies and intensities have been projected, with very variable confidence statements (Australian Bureau of Meteorology and CSIRO, 2014). For the Indian Ocean, Cai *et al.*, (2014) found that anomalously dry condition events due to decadal occurrences of wind and oceanic current reversals (positive Indian Ocean Dipole events) will appear nearly three times more often in the 21st compared to the 20th century. This will probably result in higher frequencies of extremely high precipitation events in north-western parts of the Indian Ocean but negative rainfall anomalies especially in equatorial western and in tropical and sub-tropical regions of the southern hemispheric Indian Ocean.

Tropical cyclone activity and intensity especially in the Pacific is influenced by the ENSO (Chand *et al.*, 2013). Generally, surface temperatures and the resulting availability of more energy directly affect tropical cyclones. There is remaining uncertainty about the future frequency but large consensus that these storm systems (also known as hurricanes or typhoons) will increase in intensity (2–11 % by 2100; Knutson *et al.*, 2010) and shift polewards (Graff and LaCasce, 2012; see also Arozena Concepción *et al.*, 2008 for effects of a storm surge on laurel forests on Tenerife and Whinam *et al.*, 2014 for damage on the Macquarie Island endemic *Azorella macquariensis* by changing wind conditions). Besides tsunamis (which occur much less often), tropical cyclones are associated with the most extreme temporal sea level rises (Walsh *et al.*, 2012) and can strongly affect species composition and ecosystem dynamics on oceanic islands (Terry and Chui, 2012; Webb *et al.*, 2014).

Most of the overall biodiversity of oceanic islands is located on high elevation islands in the tropics and subtropics. On many of those islands, trade wind-induced orographic uplift of humid air masses and orographic cloud formation provide considerable direct or indirect (fog drip) precipitation (Scholl *et al.*, 2007) and thus account for (elevational) zonation of ecosystems, each with own species assemblages. However, cloud layer elevations and orographic cloud formation (and thus rainfall, solar radiation, temperature and humidity patterns on islands) depend on the trade wind inversion layer, which shows increasing variability in its occurrence and elevation (*e.g.* Cao *et al.*, 2007; Lauer *et al.*, 2013). Concomitant changes of precipitation distributions on these islands pose the most significant uncertainty for ecosystems and could be main drivers of biodiversity change on high oceanic islands (Loope and Giambelluca, 1998; Diaz *et al.*, 2011). Especially windward cloud forests with their high endemism do strongly depend on constant humid or wet conditions (Crausbay and Hotchkiss, 2010). Locally there are contrasting projections about whether the thermal inversion layer and the associated cloud formation is shifting upward (Still *et al.*, 1999) or downward (Sperling *et al.*, 2004; Lauer *et al.*, 2013), both of which would endanger highly adapted ecosystems. Again, the ENSO and fluctuations of the ITCZ have a large influence on the occurrence and height of trade wind inversions and have been shown to strongly affect regional climate variation and local plant life (Trueman and d'Ozouville, 2010; Crausbay *et al.*, 2014b).

Sea level rise

Rising sea levels due to thermal extension of oceanic water volumes and melting glaciers and polar ice caps probably pose the most apparent threats for flat islands like atolls but also for coastal plains of higher elevated islands. IPCC projections range from 0.26 m to 0.82 m with regional differences until the end of this century compared to the 1980 – 1999 period (IPCC, 2013b). Other estimates indicate sea level rises between 0.7 – 1.2 m (Horton *et al.*, 2014) and 0.75 – 1.9 m (Vermeer and Rahmstorf, 2009; Jevrejeva *et al.*, 2014) in high warming scenarios. Assuming a rapid melt of global ice-sheets, which is also reported for past global warming periods in the Pleistocene, even up to more than 6 m to the end of the century can be possible (Overpeck *et al.*, 2006). Also, recent satellite-based actual ice loss detections (McMillan *et al.*, 2014), updates of glacier retreat and ice drainage velocity in Antarctica (Rignot *et al.*, 2014) as well as new data on glacial valley depth and subaqueous glacier melting in Greenland (Morlighem *et al.*, 2014) suggest that sea level rise projections might have to be corrected upwards.

Landward shifts of shorelines and concomitant loss of habitat area would have consequences for coastal island ecosystems (Menon *et al.*, 2010; Wetzel *et al.*, 2012; Bellard *et al.*, 2014). Sea level rises will most likely lead to the total and permanent inundation of low elevation islands and island states (such as Kiribati, Maldives, Marshall Islands, Tuvalu; Wong *et al.*, 2005). Wetzel *et al.*, (2013) calculated for islands the Southeast Asian Pacific region that a sea level rise of 1 m leads to complete inundation losses of 14.7 % (61.7 % with 6 m sea level rise) of the considered islands, and that 18.9 % (20.5 %) of all islands would lose 50 – 99 % of their terrestrial area. For Oceania (with major Pacific island regions, including Hawaii) this would mean a 3.9 % (14.5 %) loss of island area in total (Wetzel *et al.*, 2013). It has to be noted though that the most affected low islands and atolls host comparably low numbers of endemics, which might limit direct effects of sea level rise to global biodiversity changes. However, affected islanders might have to translocate their settlements and land-use to higher regions (Wetzel *et al.*, 2012) or congregate on other (higher elevated) islands, potentially causing secondary effects to primarily unaffected regions.

Factors contributing to oceanic island plant vulnerabilities to global climate change

Oceanic islands vary in geographical, ecological and anthropogenic settings, which determine the specific risks and vulnerabilities towards different climate change impacts. In the following subchapters we discuss the most important factors and their interactions with climate change impacts regarding oceanic island floras. Tab. 1 gives a summary of the introduced concepts.

Vulnerability is defined here as the degree to which climatic changes are capable to result in significant (mostly negative) responses and modifications of a system, applying for individuals, populations, species, ecosystems or entire islands and regions, and being determined by the system's exposure to climate change impacts, its sensitivity to these impacts, and its capacity to respond (*e.g.* tolerate, adapt, migrate).

Table 1: Concepts of climate change vulnerability of island floras and species. Main factors, their potential influence on or interaction with climate change impacts, as well as the underlying mechanisms are depicted, respectively. In the second column the potential effects of the respective factor are shown, either lowering climate change impacts on ecosystems and species (+) or increasing vulnerability and risks (–). Additionally, important references and research potential on the different issues are given. SDMs: species distribution models; VAs: viability analyses (populations and/or species).

Factor	**Potential influence or interaction**	**Effect on climate change impacts on plants**	**Framework references / Consultable research**	**Research potential for species vulnerability estimation**
Island area	Habitat area and diversity +	Potential refuge habitats and habitat space for range shifts	Foufopoulos *et al.*, 2011: lizards on Greek islands; Biber, 2002: extinction and threatening patterns of bird species	Palaeoecological and palynological comparisons of past climatic change effects among islands of different sizes, phylogeographic analyses, SDMs
	Carrying capacity +	Large populations less vulnerable to stochastic events and inbreeding; more resilient/adaptive to environmental changes	Frankham, 1998; Ditto and Frey, 2007; Gillespie *et al.*, 2008	VAs of oceanic islands endemic species and populations (including genetic characterisation, *e.g.* bottleneck detections) comparative for different island types
Island age and geology	Elevation +	Less inundation and coastal erosion, less impacts from saltwater intrusion into groundwater	Woodroffe, 2008; Ross *et al.*, 2009; Mandal and Zhang, 2012; Rotzoll and Fletcher, 2012; Forbes *et al.*, 2013	Combination of comparative geomorphological and geohydrological analyses with species distributions
	Elevation +	Potential upward range shift target areas	Biber, 2002: extinction and threatening patterns of bird species	SDMs
	Geological heterogeneity, topographical structuring +	Amount of potential micro-refugia	Ashcroft *et al.*, 2009: general micro-refugia identification; Fortini *et al.*, 2013 for Hawaii	Comparative fine scale SDMs, micro-refugia identification, palaeoecological and palynological comparisons of past climatic change effects

Ecological complexity	Species richness, functional redundancy and ecosystem resilience +	Stable ecosystem processes, less extinctions	Cushman, 1995; van der Putten *et al.*, 2010; no research on climate change vulnerability of island ecosystems according to ecosystem complexity yet (but see Smith and Steenkamp, 1990)	Species removal/addition experiments, use of "natural experiments" (*e.g.* extreme events), understanding of ecosystem processes and potential resilience
	Ecosystem interactions –	Negative influences from proximate ecosystems, potential co-degradations	Polis and Hurd, 1996; Anderson and Polis, 1999; Gauthier *et al.*, 2011 for continental islands	Understanding of oceanic island ecological systems and potential effects of ecotones-crossing climate change impacts
	Species richness – / +	-: Community interaction challenges for shifting species, dispersal/establishment filters +: Functional redundancy for species interactions	Caplat *et al.*, 2008; Roux and McGeoch, 2008; Hsu *et al.*, 2012; Norberg *et al.*, 2012 and Urban *et al.*, 2012 for general models of competition effects between resident and range shifting species; Gilman *et al.*, 2010; Aslan *et al.*, 2013	Comparative SDMs of interaction partner species, experimental approaches on potential new species combinations, understand effects of recent species range shifts
Species characteristics	Niche size / habitat specialist –	Low tolerance against environmental change, low range shift capability, high risk of extinction	Frankham, 1997; Jansson, 2003; Roux and McGeoch, 2008; Fortini *et al.*, 2013; Krushelnycky *et al.*, 2013	Climate experiments, field studies on range shifts, comparative SDMs
	Dispersal capability +	High dispersal capacity enables species to rapidly track climatic alterations to new habitats	Fordham and Brook, 2010; Fortini *et al.*, 2013	Data on dispersal capabilities are needed (fruit set analyses, gene flow analyses); inclusion of dispersal capability estimates or of different dispersal capability scenarios into SDMs
	Short life cycles, genetic variation +	High dispersal rates, adaptational potential, lower genetic risks (inbreeding, genetic drift)	Díaz and Cabido, 1997; Jump and Peñuelas, 2005; Jump *et al.*, 2009; Buckley and Kingsolver, 2012; Shapcott *et al.*, 2012	Population genetic studies, evolutionary studies (*e.g.* screening for climate-relevant genes and tests for adaptability)
	Dependence on mutualistic interactions –	Dependence on persistence or co-migration of interaction partners	Memmott *et al.*, 2007; Tylianakis *et al.*, 2008; Kaiser-Bunbury *et al.*, 2010; Aslan *et al.*, 2013	Understanding of species interactions and their susceptibility to climatic change effects; incorporation of interaction partners and their threats in SDMs
Human influences	Prior and parallel human impairments on ecosystems and species –	Higher prior vulnerability towards climatic changes; interactions between climate change impacts and additional stressors	Brook *et al.*, 2008; Caujapé-Castells *et al.*, 2010; Fordham and Brook, 2010; Fortini *et al.*, 2013; Vorsino *et al.*, 2014	VAs; understanding of interactions of human disturbance and climate change impacts; assessments of future human land use; link of SDMs for invasive neobiota and endemic species SDMs
	Human climate refugees and climate-induced land use or subsistence changes –	Further habitat destruction, fragmentation, degradation	Fordham and Brook, 2010; Wetzel *et al.*, 2012	Combinations of SDMs, inclusion of human population and land use forecasts and scenarios in risk studies
	Conservation measures +	Decreased extinction risk	Küffer *et al.*, 2007; Caujapé-Castells *et al.*, 2010; Fordham and Brook, 2010	Understanding of conservation potential and possibilities in the face of global climate change on small islands

Island area

The role of area and habitat space for intra-island range shifts

Species' distributions and abundances depend on climatic factors, forcing species to track their climatic demands by range shifts during periods of climate changes (Parmesan, 2006; Thomas, 2010). The most fundamental prerequisite for successful dispersal is the availability of suitable habitats including adequate substrate or biotic interactions (*e.g.* pollinators), and without anthropogenic restrictions (*e.g.* fragmentation, settlements, land use). Referring to the large-scale climatic changes and the comparably small spatial extent of islands, the opportunities for island species to shift their ranges in climate-relevant latitudinal (or longitudinal) extents and to maintain population size and genetic variability are very restricted. Nevertheless, the existence of old (pre-Pleistocenic) relict species (*e.g. Laurus novocanariensis* and *Woodwardia radicans* on the Macaronesian Islands) and neo-endemic lineages document the long-term persistence of island populations (see also Fernández-Palacios *et al.*, 2011). Larger islands often contain more habitat space and diversity, likely providing more spatial and ecological opportunities for species to escape unfavourable conditions than small islands (see Triantis *et al.*, 2003 and Kisel *et al.*, 2011 for general consideration of area and habitat diversity).

Large islands also tend to have higher elevations than small islands (Weigelt *et al.*, 2013). Referring to rising temperatures, upward shift on mountainous elevations can provide compensation of lost habitats. However, area declines with elevation, wind speed may increase, and precipitation patterns may differ from original habitats, which makes the finding of suitable conditions in high elevations uncertain for species from lower zones. It has been shown that water stress is an important determinant of high elevation species distributions on oceanic islands (*e.g.* Brito *et al.*, 2014; Crausbay *et al.*, 2014b). Considering strong temperature increases in high elevations (Giambelluca *et al.*, 2008; Keener *et al.*, 2012), stable or decreasing moisture conditions in the future thus can powerfully restrict upslope migration, generating great vulnerabilities of high elevation island species to climate change (Crausbay *et al.*, 2014b). Also, on many islands in the tropics and subtropics, trade wind inversion layers create a strong elevational zonation in precipitation, including a sharp transition on the windward sectors from very humid conditions in cloud contact elevations to dry conditions above those cloud banks. These ecotones potentially act as barriers for plant species' upward shifts.

Island area shaping population sizes

An island's carrying capacity for a particular species (*i.e.* its maximum population size) is determined, among other factors, by habitat availability, which in turn is a function of the area of the island. Therefore, island area controls overall population sizes and, thus, the response or adaptation capacity of populations (Hanski, 2010). Small populations are more exposed to risks of decline or extinction than larger populations, mainly due to demographic and environmental stochasticity (Gilpin and Soulé, 1986; Marrero-Gómez *et al.*, 2007; Caujapé-Castells *et al.*, 2010). Also, low genetic variability and an associated limitation of adaptational potential (Willi *et al.*, 2006), higher inbreeding rates (homozygosity, genetic erosion) and higher impacts of genetic drift may cause instability in small populations (Ellstrand and Elam, 1993; Frankham, 1997; Schaal and Leverich, 2005). In consequence, rare or spatially very restricted species are expected to be more vulnerable to climate change impacts (Ditto and Frey, 2007; Williams *et al.*, 2008; Thomas *et al.*, 2011; Fortini *et al.*, 2013). This suggests that the area of an island correlates negatively with vulnerabilities of inhabiting plant species to climate change, especially for single island endemics. However, small islands host few endemic plants (often none at all, but see Junak and Philbrick, 1999; Florens and Baider, 2006), limiting their general contribution to potential global biodiversity loss. It should also be mentioned that rarity (*i.e.* small population sizes) does not necessarily imply proneness to extinction (Gaston, 1994; Mace and Kershaw, 1997). Some naturally rare species have evolved resistance to inbreeding and mutational load and show traits that can be seen as adaptations to conditions and processes associated with their rarity, *e.g.* increased self-compatibility or asexuality, reduced dispersal or specialised floral traits (Karron, 1997; Orians, 1997).

Island origin and topography

Geological, topographic and edaphic structuring

Although originally based on volcanism, oceanic islands embrace diverse island types with different geological origination. Generally, the following formation histories are shared: (i) volcanic birth and submarine formation of a seamount, (ii) further growth by lava eruption above the sea level, (iii) reaching of maximum elevational extent dependent on duration and intensity of volcanic activity and expiration of the (main) volcano, (iv) subsequent erosion and radial drainage channels, (v) ongoing flattening and reduction to a low-altitude plain and (vi) terminal decline and subsidence beneath sea level to shape a guyot (Menard, 1986; Nunn, 1994; Fernández-Palacios *et al.*, 2011). In tropical regions, coral accretion is likely to occur in the island periphery during the last stages and even after submergence, and atolls or reef islands will originate as secondary islands (Menard, 1986; Nunn, 1994; Forbes *et al.*, 2013). With the exception of tectonically raised atolls (*e.g.* Makatea, Tuamotus; Aldabra, Seychelles or Henderson Island, Pitcairn Islands) and emergent limestone islands (*e.g.* Bahamas), atolls and most reef islands exhibit little topographical structures and are less heterogeneous in terms of habitat types. However, structural heterogeneity, depending on the geological composition, elevation, topography, soil characteristics and zonation along different geographic and ecological gradients of an island increases the diversity of micro- and mesoclimatic opportunities, habitats and ecosystems (Fernández-Palacios and Andersson, 2000; Whittaker and Fernández-Palacios, 2007). In turn, structural heterogeneity on elevated islands will likely decrease the vulnerability to climate change impacts by providing micro-refugia for endangered plant populations (Fig. 3). For example, mesic plants may evade increases of temperature and/or decreasing moisture conditions by finding sheltering habitats in gorges or at cloud-affected locations in higher elevations as indicated for droughts during the Quaternary on Santa Cruz, Galápagos (Collins *et al.*, 2013). Additionally, specific geomorphological and micro-climatic conditions have generated different ecological and evolutionary histories among islands types (Stuessy *et al.*, 2006; Stuessy, 2007; Whittaker *et al.*, 2008), implying very different species adaptations and variable (but unknown) ecological tolerances and response capacities towards climatic shifts.

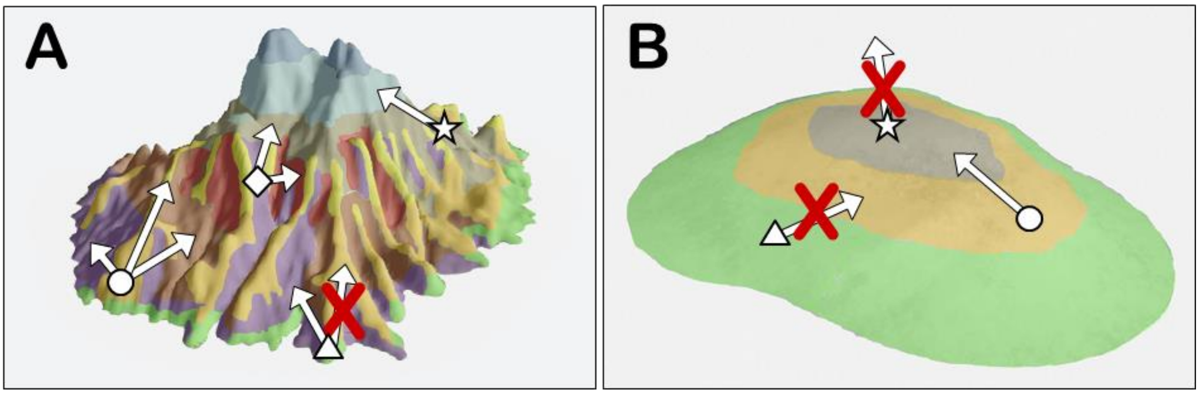

Fig. 3: Effects of island elevation, topography and habitat diversity on range shift potentials of plant species. Habitat types are symbolised by different colours; arrows indicate migration into new habitats in response to climatic alterations. Red crosses symbolise impossibility of an indicated migration into a certain target habitat due to ecological or spatial constraints (*e.g.* predominant competitor species, lacking interaction partners, absence of specific soil properties, etc.). On highly elevated and topographically structured islands with greater habitat diversity (A), plant species have different options to find adequate micro-refugia (note movements into different types of habitat). On flat and less structured islands (B) many species will face limited possibilities to find adequate and accessible micro-refugia. Insuperable dispersal or establishment filters in a certain direction, or lacking higher elevations might cause problems to species if they fail to adapt to the novel climatic conditions in their original habitats.

The role of elevation

Sea level rise during the 21st century results in increased coastal erosion and temporal or permanent inundations of flat coastal zones or even entire islands and facilitates salt water intrusion into island groundwater (Wong *et al.*, 2005; Woodroffe, 2008; IPCC, 2012; Terry and Chui, 2012; Nurse and McLean, 2014). Degradation and subsidence of whole islands and substantial habitat loss in low-elevated areas can be expected, potentially resulting in extinctions of endemics if upward shifts of species are not possible (Heywood, 2011; IPCC, 2012; Bellard *et al.*, 2014).

Steep coastlines built of firm rock are less affected by rising sea levels or substantial wave erosion (Forbes *et al.*, 2013). However, higher elevated oceanic islands can also suffer from sea level rise if coastal plains or terraces lie within the elevational range of rise. Accordingly, it has been shown for O'ahu and Maui (Hawaii) that considerable areas in coastal plains are under risk from direct (marine) flooding, and even more from groundwater inundation, *i.e.* localised flooding due to sea level-driven uplift of groundwater tables (Rotzoll and Fletcher, 2012; Cooper *et al.*, 2013). Although indicated for urban regions, the risk is also apparent for natural areas, including critical habitats and protection areas (Kane *et al.*, submitted for publication). However, if affected urban areas cannot be adaptively protected from inundation, secondary effects on hinterland ecosystems are possible due to potential habitat destruction by settlement relocations (*e.g.* Wetzel *et al.*, 2012).

Increases in sea levels, solely and in combination with storm surges, are projected to affect freshwater lenses on islands (Kundzewicz and Döll, 2009; Terry and Chui, 2012). This will have serious effects on flat atolls and reef islands or on coastal plain ecosystems where saltwater intrusion can affect upper soil water quality for root water uptake (*e.g.* Spennemann, 2006; Ross *et al.*, 2009; Greaver and Sternberg, 2010). Consequently, ecosystem degradation and plant population declines due to climate change-driven changes of water supply and quality are less likely on elevated volcanic oceanic islands where freshwater aquifers are more salt water-independent and a larger and more resistant groundwater body is present (Menard, 1986; Kundzewicz and Döll, 2009). However, high elevation islands may also be threatened by saltwater intrusions if precipitation patterns change and groundwater recharge decreases. This can result in upward shifts of the fresh groundwater-saline water boundaries as indicated for Rishiri Island, Japan as a consequence of changing snow fall amounts and altered temporal snowmelt patterns (Mandal and Zhang, 2012).

High elevation islands (like most Hawaiian islands, the Marquesas, some Macaronesian Islands, Society Islands, Samoa, Fiji, Granitic Seychelles, etc.) can induce barrier effects on rain clouds and orographic cloud formation and hence often generate higher precipitation and more freshwater for their ecosystems than low islands (Basist *et al.*, 1994; Scholl *et al.*, 2007). In the course of climate change, potential shifts of cloud layer altitudes might result in modified elevational zonation of vegetation types and species assemblages, *e.g.* of cloud forests, including species losses (Loope and Giambelluca, 1998; Still *et al.*, 1999; Sperling *et al.*, 2004; Scholl *et al.*, 2007; James, 2008; Lloret and González-Mancebo, 2011). It is yet unclear if and how cloud layer altitudes will respond to climate change and how this could differ regionally. Cao *et al.*, (2007) found only weak and inconsistent (slightly increasing to slightly decreasing) trends of altitudinal change of the trade wind inversion in Hawaiian Islands in observational data for the period 1979–2003. Nevertheless, recent downscaling of 10 CMIP5 models for the two warming scenarios RCP4.5 and RCP8.5 done by Lauer *et al.*, (2013) indicates a significant and robust downward shift of the trade wind inversion in the Hawaiian region until the end of the century (2090–99 compared to 1990–99). In RCP4.5 experiments, elevational changes of the trade wind inversion ranged between 12 and –167 m (multimodel mean –52 m), while in RCP8.5 experiments a decrease between –46 and –355 m (multimodel mean –157 m) was projected (Lauer *et al.*, 2013). Increased occurrence of low-level clouds in the past and future downward trends of cloud layer altitudes are also indicated for the Canary Islands with modelled cloud base level shifts of 15 to –58 m (Sperling *et al.*, 2004). Crausbay *et al.*, (2014a) suggested a subsidence of the trade wind inversion during periods of increased ENSO frequencies in the past three millennia for Maui, Hawaii. Decreases in cloud base altitudes may suggest that humid-adapted ecosystems can extend their range downslope where more area is available, resulting in a beneficial situation. However, on islands with elevations above the cloud layer, observations of drying conditions across the trade wind inversion (Sperling *et al.*, 2004) and projected trade wind inversion subsidence (Lauer *et al.*, 2013) imply a future loss of suitable habitat in the upper ranges of cloud contact-dependent species and ecosystems and may reduce the possibility of elevational movements to keep within thermal limits of species ranges. Also, potential soil characteristics constraints or competing human land use might hinder downslope migration, meaning that lower cloud layers do not necessarily mean a benefit for species of humid-adapted ecosystems (Sperling *et al.*, 2004). However, for dry-adapted high elevation endemics living beyond the trade wind inversion, subsidence of cloud layers might increase potential habitat ranges.

In contrast to downward shift projections, indications for cloud layer upward shifts under warming conditions and associated drastic changes in precipitation are reported for past glacial cycles (Suchodoletz *et al.*, 2010). Higher cloud layers (upward shifts of up to over 200 m) were also projected for some tropical montane cloud forest sites on continents under increased CO_2 concentrations and climate change conditions (Still *et al.*, 1999). In such scenarios, humid-adapted species might profit from the provision of valuable habitat and temperature conditions for upward shifts in a warming climate. Concomitantly, this would cause risks for dry-adapted high elevation ecosystems above the primary cloud contact elevation (*e.g.* above trade wind inversion layers) due to increasing humidity and invasion of upward-shifting humid-adapted species and ecosystems, as indicated in climate change scenarios for Madeira (Cruz *et al.*, 2010). It is also imaginable that some islands could completely lose humid elevational zones that depend on cloud contact if cloud layer shifts above the island's maximum elevation occurred (potentially applying for some mid-elevation islands, *e.g.* Pohnpei, Kosrae Rapa Iti, Lord Howe and others).

Ecological complexity

Lacking functional redundancy

Generally, biotic interactions, niche overlap, resource competition and facilitation effects between sympatric species, which are more probable to occur with high species numbers, are factors that can facilitate ecosystem resilience to perturbations and increasing environmental variability (Chapin, III *et al.*, 1997; Yachi and Loreau, 1999; Beierkuhnlein and Jentsch, 2005; Hooper *et al.*, 2005).

Compared to continental ecosystems, oceanic islands have relatively low species numbers, simple biotic community structures and can be characterised as 'closed communities' with limited biotic exchange (Whittaker and Fernández-Palacios, 2007; Gilman *et al.*, 2010). Thus, changes in the abundance of single members of functional groups (*e.g.* pollinators, diaspore dispersers, herbivore control) can affect the structure and functional integrity of an island's ecosystem more strongly than is the case in more species-rich continental systems. According to this lack of functional redundancy, the ecological integrity of island ecosystems is relatively fragile. Concomitant to that, a higher proportion of species acts as 'keystone' or 'umbrella' species. Alterations on these species' abundances or functional performance will have dramatic effects for island species (Cushman, 1995; Aslan *et al.*, 2013; Boyer and Jetz, 2014).

Several examples are documented where extinctions of single bird species induced co-extinctions of a number of endemic plant taxa on oceanic islands due to lost mutualism (*e.g.* Cox and Elmqvist, 2001; Fall, 2005; Kingston and Waldren, 2005). Regarding the great role of birds on oceanic islands as pollinators and dispersers, Şekercioğlu *et al.*, (2004) projected that by 2100, 28 – 56 % of all native bird species on oceanic islands worldwide might be functionally extinct (*i.e.* without appreciable contribution to ecosystem processes, see also Boyer and Jetz, 2014). One specific risk from climate change comes from the facilitation of spread and transmission of introduced avian malaria for which endemic birds of oceanic islands often lack adaptation (Garamszegi, 2011). With rising temperatures, spread of the avian malaria mosquito vector to the high elevation thermal refugia of affected birds, as indicated for Kaua'i, Hawaiian Islands (Atkinson *et al.*, 2014), can further diminish their remaining diversity and ecological functions (pollination, diaspore dispersal, herbivore predation, etc.). At least in such high elevation refuge regions, which often also harbour endangered plant endemics, the loss of ecological services from birds might cause indirect climate change impacts for depending plant species.

It is likely that abundances, spatial distributions, behaviour and competitive capacities of species that interact with plants will be altered by climate change impacts, including potential extirpations (Aslan *et al.*, 2013). Trophic and competitive balances or demographic proportions in mutualistic interactions might be changed, thus affecting community structures, ecosystem functioning and therefore, single plant species (Jump and Peñuelas, 2005; Roux *et al.*, 2005; Gilman *et al.*, 2010; Cahill *et al.*, 2013). Various ecological interactions have been shown to be vulnerable to climatic changes, which likely can be transferred to oceanic island ecosystems: Dependence on single pollinator species and phenology shifts (Memmott *et al.*, 2007) or species declines (for mutualism disruptions on oceanic islands see Caujapé-Castells *et al.*, 2010, Kaiser-Bunbury *et al.*, 2010; Aslan *et al.*, 2013), expiration of control of herbivore or parasite populations by particular predators (Wilmers *et al.*, 2006; Spiller and Schoener, 2007; Thomson *et al.*, 2010), alteration of plant community structures essential for microclimate (Roux *et al.*, 2005; Royer *et al.*, 2011), lack of maintenance of substrate characteristic by soil organisms, bioturbation or nutrient decomposers (Smith and Steenkamp, 1990; Blankinship *et al.*, 2011) and pest and disease facilitation induced by climatic alterations (Pautasso *et al.*, 2010; van der Putten *et al.*, 2010).

Depending on the differences in species richness, ecological complexity and functional redundancy of plant interaction partners, impacts of climatic alterations on native floras will differ among oceanic islands. Islands of higher numbers of ecologically closely related species will undergo less climate change-induced extinction cascades than species-poor islands of low functional redundancy. Trøjelsgaard *et al.*, (2013a) showed that plant specialisation on particular pollinator species increases with island age, potentially indicating a higher vulnerability to co-modification. However, rescue from lacking functional redundancy can come from alien species. Neobiota may integrate themselves into local ecosystems and build up novel interactions. This may lead to dependable performances of mutualistic interaction services or even prevent species from co-extinctions (Kaiser-Bunbury *et al.*, 2010; Traveset *et al.*, 2013).

Species richness and migration within islands

Species have to bear up against challenges of new habitats during migratory shifts. Often this means lacking ecological interaction partners that did not follow, for example, adequate tree hosts for epiphytes (Hsu *et al.*, 2012). Another challenge is an altered competitive setting for the shifting species as well as for the species in the receiving community (Caplat *et al.*, 2008; Lavergne *et al.*, 2010). This can be a deleterious or at least strongly filtering factor (Norberg *et al.*, 2012; Urban *et al.*, 2012; Corlett and Westcott, 2013). Species under climatic range shift pressure and potentially resulting new ecological interactions along migration routes, including competition and exclusion, can be assumed to increase with species number on an island. This means that islands rich in species may hold more biotic filters for potential climate change-induced migrations into new habitats than species-poor islands. However, filtering functions of biodiversity in target areas of shifting species are widely unclear (see Corlett and Westcott, 2013 and references therein). Further on, invasibility of ecosystems by migrating plant species could change due to changing disturbance regimes like wildfire frequency (Wong *et al.*, 2005; James, 2008, see also Angelo and Daehler, 2013) or new forest gap dynamics due to more frequent and/or severe tropical storms (*e.g.* Arozena Concepción *et al.*, 2008; Graff and LaCasce, 2012). In rare cases, asynchronous range shifts also might offer chances for newly co-occurring species to benefit from each other by being driven into new ecological interactions and micro-evolutionary trajectories (Corlett and Westcott, 2013). For example, novel pollinator interactions (see *e.g.* Hembry *et al.*, 2013) or new mycorrhiza alliances could evolve. In the long run, trait adaptations to the new environmental conditions might occur (Lavergne *et al.*, 2010; Hoffmann and Sgrò, 2011; Franks and Hoffmann, 2012).

So far, empirical evidence for novel species interactions induced by climate change-driven range shifts, including establishment rates, the development of novel ecosystems or novel evolutionary pathways of affected species, has not been provided for oceanic islands. Nevertheless, novel community compositions according to species-specific variation in temperature rise-mediated upward range shifts are evidenced by le Roux and McGeoch (2008) on Marion Island.

Interactions between ecosystems

Ecosystems interact with each other especially via matter and energy flows and organismic exchanges (Gauthier *et al.*, 2011 and references therein). On some oceanic islands, the integrity of high elevation forests is an important factor for the hydrologic budget and discharge to lower elevational zones (Izquierdo *et al.*, 2011; Pryet *et al.*, 2012). These ecosystems are especially vulnerable to climate change (*e.g.* by shifting precipitation regimes, see above; Loope and Giambelluca, 1998; Foster, 2001;

Sperling *et al.*, 2004). Erosion control by vegetation cover in mountainous zones is also an essential ecotone-crossing service for lowland ecosystems. On some islands mangrove belts protect shorelines and coastal plains from coastal erosion and interact with coral reefs and other marine ecosystems, which in turn deliver their own suite of services to an island`s ecological (and human socio-economic) processes (Field, 1995; Barbier *et al.*, 2011).

Interactions between ecosystems are of relevance for the persistence of their species. However, resource flows and trophic connections across ecotones are themselves vulnerable to climatic alterations. For example, nutrient allocation by piscivorous sea birds affects coastal plant communities (Polis and Hurd, 1996; Anderson and Polis, 1999). Such communities are dependent on the abundance of bird prey, which can be affected by climatic alterations (*e.g.* Roessig *et al.*, 2004; Harley *et al.*, 2006). Decomposition of hurricane-deposited seaweed also increases nutrient availability for coastal plants (Spiller *et al.*, 2010), indicating a potential effect of increasing hurricane frequency spreading across ecosystems. Therefore, ecosystem interdependencies must be included when considering possible climate change impacts (*e.g.* Gauthier *et al.*, 2011). Especially floras of islands rich in ecological gradients and ecotones, such as high elevation volcanic islands, might receive ecosystem co-modifications. Species living in primarily unimpaired ecosystems thus can be at risk from climate change impacts on interacting ecosystems.

Species characteristics

The risk to sustain negative impacts or to become extinct from climate change is not equally distributed among species (Díaz and Cabido, 1997; Buckley and Kingsolver, 2012; Fortini *et al.*, 2013). It rather varies with specific external (*e.g.* affiliation to specific island types, regional type and strength of climatic alterations, ecosystem and biotic interaction dynamics) and inherent factors (*e.g.* species traits, eco-physiological tolerance, plasticity, dispersal capability). Island endemics are especially affected due to their limited opportunities to escape unfavourable conditions.

Spatial distribution and environmental niche

Endemic plant species are not only geographically isolated but are often also strongly bound to certain environmental conditions (Kruckeberg and Rabinowitz, 1985; Hermant *et al.*, 2013). At the global scale, high levels of endemism are associated to stable climatic conditions since the last glacial maximum (Jansson, 2003). Many island endemics have a small distribution and are confined to specific climatic and edaphic conditions and realise narrow ecological niches (Bramwell, 2011), which generally increases their risk of extinction under changing climatic conditions. For example, *Cheirolophus junonianus*, a single island endemic of La Palma (Canary Islands), is restricted to a particular rocky outcrop and counts only about a hundred individuals (Garnatje *et al.*, 1998). Its area of distribution was minimised to its present extent by recent volcanic activity changing the surrounding soil conditions and geologic properties and thus prohibiting a recuperation of its range to its previous extent (Garnatje *et al.*, 1998). However this also prohibits suchlike species from migrating to track changing climates within their islands, making them highly dependent on more or less consistent habitat conditions, high eco-physiological tolerances, or on rapid evolutionary adaptation. Also, cloud forest species on island mountain tops probably are threatened by their ecological restrictions to very specific, stable and spatially restricted climatic conditions. Even if some adaptive potential is existent, changes in precipitation (or cloud contact) and temperature could alter growing conditions beyond the tolerances of these species (see *e.g.* Shapcott *et al.*, 2012 for the palm species *Lepidorrhachis mooreana* endemic to one single mountain top on Lord Howe Island).

Endemic species are not necessarily specialists on their respective islands. Rather, they can represent ‘keystone’ species in their inhabited insular environments and ecosystems, often dominating vegetation composition and structure. On all Hawaiian islands, the endemic *Metrosideros polymorpha* is the predominant tree species, being distributed from sea level to the tree line at around 2500 m asl., and along a mean annual precipitation gradient ranging from less than 400 to more than 6000 mm (Cordell *et al.*, 1998). *M. polymorpha* is capable of morphologically and physiologically adapting to a large variety of environments (Cordell *et al.*, 1998). Similarly, the endemic Canary Pine (*Pinus* *canariensis*) is in large parts of the Canary Islands virtually the only occurring tree species. Potentially it occupies about 15 % of the total archipelagic area (del Arco Aguilar *et al.*, 2010). The species can tolerate fire and in addition, its long needles can comb out water from passing clouds, improving the water supply for the ecosystem (del Arco Aguilar *et al.*, 2010). Generalist species are less likely to be threatened by climatic alterations, as indicated in several studies of continental plant species (*e.g.* Thuiller *et al.*, 2005; Broennimann *et al.*, 2006), but also in comparative modelling of Hawaiian species' niche projections (Fortini *et al.*, 2013). Supporting this, le Roux and McGeoch (2008) have found higher rates of upward shift in generalists related to rising temperatures since 1966 on Marion Island, contrasting to specialist species with lower opportunities of finding appropriate habitats. However, it is not clear yet, if generalist species might even be facilitated by climatic alterations on oceanic islands (*e.g.* range extractions or increases in abundance)

The relative contribution of endemic species increases with elevation (Steinbauer *et al.*, 2012), owing to geographical and environmental isolation to areas with comparable conditions (Steinbauer *et al.*, 2013). High elevation ecosystems on oceanic islands are small, rare and isolated, yet they significantly contribute to the endemic species richness of a given island and are often still undisturbed. However, these ecosystems are also particularly threatened by climate change. An example from Maui on the Hawaiian archipelago shows that the abundance decline of the iconic silversword *Argyroxyphium sandwicense* ssp. *macrocephalum*, which is restricted to the highest parts of the Haleakala crater, is associated with decreasing precipitation (Krushelnycky *et al.*, 2013). Consequently, the future outlook for this species is bleak as drought conditions in the associated alpine ecosystem are projected to increase. Similarly, the high-elevation single island endemic *Helianthemum juliae* found on Tenerife, Canary Islands is highly at risk of extinction if precipitation decreases as predicted (Marrero-Gómez *et al.*, 2007). Marrero-Gómez *et al.*, (2007) even suggest translocating this species to more humid areas of the island to ensure its survival.

Indeed, alpine insular ecosystems and their specialised species are especially endangered by climate change due to changes in precipitation patterns (Sperling *et al.*, 2004; Cao *et al.*, 2007; Diaz *et al.*, 2011), disproportional high temperature increase (Giambelluca *et al.*, 2008; Diaz *et al.*, 2011) and the fact that further upward shift is not possible for species already at the highest elevations. Furthermore, a study from Taiwan (Jump *et al.*, 2012) suggests that potential climate change-related upward shifts of plant species from lower elevational zones into summit ecosystems might threaten high elevation endemics on islands. However, for oceanic islands, indications for substantial changes in elevational zonation of ecosystems in summit areas are still lacking.

Life history effects on migration and adaptation abilities to climatic changes

Several species-specific life history traits are likely to influence a given species predisposition to cope with climatic changes. One of the striking trends in island endemics is the tendency towards decreased dispersal capabilities (*e.g.* Carlquist, 1974; Whittaker and Fernández-Palacios, 2007; Bellemain and Ricklefs, 2008, but see also Vazačová and Münzbergová, 2014). However, the capability to quickly reach adequate new growing sites when the original habitat conditions change to unsuitability is of fundamental importance for climate change-induced range shift of endemics (Fordham and Brook, 2010). Species with poor dispersal capability will have trouble tracking rapidly increasing temperatures or changes in precipitation patterns (Roux and McGeoch, 2008; Bramwell, 2011). Consequently, endemics will have to migrate upward within an island following the temperature isocline at an adequate speed, diminish to refugia (*e.g.* sheltering gorges), or, if possible, move to small-scale micro-refugia "around the bend" (*sensu* Scherrer and Körner, 2009; see also Fig. 3). Indeed, le Roux and McGeoch (2008) demonstrated species-specific responses in the flora of a sub-Antarctic island to increased temperatures during the last 50 years. They found novel communities with non-analogue species assembly at mid and high elevations due to different range expansion rates among species. Species abundance increases and compositional changes over the last 80 years have also been demonstrated by Kapfer *et al.* (2012) on the sub-Arctic island of Jan Mayen.

Furthermore, it is plausible that species' life cycles influence their ability to cope with climatic changes, regarding both range shifts and local persistence. Generation times vary considerably in island floras. Many island floras are characterised by the longevity of endemic woody species belonging to genera or families that are dominated by herbaceous species (Carlquist, 1974), which often correlates with prolonged individual generation times (Bramwell, 2011). Carlquist (1974) argues that the increased longevity resulting in island woodiness is driven by the adaptation to comparably stable past island climates and low seasonal fluctuations. Increasing extreme events and climatic variation on islands (Mimura *et al.*, 2007; IPCC, 2012; Nurse and McLean, 2014) thus may select negatively for island endemics adapted to stable environments (Bramwell, 2011). In contrast, short life cycles, mediated by higher reproductive rates, are advantageous according to dispersal capacities and for evolutionary adaptations to altering environmental conditions (Jump and Peñuelas, 2005; Donnelly *et al.*, 2012). However, the proportion of annual species in island floras is relatively small, *e.g.* only roughly 7% of the endemics of the Canary Islands are annuals (Shmida and Werger, 1992).

Adaptation to changing climatic conditions is more difficult to assess than range shifts, although not less important (Jump and Peñuelas, 2005). Climatic alterations have repeatedly taken place on most oceanic islands and the majority of existing species have endured these environmental changes. According to the special nature of isolated oceanic islands this evidently shows that adaptational processes must have occurred. However, in comparison to Tertiary and Quaternary climatic shifts and oscillations, which have resulted from geological and geophysical processes, the recent anthropogenic changes are proceeding much more rapidly (Diffenbaugh and Field, 2013), making species' adaptations in necessary rates difficult if not impossible (Huntley, 1991; Jump and Peñuelas, 2005). This is especially true for oceanic island plants due to two main reasons. First, numerous co-occurring (anthropogenic) stressors and impairments are causing additional pressure. Second, island species tend to have low genetic variability (Frankham, 1997; Johnson *et al.*, 2000; Crawford *et al.*, 2001; Stuessy, 2007; Stuessy *et al.*, 2013), potentially contributing to low adaptive capacities by lacking 'option values' *sensu* Jump *et al.* (2009). In combination with reduced dispersal capability, those two aspects are likely to disproportionally increase threats and extinction risk of many endemics facing global climate change. However, some endemics on very isolated and highly dynamic islands show surprisingly large intraspecific ecological variation and/or phenotypic plasticity as a consequence of niche breadth and frequent disturbances (*e.g. Metrosideros polymorpha* in Hawaii; Cordell *et al.*, 1998). Such species can be expected to cope with climatic changes (Jump and Peñuelas, 2005).

Mutualistic species interactions

Many island endemics, too, have developed mutualistic species interactions and dependencies and will only be able to perform range shifts or adapt to a changing environment if their mutualistic partners are able to do the same (Memmott *et al.*, 2007; Tylianakis *et al.*, 2008; Aslan *et al.*, 2013, but see also Hembry *et al.*, 2013). Due to the relatively low species richness of islands the probability is low that if one mutualistic partner is lost, another species will be able to take over its function, possibly resulting in indirect impacts on island plant communities. However, some island endemics have been described as 'super generalists' (Olesen *et al.*, 2002; Traveset *et al.*, 2013; Trøjelsgaard *et al.*, 2013b), meaning species that have very high linkage levels (Olesen *et al.*, 2002). At least for some island plants, certain ecological interactions with other species thus are not as important as in more obligate species interdependencies.

Local human influences

Since human arrival (especially since colonisation by Europeans), many oceanic island species and ecosystems have suffered from anthropogenic influence, drastically changing environmental settings and ecological integrities (*e.g.* Whittaker and Fernández-Palacios, 2007; Caujapé-Castells *et al.*, 2010; Triantis *et al.*, 2010; Heywood, 2011; Walker and Bellingham, 2011). Besides high direct and primary impacts on island systems and island species extinctions, it is likely that past and present human impacts can amplify vulnerabilities towards additional disturbances, and that human activity can synergistically intensify impacts of climate change on species and ecosystems (*e.g.* Brook *et al.*, 2008; Caujapé-Castells *et al.*, 2010). Among the most affecting human activities on oceanic islands are non-sustainable land use, overexploitation, pollution and introduction of invasive neobiota (Millenium Ecosystem Assessment, 2005; Wong *et al.*, 2005; Caujapé-Castells *et al.*, 2010).

Land-use, overexploitation and habitat degradation

Land-use (settlements, infrastructure, agriculture, etc.) often leads to habitat destruction or alteration, fragmentation, and pollution by eutrophication, pesticides or waste. These factors can lower natural species' population sizes, affect their ecological integrity and modify their vulnerability to additional stressors (Gilpin and Soulé, 1986; Gillespie *et al.*, 2008; Caujapé-Castells *et al.*, 2010). Human-induced increases of fire frequency or intensity lead to non-natural disturbance regimes and can cause ecosystem impoverishments and the decline and weakening of native species and populations on oceanic islands (Walker and Bellingham, 2011; Connor *et al.*, 2012; Garzón-Machado *et al.*, 2012). Over-exploitation of freshwater resources for agriculture, settlements and tourism demands can lower groundwater tables, deteriorate groundwater quality, and facilitate saltwater intrusion (Gössling, 2001; Praveena and Aris, 2009; Abdullah *et al.*, 2010), leading to habitat degradation and decreased stress tolerance and resilience of species and ecosystems. The same is true for the effects of non-sustainable exploitation of economically valuable taxa. In species with limited population sizes and restricted habitats (like many island species), reduction of population sizes can diminish genetic variation and adaptive potential (Crawford *et al.*, 2001; Willi *et al.*, 2006; James, 2008; Jump *et al.*, 2009; Stuessy *et al.*, 2013). Fragmentation often leads to subsequent Allee effects and higher inbreeding (see Frankham, 1998; Gigord *et al.*, 1999; Ricci, 2006; Caujapé-Castells *et al.*, 2008; James, 2008; Finger *et al.*, 2012; Morrison *et al.*, 2012 for island plants). As a consequence, the vulnerability to genetic erosion, but also to stochastic extinction events is raised (Gilpin and Soulé, 1986; Kingston and Waldren, 2005; Gillespie *et al.*, 2008). Furthermore, potential range shift pathways or target areas for species are often obstructed by human presence or activity, lowering their prospects to escape deteriorating conditions (*e.g.* James, 2008). Disproportionally high proportions of island endemic plants show outcrossing reproduction behaviour and limited dispersal capabilities, which intensifies the impacts of fragmentation (Carlquist, 1974; Francisco-Ortega *et al.*, 2000; Bramwell, 2011).

Reductions of island species populations thus decrease their viability, adaptability and resistance potential against additional threats such as climatic alterations (Frankham, 1997; Caujapé-Castells *et al.*, 2010; Heywood, 2011). Supporting this, Sedlacek *et al.*, (2012) experimentally showed that shifting precipitation regimes (drought stress) interact with inbreeding due to small population sizes towards higher extinction risks in the case of a rare and threatened endemic species on Tenerife, Canary Islands. Fortini *et al.*, (2013) projected that rare and threatened plant species in the Hawaiian archipelago show the highest vulnerability to climate change threats. However, common species (especially obligate outcrossers) might suffer even more from potential population size reductions, habitat fragmentation and resultant genetic erosion (Honnay and Jacquemyn, 2007; Aguilar *et al.*, 2008). Mating systems and life histories of such species often are not adapted to small population sizes, decreased connectivity and/or low individual density, which can cause extreme endangerment when their numbers are suddenly reduced (Aguilar *et al.*, 2008). Contrastingly, naturally rare species might be adapted to genetic disadvantages of small population sizes (Karron, 1997; Orians, 1997; see above). Therefore, population histories have to be considered when assessing ecological or conservational consequences of rarity in species or populations (naturally small or fragmented populations *vs.* originally common but recently declined or fragmented species/populations; Huenneke, 1991; Aguilar *et al.*, 2008).

Only little is known about relationships between population sizes and extinction risks for oceanic island species, mostly due to lacking knowledge on breeding systems and the histories of species and populations, making direct inferences difficult (Caujapé-Castells *et al.*, 2010). Nevertheless, declines of common species (by local human action, but prospectively also by climate change impacts) should be taken more into consideration for conservation plans as those species can be inherently prone to genetic erosion (Aguilar *et al.*, 2008). Consequential reductions of their biological fitness, resistance capability, viability and thereby population sizes might result in losses of their ecological functions and cause structural risks for ecosystems (Chapin, III *et al.*, 1997; Hooper *et al.*, 2005). It is thus crucial for considerations of climate change impacts on islands to include preceding human detrimental impacts and the degree of human presence and/or influence. Regarding prior degradation of species and ecosystems and the potential of amplifications of climate change threats by local human perturbation (Brook *et al.*, 2008; Fordham and Brook, 2010), densely populated islands with high human impacts on ecosystems are presumably more prone to plant species loss due to climate change influences than more pristine islands.

Invasive neobiota

Invasive neobiota (plants, animals, fungi and microbes) constitute one of the greatest threats to island floras and ecosystems (*e.g.* Cushman, 1995; Millenium Ecosystem Assessment, 2005; Gillespie *et al.*, 2008; Sax and Gaines, 2008; Caujapé-Castells *et al.*, 2010; Küffer *et al.*, 2010; Irl *et al.*, 2014). Many invasive neobiota can directly or indirectly affect island taxa (*e.g.* as competitors, herbivores, predators, parasites, hybridising congeners, diseases or disease vectors). Climate change impacts are expected to interact with species invasions (Walther *et al.*, 2009; Mainka and Howard, 2010) which will likely intensify impacts on island ecosystems and the challenges of management and control of invasives (Hellmann *et al.*, 2008; Vorsino *et al.*, 2014). In a simple example, moderate changes in temperature or precipitation may only lead to mild modifications of a particular ecosystem and may lie within the capabilities of response of its species (*e.g.* tolerance, plasticity, slight range shifts of populations or even adaptation). However, additional stressors, such as a newly facilitated alien species (*e.g.* a plant competitor) that is now able to establish in the already affected ecosystem can set up profound synergistic effects on climatically weakened native plant species. Such effects are likely to be greater than simple additive effects of single stressors (Brook *et al.*, 2008) and can cause important ecological consequences of climatic changes to oceanic islands.

In contrast to many island endemics, invasive species often show higher rates of dispersal and reproduction, or possess high phenotypic plasticity and climatic tolerance, resulting in comparably high adaptive potentials and relative performance advantages over specialised endemics, especially when confronted with environmental alteration like climatic disturbances and change (Hellmann *et al.*, 2008; Mainka and Howard, 2010; Willis *et al.*, 2010; Chuine *et al.*, 2012). Specialisation in oceanic island endemics and their reduced competitiveness against alien species due to their evolution in isolation raises a particularly high threat potential from interactions and synergies between climate change and species invasion to oceanic islands.

Climatic changes in habitats or higher intensity and/or frequency of extreme events can facilitate the introduction and colonisation of alien species by increased transport of propagules, opening of new areas and increased growth or survival of colonising neobiota (Loope and Giambelluca, 1998; Walther *et al.*, 2009; Huang *et al.*, 2011; Chuine *et al.*, 2012). Further on, reproduction, competitiveness relative to natives, and thus establishment rates of alien species after colonisation in ecosystems might be enhanced (Chown *et al.*, 2008; Sax and Gaines, 2008; Walther *et al.*, 2009), and finally, climate change impacts might extend suitable areas for alien colonisers, enabling spread and invasion into larger areas of an island (Loope and Giambelluca, 1998; Walther *et al.*, 2009; Vorsino *et al.*, 2014). However, some invasive species on oceanic islands might also experience range contractions due to climatic alterations (Taylor and Kumar, 2014; Vorsino *et al.*, 2014). Nevertheless, the presence of invasive neobiota or neobiota that have potential to become invasive is one of the most important co-factors for the climate change vulnerability of an island flora (*e.g.* Sax and Gaines, 2008; Bramwell, 2011; Vorsino *et al.*, 2014). Regarding this particular relevance for oceanic islands, it is thus surprising that only few studies exist on neobiota-climate change interactions in such systems.

Human-mediated secondary effects of climate change

Feedbacks can also come from interacting stressors related to the presence and climate change-response of human populations. Climatic alterations and sea level rise may force human islanders to relocate low-lying coastal dwellings, agriculture or infrastructure upwards into the hinterland or to other, *e.g.* larger, higher-elevated or less degraded islands (Nunn, 2013). Islands have high portions of their human populations living in coastal areas, bearing large potential for climate change migration (McGranahan *et al.*, 2007; Nunn, 2013).

Wetzel *et al.*, (2012; 2013) stated for islands of Southeast Asia and the Pacific region that under different sea-level rise scenarios (1 m, 3 m, or 6 m) considerable portions of the coastal zones and their ecosystems could be lost (inundation and erosion). This would cause 8 – 52 million people to become climate change migrants and force them to reclaim formerly unaffected area in the interior of their island, impairing local ecosystems and species' actual or potential habitat (secondary effects; Wetzel *et al.*, 2012). These investigations are based solely on land loss and human land occupancy. Potential side-effects that might affect adjacent areas, such as increased exploitation of resources, pollution, eutrophication and spread of neobiota are likely to encroach plant species and biodiversity as well (Gillespie *et al.*, 2008; Fordham and Brook, 2010; Heywood, 2011).

Climatic alterations may force (or enable) island societies to drastically rearrange their economies. Besides tourism and fishery, agriculture is the main economic sector. Water availability, soil fertility, salinisation, temperature, length of growing season, extreme events, increase of CO_2-concentration, weeds, pest and disease risks are factors potentially affected by, or co-occurring with, climate change. Performances of crop species and livestock can change (Fuhrer, 2003; Chartzoulakis and Psarras, 2005). Adaptations in agricultural systems are then required, concerning the selection of crop species and cultivars, demands and distributions of farmland, use of fertilisers and pesticides or freshwater withdrawal (Wong *et al.*, 2005; Mimura *et al.*, 2007; Nurse and McLean, 2014). Additionally, on formerly unsuitable islands or in previously unfavourable elevational zones, climatic changes can improve the conditions for farming or livestock breeding (Mimura *et al.*, 2007). This would especially affect high-latitude islands with warming climates or originally dry islands that receive more precipitation in the course of climate change. However, it is also imaginable that climate change impacts force islanders to abandon agricultural sites or even settlements, potentially resulting in local recoveries of natural processes and conditions.

Another effect of climate change impacts on island societies might be a reduction of conservation efforts due to changing socio-economic priorities. Currently, a lot of threatened species on oceanic island persist only in refugial habitats under continued conservation efforts, *i.e.* 'conservation-reliant species' (*e.g.* Whittaker and Fernández-Palacios, 2007). If island societies find themselves faced with climate change impacts, the viability of these long-term conservation efforts is doubtful (Heywood, 2011). Resource allocations to afford climate change mitigation measures may lead in turn to severe reductions or even complete cessation of conservation expenditures and therefore could indirectly result in species extinctions.

Conservation and adaptation measures to climate change impacts

On islands with greater possibilities and political will to continuously allocate socio-economic resources to protection and management efforts, some climate change impacts on species and ecosystems may be mitigated or even forestalled. Well-designed (in the best case pre-emptive) conservation measures could lessen the vulnerability of species to climate change.

Strategies might include recovery from preceding anthropogenic encroachments in order to increase resistance potentials of threatened species or ecosystems against disturbances, for example by habitat rehabilitation and establishment of protected areas (Whittaker and Fernández-Palacios, 2007; Caujapé-Castells *et al.*, 2010). Regional modelling (downscaled climate projections and ecological niche modelling) as well as identification of emerging threats must be included in planning and area prioritisation for such reserve-based measures to adapt conservation strategies to the challenges and dynamic nature of climate change impacts (Hannah *et al.*, 2002; Küffer *et al.*, 2007; Heywood, 2011). For oceanic islands, it might be particularly reasonable to establish protected areas and managed dispersal corridors along relevant environmental gradients to enable species range shifts (*e.g.* elevational or rainfall gradients). In contrast to large continental systems, gradients on oceanic islands are often very steep, potentially lowering efforts in establishment and management, and less likely to exceed natural dispersal capabilities of species trying to track their climatic habitats. However, it might be needed to control potential negative effects of such corridors, like facilitation of invasive species spread (see *e.g.* Haddad *et al.*, 2014). Alagador *et al.*, (2014) also suggested dynamically shifting conservation areas, including sequential scheduling of conservation area designation, followed by the release of areas that stopped contributing to long-term conservation goals. Such approaches would account for the expectable dynamics in niche shifts during climatic changes and might be especially adequate for oceanic islands, where budgets and available area for conservation are often very limited.

On a larger scale, management of the matrix (areas outside and between protected areas) and improved regional coordination are important to improve range shift potentials and species persistence during climatic changes (Hannah *et al.*, 2002). On oceanic islands, this particularly includes the control of invasive species (*e.g.* competitive alien plants or herbivores; de la Luz *et al.*, 2003; Kingston and Waldren, 2005; Whittaker and Fernández-Palacios, 2007; Cole and Litton, 2014). Also, augmenting population connectivity and gene flow (*e.g.* by decreasing habitat fragmentation), active genetic management ('genetic rescue'), and population reinforcements by (re-)introductions of plant individuals into natural habitats from *ex situ* breeding programmes ('demographic rescue') would benefit from regional coordination and may help to increase adaptive potential and resistance against disturbances in natural populations (*e.g.* Küffer *et al.*, 2007; Caujapé-Castells *et al.*, 2008).

However, for species of particularly narrow ecological niches, climate change impacts might exceed physiological tolerances or include overwhelming pulse disturbances (*e.g.* fire frequency), precluding sustainable *in situ* conservation (Cahill *et al.*, 2013; Fortini *et al.*, 2013; Krushelnycky *et al.*, 2013). If affected species cannot escape by natural migration to refugia, human-mediated relocations could save them from being decoupled from their climatic niche (see Vitt *et al.*, 2010 for a general consideration). Also, permanent habitat loss (*e.g.* by sea level rise) might call for relocation strategies, including translocations to other islands (for general considerations and case studies from the Florida Keys see Maschinski *et al.*, 2011). Generally, establishments of seed banks and other *ex situ* collections in time would avert total loss of species (Caujapé-Castells *et al.*, 2010). Some efforts in the past were successful in preventing island species from extinction (*e.g.* summarised in Whittaker and Fernández-Palacios, 2007). From a global perspective, however, it is likely that in the face of climatic deteriorations and limited resources for human subsistence on oceanic islands, sustainability and conservation will be sacrificed in many places (see above; Bramwell, 2011; Heywood, 2011).

It is imaginable, however, that some native species could profit from human adaptation measures. Agricultural land, settlements or infrastructure can be abandoned or transformed to restore or conserve ecosystems and their services. These could include coastal preservation (*e.g.* through restoration of mangrove belts), slope reinforcement against landslide and soil erosion (reforestation of hill slope forests), preservation of water accumulation, retention and filtering in watershed areas (groundwater recharge effects; *e.g.* by forest restoration, *e.g.* Perkins *et al.*, 2014), and general services like the supply of wood, food or medicine, *e.g.* by agroforestry (Hills *et al.*, 2013). Conservation of biodiversity also contributes to maintain or restore the natural attractiveness of an island for lucrative tourism. Here, particular species or ecosystems are likely to be favoured due to their rarity, popularity or iconic and charismatic value (Duarte *et al.*, 2008; Hambler and Canney, 2013; Krushelnycky *et al.*, 2013).

Outlook

General patterns

Global climate change will most likely have direct and dramatic consequences for oceanic island plants and ecosystems. In many cases climate change will also affect island biota indirectly or interact with other drivers, leading to habitat degradation, population decrease and extinction of endemic species. However, oceanic islands are not homogenous in terms of climatic changes and vulnerability. They differ in abiotic, biotic and anthropogenic aspects. Oceanic island plants also differ in their vulnerability and threats due to specific ecological interactions, traits, population viability and response capability. Some patterns can thus be assumed to have general relevance for impacts of global climate change on island floras, although large uncertainties and knowledge gaps are present that preclude more distinct statements about their vulnerabilities. In the following paragraphs, the most important considerations are given, and a conceptual summary of processes related to island characteristics is provided in Tab. 1, including research challenges.

1. The estimated change in climate is not evenly distributed but depends on geographic location and the associated atmospheric circulation patterns of an island. While temperature is

generally increasing (although with variable magnitude), strong differences are assumed for trends in precipitation. There is also a tendency towards intensifying and more frequent extreme events for some island regions.

2. We expect the greatest flora vulnerabilities on oceanic islands of small area, low elevation and simple topographic structuring (with flat atolls and reef islands being threatened most, due to high inundation risks and saltwater intrusions). Nevertheless, these island types possess relatively low numbers of native plant species and only few endemics (Kreft *et al.*, 2008), limiting their role for potential terrestrial plant biodiversity loss in the face of global climate change. In addition, low functional redundancy and strong interactions between ecosystems are likely to increase risks of co-modifications on different organisational levels.
3. In contrast, we presume islands of high topographic and ecological heterogeneity to provide potential refugial habitats. Also, species-rich island communities are probably more resistant or resilient to climatic disturbances and the loss of single species functions.
4. Probably the greatest challenges for island species from climatic alterations are to be expected from interactions and synergies with local anthropogenic pressures. Previous ecosystem alterations, as well as current human impacts parallel to or, as a consequence of, climatic alterations can be fatal for an island's flora and its endemics. Alien species invasions and their detrimental effects are likely to intensify with climate change. Human encroachments in general can be assumed to play major roles in threatening oceanic island floras and may even supersede effects of climatic alterations in some ecosystems or entire islands (*e.g.* Caujapé-Castells *et al.*, 2010).
5. Besides local anthropogenic impacts and synergistic factor-interactions, we suggest that secondary (indirect) effects (*e.g.* trophic cascades, co-extinctions, problems arising from climate-induced human migration) are major threats to oceanic island floras facing climatic alterations.
6. On the species level, low dispersal capacity and high ecological specialisation (narrow ecological niches, including strict dependencies on single interacting species) amplify the risks of decline and extinction. The potentials of rapid evolutionary adaptation to novel climates are not clear, but likely to be negligible.

Main drivers and their interactions are summarised in a flowchart (Fig. 4), providing an overview on the inter-relatedness of factors and mechanisms. Self-amplifying mutual inter-relationships between different factors will probably have more impact than additive singular effects. Moderate primary effects of a single threat factor can turn into harsh encroachments if further interacting stressors come into play.

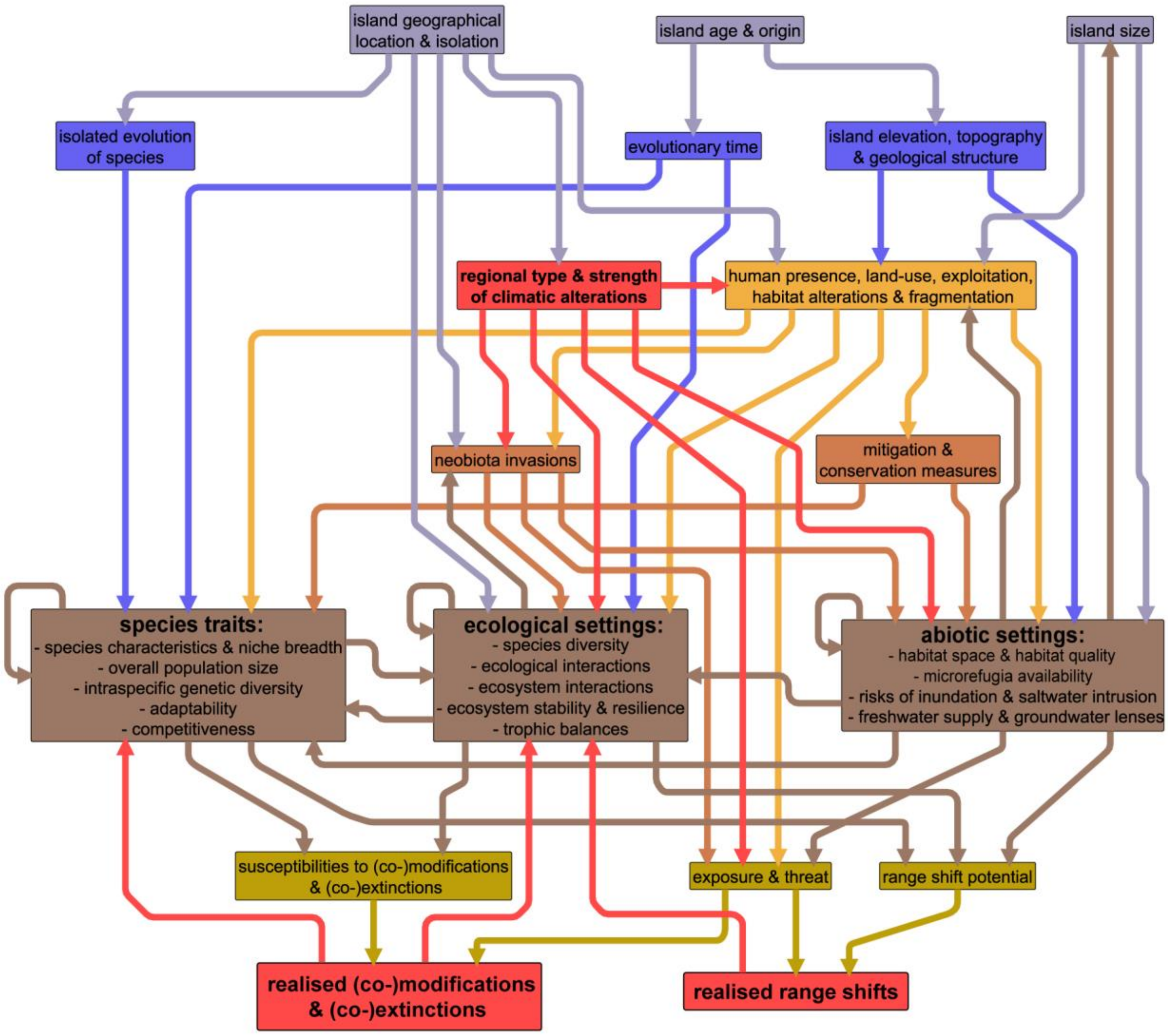

Fig. 4: Flowchart depicting the influences of the most important factors and interaction pathways on species susceptibilities to climatic changes on oceanic islands. Arrows symbolise the direction of influence. Roughly, higher order factors (independent parameters) are positioned at the top, whereas factors of lower order (dependent on others) are positioned towards the bottom. Modifications and co-modifications mean all potential changes in population sizes, structures and viabilities of species and ecosystems, including extinctions and co-extinctions.

Suitability and potential of oceanic islands for climate change research

Albeit following logical considerations and being supported by island case studies as well as research on continental systems, various assumptions on climate change vulnerability of island floras are hypothetical and need validation. On oceanic islands, research on ecological consequences of climate change is lagging far behind continental systems, making empirical inference on patterns and processes difficult. However, the disproportionate importance of oceanic islands for global phytodiversity in combination with their special sensitivity calls for comprehensive risk assessments. These are urgently needed for adequate decision-making on counteraction and conservation programmes.

Oceanic islands stimulated the development of fundamental and seminal theories in the fields of ecology, evolution and biogeography that have significantly improved our understanding of natural systems (Whittaker and Fernández-Palacios, 2007). It is therefore surprising that the opportunities of studying oceanic islands to gain general insights and understanding of climate change outcomes on natural systems have been largely unexploited so far. Many aspects of oceanic islands make them best suited model systems for constitutive research on climate change ecology with potential relevance and applicability for the understanding and conservation of non-island systems as well (see *e.g.* Küffer *et al.*, 2014):

i) The clear spatial and ecological delimitation combined with the large number and variability of oceanic islands provides possibilities for replications and comparisons in multi-island data sets. Setting up sound hypotheses and controlling for island idiosyncrasies would make it possible to generate knowledge on basic processes and mechanisms of climate change impacts on plant communities and ecosystems.

ii) Oceanic islands host relatively species-poor and simple ecosystems compared to most continental regions (Whittaker and Fernández-Palacios, 2007; Kreft *et al.*, 2008). As it is difficult to disentangle influencing factors and interactions in more complex systems, this brings great advantages. Straightforward interpretation of ecological processes in field study data as well as target-oriented experimental designs and modelling approaches can be implemented.

iii) Volcanic islands often exhibit high elevations with steep and fissured slopes. This causes outstanding ecological heterogeneity and sequences of different ecosystems within small spatial scales (elevational zonation, island side differences, topographical structure, etc.). Steep ecological gradients and small-scale heterogeneity provide excellent natural experiments to researchers, for example by translating these gradients into potential climatic alteration series (Crausbay and Hotchkiss, 2010).

iv) Considerable portions of plants on oceanic islands are endemic, meaning that the area of their island represents their entire global distribution. This might benefit analyses and inferences on potential evolutionary and biogeographic responses during past climatic changes. Species distribution (or ecological niche) modelling, species and population viability analyses, population genetic and phylogeographic studies, as well as combinations of these, can yield more meaningful results compared to continental species of wider distribution.

v) In contrast to most continental systems, oceanic islands are ecologically sharply delimited without significant natural biotic exchange to other regions, simplifying hypotheses and their testing in many possible scientific approaches.

vi) A wide array of open questions exists concerning mechanisms, processes and consequences of the upcoming climatic alterations (Tab. 1), calling for extensive use of oceanic islands and their research opportunities by global change ecologists.

Knowledge gaps and suggestions for research approaches

Besides differences in ecological settings, types and magnitudes of climatic impacts and socio-economic structures, the availability of scientific knowledge and necessary data, as well as of resources for potential research is very heterogeneously distributed among islands and island regions. Based on these differences and capabilities, varying research efforts are necessary to set the scientific base for robust assessments of ecological climate change impacts and to enable knowledge-based prioritisation of conservation and mitigation measures. The interdisciplinary work of field biologists, climatologists, social scientists and human geographers, ecological experimenters and modellers, palaeontologists and island biogeographers is needed. Here, we provide a brief summary of research options and data required to advance the understanding of ecological outcomes of climate change on islands (see also Tab. 1 for conceptual summarisation):

i) Botanical surveys and species threats: The floras of many oceanic islands are still largely unexplored and need fundamental botanical surveys (species distributions, individual abundances, population structures, etc.). For those islands with explored floras, long-term monitoring studies may identify changes in species assemblages and abundances. Species and populations at risk need to be identified and prioritisation regarding needs of research and conservation measures must be done. For selected taxa, demographic and population genetic analyses or viability assessments should be carried out.

ii) More detailed climatic data: Baseline data on current and future climatic conditions are certainly needed for almost any study on potential climate change impacts. The amount and spatial distribution of weather stations is not satisfactory on many islands, making consistent and spatially well-arranged long-term data hardly available. Additionally, most oceanic islands are rather small and existing spatial climate data and projections offer insufficient resolutions (grid side lengths of one to several hundreds of kilometres; *e.g.* WorldClim: Hijmans *et al.*, 2005) with some islands even completely slipping through the grid of climate models and being 'unseen' by them (Heywood, 2011). Considering spatial climate patterns and related ecological processes within topographically structured islands (*e.g.* by elevational differences, luv/lee-effects, seasonality differences) is often not yet possible. Thus, there is a need for more and better climate data and models on the island scale.

iii) Anthropogenic factors: Future scenarios for human demographics, land use and socio-economic changes and settings on islands are needed. These will allow for ecological assessments and modelling approaches on interacting effects between climatic alterations and local anthropogenic disturbances on species and ecosystems (Brook *et al.*, 2008; Heywood, 2011). Combinations of species distribution models with scenarios of human population, socio-economic structure, tourism, and potential adaptation strategies to climate change will yield more realistic vulnerability assessments of oceanic island floras. For example, Wetzel *et al.*, (2012) assessed land loss and forced human migration on islands due to sea-level rise. Beck (2013) modelled ecological suitability for agriculture under future climate conditions, which could be adapted to island scales. Such approaches can identify potential conflicts between human responses to climate change and range developments of island plant species.

iv) Experiments and field studies: Response capacities towards disturbances from climatic alterations is widely lacking for oceanic island species and their ecological interactions, impeding the mechanistic understanding of climate change outcomes on island ecosystems (Fordham and Brook, 2010; Heywood, 2011). Different experimental approaches are conceivable to gain knowledge about the susceptibility of species and ecosystems to climate change impacts and their potential responses. *In situ* experiments might profit from different island settings, simple ecosystems and steep ecological gradients ('natural experiments') (*e.g.* Roux *et al.*, 2005; Ross *et al.*, 2008; Levine *et al.*, 2010; Anderson *et al.*, 2011 for consultable research on continental islands). Experimental manipulations of climatic parameters and/or adding or removal of potentially important species and invasibility experiments would provide important insights into biodiversity effects under climate change and general response potentials. Field studies and molecular data can bring knowledge on basic ecological demands, demographical dynamics, dispersal capacities, gene flow patterns and adaptability of insular plant species and populations (see *e.g.* Levine *et al.*, 2008; Roux and McGeoch, 2008; Sedlacek *et al.*, 2012; Shapcott *et al.*, 2012; Krushelnycky *et al.*, 2013). Specific studies are most important for rare and presumably threatened species or ecosystems (*e.g.* high elevation ecosystems, ecosystems under inundation risk, rare specialist species) but the climate change response of 'keystone' species might also be of great interest. Potential impacts of climate change on species interactions and ecosystem processes could be analysed by benefitting from the ecological outcomes of 'natural experiments'. Extreme weather events such as abnormal heat waves, droughts, heavy rainfalls, hurricanes, storm surges, inundations and saltwater intrusions might be seen as frequent or even constant future conditions for particular islands or archipelagos. Moreover, adaptabilities of neobiota and their potential to affect island endemic plants can be investigated in experimental settings, enabling adequate decision making for conservation approaches.

v) Modelling approaches: One of the most powerful tools for analysing susceptibilities and range shift prospects of species and ecosystems towards environmental changes is niche distribution and viability modelling (for island studies see *e.g.* Kingston and Waldren, 2005; Attorre *et al.*, 2007; Fordham and Brook, 2010; Fortini *et al.*, 2013). However, biotic, landscape and anthropogenic factors have to be included in such models to cover the whole complexity of potential ecological and distributional changes of species and ecosystems in the future (see Fortini *et al.*, 2013 for a straightforward and comprehensive approach to quantify Hawaiian plant species' climate change vulnerability). Among the often neglected (mainly due to lack of data) but very important factors are dispersal capacities (*e.g.* Corlett and Westcott, 2013; Fortini *et al.*, 2013), ecological interactions (dependencies on particular ecosystem processes and structures, availability or co-migration potentials of interacting partner species, competition; *e.g.* Wisz *et al.*, 2013), migration path suitability (*e.g.* Fortini *et al.*, 2013), and availability of micro-refugia (*e.g.* Ashcroft *et al.*, 2009; Fortini *et al.*, 2013). Furthermore, island populations, economies and land use can be expected to change during global change. Thus we have to include current patterns and future scenarios of human impacts (*e.g.* habitat degradation and fragmentation, resource exploitation, effects of invasive species, pollution) into species models to get the best possible projections of species' susceptibility and range shift potentials (*e.g.* Fordham and Brook, 2010; Vorsino *et al.*, 2014).

vi) Island biogeographic analyses: Comparative studies between islands (*e.g.* within archipelagos) on past ecological responses to climatic changes can reveal potential influences of island biogeographical parameters (area, topography, elevational range, species richness, etc.). Linking vegetation histories to past climatic changes could help to assess future impacts of climate alterations on species and ecosystems. Pollen records can be used to document past extinctions and rates of species turnover (*e.g.* Nascimento *et al.*, 2008; Connor *et al.*, 2012; Boer *et al.*, 2013; Collins *et al.*, 2013; Nogué *et al.*, 2013). Additionally, phylogeographic analyses can detect species histories and responses to past climatic shifts (Davison and Chiba, 2008; Rodríguez-Robles *et al.*, 2010; Bisconti *et al.*, 2013 for studies on island animals). Palynological and phylogeographic results can then be related to the characteristics of specific islands. However, time scales and island histories must be considered to adequately take the dynamics of island areas, topographies and geographical settings (isolation, fusions, fissions etc., see Rijsdijk *et al.*, 2014) into account in order to relate species dynamics to past climatic alterations.

Between-island comparisons of (modelled) intra-island species range shift potentials may reveal benefitting or impeding island features for species responses to climatic changes. Islands that are geographically close to each other and share similar macro-climatic conditions, but differ in an island feature of hypothesised relevance (*e.g.* topographical structure), would provide suitable study systems. This would be possible with species that realistically occur on the islands under comparison. However, meaningful insights into the role of island features would also be possible with species that occur only on one island but whose climatic niche is also existent on another, *i.e.* with comparing "half-hypothetical" species distribution models.

In conclusion, oceanic islands and their unique and diverse flora are at particular risk from global climate change, which poses a high potential for global biodiversity loss. Thus, climate change impacts on islands deserve more scientific interest, not least because of the special suitability of oceanic islands for diverse climate change-related scientific approaches, and their potential to extend their established function as 'test tubes' in ecology and evolution to model systems for basic and applied climate change research.

Climate change impacts on oceanic island ecology have not been comprehensively examined before. This review offers an initial conceptual framework by (i) providing considerations to generalise the findings of case studies and single aspects of island ecology for the climate change vulnerability of oceanic island floras, (ii) categorising relevant known and presumable factors and interactions, and (iii) depicting uncertainties and research potentials. The paper may thus help to identify specific research needs for different islands, which is important considering the complex and variable risks from climatic alterations, the lack of necessary data for adequate conservation planning and prioritisation, as well as the limited resources for research and conservation in most islands. We hope to stimulate an integrative and multi-disciplinary scientific discussion aiming to better understand and protect the unique biodiversity on oceanic islands with respect to climate change.

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Appendix

Spatial pattern analysis

For describing the hump-shaped relations between latitude and changes of temperature and precipitation, we implemented illustrative regression models. In other words, we intended not to predict climate change but to describe the relations. We opted for Generalized Least Squares (GLS) framework due to heteroskedasticity of dependent variables against latitudinal gradients found by Breusch-Pagan test (*p*-value < 0.001).

For temperature change, we modelled the potential relationships to simple latitude to account for the full south to north-gradient (Fig. A.1A). Precipitation change projections showed a more consistent trend among both hemispheres, thus absolute latitude (equivalent to distance to the equator) was used as predictor. For better comparability with the 787 island values and to maintain clarity in the regression plots, we randomly sampled the same number of coastal and continental grid cells, respectively. The overall patterns and regression co-efficients did not change qualitatively by that approach, compared to the use of full data sets. Additionally, we fitted a temperature change regression to the islands' distance to the next continent to show potential influence of oceanic buffering on temperature changes on islands. For all the models, an exponential function was used to weight dependent variables. Functions `bptest' of the package 'lmtest' and `gls' of 'nlme' package were used on the R base system (version 3.0.2).

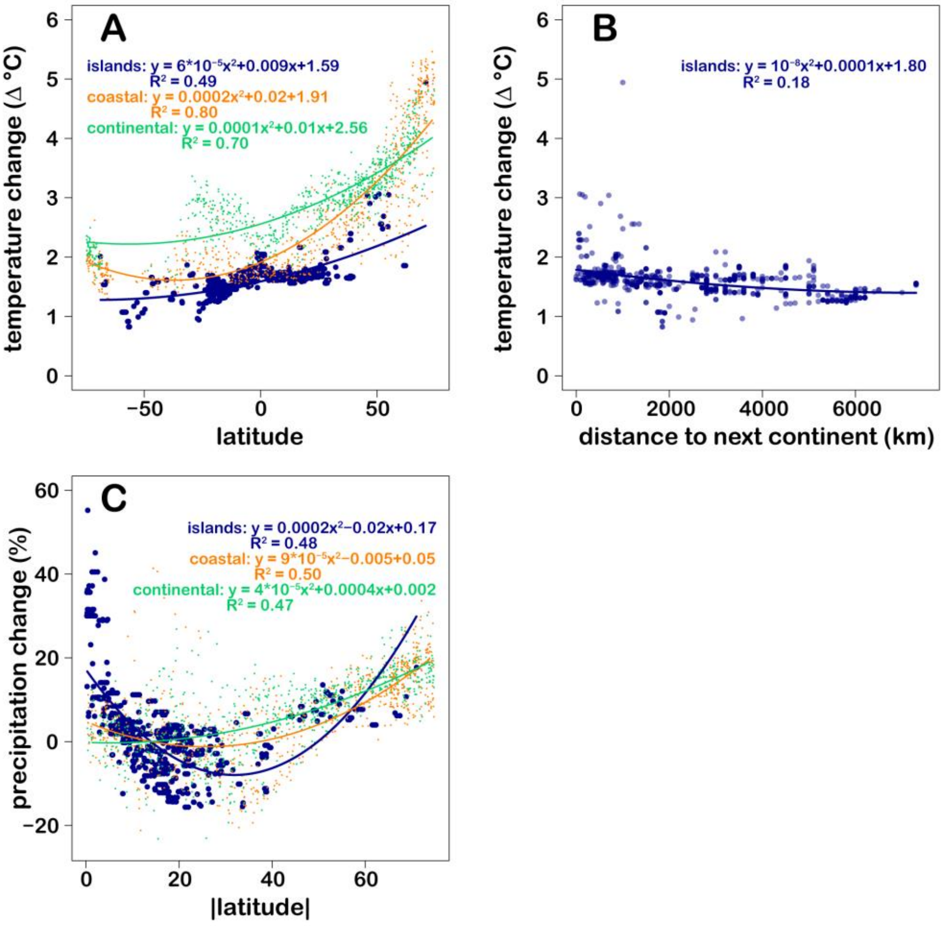

Fig. A.1: Relationship of latitude (A) and distance to the next continent (B) to the projected change of temperature and of absolute values of latitude (equivalent to distance to the equator) to changes of precipitation (C). Figures and calculations are based on 787 oceanic islands (large blue points) worldwide for the period 2081 – 2100 according to scenario RCP 6.0 and the same numbers of coastal (orange) and continental (green) values, respectively. Regression lines represent the fitted GLS models, respectively.

Manuscript 2

Transgenerational effects of extreme weather: perennial plant offspring show modified germination, growth and stoichiometry

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Summary

1. Climate change is predicted to increase the frequency and magnitude of extreme climatic events. These changes will directly affect plant individuals and populations and thus modify plant community composition. Little is known, however, about transgenerational effects (*i.e.* the influence of the parental environment on offspring phenotype and performance beyond the effects of transmitted genes) of climate extremes and community composition. Perennial plants have been particularly neglected. This impedes projections on species adaptations and population dynamics under climate change.

2. Maternal plants of two widespread dwarf-shrub species (*Genista tinctoria* and *Calluna vulgaris*) recurrently experienced extreme weather event manipulations each year (drought and heavy rain). To test for transgenerational effects of community composition, *C. vulgaris* maternal plants were grown in communities differing in the number of neighbouring species. After 6 years, seeds of maternal plants were collected at least 2 months after the final weather treatments. We assessed transgenerational effects of the extreme events and of altered community composition on germination and monitored the development of offspring over 2 years.

3. We show that extreme events experienced by maternal plants influence offspring germination and growth beyond the seedling stage. Seeds produced by maternal plants experiencing stress, indicated by increased tissue dieback, germinated earlier in both observed species. We observed differences in leaf stoichiometry and growth rates for *G. tinctoria* offspring throughout the first year: Offspring from heavy rain-treated mothers showed reduced leaf C:N ratio and higher growth rates. Results further indicate that not only community density, as investigated in prior studies, but also community composition trigger transgenerational effects.

4. *Synthesis*. Our findings show that variation in the maternal environment not only affects the number, but also the performance of offspring. Extreme climatic events, terminated before seed set, induce transgenerational effects. Species richness of mother communities can affect the stress level of target species and thereby germination regardless of community density. In contrast to prior studies, which revealed direct effects of chronic stress on plant individuals, this study emphasizes the

importance of addressing transgenerational effects of extreme weather events when projecting future ecological responses and adaptation to climate change.

Key words

Disturbance, dwarf-shrub, environmental maternal effects, germination, heath, microevolution, plant–climate interactions, precipitation change, transgenerational plasticity

Introduction

Transgenerational effects are defined as the influence of the parental (usually the maternal) environment on offspring phenotype and performance beyond the effects of transmitted genes (Herman & Sultan 2011; Salinas & Munch 2012). Besides phenotypic plasticity and genetic adaptation, they might be one important, but less investigated mechanism to cope with rapidly changing environmental conditions. Transgenerational effects can be adaptive: in this case, offspring which experience a similar environment to their parents reveal higher lifetime fitness (Galloway & Etterson 2007; Herman & Sultan 2011). Transgenerational effects, however, can also decrease offspring or maternal lifetime fitness as a result of resource restrictions (Galloway 1995; Marshall & Uller 2007). Irrespective of their adaptive potential, could transgenerational effects be crucial for community and population dynamics under a changing climate (Hovenden *et al.* 2008; Germain & Gilbert 2014). These effects drive germination, recruitment, seedling and probably later life stage performance, and as such influence the genetic and phenotypic structure of subsequent generations (see, *e.g.* Walck *et al.* 2011 and references therein).

Global climate change not only causes warming, but also changes in precipitation patterns (IPCC 2013). Little is known on the transgenerational effects of altered precipitation. Germain and Gilbert (2014) found maternal effects of a chronically dry environment in 40% of 29 tested annual species. Thus, in many cases no effects of changed soil moisture on offspring performance could be shown (Riginos, Heschel & Schmitt 2007). Chronically dry conditions for mothers sometimes benefitted offspring by increasing seed resource provisioning (Sultan 1996) or increasing offspring germination, growth and fitness (Germain, Caruso & Maherali 2013). Furthermore, root growth was altered in two species after maternal drought, which was adaptive for one of these species (Sultan, Barton & Wilczek 2009).

In the future, many ecosystems will have to cope with an increased frequency and magnitude of extreme, pulsed precipitation events, including prolonged drought periods and heavy rain spells (IPCC 2012). All studies on transgenerational effects of soil moisture regimes, except for Riginos, Heschel & Schmitt (2007), to our knowledge focused on chronically changed precipitation or soil moisture regimes and not on discrete, pulsed events. Transgenerational effects of *increased* precipitation have only been investigated by Li *et al.* (2011), who found higher seed production rate when mothers received 30% more rain during summer, with no effects on seed mass or germination success.

Two aspects of transgenerational effects of climate change remain unknown: first, transgenerational effects of extreme precipitation events and particularly of extreme rain spells are largely unstudied. Secondly, the role of transgenerational effects of precipitation for perennial species, in contrast to annuals, is still unclear. Perennial species with their long life cycles might particularly rely on transgenerational effects, as genetic adaptation by natural selection could be too slow to keep pace with rapid climate change (Herman & Sultan 2011; Salinas & Munch 2012).

Global change not only alters climatic conditions that are affecting species population performance, but also shifts community composition towards new species assemblies and interactions, respectively. In consequence, such community shifts result in changes in the competitive environment of species, which might affect offspring performance. Offspring originating from dense and productive communities with high competition intensity revealed reduced germination (Tielbörger & Petru 2010) and growth (Heger *et al.* 2014), but higher phenotypic plasticity (Heger *et al.* 2014). Graminoids have been shown to become more dominant in dwarf-shrub-dominated heathland, for instance after nitrogen deposition (Aerts & Heil 1993) and after nutrient addition combined with warming (Klanderund & Totland 2005). Further to this, grasses expand in heathland after soil disturbance (Ransijn *et al.* 2015). However, the contribution of community composition and species richness, rather than vegetation density or productivity (Tielbörger & Petru 2010; Heger *et al.* 2014), has until now been neglected when studying transgenerational effects in plants.

The objective of our study was to study transgenerational effects of *extreme weather events* and *community composition*. In contrast to existing studies, we focus on both *drought* and *heavy rain* pulses applied to *perennial* species on maternal plants *before seed set and flowering*. Besides seed parameters and germination rate, the main response reported in other studies on transgenerational effects of climate change (*e.g.* summarized in Hovenden *et al.* 2008 or Latzel *et al.* 2014), we also monitored progress of germination, seedling establishment, growth, flowering phenology and leaf stoichiometry (C:N ratio) of offspring from pre-exposed maternal plants. These responses can be informative about growth and tolerance strategies under recurrent disturbance regimes in the offspring generation. A higher C:N ratio indicates a higher proportion of more structural and defensive tissue, which can be seen as an adaptation towards drought and as defence mechanisms (Herms & Mattson 1992; Sardans *et al.* 2008). Using the two widespread European dwarf-shrub species *Calluna vulgaris* and *Genista tinctoria*, we addressed the following research questions:

1. How do extreme precipitation events (drought and heavy rain) experienced by maternal plants before seed set affect germination, seedling growth and juvenile development in the next generation of woody species?
2. How does the community composition (species richness) in the maternal environment affect the performance of offspring?

Material and Methods

Study organisms and experimental treatment of maternal plants

We chose the two common European dwarf-shrub species *C. vulgaris* L. (HULL) (common heather; Ericaceae) and *G. tinctoria* L. (dyer's greenweed; Fabaceae) as study species. *Genista tinctoria* is an insect-pollinated, deciduous legume (Floraweb, 2015) and prefers fresh to moist soils. Within the EVENT I experiment (Jentsch, Kreyling & Beierkuhnlein 2007), it flowered twice, in early and in late summer (Jentsch *et al.* 2009). Seeds were collected in late summer, to ensure to have seeds from the late flowering period. Seeds disperse ballochor, with short dispersal distances (Botanischer Informationsknoten Bayern, 2015). *Calluna vulgaris* flowers in late summer and autumn, is wind- and insect-pollinated and grows on acidic and nitrogen-limited sites. It is indifferent towards moisture supply and can be dominant in bogs, heathland and moorland vegetation across Europe. It is also found in sandy forests (Ellenberg & Leuschner 2010). The light seeds are dispersed by wind, often over long distances (Floraweb, 2015). Both species therefore differ in traits that might be relevant for the transmission of transgenerational effects (Galloway 2005; Kuijper & Hoyle 2015; Leimar & McNamara 2015): Short-distance dispersal is predicted to favour transgenerational effects, as offspring are likely to experience the same conditions as maternal plants. Additionally, constant moisture supply is relevant to *G. tinctoria*, but not to *C. vulgaris*, and therefore, extreme events should cause transgenerational effects for *G. tinctoria* only, as they are not likely to alter the fitness of *C. vulgaris* and should thus not be a strong selective force.

Maternal plants from which seeds were collected grew in the EVENT I experiment, which investigated the effects of extreme weather events on ecosystem functioning (Bayreuth, Germany, Central Europe; 49°55'19" N, 11°34'55" E; 365 m a.s.l.; see Jentsch, Kreyling & Beierkuhnlein 2007). The maternal plants grew under three different, annually recurrent weather treatments (drought, heavy rain and control). *Calluna vulgaris* maternal plants were additionally grown in different plant communities with differing species richness (two- *vs.* four-species communities) and either including or excluding grasses, to test for effects of community composition. *Calluna vulgaris* was accompanied by the dwarf-shrub *Vaccinium myrtillus* in the two-species community, and by *V. myrtillus* and the two graminoids *Deschampsia flexuosa* and *Agrostis stolonifera* in the four-species community. The latter composition thus reflects predicted future scenarios under global change with a higher abundance of graminoids (Aerts & Heil 1993; Klanderund & Totland 2005). *Genista tinctoria* was grown in the four-species community with *V. myrtillus*, *D. flexuosa* and *A. stolonifera*.

The experimental plant communities consisted of 100 planted individuals per 2 m 9 2 m, initially arranged in a regular grid with 20 cm distance between neighbouring individuals. Plots were installed in April 2005. Dwarf-shrubs were planted as 2.5-year-old individuals obtained from a nearby nursery and were randomly assigned to the different experimental plots. The soil texture of the previously homogenized soil was loamy sand (82% sand, 13% silt and 5% clay). Each community was planted equally dense, resulting in 50 individuals per species in two-species communities and in 25 individuals per species in four-species communities. The two crossed factors weather treatment and community composition were applied in a split-plot design, with community diversity nested within weather treatments. Each factorial combination was replicated five times, resulting in 15 plots with *G. tinctoria* maternal plants and 30 plots with *C. vulgaris* maternal plants.

As increased drought frequency and intensity, and more extreme heavy rain spells are expected under climate change (IPCC, 2012), we simulated both of these events. Extreme maternal drought was induced using transparent foil rainout shelters, starting from a height of 80 cm to avoid strong greenhouse effects. This resulted in a drought period with a length of 32 days from 2005 to 2007 (event with a probability of occurring once in 100 years, see Gumbel 1958). Since 2008, a drought period with a length of 42 days was applied (probability of occurring once in 1000 years). Drought started in mid-June in 2005 and at the end of May since 2006. Heavy rain was applied using portable irrigation systems with standard drop size simulating natural rain events. In the years 2005 to 2007, 170 mm of rain was applied within 2 weeks (twice per day; *i.e.* a 100-year recurrence probability). Since 2008, 260 mm of rain was applied within 3 weeks (1000-year recurrence probability). The heavy rain treatment was started at the end of June each year. The control treatment remained under natural conditions without any manipulation. Figure S1 in Supporting information shows the level of soil moisture assessed between −2 and −7 cm using frequency domain sensors (ECH2O, Decagon devices, Pullman, WA, USA) in plots adjacent to the study plots and treated with the same weather extremes. Extreme weather treatments resulted in marked changes in soil moisture during the treatment periods, but recovered quickly to control values afterwards.

Offspring generation

To test for effects of the maternal environment on germination and offspring development, seeds of maternal plants growing in different communities and treated with extreme weather events were collected. Seeds of *G. tinctoria* were collected in September 2010 and seeds of *C. vulgaris* were collected in October 2010. Fifty mature seeds per plot from at least 10 plants were collected and combined to produce a single sample per plot. Seeds were dried, stored at room temperature in paper bags and then stratified at 3 °C from January until March 2011. Seeds were sown on soil containing equal parts of sand, turf and a prepared cultivation soil mixture (Frux; Einheitserde Werkverband e.V., Sinntal-Altengronau, Germany). *Calluna vulgaris* seeds of each parental plot were sown together in seed bowls, while *G. tinctoria* seeds were large enough to place them individually in QuickPot® trays (Herkuplast Kubern GmbH, Ering/Inn, Germany). All seeds germinated in the greenhouse in March and were transferred to hotbeds in early spring. Ten well-established and healthy saplings per source plot were carefully removed from seed bowls or QuickPots® and planted into single pots (18.7 cm diameter) on 15th of June (*G. tinctoria*) and 15th of July (*C. vulgaris*). For *C. vulgaris*, heathland soil substrate (black peat, white peat and sand in equal parts plus long-term fertilizer Osmocote (N 15, P 9, K 12, Mg 2); Everris GmbH, Nordhorn, Germany) and for *G. tinctoria*, sandy loam was used for the long-term cultivation. This resulted in 150 pots containing *G. tinctoria* plants and 300 pots with *C. vulgaris*. Pots were overwintered in hotbeds from November 2011 until April 2012 and then again placed outside at the experimental field in spring 2012. During 2011, the offspring plants were regularly watered to ensure sufficient and equal moisture supply. In summer of 2012, irrigation mistakes occurred, which led to very dry conditions, especially for *C. vulgaris*. Nevertheless, conditions were equal for all plant individuals, as they received always the same amount of irrigation and precipitation. Figure S2 shows the daily rain sums over the 2 years of the offspring study.

Response parameters

Performance of maternal plants prior to the offspring experiment (2006 –2010)

The performance of maternal plants and maternal plant communities was assessed each year after annually pulsed extreme weather treatments (2005–2010) using the point-quadrat method to estimate the dead and living covers of all species (Goodall 1952). Biomass of individuals and communities was estimated using non-destructive biometric measures that were calibrated by multiple regression analysis against harvested individuals before the experiment, as described in detail by Kreyling, Beierkuhnlein & Jentsch (2010).

Performance of offspring (2011 –2012)

Offspring of seeds collected in 2010 stemmed from mothers growing within the EVENT I experiment under recurrent pulsed drought, heavy rain and ambient conditions since 2005. Offspring grew in pots containing one individual, respectively, and all pots received the same amount of precipitation or irrigation. Weight of 50 seeds of *G. tinctoria* from each plot was assessed. Seeds of *C. vulgaris* were too small and light for reasonably assessing differences in weights due to treatments.

Germination started 28 days and 10 days after sowing for *C. vulgaris* and *G. tinctoria*, respectively. Germination was monitored every 2 days until no further germination occurred. Maximum height and number of branches larger than ten centimetres were measured for *G. tinctoria* offspring in June and October in 2011 and in September in 2012. Maximum shoot length and maximum and minimum plant width were measured for *C. vulgaris* on August 23rd and October 21st in 2011 only, as many plants died between May and August 2012, due to dry and hot weather condition. We calculated relative rates for the growth parameters, including branching, using the following formula (Crawley 2009):

$$RGR = \ln(\frac{final\ size}{initial\ size})$$

Analyses of carbon to nitrogen ratios in leaf tissues (C:N analyses, Vario Max C:N Analyser, EVISA, Germany) were conducted in October 2011 (see Data S1).

The day when first flowers opened was monitored and recorded every second day for each plant in 2012. Above- and below-ground biomass of G. tinctoria was assessed by cutting the whole plants on September 2012 and by washing the roots. All plant parts were dried at 70 °C until constant weight and weighed. Formation and opening of flowers were recorded during 2012. As the lifetime of the two species is much longer than the 6 years running time of the experiment, and as there was little establishment of new individuals after the start of the experiment, we are confident that no genetic adaptation has occurred within communities yet.

Statistical analyses

Data on maternal plants for the years 2006 until 2010 were analysed using mixed-model ANOVAs, with maternal weather treatment and, for *C. vulgaris*, community composition as fixed factors and year of the experiment as random factor. To analyse tissue dieback, we used generalized linear (mixed effects) models with a binomial error distribution. The response was created as an odds ratio of dead and alive covers (Crawley 2009).

For *G. tinctoria* offspring, we used one-factorial ANOVAs, with maternal weather treatment as fixed factor and means of offspring derived out of one plot of maternal plants as a response variable to avoid pseudo-replication. Response variables for *C. vulgaris* offspring were analysed by a mixed-model ANOVA with weather treatment and community of maternal plants as fixed factors and with weather treatment block, in which plots of different community composition were nested as random effect to account for the split-plot design. Again, we took means for offspring originating from mothers out of the same plot to avoid pseudo-replication.

The progress of germination was assessed using generalized linear mixed-effects models with a Poisson error distribution (count data) and with weather and community treatments and time as fixed effects. The number of days from first to last germinations was divided into three parts, and the number of seedlings that germinated within this time was used for the analyses. A significant interaction between weather extremes or community composition and time shows that treatments differed in the progress of germination. Overall germination proportion was analysed using generalized linear (mixed effects) models with binomial error distribution (*C. vulgaris*) and quasi-binomial error distribution (*G. tinctoria*) as described for the analyses of tissue dieback. *P*-values for generalized linear models were obtained by stepwise-backwards model simplification and by likelihood ratio tests between full and simplified models (Crawley 2009).

Response variables were transformed accordingly, to ensure homoscedasticity and normality of residuals, which was assessed graphically (see Table 1 for transformations of the response variable). C:N ratios of *G. tinctoria* offspring were analysed using a Kruskal– Wallis test, as assumptions for an ANOVA could not be met with transformations. All statistical analyses were performed using R 3.0.2 (R Core Team, 2013). For mixed-effects models, we used the software packages lme4 (Bates *et al.* 2013) and lmerTest (Kuznetsova, Brockhoff & Christensen 2011). For Tukey's multiple post hoc comparisons to evaluate differences between extreme weather events the package multcomp was used (Hothorn, Bretz & Westfall 2008). Package sciplot was used for graphs (Morales 2012).

Results

Effects of extreme weather events on Genista tinctoria maternal plants

Genista tinctoria maternal plants benefitted from heavy rain and yielded consistently more biomass than control plants (Tukey's HSD: $P = 0.012$; main treatment effect over years 2005 until 2009 $P = 0.014$). In 2009, the year preceding the study, maternal biomass under the extreme rain treatment was increased by 44% compared with the control mothers. Drought, on the other hand, did not significantly change biomass production (Tukey's HSD: $P = 0.16$), but apparently stressed maternal plants as indicated by increased tissue dieback under drought treatment compared to control over all years (Fig. 1a; likelihood ratio test, $\chi^2_{3\,\text{d.f.}} = 1202$, $P < 0.001$).

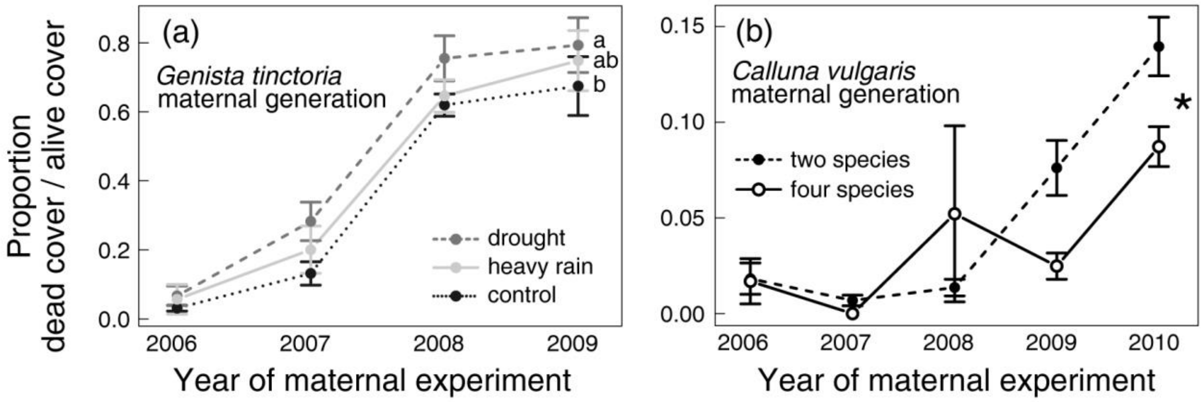

Fig. 1. Dead cover of maternal plants of *Genista tinctoria* for different weather treatments (a; black = control, light grey = heavy rain, dark grey = drought) and of *Calluna vulgaris* maternal plants growing in communities including two (black)- *vs.*- four-species (open) (b) in the years before collecting seeds for offspring. Different letters or stars indicate significant differences between the groups over all years. Means ± SE are given.

Effects of maternal extreme weather events Genista tinctoria offspring performance

Maternal weather treatments did not affect seed weights of G. tinctoria (statistical details in Table 1), but did affect progress of germination (significant weather treatment × time interaction: $P < 0.001$) and germination rate ($P = 0.027$). Seeds of drought-treated mothers germinated earlier than seeds of control and heavy rain-treated mothers (Fig. 2). Furthermore, less seeds germinated overall from heavy rain-treated mothers (52 ± 14% SD) compared to seeds from mothers growing under control (69 ± 7% SD) or drought (68 ± 5% SD; Fig. 2). Offspring of heavy rain-treated mothers revealed a lower C:N ratio than offspring of control plants in October 2011 (Fig. 3a; $P = 0.026$). Additionally, juveniles from heavy rain-treated mothers grew significantly faster between June and October of the first year ($P = 0.043$; Fig. 3b) and also developed branches faster during the time assessed in the second year ($P = 0.05$; Fig. 3c). There were no significant differences in overall and final height ($P = 0.6$), number of branches ($P = 0.9$), shoot biomass ($P = 0.7$) or root biomass ($P = 0.4$). Maternal treatments did not affect the flowering onset of offspring ($P = 0.9$).

Table 1. ANOVA results for the effects of maternal weather treatments on *Genista tinctoria* seeds, germination and offspring. Transformations to ensure homogeneity of variances and normally distributed residuals are given for the response variables, if necessary. Significant *F*-values are shown in bold; *P*-values are given in the text.

Genista tinctoria	*F* or χ^2
Seed weigth^2	$F_{2,12} = 0.008$
No. of germinated seeds: weather × time interaction	**$\chi^2_2 = 16.8$**
Germination rate	**$F_{2,14} = 4.9$**
C/N ratio	**$\chi^2 = 7.28$**
Relative growth rate 1st year	**$F_{2,12} = 4.12$**
Branching rate 2nd year	**$F_{2,12} = 3.88$**
Height	$F_{2,40} = 0.46$
No. of branches	$F_{2,12} = 0.07$
Root mass	$F_{2,12} = 0.93$
Shoot mass	$F_{2,12} = 0.32$
Flowering onset	$F_{2,12} = 0.15$

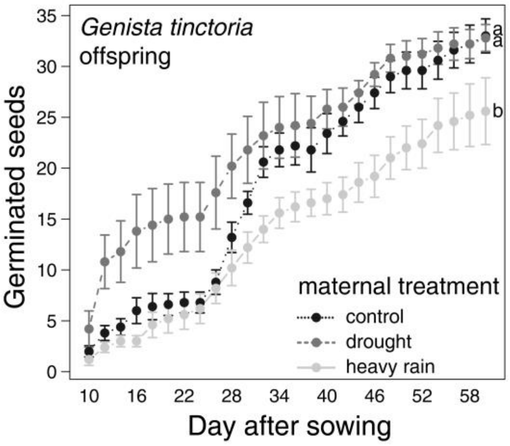

Fig. 2. Progress of germination of *Genista tinctoria* seeds stemming from mothers treated with different experimental extreme weather events (black = control, light grey = heavy rain, dark grey = drought). Number of germinated seeds is shown from first germinations until no more germination occurred. Different letters show significant differences ($P < 0.05$) in the final germination rate. Means ± SE are given.

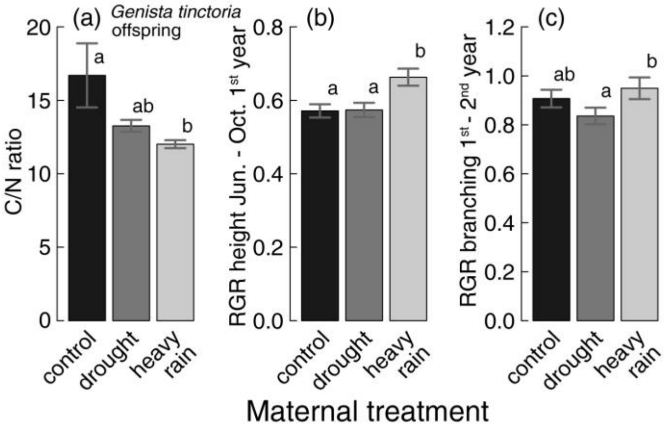

Fig. 3. Long-lasting transgenerational effects on C:N ratio after one growing season (a), relative growth rate in height during the first year (b) and relative branching rate from the first to the second year of *Genista tinctoria* offspring. Plants stem from mothers treated with different experimental extreme weather events (black = control, light grey = heavy rain, dark grey = drought). Different letters show significant differences in Tukey's HSD post hoc tests ($P < 0.05$).

Effects of extreme weather events and of community composition on Calluna vulgaris maternal plants

Extreme weather events did not affect C. vulgaris tissue dieback (Fig. 1b; likelihood ratio between models with and without weather treatment χ 22 = 3.2; P = 0.2) or biomass production (F3.35 = 0.54; P = 0.66). As expected, C. vulgaris yielded higher biomass in the two-species communities from 2006 until 2009, as twice as much individuals were planted compared to four-species communities (Fig. S3). However, differences between communities got smaller over the years, and in 2010, there was no difference between the two communities, indicating smaller individuals or higher mortality in two-species communities (although biomass of individual plants was not assessed) (Table 2; main interaction effect between year and community: P < 0.001). Concomitant with the decrease in differences of C. vulgaris biomass between the two communities, indicating a higher stress level on individuals in two-species communities, tissue dieback in two-species communities was higher in the two years preceding the offspring experiment compared to four-species communities (likelihood ratio test between model with and without community composition over all years: χ 21 = 3.3; P = 0.07; Fig. 1b).

Table 2. Overall biomass [gm^{-2}] and biomass per initially planted individual [g] of *Calluna vulgaris* maternal plants in two- *vs.* four-species communities during the years of the mother plant experiment. Values in bold revealed significant differences in the Tukey's post hoc comparison between four- *vs.* two-species communities in the respective year (*P* > 0.05). Means and standard errors are given. Note that twice as much individuals were planted initially in two-species as compared to four-species communities. Individual biomass values were simply derived by dividing overall *C. vulgaris* biomass within the four square metre of the experimental plots by the number of individuals that were initially planted within these plots and thus only shown here without further statistical analysis. Alternatively, shrinking difference between the two communities over the years could have been caused by higher mortality in two-species communities.

Year	*C. vulgaris* biomass Two-species	*C. vulgaris* biomass Four-species	Per individual Two-species	Per individual Four-species
2006	**229.9 ± 16.9**	**74.1 ± 6.5**	**18.4**	**11.9**
2007	**382.5 ± 22.6**	**243.2 ± 21.3**	**30.6**	**38.9**
2008	**933.8 ± 52.2**	**562.1 ± 43.6**	**74.7**	**89.9**
2009	**975.4 ± 57.7**	**673.5 ± 61.6**	**78.0**	**107.8**
2010	1134.8 ± 94.9	1176.3 ± 64.9	90.8	188.2

Effects of maternal extreme weather events and of maternal community composition on Calluna vulgaris offspring performance

Maternal community composition affected the progress of germination of *C. vulgaris* offspring (significant community × time interaction: *P* < 0.001), but not germination rate (*P* = 0.45; Fig. 4; statistical details in Table 3). Maternal weather treatments did not affect the progress of germination (non-significant weather × time interaction: *P* = 0.63). Germination rate, however, was affected by maternal weather experience (*P* < 0.001) with less seeds germinating when mothers grew under ambient conditions (28 ± 9% SD) compared to seeds from heavy rain-spell (33 ± 22% SD) or drought-treated mothers (33 ± 15%) (Fig. S4).

Both maternal weather treatment and maternal community composition only marginally affected C:N ratio in offspring tissue (*P* = 0.062 and *P* = 0.077, respectively), with no interaction between the two factors: Offspring of heavy rain-treated mothers revealed a lower C:N ratio (16.9 ± 0.97 SD) than offspring of drought-treated mothers (18.3 ± 1.11SD; Tukey's HSD: *P* = 0.01). Offspring of mothers growing in two-species communities revealed a marginally lower C:N ratio than offspring of mothers of four-species communities (17.3 ± 1.25SD *vs.* 17.8 ± 0.93; Fig. S5).

The maternal environment did not affect growth rates of *C. vulgaris* offspring (*P* = 0.67 for weather treatments and *P* = 0.15 for community composition). Neither maternal weather treatment nor community composition affected final offspring height (*P* = 0.87 and *P* = 0.93, respectively). Maternal treatments did not affect flowering onset of offspring (*P* = 0.52 for weather treatments and *P* = 0.57 for community composition).

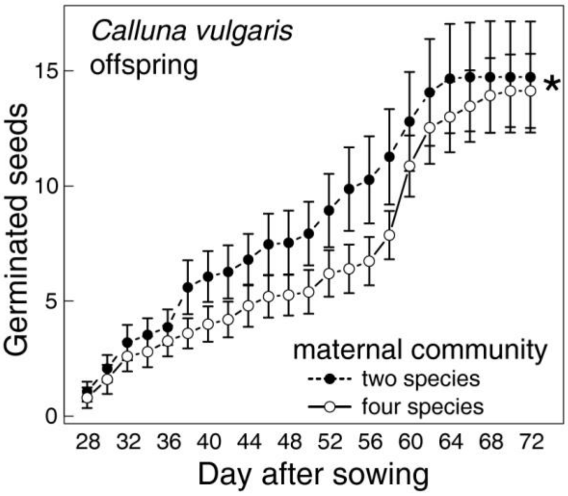

Fig. 4. Progress of germination of *Calluna vulgaris* seeds stemming from mothers grown in different species communities (black = two-species communities, open = four-species communities). Number of germinated seeds is shown from first germinations until no more germination occurred. Means ± SE are given. Asterisk shows significant differences between the maternal communities in the progress of germination.

Table 3. ANOVA results for the effects of maternal weather treatment and maternal community composition on *Calluna vulgaris* germination and offspring. Significant *P*-values below 0.05 are shown in bold.

Calluna vulgaris	*F* or χ^2
No. of germinated seeds:	
Weather x time	$\chi^2_2 = 0.93$
Community × time	$\boldsymbol{\chi^2_1 = 19.3}$
Germination rate	
Weather	$\boldsymbol{\chi^2_2 = 15.1}$
Community	$\chi^2_1 = 0.58$
Weather × community	$\chi^2_2 = 4.7$
C/N ratio	
Weather	$F_{2,12} = 3.69$
Community	$F_{1,74} = 3.13$
Weather × community	$F_{2,74} = 0.74$
Relative growth rate first year	
Weather	$F_{2,12} = 0.41$
Community	$F_{1,12} = 2.4$
Weather × community	$F_{2,12} = 1.76$
Height	
Weather	$F_{2,12} = 0.14$
Community	$F_{1,9} = 0.006$
Weather × community	$F_{2,9} = 1.6$
Flowering onset	
Weather	$F_{2,107} = 0.67$
Community	$F_{1,107} = 0.32$
Weather × community	$F_{2,107} = 0.38$

Discussion

Our study shows that extreme events experienced by maternal plants and eventually causing only minor effects on maternal phenotypes, dramatically can influence offspring germination and growth beyond the seedling stage. We show for the first time that environmental impacts that were experienced by maternal plants long before seed set caused transgenerational effects. Seeds produced by maternal plants experiencing stress, indicated by increased tissue dieback, germinated earlier in both of the observed species. *Genista tinctoria*, the more moisture-sensitive of two investigated species, generally showed stronger and longer term transgenerational effects compared with *C. vulgaris*. We could even observe differences in leaf stoichiometry and growth rates for *G. tinctoria* offspring throughout the first year. Our results further indicate that community composition and species richness trigger transgenerational effects, which adds on previous studies showing effects of community density or productivity on offspring germination and performance (Tielbörger & Petru 2010; Heger et al. 2014).

Effects of extreme events and community composition on maternal plants

Genista tinctoria mothers profited more from rain (more biomass, less senescence) and suffered more under drought (higher senescence), which is in line with their preference for fresh to moist soils. Higher density of *C. vulgaris* individuals (with overall planting density kept constant) possibly caused higher intraspecific competition in less species-rich communities. This is indicated by increased tissue dieback in maternal plants of the two-species communities as well as by the decreasing difference in *C. vulgaris* biomass between the two communities over the years, despite doubled planting density in the low diversity two-species communities. This indicates higher mortality or smaller individuals and thus a higher stress level in two-species communities. *Calluna vulgaris* mothers were largely unaffected by weather extremes, possibly reflecting the species' indifferent soil moisture preferences (Ellenberg & Leuschner 2010).

Transgenerational effects on germination

The results obtained for germination of G. tinctoria offspring are in accordance with studies on annual species in showing that moist maternal conditions decreased germination compared with offspring from mothers growing under dry conditions (Tielbörger & Valleriani, 2005; Germain, Caruso & Maherali 2013). In accordance with other findings (Sultan 1996; Sultan, Barton & Wilczek 2009) and the findings for *G. tinctoria*, maternal beneficial conditions lowered germination rates in contrast to stressful maternal conditions. Tielbörger and Valleriani (2005) interpreted their findings of decreased germination after beneficial, productive years as a strategy to avoid stress under high competition in offspring. Our findings show that effects of modified water supply on germination vary species-specifically and might depend on a species' ecological preference. Also Germain and Gilbert (2014) found the direction of transgenerational effects of moisture supply to differ between species.

Particularly, our results show that the timing of germination was advanced in both, *G. tinctoria* offspring from drought-stressed mothers, and *C. vulgaris* offspring from competition-stressed mothers. These responses can reflect two strategies. First, advanced germination might be a transgenerational response to avoid unfavourable conditions that have been experienced by the previous generation in particularly sensitive early developmental juvenile stages. Secondly, offspring individuals can gain advantage over individuals that germinate later. This might increase fitness as offspring is already more matured and thus less vulnerable when experiencing the next stress exposure or disturbance event.

In our experiment, seed mass was not modified in *G. tinctoria* as a response to extreme weather events, possibly because these events were applied before seed set. For this reason, factors other than seed resource provisioning must be responsible for the observed transgenerational effects such as advanced germination timing. One possible explanation for altered germination is that the seed coat, which is maternal tissue, can shape germination timing via altered permeability (Donohue 2009). Another potential mechanism behind phenotypic variation in offspring is inherited epigenetic modification, such as DNA methylation or histone modification causing altered gene expressions (see, *e.g.* Bossdorf, Richards & Pigliucci 2008; Angers & Castonguay 2010). To conclude, stress accelerated germination in the tested species. Effects of water supply on germination rate were less evident and differed between both species.

Transgenerational effects on seedling and juvenile performance

To our knowledge, this is the first study showing transgenerational effects on leaf stoichiometry and growth rates that persist more than one offspring growing season. The observed lower C:N ratio in offspring of heavy rain-treated mothers may be due to leaves containing less structural, C-rich tissue and more metabolic, N-rich tissue. This might be an adaptation to sustain high growth rates under sufficient water supply, in opposition to the often observed higher C:N ratio under drought stress (Bussotti *et al.* 2000; Sardans *et al.* 2008). The slightly higher growth rates in offspring of heavy rain-treated mothers support this interpretation for *G. tinctoria*. Although it is interesting that both species revealed the same response pattern regarding C:N ratio, differences for *C. vulgaris* were smaller and cannot be explained by differences in germination timing or growth rates. In contrast to the findings of Heger *et al.* (2014) for annual plants, we could not document alterations of offspring growth performance in perennial plants after changes in the maternal community composition and thus modified competition intensity.

Our findings suggest that transgenerational effects are more pronounced in species with short-distance dispersal, and for the factors relevant for maternal fitness, such as water supply for *G. tinctoria* and competition for *C. vulgaris* (compare Kuijper & Hoyle 2015; Leimar & McNamara 2015). By modifying demographic structure and phenotypic composition in plant populations, transgenerational effects are important ecological and evolutionary factors for adaptation and population persistence during environmental alterations (Galloway 2005; Bossdorf, Richards & Pigliucci 2008).

To conclude, our study shows that climate change can trigger transgenerational effects not only by changing resource availability for plants, but also by changing the competitive environment. We show that changes in community composition initiate transgenerational effects that in turn can retroact on community composition. Thus, transgenerational effects might initiate a cascade of further transgenerational effects. For two ecologically important species (*C. vulgaris* and *G. tinctoria*), suddenly changed maternal resources or disturbance events led to marked changes in the seed germination rate and germination timing. This was found despite the treatments often had only minor evident effects on the mother plants. We further show persistent transgenerational effects on offspring growth and C:N stoichiometry for *G. tinctoria* offspring. Future research should investigate whether transgenerational effects even persist across multiple generations. Further on, future studies should scrutinize transgenerational effects for larger sets of plant species.

Transgenerational effects may have evolved as a mechanism to adapt to environmental change. As they are shown here not only to affect offspring abundance (number of germinated seedlings), but also offspring phenology and performance, they will affect population dynamics and species responses through processes that are currently neglected. Optimized parameterization of population models would yield more realistic conclusions on future species performance under climate change.

Acknowledgements

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/ dryad.503nd (Walter et al. 2016).

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Supporting information

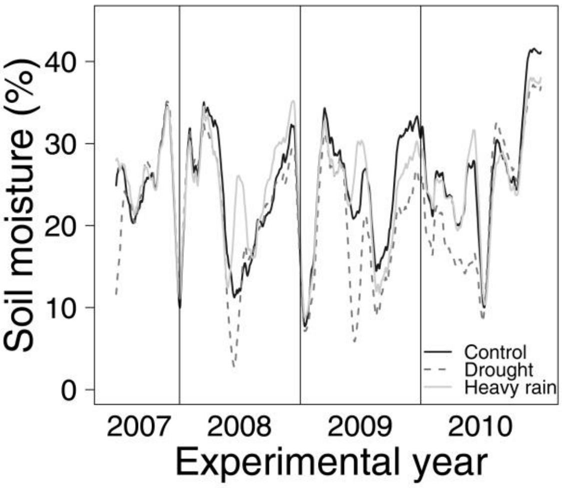

Fig.S1. Course of soil moisture in the years before seed harvest (2010) in the mother plant experiment.

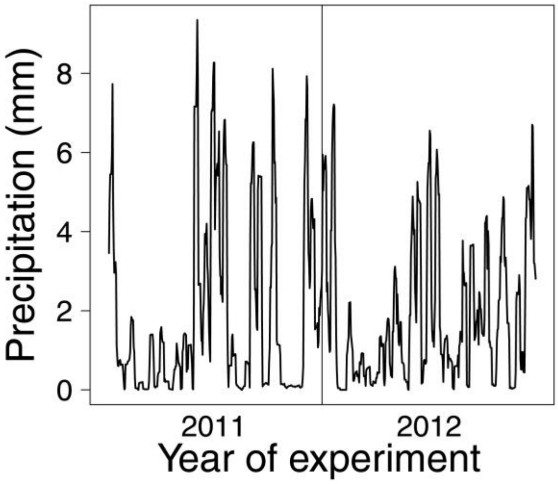

Fig.S2. Weekly precipitation sums during the years of offspring growth 2011-2012. Note that in addition to natural precipitation, all pots received the same amount of irrigation when outside conditions were very dry in 2011. In 2012, some irrigation mistakes led to high mortality of *C. vulgaris* during summer.

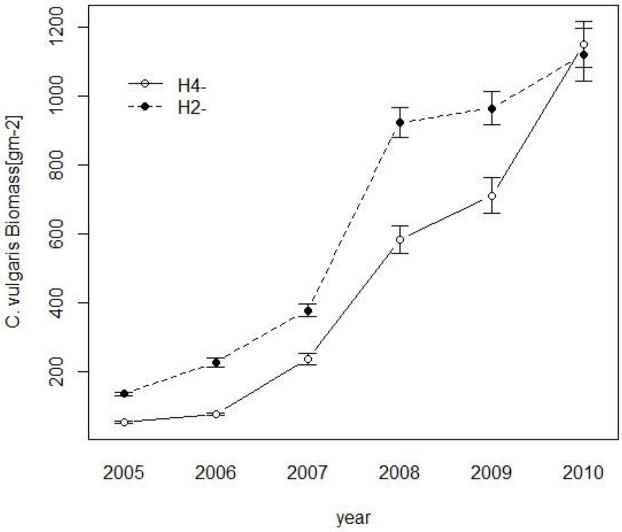

Fig. S3 Overall biomass [gm-2] of *Calluna vulgaris* mother plants in two- *vs.* four-species communities during the years of the mother plant experiment. Means and standard errors are given. Note that twice as much individuals were planted initially in two-species as compared to four-species communities.

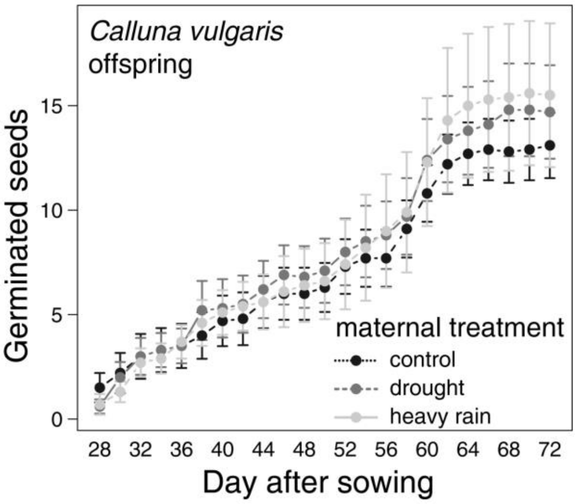

Fig. S4 Progress of germination of *Calluna vulgaris* seeds stemming from mothers treated with different experimental extreme weather events (a; black = control, light gray=heavy rain, dark gray = drought) or cultivated in different species communities (b; black = two-species communities, open=four-species communities). Number of germinated seeds is shown from first germinations until no more germination occurred.

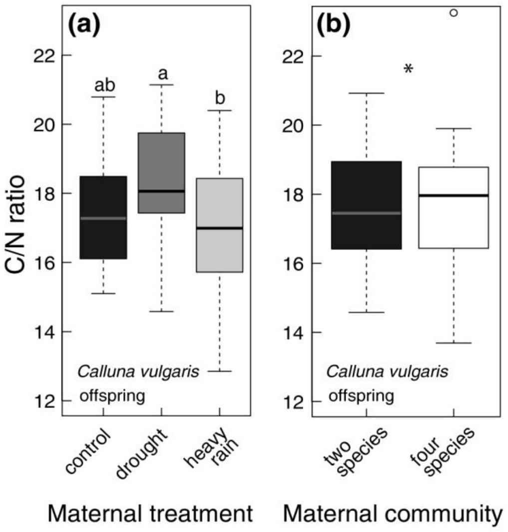

Fig. S5 C:N ratio of *Calluna vulgaris* offspring after one growing season under the same control conditions. Plants stem from mothers treated with different experimental extreme weather events (a; black = control, light gray = heavy rain, dark gray = drought) or cultivated in different species communities (b; black = two-species communities, open = four-species communities). Different letters show significant differences in TukeyHSD post-hoc tests ($P < 0.05$) and asterisk shows marginal significant difference ($P < 0.1$) between communities.

Manuscript 3

A comparison of genetic diversity and phenotypic plasticity among European beech (*Fagus sylvatica* L.) populations from Bulgaria and Germany under drought and temperature manipulation

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Abstract

Premise of research. In the future, ecosystems will have to deal with climate warming in combination with increasing frequency and magnitude of extreme weather events such as drought. Adaptive phenotypic plasticity enables plants to respond to environmental variability and is likely to buffer impacts of climate change. Therefore, factors that influence the phenotypic plasticity of plant populations must be identified to assess climate change outcomes and support conservation measures. Genetic diversity in many temperate plant species is known to vary among regions and populations, largely as a result of their phylogeographic history during the late Pleistocene and Holocene. Here, we argue that high (neutral) genetic diversity of populations might represent increased probability of possessing alleles or allele combinations that are advantageous or more capable in terms of average response capacities to environmental change.

Methodology. We test this idea for European beech (*Fagus sylvatica*) by investigating response patterns of plant growth and leaf phenology to drought and warming treatments in a common garden experiment with seedlings of six populations from Bulgaria and Germany. Phenotypic plasticity of populations was assessed and correlated with allozyme diversity.

Pivotal results. Populations differed in their plasticity to warming with respect to timing of leaf unfolding and senescence as well as in their drought plasticity in terms of height increment (marginally not significant), with some populations showing consistently high plasticity among traits. Measures of genetic diversity showed an inter-regional structure according to known phylogeographic patterns. Height increment plasticity showed a significant positive correlation with genetic variation (allelic diversity) at the population level.

Conclusions. Our results suggest general differences in phenotypic plasticity among populations and a potential influence of genetic diversity on the average plasticity. Besides its evolutionary value, genetic diversity might thus be an important property of plant populations for their short-term response capability against adverse effects of climate change.

Key-words

Genotype diversity, isozymes, phenotypic buffering, phylogeography, provenance trial, reaction norm

Introduction

Climatic extreme events are expected to increase in frequency and magnitude as a consequence of global warming and changing precipitation patterns (Min *et al.* 2011; IPCC 2012). Growing conditions for plants will therefore become more variable with an increased risk of extreme events, such as drought periods, which result in increased mortality in plant communities such as forests (Allen *et al.* 2010) and are further exacerbated by the general warming trend (Parmesan and Yohe 2003; Root *et al.* 2003). Thus, increasing attention is directed at the ability of long-lived species to cope with environmental variability in the face of climate change. An important way for organisms to respond to unstable conditions is phenotypic plasticity, *i.e.* the capacity of a genotype to render different phenotypes under diverse environmental conditions (Garland and Kelly 2006). When plasticity of traits correlates with fitness advantages in the face of variable environmental conditions, this is termed adaptive phenotypic plasticity (Ghalambor *et al.* 2007). In particular for immobile organisms such as plants, adaptive plasticity allows for direct and rapid responses to environmental changes or climatic extremes and may lead to a decreased risk of species loss due to climate change (Nicotra *et al.* 2010; Thomas 2011; Richter *et al.* 2012).

Available genetic diversity within a population increases the possibility of possessing alleles or allele combinations that are advantageous or even more capable in terms of response capability to environmental change (Jump *et al.* 2009; Nicotra *et al.* 2010). Thus, a high mean response capability towards environmental alterations can be assumed for a genetically diverse population (averaged over its individuals) compared to populations of lower genetic diversity, or at least a higher within-population variation in individual response strength. Some studies suggest that diversity of alleles and genotypes can facilitate plasticity and response capacity cumulatively at the scale of species or populations (Ehlers *et al.* 2008; Hughes *et al.* 2008; Jump *et al.* 2009; Doi *et al.* 2010 but see also Arnaud-Haond *et al.* 2010). Nonetheless, it is not yet clear whether genotypic and genomic diversity directly correspond to phenotypic plasticity and different response patterns among populations (*e.g.* Meier and Leuschner 2008; Nicotra *et al.* 2010; Wortemann *et al.* 2011). Such interrelations between genetic diversity and response capacity on the population scale, however, would be of high relevance for conservation and forest management in the face of climate change and their study can improve the understanding of phenotypic plasticity and adaptive potential of populations. Here, we tested the potential relationship between genetic diversity and phenotypic plasticity for a set of populations of *Fagus sylvatica* (European beech), based on their natural differences in genetic diversity according to the phylogeographic history of this species in Europe.

In Central Europe, *F. sylvatica* is the dominant native forest tree species. Like in many other European tree species, regional differences in genetic diversity within the current natural distribution range of *F. sylvatica* are mainly caused by its biogeographical history during and after the Pleistocene climate oscillations (Magri *et al.* 2006). Genetic diversity is higher in areas that have likely acted as glacial refuges than in later re-colonized peripheral regions (*e.g.* Comps *et al.* 2001; Magri *et al.* 2006). This is due to the fact that in refugia lineages from different regions merged and combined their genetic make-ups (including their potentially differentiated genes that control for phenotypic responses to environmental variability). During post-glacial re-colonization originating from these refugia, genetic variation decreases due to fixation and loss of alleles by sequential genetic drift events along dispersal routes (*e.g.* Hewitt 1996). Nevertheless, in some re-colonized regions genotypes of different refugia admixed during their spread, leading to a regional increase of genetic diversity (Petit *et al.* 2003). Both underlying mechanisms, admixture and genetic drift (*i.e.* bottlenecks and sequential founder effects along re-colonization routes), work irrespective of adaptive potentials of genotypes and alleles. Considering the relative short time period since the last glacial maximum (LGM; approx. 19-20 ka bp, Clark *et al.* 2009), regional differences in genetic diversity of European beech populations can therefore be assumed to originate primarily from these neutral phylogeographic processes and to a much lesser extent from the development of new alleles by mutation and local adaptations (Comps *et al.* 2001).

Intraspecific genetic structures predominantly measured with neutral markers also represent genomic variation relevant for variability in quantitative trait expression and adaptive potential (Merilä and Crnokrak 2001; Jump *et al.* 2009; Nicotra *et al.* 2010; Frankham 2011, but see also Reed and Frankham 2001; Bonin *et al.* 2007). Adaptive plasticity of traits is known to vary among genotypes within and among populations and regions (for some traits of potential relevance for *F. sylvatica* such as root growth, leaf phenology, photosynthetic performance or water-use efficiency see Meier and Leuschner 2008; Kraj and Sztorc 2009; Robson *et al.* 2012; Sánchez-Gómez *et al.* 2013). Generally, genetic variation is the basis for the expression and potential reaction norms of morphological traits (Scheiner 1993; Nicotra *et al.* 2010). Based on this, epigenetic, transcriptational or post-transcriptational regulation determines the final phenotype depending on the environment (*e.g.* Nicotra *et al.* 2010; Zhang *et al.* 2013).

F. sylvatica is drought-sensitive (Jump *et al.* 2006; Seynave *et al.* 2008), with drought primarily influencing the establishment of seedlings (Madsen and Larsen 1997; Aranda *et al.* 2002) but also long-term growth performance of older individuals (*e.g.* Bréda *et al.* 2006; Castagneri *et al.* 2014). Drought conditions are often correlated with high temperatures, which can cause stress on their own (*e.g.* higher respiration rates compared to photosynthesis; see *e.g.* Wittmann and Pfanz 2007; Robson *et al.* 2012). Furthermore, temperature is an important driver of spring leaf phenology in *F. sylvatica*, thereby controlling growing season length (*e.g.* Kramer 1994; Fu *et al.* 2013). Environmental variability is expected to be increased by climate change (IPCC 2012) and correspondent temporal resource limitations related to water availability and temperature are likely to cause high selective pressures on forests (Allen *et al.* 2010). For trees, this is particularly relevant at the seedling stage (Reich *et al.* 2003; Silvertown and Charlesworth 2007) and thus crucial for natural regeneration and genetic composition of future tree stands.

Population trials have revealed variable performances of *F. sylvatica* populations from diverse geographic origins in relation to different abiotic stressors such as drought (*e.g.* Nielsen and Jørgensen 2003; Thiel *et al.* 2014), warming (*e.g.* Wuehlisch *et al.* 1995) and late frost (Kreyling *et al.* 2012). Combinations of population trials with realistic climate manipulations are required to quantify short-term phenotypic adaptive potentials of different populations to future climate conditions. Phenotypic plasticity at the population level can then be assessed as a population's response variation in different environments (Valladares *et al.* 2006).

We expected that Bulgarian populations possess a more diverse composition of genotypes and alleles than German populations as the former provenance represents a glacial refuge region and the latter provenance originated from post-glacial re-colonization (Comps *et al.* 2001; Konnert and Ruetz 2001; Magri *et al.* 2006). We hypothesized that these differences correspond to differences in the response capability to drought and/or warming. Thus, we compared the phenotypic plasticity of *F. sylvatica* populations from Bulgaria and southern Germany (three populations, respectively) to warming and extreme drought in a common garden experiment. Calculated indices of phenotypic plasticity were related to genetic diversity at the population scale. We addressed the hypotheses that populations differ in their response to warming and drought. Specifically, we expected differences in growth, leaf phenology, response capability to the treatments and in genetic diversity between the regions (Bulgaria *vs.* Germany). Furthermore we predicted correlations between genetic diversity within populations and population phenotypic plasticity to drought and warming.

Material and Methods

Experimental design

This common garden experiment was established in March 2010 in the Ecological-Botanical Garden of the University of Bayreuth, Germany (E 11.571, N 49.94d; Fig. 1).

A comparison was made among six *F. sylvatica* populations from southern Germany (DE) and Bulgaria (BG; coordinates given in decimal degree, respectively): Hengstberg (DE1; E 12.183, N 50.133), Elchingen (DE2; E 10.063, N 48.456), Weildorf (DE3; E 12.882, N 47.846), Gotze Delchev (BG1; E 23.586, N 41.636), Petrochan (BG2; E 23.239, N 43.255), Strumjani (BG3; E 23.012, N 41.682), *i.e.* from a re-colonized region at the center of the species' distribution range and from a region at the southeastern periphery of the species' range that served as a glacial refuge, respectively (Fig. 1). The seeds were systematically collected in autumn 2008 from at least 20 mother trees into pooled samples for each target population. Sampling was done throughout the whole distribution of the population, respectively, ensuring sufficient distance between mother trees and avoiding bias from individual relationships within the seed pools. Based on these mixed samples, haphazardly chosen seeds were sown. From the germinated seedlings, individuals were randomly selected and assigned to the different experimental treatments for each population. All plants were cultivated at the *Bavarian Institute for Forest Seeding and Planting (ASP)* in Teisendorf, Germany from February 2009 to March 2010. The seedlings were then transported to Bayreuth and individually planted into 12-litre plastic pots filled with sandy silt from a typical local *F. sylvatica* habitat (but not of one of the analyzed populations) on 22nd of March (pH 7.27, total C 1.58 %, total N 0.13 %).

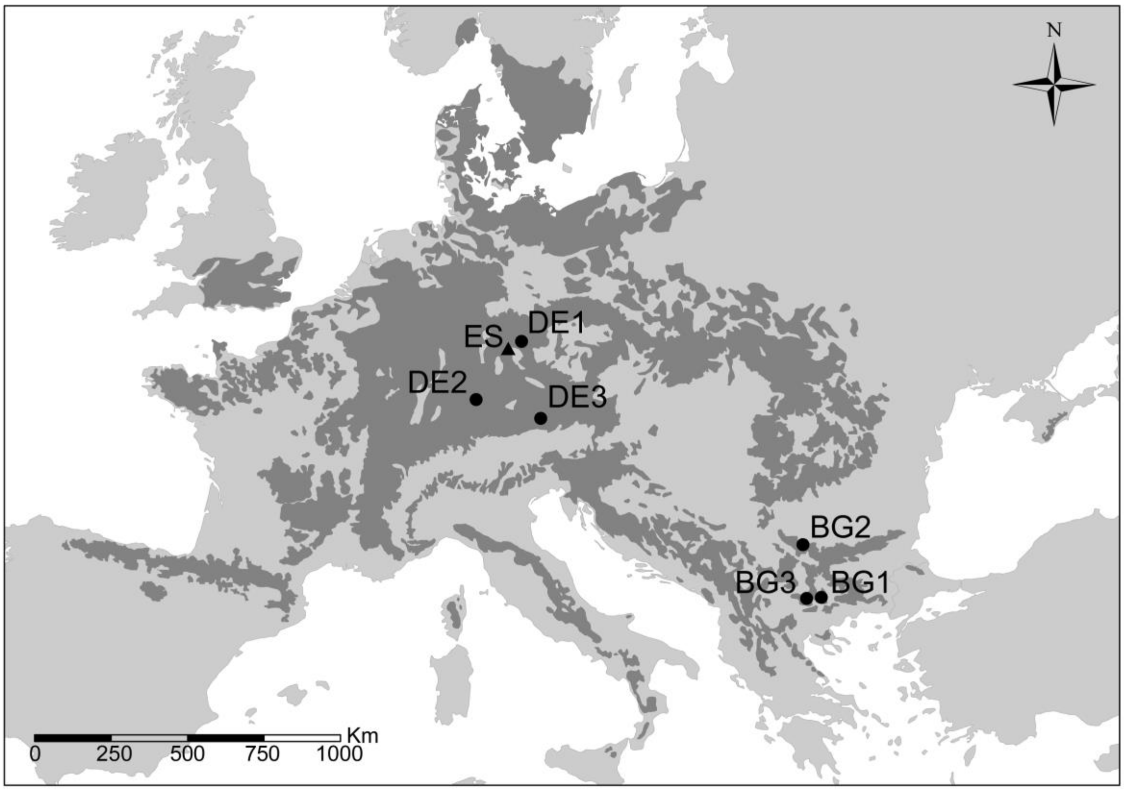

Fig. 1. Location of the experimental site and target populations of Fagus sylvatica in Germany and Bulgaria. DE1: Hengstberg, DE2: Elchingen, DE3: Weildorf, BG1: Gotze Delchev, BG2: Petrochan, BG3: Strumjani, ES: experimental site. The distribution of Fagus sylvatica is displayed in dark grey (EUFORGEN 2009).

The potted individuals were exposed to the fully crossed threefold factorial combination: (1) A drought manipulation and (2) a continuous warming manipulation (resulting in four different climate treatments: control, drought, warming, drought × warming) were each applied on (3) three Bulgarian and three German populations, respectively (the population factor).

Each climate treatment was replicated three times, which resulted in 12 experimental units in total, randomly arranged within separated rows, respectively. Per experimental unit each population was represented by three plants (nested replicates) which resulted in 9 individuals per population-treatment-combination, 36 individuals per population, and 216 seedlings in total. Individual plants of each population were randomly assigned to the experimental units. Spatial arrangements within the single experimental units were also random and a minimum distance of 1.5 m to the edge of the units minimized potential edge effects of the climate treatments. Each experimental unit was covered by a single rain-out shelter constructed from a steel frame and covered with a greenhouse plastic sheet (0.2 mm transparent polyethylene, SPR5, Hermann Meyer GmbH) with the lower edges of the rain-out shelters being 80 cm from the ground to allow for air circulation. The film permitted nearly 90% penetration of photosynthetically active radiation. Shading nets reduced radiation by another 30% for all experimental units as *F. sylvatica* seedlings usually occurs below the tree canopy.

The warming manipulation was achieved by a combination of passive warming (wind-shelters reducing wind speed by 70%, and black floor covers versus white floor covers) and active warming (IR-radiation from April 1st to the end of the experiment with approximately 30 W per m² (Elstein IOT/90 à 250W)), which increased the average air temperature at plant height by 1.6 °C (Fig. 2). The pots were watered after planting on 23rd, 25th and on 29th of March until they were saturated. Afterwards the irrigation simulated the local daily 30-year average precipitation applied twice a week with collected rain water (Tab. A1). Plants subjected to the drought manipulation received no water from 17th of May until 15th of July 2010 (60 days, days of year 137 to 196): Drought manipulation was ceased when 20% of the plants showed strong damage (76-100% of the leaves wilted) in order to ensure an effect of the drought on plant performance, which is an essential part in the definition of an extreme climatic event in ecology (Smith 2011). After the drought, the plants were initially watered with 600 ml per pot on 16th, 19th and 23rd of July and afterwards treated like those in the non-drought treatments. Air temperature at plant height (2 thermistores B57863-S302-F40, EPCOS, per experimental unit connected to a dl2 datalogger, Delta) and soil moisture (3 ECH20 EC-5 moisture sensors, Decagon Devices, Inc.) per climate treatment were logged hourly throughout the experiment.

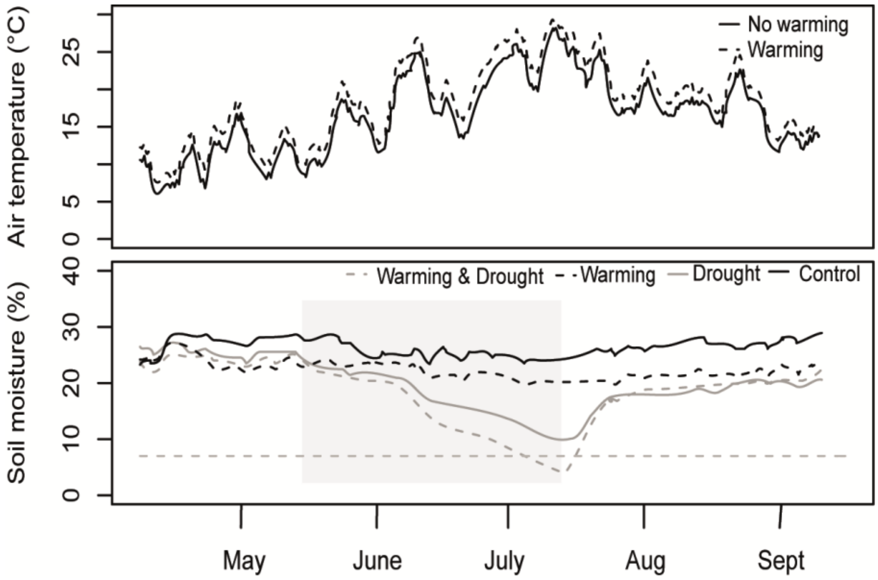

Fig. 2. Air temperature and soil moisture over the course of the experiment. Permanent wilting point (pF = 4.2 means 7 % soil moisture) is indicated by the dashed grey line in the lower panel. Time of drought is highlighted with a grey background in the lower panel.

Response parameters and phenotypic data analysis

Plant height increment was measured in May and October 2010 from root crown to plant tip and percentage increments between these dates were calculated per plant. Leaf phenology was recorded weekly for each individual and averaged within treatment groups which resulted in data at the daily scale (Jentsch *et al.* 2009). With regard to leaf unfolding phenology, we focused on its beginning, *i.e.* the date when the bud of a leaf broke open and the first green leaf was visible. Furthermore, leaf senescence in autumn was recorded, *i.e.* the date when all leaves turned fully brown.

All statistical analyses were conducted with the software R 3.0.2 (R Development Core Team 2013). For height increment, three-way analysis of variance (ANOVA) based on linear mixed effect models (lmer; package lme4; Bates *et al.* 2014 and package lmerTest; Kuznetsova *et al.* 2014) were applied to test for the main and interactive effects of the three factors: population, drought, and warming. Including the experimental unit as a random factor accounted for the split-plot design and the nested replicates (Pinheiro and Bates 2009). The same models were run a second time with the region (Bulgaria and Germany) as a fixed factor, in order to determine if potential effects were visible between the two regions. Here, population nested in region was included as an additional random factor. Height increment data were square root-transformed to improve the homogeneity of variances and the normality of residuals (Faraway 2005). For leaf unfolding and leaf senescence we applied rank-based ANOVAs because distributions of variances and residuals did not allow for parametrical models. Calendar dates were transformed to day of year to obtain a continuous variable.

Genotyping and analysis of genetic data

Allozyme variation was investigated to measure genetic parameters. This method is suitable to detect co-dominant genetic variation within samples (heterozygosities) as well as genetic variation within and among populations with relatively low investments of resources and time.

Plant buds were sampled in October 2010 and analyzed for their allelic structure of ten enzyme systems (*i.e.*, AAT = aspartate-aminotransferase, ACO = aconitase, IDH = isocitrate dehydrogenase, MNR = menadione reductase, MDH = malate dehydrogenase, PGI = phosphoglucose isomerase, PGM = phosphoglucomutase, PEX = peroxidase, 6PGDH = 6-phosphogluconate dehydrogenase, SKDH = Shikimate dehydrogenase) with 16 gene loci, commonly used to assess allozyme variation in European beech (*e.g.* Müller-Starck and Starke 1993) (AAT-A, AAT-B, ACO-A, ACO-B, IDH-A, MNR-A, MDH-A, MDH-B, MDH-C, PGI-B, PGM-A, PEX-B, 6-PGDH-A, 6-PGDH-B; 6-PGDH-C, SKDH-A). In addition to the samples from our experiment, we increased sample size to n = 378 in total (*i.e.* 63 samples per population) by including samples from another set of the same populations, for which seed collection and seedling cultivation were performed identically and at the same time.

Enzymes were analyzed on starch gels and zymograms were genetically interpreted as described by Müller-Starck and Starke (1993). Quality of bands was checked visually and only clear and distinct bands were analyzed.

We tested the non-selective neutrality of alleles based on their distributions within the analyzed loci to prove unbiased inferences in genetic structuring and diversity estimates by Ewens-Watterson tests on allele frequency distributions for each population in Arlequin v. 3.5 (Excoffier and Lischer 2010) which would detect deviations from the neutral equilibrium model. Two different test-statistics were applied, the homozygosity test-statistic (Watterson 1978) and the exact test (Slatkin 1994; Slatkin 1996) with 99999 permutations ($\alpha = 0.05$). Tests for Hardy-Weinberg-equilibrium (HWE) were done with Genepop (Rousset 2008), applying the Probability test (exact HW test) with standard settings and allowing for complete enumeration for loci with less than 5 alleles.

Computations of descriptive genetic parameters and genetic structure (AMOVA) were carried out with GenAlEx 6.5 (Peakall and Smouse 2012). The degree of genetic diversity within populations was quantified using the mean number of alleles over all loci (A), the percentage of polymorphic loci (P_{tot}), the observed (H_o) and the expected (H_e) heterozygosity, and the within-population inbreeding coefficient (F_{IS}). Additionally, the numbers of private alleles (alleles occurring only in one population) were calculated. To compare the proportions of genetic differentiation among populations and between regions, analyses of molecular variance (AMOVA) related to Wright's *F*-Statistics were conducted with 9999 permutations for significance tests.

Correlation of phenotypic plasticity and genetic diversity

We applied the widely used PI_{md} (phenotypic Plasticity Index; Valladares *et al.* 2006) as a parameter to quantify phenotypic plasticity of traits. It is based on maximum and minimum medians among the responses of the different treatments of a respective population: PI_{md} = (maximum median – minimum median) / maximum median, and thereby incorporates the responses of a population's individuals to different treatments. It thus fits well to the concept of phenotypic plasticity according to our study design. The median-based index PI_{md} is recommended in case of small sample sizes and non-Gaussian distribution of response parameter values. However, for our phenological parameters it performed poorly, due to the relatively small ranges and the integer nature of values (day of year). Despite obvious differences between populations and treatment effects, medians were identical among many treatments and populations, resulting in invariable plasticity indices. Therefore, we used the mean-based equivalent index PI_v (Valladares *et al.* 2006) for the assessments of phenotypic plasticity in phenological traits.

Indices of phenotypic plasticity among the different treatments for all single populations were correlated with their genetic diversity (allelic diversity A as well as expected and observed heterozygosity H_e and H_o).

Additionally, we tested if variation ranges (coefficients of variation – CVs) among individual phenotypic responses within treatment groups (population × treatment) were correlated with genetic diversity measures within treatment groups.

All correlations with genetic diversity measures were tested via Spearman's rank correlation coefficient. To account for the low number of data points (six populations) and thereby potentially strong influences of sample sizes on Spearman's *P*-values, significances of the correlations were calculated with a Monte-Carlo permutation procedure implemented in the R-package simba (Jurasinski 2012). 10000 permutations were done to test the correlations against a null model with zero slope, respectively.

Finally, in order to test the alternative hypothesis that differences in trait values and plasticity might originate from local adaptations of populations to their respective climatic site conditions rather than phylogeographic processes; we related the trait values to the climate at the origins in further correlation analyses. The calculated plasticity indices as well as means of the tested phenotypic parameters per population (averaged over all treatments, respectively) were correlated with climatic parameters of the original population sites. We tested 11 potentially relevant variables, derived from WorldClim (Hijmans *et al.* 2005; data for the period 1950–2000): annual mean temperature, temperature seasonality, mean temperature of driest quarter, mean temperature of warmest quarter, annual precipitation, precipitation of driest month, precipitation seasonality, precipitation of driest quarter, precipitation of warmest quarter, mean temperature from April to October (period of our warming treatment) and precipitation from May to July (duration of our drought treatment). See Tab. A2 for population values of these parameters. Again, Spearman's rank correlation coefficient and the above-described permutation approach to calculate significances were applied.

Results

Phenotypic variation among populations and regions

Height increment differed among populations (F = 6.55, P < 0.001), with BG1, BG2 and DE1 performing best, whereas no difference between the two regions could be found (F = 0.57, P = 0.499; Fig. 3, Tab. 1).

The populations differed in the timing of leaf unfolding (*F* = 4.47, *P* < 0.001, Tab. 1). On average, DE2 and BG2 flushed 1 day and 2 days earlier than the other populations, respectively (Fig 4A). The populations did not differ in the timing of leaf senescence (*F* = 1.12, *P* = 0.355). Average leaf unfolding and average leaf senescence did not differ significantly between the two regions, (*F* = 6.42, *P* = 0.484; *F* = 1.92, *P* = 0.217, respectively).

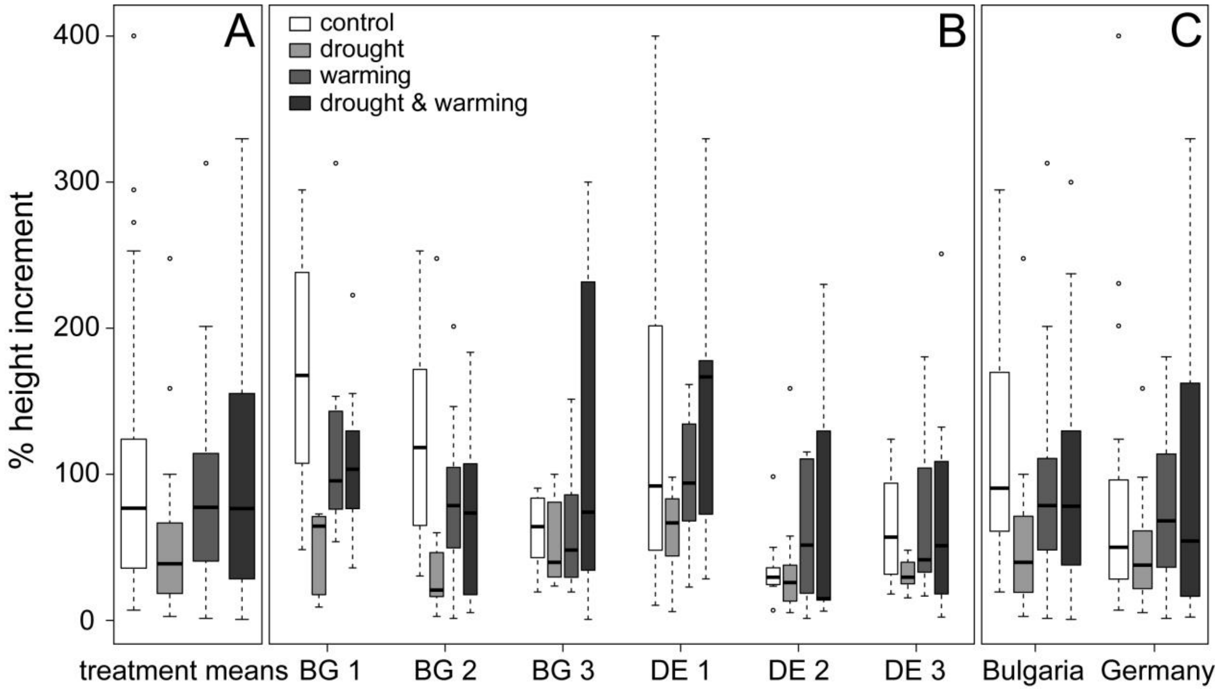

Fig. 3. Height increment of Fagus sylvatica populations over one growing season in response to warming, extreme drought, and origin of populations. (A) responses over all populations to the climate manipulations (B), response of the single populations to the climate manipulations, (C) responses over all populations split by the two regions to the climate manipulations. n = 216. Boxplots include median and upper and lower quartiles; whisker ranges in both directions include data points within the 1.5-fold interquartile range, maximally.

Response to warming and drought

Height increment of the plants was reduced by 19 % due to the drought manipulation (seedling mean height increment under control treatment: 90.4 % ± 6.9 (standard error), mean height increment under drought treatment: 73.4 % ± 6.9; *F* = 4.67, *P* = 0.063; Fig. 3A). Marginally significant population-specific differences in drought effects with respect to height increments were observed (BG1 showed a height increment reduction of 47 %, BG2 of 40 % and DE3 of 24 %; drought × population *F* = 1.98, *P* = 0.084; Tab. 1, Fig. 3B). BG1 and BG2 with the highest values of height increment in the control treatment showed the strongest negative response to drought. No significant difference in height increment responses on drought could be detected on the region scale (drought × region *F* = 1.63, *P* = 0.204, Tab. 1 and Fig. 3C).

Height increments were not affected by warming (*F* = 3.29, *P* = 0.107; Tab. 1). Accordingly, neither populations (*F* = 0.29, *P* = 0.919) nor regions (*F* = 0.68, *P* = 0.410) showed differences in response to warming with regard to height increment.

Interactive effects of drought and warming were detected for height increment (*F* = 8.96, *P* = 0.017; Tab. 1 and Fig. 3A). Plants exposed to the 'warming & drought'-treatment showed a higher height increment than those exposed to 'drought' only.

Leaf unfolding date was advanced by 3 days (*F* = 93.35, *P* < 0.001; Tab. 1 and Fig. 4A) for plants that experienced warming. Populations BG1, BG3 and DE3 had late leaf unfolding dates under control conditions but earlier leaf unfolding dates under warming, while the other populations showed no effect (*i.e.* kept their early leaf unfolding more or less constant; population × warming F = 5.11, P < 0.001; Tab. 1 and Fig. 4A). There were no differences in warming response between the regions (region × warming F = 0.06, P = 0.813).

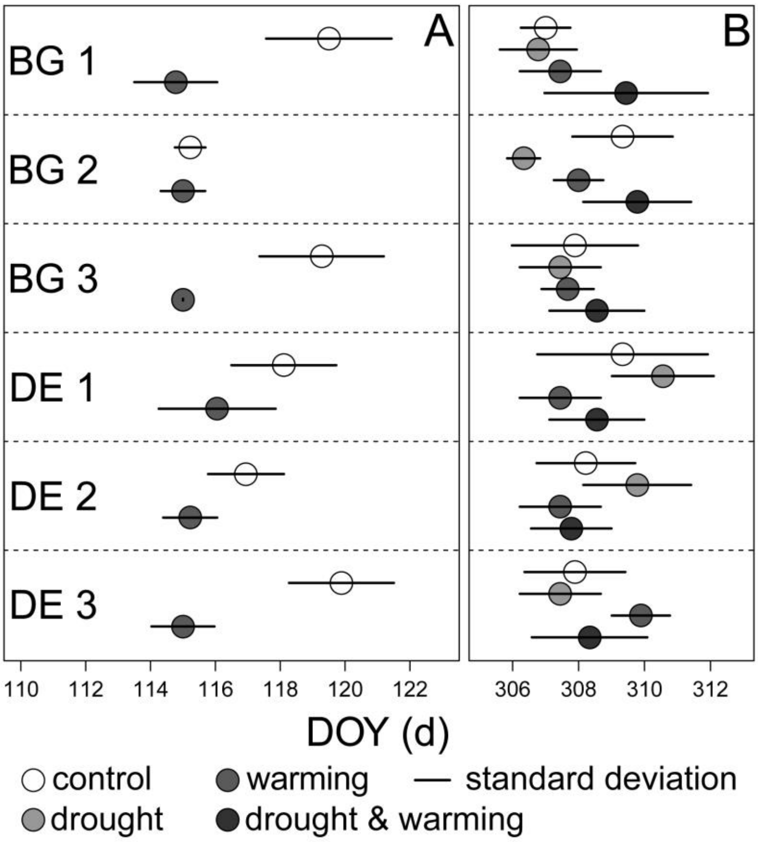

Fig. 4. Dates of leaf unfolding (A) and leaf senescence (B) for different Fagus sylvatica populations in response to the climate manipulations. Note that the drought manipulation started after leaf unfolding and therefore cannot be considered for (A). Displayed are mean values and standard deviations per group. DOY: day of year.

Leaf senescence date was neither affected by drought manipulation (F = 0.04, P = 0. 843) nor by warming (F = 0.75, P = 0.412; Tab. 1 and Fig. 4B) or by the interaction of drought and warming (F = 0.15, P = 0.705). However, populations responded differently to warming (population × warming F = 2.31, P = 0.046; Tab. 1), with population DE3 senescing later than other populations (Fig. 4B). No population effect was found for the responses to drought or the interaction of drought and warming (Tab. 1). The two regions showed no significant response differences to the drought treatment, however, the warming treatment generated different responses (region × warming F = 3.95, P = 0.048), with DE3 senescing very late and all Bulgarian populations senescing at earlier dates. Also, regional responses on the interaction of drought and warming differed significantly (region × drought × warming F = 5.67, P = 0.018; Tab. 1), with the German populations senescing earlier than the Bulgarian populations (Fig. 4B).

Table 1 ANOVA results of the applied mixed models for height increment, leaf unfolding date and leaf senescence date on the population scale and on the region scale.

Parameter	% Height increment			Leaf unfolding date			Leaf senescence date		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
Population	6.55	5	<0.001	4.47	5	<0.001	1.12	5	0.355
Warming	3.29	1	0.107	93.35	1	<0.001	0.75	1	0.412
Drought	4.67	1	0.063				0.04	1	0.843
Warming × drought	8.96	1	0.017				0.15	1	0.705
Population × warming	0.29	5	0.919	5.11	5	<0.001	2.31	5	0.046
Population × drought	1.98	5	0.084				1.69	5	0.139
Population × warming × drought	0.72	5	0.606				1.71	5	0.134
Region	0.57	1	0.499	0.64	1	0.484	1.91	1	0.217
Warming	3.30	1	0.107	84.60	1	<0.001	0.75	1	0.412
Drought	4.64	1	0.063				0.04	1	0.843
Warming × drought	8.84	1	0.018				0.15	1	0.705
Region × warming	0.68	1	0.410	0.06	1	0.813	3.95	1	0.048
Region × drought	1.63	1	0.204				0.57	1	0.450
Region × warming × drought	0.68	1	0.411				5.67	1	0.018

Note. ANOVAs for leaf unfolding date and leaf senescence date were calculated with ranked response parameters to account for insufficient distributions of variances and residuals in the regular models. For population scale, fixed factors: population, warming, drought; random factor: experimental unit; for regional scale, fixed factor: region, warming, drought; random factors: experimental unit, population.

Genetic diversity patterns and genetic structure

Genetic analyses were made for 16 allozyme loci of 378 individuals (Tab. 2). The locus MDHA was monomorphic for all populations. The neutrality tests revealed no departure from non-selective neutral allele distributions within populations, proving the applicability of the used allozyme loci for our study: Watterson's *p*-values ranged between 0.143 (DE2) and 0.999 (BG1) and Slatkin's exact test *p*-values elevated from 0.107 (DE2) to 0.999 (BG1). Allele frequencies per locus and population are given in the appendix (Tab. A3).

Table 2 Descriptive population genetics and plasticity indices of the analyzed populations.

Population	*N* (genetic analyses)	no. of private alleles	P_{tot} (%)	H_e	H_o	*A*	F_{IS}	PI_{md} height increments	PI_v leaf unfolding	PI_v leaf senescence
BG1	62	4	93.8	0.18	0.18	2.44	0.00	0.615	0.040	0.009
BG2	63	3	93.8	0.22	0.20	2.63	0.05	0.825	0.002	0.011
BG3	64	0	87.5	0.19	0.19	2.25	0.00	0.464	0.036	0.004
DE1	63	0	93.8	0.25	0.23	2.38	0.03	0.60	0.017	0.010
DE2	62	0	87.5	0.26	0.25	2.25	0.02	0.711	0.015	0.008
DE3	64	0	81.3	0.22	0.21	2.13	0.02	0.482	0.041	0.008

Note. *N* = genetically analyzed samples; no. of private alleles = number of alleles unique to a single population; P_{tot} = mean percentage of polymorphic loci; H_e = mean expected heterozygosity; H_o = mean observed heterozygosity; *A* = mean no. of different alleles per locus; F_{IS} = mean fixation index; PI_{md}, PI_v = plasticity indices based on medians or mean values, respectively.

The only populations with private alleles were BG1 (4 alleles) and BG2 (3 alleles). However, the frequencies of these alleles were less than 3 %. In the regional comparison, eight alleles were unique to Bulgaria and absent in the German populations and no alleles unique to the German region were found. Mean percentage of polymorphic loci (P_{tot}) varied between 81.25 % (DE3) and 93.75 (BG1, BG2 and D1), showing a tendency of slightly higher genetic diversity within Bulgarian populations (no test possible, see discussion). Expected (H_e) and observed (H_o) heterozygosity showed almost equal values and patterns, and there were no deviances from HWE detectable within populations (Fisher's method population probabilities between 0.19 and 1), supporting an un-biased seed sampling within original populations. Heterozygosity was higher in the German region (populations H_o ranging from 0.208 to 0.249) than in the Bulgarian region (populations' H_o varying between 0.177 and 0.204) with DE2 and BG2 being the populations with the highest heterozygosities for their region, respectively. Contrastingly, the average number of different alleles per locus (A) had a tendency to be slightly higher in the Bulgarian (ranging from 2.250 to 2.625) compared to the German populations (A ranging from 2.125 to 2.375), except for BG3, which showed the same or a lower value than two of the German populations (DE2: 2.250, DE1: 2.375, respectively). A large distance trend of inbreeding was not detected as the within population fixation indices (F_{IS}) were quite low, suggesting strong outbreeding behavior within populations, and had both, the highest (0.049, BG2) and the lowest (–0.003, BG3) values in populations from Bulgaria.

Overall AMOVA revealed the highest portion of molecular variance within the populations (89.94 %), and considerably lower portions among (all six) populations (5.57 %, $F_{ST} = 0.101$, $P < 0.001$) and among the two regions of Bulgaria and Germany (4.50 %, $F_{RT} = 0.045$, $P < 0.001$). Differentiation of populations within regions ($F_{SR} = 0.058$, $P < 0.001$) was higher than differentiation between regions ($F_{RT} = 0.045$, $P < 0.001$). Single-region AMOVAs indicated that Bulgarian populations were genetically more differentiated within their region (8.56 %, $F_{ST} = 0.086$, $P < 0.001$) than the German populations (2.79 %, $F_{ST} = 0.028$, $P < 0.001$).

Correlations of genetic diversity and phenotypic plasticity

The calculated plasticity indices showed contrasting patterns at the population level regarding the different phenotypic parameters (Tab. 2). While BG2, DE2 and BG1 showed the highest plasticity values for height increment ($PI_{md} = 0.825$, $PI_{md} = 0.711$ and $PI_{md} = 0.615$, respectively), leaf senescence plasticity was highest in BG2, DE1 and BG1 ($PI_v = 0.009$, $PI_v = 0.011$ and $PI_v = 0.01$, respectively) and leaf unfolding plasticity was strongest in DE3, BG1 and BG3 ($PI_v = 0.041$, $PI_v = 0.040$ and $PI_v = 0.036$, respectively) and lowest in BG2 ($PI_v = 0.002$).

Correlation analyses revealed a significant positive relationship between the number of alleles (A) and height increment plasticity (Spearman's rank correlation coefficient $\rho = 0.67$, significance of slope equality to null model $P = 0.042$; Fig. 5A). The relationships of A with the plasticity indices on phenology parameters were not significant for leaf unfolding ($\rho = -0.55$, $P = 0.128$, Fig. 5B) and marginally significant for leaf senescence ($\rho = 0.75$, $P = 0.0502$, Fig. 5C). However, slight tendencies were visible of leaf unfolding plasticity being negatively related and leaf senescence plasticity being positively related to allelic diversity.

Correlations of plasticity indices to H_o and H_e yielded no significant relationships ($P > 0.2$, not shown).

No significant correlation could be found between genetic diversity parameters and response variation within treatment groups (CVs). Also, neither significant correlation was detectable between mean phenotypic trait values per population and the tested climatic parameters at the sites of origin of the populations, nor between plasticity indices and the climatic parameters at the sites of origin.

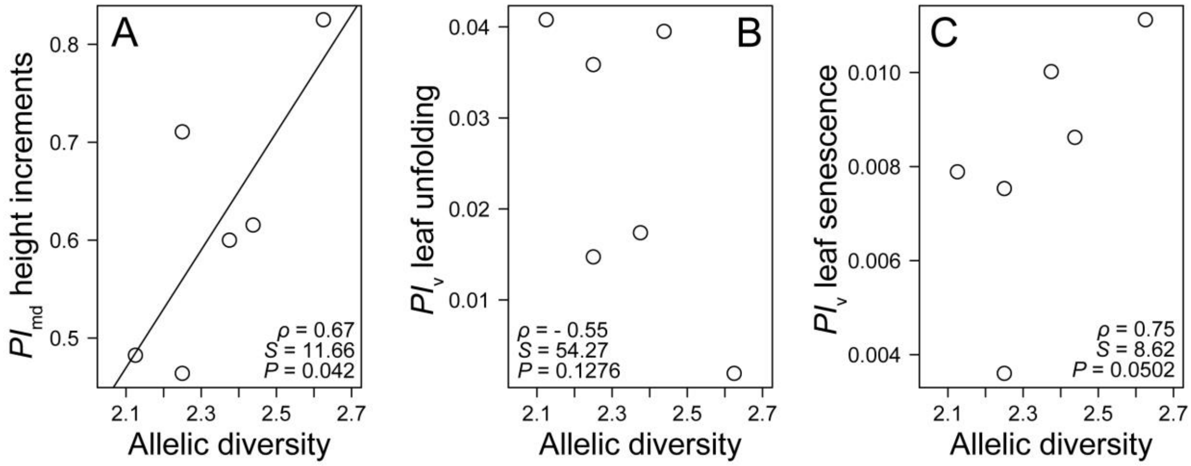

Fig. 5. Relationship between allelic diversity (mean number of alleles over all loci) and plasticity index for height increments (A), the leaf unfolding plasticity index (B) and the plasticity index for leaf senescence date (C) of the analyzed populations. Spearman's rank correlation coefficient and significances based on Monte-Carlo permutations are depicted.

Discussion

Regional and population phenotypic variation

We found variation in height increment and leaf unfolding among the tested populations of *Fagus sylvatica*, but not between the two regions. Nielsen and Jorgensen (2003) also showed general differences in absolute height increment, leaf flushing and cessation of growth in autumn among 14 European *F. sylvatica* populations. In their study, however, clear regional differences were visible in both leaf flushing and cessation of growth (the associated trait leaf senescence showed no difference among populations in our study at all). These differences followed the latitudinal gradient but were not related to temperature of the original sites (which supports the missing correlations to temperature in our analyses). This indicates that latitudinal variation in photoperiod is an important factor for leaf phenology differences within European beech (at least for leaf unfolding, see also Körner and Basler 2010; Vitasse and Basler 2013), which, however, could not be detected in our study for the analyzed mean traits, probably because of the high intraregional variation. Accordingly, Robson *et al.* (2013) analyzed 32 *F. sylvatica* populations from across Europe in a common garden in Spain and found regional differences in spring phenology and growth performance, but also strong variation among geographically close populations. In line with our results on phenotypic traits, genotypic data (AMOVA results) showed only minor regional differentiation between South-Germany and Bulgaria compared to intraregional differentiation. Taken together, these findings emphasize the need to consider small to medium geographical scales (regions and below) with the same attention as this is done with larger (continental) scales when exploring within-species variation of *F. sylvatica*.

Population differences in response to warming and drought

The analyzed traits, in particular height increment, integrate various processes such as efficiencies of the root system or numerous physiological processes, mainly photosynthesis. Thus, plasticity in the analyzed traits represents response capabilities of various important life processes (and their interactions) in trees and therefore provides information on general performance variability, which is essential for assessments of upcoming climate change outcomes on forest ecosystems.

Apart from an advancement of leaf unfolding dates, no direct general effect of warming was found for the tested *F. sylvatica* populations. The relative small temperature increase of 1.6 °C and short treatment duration (only one vegetation period) in our experiment might have been too mild to result in significant effects on growth height increment. Accordingly, the missing effects in leaf senescence also might be a result of our moderate warming treatment as other studies that imposed greater temperature differences found temperature effects on the leaf senescence of *F. sylvatica* (*e.g.* a hyperbolic response in common garden experiment in Vitasse *et al.* 2010 and the delay in leaf senescence by 5.6 ± 0.6 days $°C^{-1}$ along an elevational temperature gradient in Vitasse *et al.* 2009). The weak differences in warming response between populations or regions in our study without consistent response direction seem to speak against this temperature sensitivity. However, the variance among and within populations in leaf senescence at the single measuring dates in Vitasse *et al.* (2009) is comparable to the inconsistent leaf senescence variation measured in our study in a common garden and a mere mean temperature difference between treatments of 1.6 °C. For many temperate tree species a limited plasticity of bud set timing due to local adaptations of populations to latitudinal photoperiod conditions has been shown (*e.g.* Howe *et al.* 2003; Ingvarsson *et al.* 2006), while for *F. sylvatica* only minor contributions of photoperiod to autumn phenology are stated (Vitasse *et al.* 2011).

The observed warming sensitivity of leaf unfolding is in line with other studies on European beech (*e.g.* Vitasse *et al.* 2009; Fu *et al.* 2013). For example, the average advancement by 3 days in leaf unfolding caused by a temperature increase by 1.6 °C in our experiment corresponds well with an advancement of 1.9 days per 1 °C for leaf unfolding as found by Vitasse *et al.* (2009).

The population-specific warming responses in leaf unfolding were due to differences between leaf unfolding dates under control conditions, while under warming conditions leaf unfolding was advanced to approximately the same date among populations. External factors controlling bud burst phenology in *F. sylvatica* include winter chilling, photoperiod, temperature and their interactions (Körner and Basler 2010; Vitasse and Basler 2013). The timing of bud burst is under strong stabilizing selection (Gömöry and Paule 2011) due to the trade-off between maximizing growing season length (and biomass accumulation) and avoiding late frost damage (*e.g.* Kreyling *et al.* 2012). This trade-off limits leaf unfolding plasticity, expressed by the dominance of photoperiod as a cue for bud burst (Körner and Basler 2010; Vitasse and Basler 2013). The observed advancement in leaf unfolding in our study to a very similar timing among all populations under warming might therefore indicate a lower temporal border of temperature-related plasticity for this trait according to the conditions of this experiment. In a warming world with advancing spring conditions, such limited plasticity probably entails a strong adaptive benefit for seedlings of *F. sylvatica* by enabling them to advance their photosynthetically active period, but on the other hand by protecting them from too early leaf unfolding and thus from occasional late frost damage. Optimization trade-offs between leafy season protraction (prolonged C uptake, nutrient remobilization) and frost damage risk can also be assumed for leaf senescence timing (Keskitalo *et al.* 2005), for which, however, no indications could be found in our plasticity study.

F. sylvatica is sensitive to drought (Jump *et al.* 2006; Seynave *et al.* 2008); hence, the average height increment decline by 19 % due to the drought treatment was not surprising. In general, height increment in juvenile plants represents a strong component of fitness because in this stage, growth performance is the main determinant of the competitive potential of a seedling and might be decisive on which individual plant will overcome its neighbors and reach the canopy to reproduce (Kozovits *et al.* 2005; Silvertown and Charlesworth 2007). However, strong tradeoffs exist between a large height increment per growing season and the general resource budgets to maintain the functional equilibrium and competitive capabilities in the actual but also in the following growth season(s) (Kozovits *et al.* 2005). The variability of growth responses found in our study implied high plasticity for this trait, *i.e.* large height increment under favorable conditions, and growth reduction to save resources under drought conditions. Accordingly, the plastic response to drought represents a shift towards an altered phenotypic optimum required by a change of environmental conditions. The plasticity in height increment due to drought is therefore adaptive (Ghalambor *et al.* 2007), increasing tolerance against variable moisture conditions and potentially attenuating climate change impacts on European beech stands.

Populations slightly differed in their height increment sensitivity to drought (marginally significant). The most plastic reactions to drought were visible in two populations from Bulgaria (BG1 and BG2), supporting our assumption of higher phenotypic plasticity in the Bulgarian region for this trait.

According to the overall lack of leaf senescence response to drought, no population effect in the response could be detected in this trait. The regional difference in response to the combination of drought and warming seemed to be more an effect of warming, as warming alone generated differences in leaf senescence response among regions and single populations whereas drought did not. This lack of drought effect might indicate a general tolerance of leaf senescence to drought in *F. sylvatica* but it is more likely that drought did not affect leaf senescence because of the long time span between the treatment (which ceased in July) and the measurement in autumn.

Unexpectedly, seedlings treated with warming and drought showed bigger height increments than seedlings treated with drought only, or even exceeded growth under control conditions in BG3 and DE1. This might indicate that the height increment plasticity in our study is not just a simple response to a limited resource (water) but is more complex in its functioning and control. Plasticity in response to a given environmental factor can be decreased by further (varying) factors influencing a plant (Valladares *et al.* 2007). This might explain the reduced and potentially non-adaptive or even maladaptive growth plasticity (Ghalambor *et al.* 2007) of our beech seedlings in response to the combination of drought and warming treatments. One could also speculate that the combination of drought and warming induced compensatory growth after the drought period, resulting in overcompensation for BG3 and DE1. The measurements were made three months after the drought treatments have ceased, precluding us from resolving the timing of the growth. However, compensatory growth as a delayed response to abiotic stressors such as drought has also been reported for Macadamia trees (Stephenson *et al.* 2003) and rice cultivars (Siopongco *et al.* 2006).

Trait plasticity appeared more consistent in some populations than in others, implying general differences in plasticity which is in line with our first hypotheses. Several traits of BG1 and BG2 showed strong responses to the applied treatments, whereas BG3 was only plastic in leaf unfolding and invariable in the other traits. Kreyling *et al.* (2012) used the same populations for a late frost experiment and also found BG1 and BG2 populations to have the highest plasticity in height increment in response to late frost. This suggests that plasticity might be a general trait which is unevenly distributed among populations, potentially causing differences in short-term adaptability to climate variability. Populations exhibiting a higher general plasticity can be expected to outperform less plastic populations in variable environments (Nicotra *et al.* 2010; Richter *et al.* 2012). However, a general pattern of different response capabilities to the treatments among the analyzed regions (according to our second hypothesis) could not be verified. It should be noted, though, that the lack of strong regional effects in plasticity in our study might result from the limited number of only two regions tested against each other.

Relation between genetic diversity and phenotypic plasticity of populations

Bulgarian populations exhibited a slightly higher genetic diversity than the German populations when looking at the regional mean number of private alleles, total percentage of polymorphic loci (P_{tot}) and number of alleles (A). This is in line with our second hypothesis on regional differences in genetic diversity and corresponds well with the phylogeographic history of European beech (*e.g.* Comps *et al.* 2001; Konnert and Ruetz 2001; Magri *et al.* 2006).

The lack of correlation at the population level between observed heterozygosity H_o and phenotypic plasticity in our analysis is in line with findings in the literature. Although the overdominance model states that heterozygosity facilitates genetic homeostasis of traits (Dobzhansky 1947; Lerner 1954; Gillespie and Turelli 1989), empirical evidence for an effect of heterozygosity on phenotypic plasticity is missing, as reviewed by Scheiner (1993). Instead, pleiotropy (differential expression of the same gene in different environments) and epistasis (interaction of different genes for character expression and genes that determine the magnitude of expression) are major mechanisms controlling phenotypic plasticity (Scheiner 1993).

Being an allele frequency-based measure of genetic diversity, expected heterozygosity (gene diversity) H_e is maximized when many alleles of equal (and thus medium to relatively high) frequencies exist. In our study allele frequencies within loci were unbalanced with one allele dominating over one or several other alleles (see Tab. A3). This probably explains the differences in relative parameter values and correlation behavior of H_e to phenotypic plasticity indices compared with A, which is not influenced by rarity or commonness of alleles.

Allelic diversity has been shown to better explain heterogeneous compositions of populations due to merging of different population sources during Pleistocene oscillations in glacial refugia (Comps *et al.* 2001). Considering phenotypic plasticity, allelic diversity of admixed populations might reflect a diversity of response type-related alleles and therefore increases ecological capacities or "options" (Jump *et al.* 2009), which presumably leads to greater average response capabilities to environmental variability at the population scale. Contrastingly, founder populations in re-colonized areas would likely have lost at least some of their genetic diversity due to genetic drift during dispersal processes.

We found no significant correlation between genetic diversity and the variation of responses among individuals within the different environments (CVs within treatment groups). Therefore, genetic diversity does not simply increase response variability within populations. This conclusion supports our hypothesis of non-random modulations of response capability in different environments due to high genetic diversity at the population level.

Whether an adaptive plastic response to varying environmental conditions results in a shift of phenotypic trait values or rather in a homeostatic reaction keeping the phenotype constant depends on the type of trait, its ecological function and its specific interaction with environmental variability (Schlichting and Smith 2002).

Within three Polish *F. sylvatica* stands, individuals of intermediate bud burst timing showed highest allelic diversity (based on microsatellite markers) compared to individuals of early and late bud burst timing (Kraj and Sztorc 2009). Although this does not directly account for phenotypic plasticity in variable environmental conditions at a population level, it is an indication of interrelation between allelic diversity and bud burst phenology. Genetic diversity at the population level might therefore facilitate homeostasis in spring phenology, which could explain the missing positive correlation between allelic diversity and leaf unfolding plasticity in our study. Such canalization, *i.e.* conservatism of phenotypes within populations in response to environmental fluctuations, is well known (Waddington 1959) and acts as a buffer against maladaptive phenotypic plasticity.

In contrast, plasticity of height increment and leaf senescence timing can be assumed to expose a greater benefit for *F. sylvatica* seedlings under climatic variability, suggesting a possible explanation for their positive correlations (in case of leaf senescence marginally significant) to allelic diversity. Height increment plasticity of tree seedlings is likely to be beneficial when environmental conditions vary: Growth depends on resource availability and controls metabolic equilibrium and individual success among competing seedlings. Thus, adequate short term modulation should be beneficial. Likewise, leaf senescence timing plasticity can effectively optimize the C-uptake period. For *F. sylvatica*, leaf senescence plasticity has been shown to have a greater effect on expanding growing season length than leaf flushing plasticity (Vitasse *et al.* 2009). Leaf senescence represents a late stage of winter hardening in deciduous trees, occurring before dormancy induction but after the critical growth cessation and bud set. Losing mature or already senescing leaves by early frost after bud set would probably not cause such a severe drawback for trees than losing unfolding leaves due to late frost in spring, which might have reduced purifying/stabilizing selection pressures on leaf senescence timing. For traits that experience varying selection pressures (*e.g.* by climatic variation in space and/or time), different phenotypic response capabilities can evolve (Jong 1995; Leimar 2005). Resulting functional diversity is likely to accumulate in admixed populations, which might be a plausible explanation for the found positive correlations in our study.

The existing patterns of genetic diversity among and within European beech populations are of phylogeographic origin (*e.g.* Comps *et al.* 2001; Konnert and Ruetz 2001; Petit *et al.* 2003; Magri *et al.* 2006). Genetic diversity itself, however, is indicated here to be interrelated to plastic responses to environmental variation on the population scale, although underlying mechanisms are not clear and call for further research. As neither phenotypic trait values nor their plasticity indices showed any direct correlation to the climatic conditions at the populations' origins, the detected differences among populations in their plasticity are unlikely to stem from local adaptation. This supports our hypothesis that differences in phenotypic plasticity among populations can be related to the degree of population admixture during the glacial and postglacial history of populations (estimated by genetic diversity measures). Adaptive response capacity in the form of phenotypic plasticity on the population scale may benefit from different combinations of alleles (from different regions and potential adaptive origins, including rare alleles). This may act through different mechanisms and on different levels, from the mere "option value effect" (Jump *et al.* 2009) to genotype interactions and neighboring effects within and between species of the same habitat or ecosystem (reviewed in Hughes *et al.* 2008).

While supporting the hypothesized interrelation between genetic diversity and phenotypic plasticity, our study is limited in several aspects and should therefore be seen as a starting point for further investigations rather than a definite proof. First, larger sample sizes in treatment groups and higher numbers of tested populations will allow for better assessments of phenotypic plasticity and more robust correlations. Second, treatment strengths or duration can be increased to obtain more distinct plastic reactions. Third,, polymorphisms of allozymes are limited and molecular marker systems of higher resolution and genomic coverage (*e.g.* Single Nucleotide Polymorphisms, or Amplified Fragment Length Polymorphisms), should thus be favored to assess genetic diversity. With these, future studies will be able to identify plasticity-related loci and respective frequency differences among populations (*e.g.* by Genome-Wide Association Studies or Quantitative Trait Loci analyses).

Nevertheless, keeping in mind these limitations, our study raises a new and interesting viewpoint on genetic diversity and phenotypic plasticity and discloses research potential on this issue. Genetic diversity, even if not directly evidenced to be adaptive or linked to phenotypic traits, should be taken more into account in ecological analyses, as well as in conservational and economic purposes in a changing climate and should not solely be considered as "simple" substrate for evolutionary adaptation. Trees are long-lived organisms with slow reproduction and comparably low standing genetic variation, which creates difficulty for quick genetic adaptation to rapidly changing climatic conditions (Aitken *et al.* 2008). Phenotypic plasticity may be a more direct and important approach for tree species to adapt to novel, or at least more variable climatic conditions (Nicotra *et al.* 2010). The potential interrelationship between standing genetic diversity in populations due to historical processes and phenotypic plasticity would thus imply a new quality of genetic diversity, relevant for different kinds of application (forestry, nature conservation) and the understanding of species' response to environmental change.

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Appendix

Table A1: Irrigation dates and amounts for the non-drought-treatment plants according to a simulated 30-year average precipitation

Date	Amount	30 y average	Date	Amount	30 y average
01.04.2010	0.49 l	6.83 mm	05.08.2010	0.34 l	4.78 mm
05.04.2010	0.51 l	7.11 mm	09.08.2010	0.62 l	8.70 mm
08.04.2010	0.40 l	5.55 mm	12.08.2010	0.64 l	9.00 mm
12.04.2010	0.49 l	6.90 mm	16.08.2010	0.32 l	4.51 mm
15.04.2010	0.28 l	3.90 mm	19.08.2010	0.35 l	4.86 mm
19.04.2010	0.38 l	5.36 mm	23.08.2010	0.58 l	8.05 mm
22.04.2010	0.16 l	2.28 mm	26.08.2010	0.42 l	5.90 mm
26.04.2010	0.35 l	4.84 mm	30.08.2010	0.44 l	6.17 mm
29.04.2010	0.36 l	5.07 mm	02.09.2010	0.37 l	5.13 mm
03.05.2010	0.56 l	7.85 mm	06.09.2010	0.70 l	9.82 mm
06.05.2010	0.25 l	3.43 mm	09.09.2010	0.41 l	5.69 mm
10.05.2010	0.55 l	7.69 mm	13.09.2010	0.61 l	8.54 mm
13.05.2010	0.44 l	6.15 mm	16.09.2010	0.50 l	7.02 mm
17.05.2010	0.43 l	5.99 mm	20.09.2010	0.29 l	4.02 mm
20.05.2010	0.32 l	4.53 mm	23.09.2010	0.44 l	6.20 mm
24.05.2010	0.60 l	8.46 mm	27.09.2010	0.38 l	5.38 mm
27.05.2009	0.43 l	6.03 mm	30.09.2010	0.39 l	5.50 mm
31.05.2010	0.58 l	8.14 mm	04.10.2010	0.44 l	6.12 mm
03.06.2010	0.51 l	7.19 mm	07.10.2010	0.46 l	6.45 mm
07.06.2010	0.83 l	11.67 mm	11.10.2010	0.44 l	6.10 mm
10.06.2010	0.61 l	8.49 mm	14.10.2010	0.35 l	4.91 mm
14.06.2010	0.89 l	12.46 mm	18.10.2010	0.41 l	5.67 mm
17.06.2010	0.54 l	7.52 mm	21.10.2010	0.42 l	5.94 mm
21.06.2010	0.78 l	10.91 mm	25.10.2010	0.65 l	9.04 mm
24.06.2010	0.54 l	7.50 mm	28.10.2010	0.41 l	5.77 mm
28.06.2010	0.61 l	8.57 mm	01.11.2010	0.54 l	7.49 mm
01.07.2010	0.53 l	7.45 mm	04.11.2010	0.38 l	5.32 mm
05.07.2010	0.67 l	9.31 mm	08.11.2010	0.45 l	6.32 mm
08.07.2010	0.62 l	8.71 mm	11.11.2010	0.30 l	4.26 mm
12.07.2010	0.71 l	9.99 mm	15.11.2010	0.73 l	10.17 mm
15.07.2010	0.72 l	10.06 mm	18.11.2010	0.57 l	8.02 mm
19.07.2010	0.82 l	11.48 mm	22.11.2010	0.59 l	8.28 mm
22.07.2010	0.69 l	9.63 mm	25.11.2010	0.28 l	3.92 mm
26.07.2010	0.92 l	12.82 mm	29.11.2010	0.51 l	7.07 mm
29.07.2010	0.58 l	8.12 mm	02.12.2010	0.31 l	4.30 mm
02.08.2010	0.45 l	6.31 mm			

Table A2: Populations used in the experiment and environmental conditions at sites of origins.

Provenance	Name	Region	Latitude (DD)	Longitude (DD)	Elevation (m asl)	BIO1 annual mean temperature (°C)	BIO4 temperature seasonality (standard deviation) (°C)	BIO9 mean temperature of driest quarter (°C)	BIO10 mean temperature of warmest quarter (°C)	mean temperature April to October (°C)	BIO12 annual mean precipitation (mm)	BIO14 precipitation of driest month (mm)	BIO15 precipitation seasonality (coefficient of variation)	BIO17 precipitation of driest quarter (mm)	BIO18 precipitation of warmest quarter (mm)	precipitation sum May to July (mm)
BG1	Gotze Delchev	BG	41.636	23.586	1600	7.4	6.78	12.9	16	12.4	618	31	21	110	146	173
BG2	Petrochan	BG	43.255	23.239	200	10.8	7.66	16.4	20.3	16.6	641	42	22	129	183	213
BG3	Strumjani	BG	41.682	23.012	1450	6.9	6.77	12.5	15.4	11.9	598	31	19	111	141	167
DE1	Hengstberg	DE	50.133	12.183	570	6.9	6.70	2.3	15.3	12.0	673	39	22	129	226	214
DE2	Elchingen	DE	48.456	10.063	560	8	6.66	0.6	16.4	13.0	773	44	30	138	280	272
DE3	Weildorf	DE	47.846	12.882	460	8.6	6.94	1	17.3	13.9	1127	58	34	193	433	408
ES	Bayreuth	DE	49.946	11.571	350	8.2	6.51	3.8	16.5	13.1	654	39	21	130	216	208

Note. Climatic data was taken for the period 1950 – 2000 from WorldClim (Hijmans *et al.* 2005). DD = decimal degree; CV = coefficient of variation; SD = standard deviation; BG = Bulgaria; DE = Germany.

Table A3: Allele frequencies of the 16 analyzed enzyme loci and analysed sample size per locus by populations.

Locus	Allele/n	BG1 Gotze Delchev	BG2 Petrochan	BG3 Strumjani	D1 Hengstberg	D2 Elchingen	D3 Weildorf
PGIB	N	62	63	64	63	62	64
	1	0.000	0.008	0.000	0.000	0.000	0.000
	2	0.992	0.976	0.984	0.976	1.000	0.992
	3	0.008	0.016	0.016	0.024	0.000	0.008
MNRA	N	62	63	63	63	62	64
	1	0.024	0.056	0.278	0.000	0.000	0.000
	2	0.000	0.024	0.000	0.000	0.000	0.000
	3	0.927	0.857	0.706	0.976	0.903	0.961
	4	0.000	0.008	0.000	0.000	0.000	0.000
	5	0.048	0.056	0.016	0.024	0.097	0.039
GOTA	N	60	46	64	63	59	60
	1	0.075	0.098	0.039	0.032	0.025	0.025
	2	0.925	0.902	0.961	0.968	0.975	0.975
GOTB	N	61	59	63	61	62	63
	1	0.262	0.347	0.127	0.344	0.290	0.325
	2	0.738	0.653	0.873	0.656	0.710	0.675
PGMA	N	62	61	64	63	62	64
	1	0.000	0.008	0.000	0.008	0.000	0.000
	2	0.024	0.189	0.023	0.421	0.258	0.461
	3	0.976	0.803	0.977	0.571	0.742	0.539
IDHA	N	62	61	64	63	62	64
	1	0.161	0.221	0.172	0.365	0.347	0.195
	2	0.782	0.779	0.828	0.627	0.637	0.805
	3	0.056	0.000	0.000	0.008	0.016	0.000
ACOA	N	62	36	58	63	61	64
	1	0.960	0.944	0.983	0.976	0.984	1.000
	2	0.040	0.056	0.017	0.024	0.016	0.000
ACOB	N	62	49	55	62	60	64
	1	0.258	0.378	0.282	0.145	0.342	0.086
	2	0.742	0.602	0.718	0.855	0.642	0.906
	3	0.000	0.020	0.000	0.000	0.017	0.008
SDHA	N	49	40	64	62	61	64
	1	0.010	0.000	0.000	0.000	0.000	0.000
	2	0.949	0.938	1.000	0.976	0.959	1.000
	3	0.041	0.063	0.000	0.024	0.041	0.000
P6A	N	62	60	63	62	62	64
	1	0.024	0.000	0.000	0.000	0.000	0.000
	2	0.944	0.967	0.841	0.871	0.968	0.828
	3	0.016	0.000	0.000	0.000	0.000	0.000
	4	0.016	0.033	0.159	0.129	0.032	0.172
P6B	N	61	60	64	61	62	64
	1	0.123	0.083	0.023	0.074	0.266	0.094
	2	0.877	0.917	0.977	0.926	0.734	0.906
P6C	N	61	60	64	61	62	64
	1	0.959	0.892	0.961	0.779	0.750	0.734
	2	0.016	0.042	0.016	0.016	0.000	0.023
	3	0.025	0.050	0.016	0.090	0.234	0.195
	4	0.000	0.017	0.008	0.115	0.016	0.047
MDHA	N	62	59	62	63	62	64
	1	1.000	1.000	1.000	1.000	1.000	1.000
MDHB	N	61	57	62	63	62	64
	1	0.205	0.035	0.218	0.071	0.065	0.063
	2	0.000	0.018	0.008	0.024	0.024	0.039
	3	0.705	0.842	0.613	0.762	0.742	0.805
	4	0.082	0.105	0.161	0.143	0.169	0.094
	5	0.008	0.000	0.000	0.000	0.000	0.000
MDHC	N	59	53	63	61	62	64
	1	0.034	0.123	0.040	0.320	0.298	0.242
	2	0.966	0.877	0.960	0.680	0.702	0.758
PexB	N	62	53	60	63	62	63
	1	0.153	0.132	0.292	0.135	0.266	0.206
	2	0.847	0.849	0.700	0.722	0.726	0.794
	3	0.000	0.019	0.008	0.143	0.008	0.000

Note. Locus MDHA was monomorphic.

Manuscript 4

Climate *vs.* topography – spatial patterns of plant species diversity and endemism on a high-elevation island

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Summary

1. Climate and topography are among the most fundamental drivers of plant diversity. Here, we assessed the importance of climate and topography in explaining diversity patterns of species richness, endemic richness and endemicity on the landscape scale of an oceanic island, and evaluated the independent contribution of climatic and topographic variables to spatial diversity patterns.

2. We constructed a presence/absence matrix of perennial endemic and native vascular plant species (including subspecies) in 890 plots on the environmentally very heterogeneous island of La Palma, Canary Islands. Species richness, endemic richness and endemicity were recorded, interpolated and related to climate (*i.e.* variables describing temperature, precipitation, variability and climatic rarity) and topography (*i.e.* topographic complexity, solar radiation, geologic age, slope and aspect). We used multimodel inference, spatial autoregressive models, variance partitioning and linear regression kriging as statistical methods.

3. Species richness is best explained by both climatic and topographic variables. Topographic variables (esp. topographic complexity and solar radiation) explain endemic richness and climatic variables (esp. elevation/temperature and rainfall seasonality) explain endemicity. Spatial patterns of species richness, endemic richness and endemicity were in part geographically decoupled from each other.

4. *Synthesis*. We identified several topography-dependent processes ranging from evolutionary processes (micro-refugia, *in situ* speciation, pre-adaptation to rupicolous conditions, dispersal limitations) to human-induced influences (introduced herbivores, fire, land use) that possibly shape the endemic richness pattern of La Palma. In contrast, climate mainly drives endemicity, which is connected to ecological speciation and specialization to local conditions. We highlight the importance of incorporating climatic variability into future studies of plant species diversity and endemism. The spatial incongruence in hot spots of species richness, endemic richness and endemicity emphasizes the need for an integrated conservation approach acknowledging different diversity measures to protect the complete spectrum of diversity. High-elevation islands such as La Palma are highly suitable to study drivers of diversity and endemism, as they offer environmental gradients of continental magnitude on the landscape scale of a single climatic mini-continent and a large array of *in situ*-speciated endemics.

Key-words

Canary Islands; climatic rarity; climatic stability; island ecology; landscape ecology; landscape scale; mini-continent; multimodel inference; spatial ecology

Introduction

Species and species diversity are not distributed randomly in space (Rosenzweig 1995). Rather they follow distinct spatial patterns (Orme *et al.* 2005; Stohlgren *et al.* 2005) or are distributed along differing environmental gradients (Hortal *et al.* 2013; Slaton 2015). In heterogeneous landscapes, often multiple overlapping and interacting gradients are influential, especially gradients based on topography and climate (Dewar & Richard 2007; Slaton 2015). This complexity results in a key task in ecology, biogeography and evolution: disentangling the major drivers of species distribution and biodiversity patterns from a diverse array of environmental factors.

In contrast to many other regions, oceanic islands harbour relatively low species richness but high endemism (Kier *et al.* 2009). Oceanic islands never had contact with other landmasses (Whittaker & Fernández-Palacios 2007); accordingly, most of their endemic species and especially single-island endemics (SIEs) are a result of *in situ* speciation (Whittaker, Triantis & Ladle 2008). The spatial distribution of endemism within a given oceanic island can thus give valuable information about origin and drivers of biodiversity patterns on the landscape scale, and have important implications for conservation (Richardson & Whittaker 2010).

Landscape scale studies on oceanic islands have a major advantage over comparable research on continental systems. The presence of a large share of species that have evolved *in situ* allows inferring general drivers of speciation, from spatial patterns of diversity and endemism. In continental systems, endemism is less a product of *in situ* speciation but rather the result of range contractions of formerly widespread species (Tribsch & Schönswetter 2003, Orme *et al*. 2005). In addition, the mixing of species pools due to past species movement and immigration resulting from environmental fluctuations might impede the interpretation of emergent diversity patterns in the context of speciation on the continent (Kissling *et al.* 2012).

On oceanic islands, two distinctly different mechanisms related to speciation can be inferred from patterns of endemism. First, centres of endemism (*i.e.* endemic richness; Myers *et al.* 2000) show regions and/or environmental conditions to which most species from the regional species pool are adapted. Endemic richness thus can be used as a measure to identify biodiversity hot spots (Myers *et al.* 2000, Possingham & Wilson 2005). Secondly, and in contrast to richness patterns, the percentage of endemic species (*i.e.* endemicity) is a measure of how strongly local species pools are derived from *in situ* speciation in comparison with immigration processes. High values of endemicity thus indicate environmental conditions that make it necessary for colonizing species to adapt, which can lead to ecological speciation (Nosil 2012, Steinbauer, Irl & Beierkuhnlein 2013).

On the regional scale, islands are characterized by relatively stable oceanic climate conditions (Whittaker & Fernández-Palacios 2007). However, traditionally 'stability' is mainly acknowledged for island temperature regimes, whereas a large spatio-temporal variation in precipitation is a common phenomenon within island archipelagos or even within a single island (Dewar & Richard 2007, Giambelluca *et al.* 2011). In addition, high intra-island precipitation variability increases the potential for adaptive evolutionary divergence within and among species, leading to adaptations to specific conditions within the island in focus (Mallet *et al.* 2014, Harter *et al.* in press). Thus, the concept of presumed stability (or variability) on islands might need a re-evaluation, as hot spots of neo-endemism tend to form in moderately variable conditions on the continent, indicating the importance of variability for speciation (Fjeldså *et al.* 1997).Climatic conditions often show pronounced spatial patterns in the distribution of specific climatic features within a single island (Giambelluca *et al.* 2011, Garzón-Machado, Otto & del Arco Aguilar 2014). The commonness or rarity of these climatic conditions (*i.e.* their isolation in a spatial context) might affect the pattern of diversity and endemism by restricting immigration and enabling speciation under specific climatic conditions (Steinbauer *et al.* 2012). The concept of climatic rarity suggests that in a specific area certain climatic conditions are less common than others (Ohlemüller *et al.* 2008). High climatic rarity is likely connected to spatially restricted selection pressure, which promotes adaptive differentiation processes (Nosil 2012). In this study, we transferred, for the first time, this concept to the landscape scale within a single island to take advantage of the continental-scale environmental gradients found on the landscape scale of a high elevation island. This enabled us to relate these large-scale gradients to patterns of diversity and endemism of the complete species assemblage of this island at resolutions not feasible for continental systems.

Topographic complexity positively influences species richness patterns and speciation processes (Whittaker, Triantis & Ladle 2008; Hortal *et al.* 2009; Trigas, Panitsa & Tsiftsis 2013). Increasing topographic complexity (*e.g.* variation in slope inclination and aspect) increases habitat diversity, niche space available for niche partitioning and speciation, and thus enables species coexistence (Hortal *et al.* 2009). Therefore, topographic complexity likely results in increased species richness (Hortal *et al.* 2009). In addition, complex topography offers micro-refugia during periods of environmental alterations (*e.g.* during past climatic fluctuations, or even during rare extreme weather events; Ashcroft *et al.* 2012). This can decrease extinction risks, consequently maintaining species richness and endemic richness over time (Mee & Moore 2014), which is crucial on isolated oceanic islands with low colonization rates. Thirdly, topographic complexity may cause gene-flow barriers among diverging populations, supporting reproductive isolation and hence local differentiation (Gillespie & Roderick 2014).

Our research aims were to assess, how climatic and topographic variables individually contribute to explaining species richness, endemic richness and endemicity at the landscape scale. Within this framework, we specifically focused on precipitation variability, climatic rarity and topographic complexity. We used the island of La Palma (Canary Islands) as a study system due to its strong elevational and ecological gradients (Garzón-Machado, Otto & del Arco Aguilar 2014), very complex topography (Carracedo *et al.* 2002, Irl & Beierkuhnlein 2011) and large array of endemic plant species (Acebes Ginovés *et al.* 2010).

Materials and methods

Study area

La Palma is the north-westernmost island of the Canary Islands, located in the Atlantic Ocean off the coast of north-western Africa (Fig. 1). La Palma covers an area of 706 km^2; its highest peak is Roque de los Muchachos (2426 m; Carracedo *et al.* 2002). La Palma has a subtropical-Mediterranean climate with humid winters and dry summers (Garzón-Machado, Otto & del Arco Aguilar 2014). However, climatic conditions differ considerably within the island. Annual precipitation ranges from about 170 mm to almost 1400 mm (excluding fog drip), annual temperature from about 9 °C on the island summit to around 22 °C at the leeward south-western coast (Irl & Beierkuhnlein 2011). Fog drip can locally lead to an increase of precipitation particularly relevant in summer (Marzol, Sanchez & Yanes 2011) but likely does not change the overall spatial pattern of precipitation because both, fog drip and rainfall are largely linked to topography (Walmsley, Schemenauer & Bridgman 1996). The north-eastern trade winds create an elevation-associated climatic divide of the island in a humid windward and a dry leeward side. Above the trade wind-induced cloud zone exists a thermal inversion layer, generally exhibiting dry conditions with the possibility of snow and ice in winter (Garzón-Machado, Otto & del Arco Aguilar 2014).

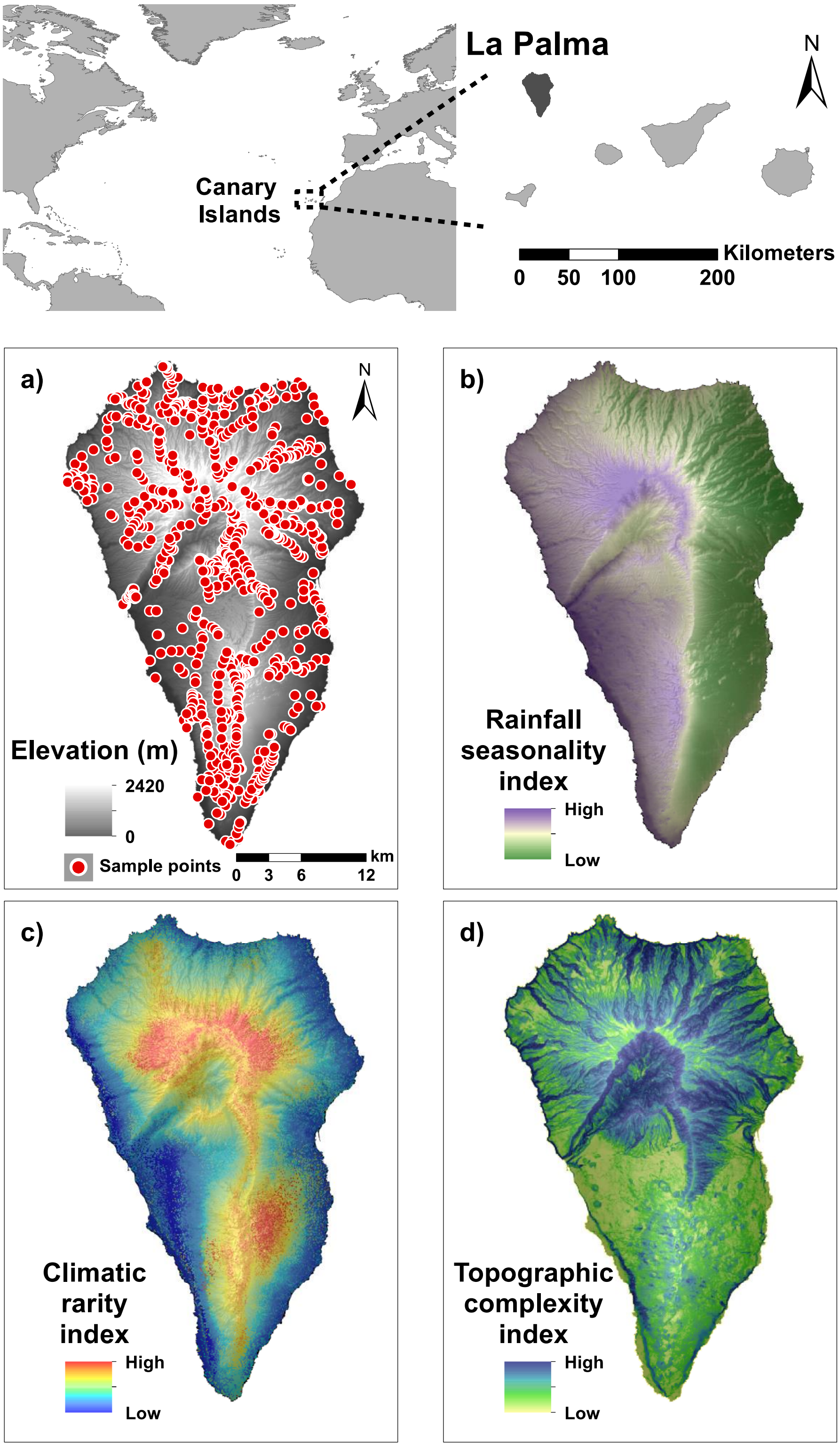

Figure 1. Maps of La Palma within the Canary Islands showing the spatial distribution of a) elevation (including the location of the sampled plots), b) the rainfall seasonality index, c) the climatic rarity index and d) topographic complexity. The large number of sampled plots (n = 890) enabled to cover the whole island and all relevant environmental conditions.

La Palma is geologically divided into two parts. The northern older part is morphologically complex with steep valleys (so-called *barrancos*), coastal cliffs and the massive Caldera de Taburiente complex, which has almost vertical cliffs in its interior reaching 1000 m or more in elevation. The volcanic activity has ceased in the northern part, which is dominated by erosive processes (Carracedo *et al.* 2002). The younger, southern part is still subject to subaerial volcanic ontogeny (the last eruption on the southern tip of the island was documented in 1971) and has gentler slopes with volcanic cones and ash fields (Carracedo *et al.* 2002).

The zonal vegetation directly reflects the climatic conditions of the island (Garzón-Machado, Otto & del Arco Aguilar 2014). These zones range from halophytic communities in arid coastal areas to succulent scrub and thermophilic woodlands in semi-arid lower elevations, through the endemic Canary Pine forest in mid elevations to a high-elevation summit scrub. On the windward side a palaeo-endemic evergreen humid laurel forest and a slightly less humid tree heath *Erica arborea-Morella faya* forest exist at mid elevations in the zone of the trade wind clouds instead of the Canary Pine forest (del Arco Aguilar *et al.* 2010, Garzón-Machado, Otto & del Arco Aguilar 2014). Endemic species are found in all natural vegetation zones (del Arco Aguilar *et al.* 2010). According to Acebes Ginovés *et al.* (2010), La Palma hosts 658 native vascular plant species (including species and subspecies; for simplification reasons hereafter referred to as *species*), of which 193 are archipelago endemics (AEs, *i.e.* endemic to the Canary Islands). Of the AEs 37 are single-island endemics (SIEs).

Sampling method

The presence and absence of endemic perennial vascular plant species (*i.e.* all SIEs and AEs) was recorded in 890 plots using a radial sampling approach with a 50 m radius (Fig. 1a; see Table S1 in Supporting Information for a list of species; to access the data used in this study, see Irl *et al.* 2015). To cover all relevant gradients, we accessed sampling sites by all means possible (*i.e.* roads, tracks, hiking paths, backcountry, non-technical climbing, etc.). In a selected area, a random point was set as centre point of each plot. This centre point was recorded with a GPS device (Garmin Oregon 550). Our sampling approach covers all relevant environmental gradients on La Palma owing to the large number of plots and the good spatial coverage, even though plots were not randomly distributed (Fig. 1a). This sampling approach is very time efficient, allowing for a large sample size, which is essential for differentiated high-resolution spatial analyses in the face of a variety of gradients (*e.g.* elevation, precipitation, geology, seasonality and solar radiation) and topographic complexity.

We focused on perennial vascular plant species including all woody species but excluding annuals. Perennials are valuable study objects for several reasons: (i) perennials are easy to identify in the field due to their relatively large growth (many are small- to medium-sized shrubs), whereas annuals can easily be underestimated as they produce above-ground biomass only for short time periods during the year. This might result in wrong absences for annuals if above-ground biomass is not present. (ii) Due to their longevity perennials integrate over long-term environmental conditions (*e.g.* climate), while annuals react very strongly to short-term weather conditions, making their appearance dependent on stochastic events. (iii) Most endemics on the Canary Islands are perennials (93% according to Shmida & Werger 1992), whereas the relevance of annuals for the flora, especially the endemic flora, of the Canary Islands is limited. In addition, this sampling focus strongly increased sampling efficiency, resulting in exceptionally high spatial coverage, which is fundamental for our research objectives.

The numbers of remaining native perennial species (*i.e.* species that were not SIEs or AEs) were identified in the field and recorded without species' identities as our focus was on endemic diversity (*i.e.* endemic richness and endemicity). We calculated total perennial species richness per plot (hereafter referred to as *species richness*, abbreviated as SR) by adding the number of recorded endemic perennial species to the remaining native perennial species.

The percentage of single-island endemics (pSIE) and the percentage of archipelago endemics (pAE) were calculated by dividing the number of single-island endemics (nSIE) and the number of archipelago endemics (nAE) through total species richness, respectively. Percentage indices are independent of richness gradients and are an indicator of taxonomic or, in our case, floristic uniqueness (Gillespie, Claridge & Roderick 2008). Nomenclature and endemic status follow Acebes Ginovés *et al.* (2010). Sampling took place in fall 2010 and in spring 2011, 2012 and 2013.

Environmental data

Basic environmental data were derived from raster data sets provided by the Cabildo Insular de La Palma (2 × 2 m digital elevation model and geologic map). The resolution of the digital elevation model was resampled to a raster resolution of 100 × 100 m. All plots were recalculated as centroids of the 100 × 100 m raster. Plots that were located within the same raster cell were homogenized (*i.e.* species lists were added together). Elevation, slope angle, micro-aspect (northernness and easternness) and macro-aspect (mean aspect per grid cell within a 5 km radius) as well as mean annual solar radiation (using a standard diffuse atmosphere and based on latitude, elevation and slope aspect) were calculated for each plot.

Mean annual precipitation and mean annual temperature were interpolated using data collected from meteorological stations for the Canary Islands (n = 214 for mean annual temperature and n = 288 for mean annual precipitation; see Fig. S1). Linear regression kriging (R package *gstat*) was applied as interpolation method, with elevation, slope, island, micro-aspect and macro-aspect as covariables (for details on linear regression kriging see Statistical analysis section). Three measures of precipitation variability were computed using monthly precipitation data from 47 meteorological stations from the Spanish Federal Meteorology Agency (AEMET) from the Canary Islands (time span: 1969 to 1998; see Fig. S1) and implementing the same linear regression kriging technique: (i) intra-annual variability represented by the 30 year mean of the annual coefficients of variation based on monthly precipitation sums, respectively, (ii) interannual variability represented by the coefficient of variation for annual precipitation over the 30 year time period, and (iii) rainfall seasonality represented by the rainfall seasonality index by Walsh & Lawler (1981); see the spatial distribution of the rainfall seasonality index for La Palma in Fig. 1a.

Climatic rarity was calculated following the method given by Ohlemüller *et al.* (2008) for two geographic entities: (i) based on the climate of the Canary Islands and (ii) based on the climate of La Palma. For both geographic entities each climatic variable was divided into equal range classes. For mean annual temperature we used a class size of 0.25 °C and for mean annual precipitation a class size of 25 mm. According to the temperature and precipitation conditions each 100 × 100 m raster cell was assigned to a respective temperature and precipitation class (*e.g.* a combination of precipitation ranging from 300 to 325 mm and temperature from 20 to 20.25 °C). Then, all cells of each combined temperature and precipitation class on La Palma were summed up, resulting in a spatial index of climatic rarity based on area (in ha). Climatic rarity increases with decreasing index values (Fig. 1b).

Topographic complexity was estimated by calculating the ratio between the 3D and 2D surface area with the following equation (Fig. 1c; Jenness 2004):

$$Topograhic\ complexity\ index\ = \frac{\sum_{100\,\times\,100\,m}(Area_{2\,\times\,2\,m}\ /\ \cos\ (Slope_{2\,\times\,2\,m}))}{Area_{100\,\times\,100\,m}}$$

where Area$_{2\ x\ 2\ m}$ is the area per grid cell from a 2 × 2 m digital elevation model (DEM), Slope$_{2\ x\ 2\ m}$ the slope of each grid cell from the same DEM in degrees, and Area$_{100\ x\ 100\ m}$ the area per grid cell from a 100 × 100 m DEM containing all 50 × 50 (*i.e.* 2500) grid cells from the 2 × 2 m DEM. This index increases with increasing topographic complexity (*i.e.* from 1 = absolutely flat surface to ∝).

The linear regression kriging method and all other statistical methods were implemented in R Statistics (version 3.0.1; R Core Team 2013). All other geo-information was calculated using ArcGIS software by Esri (Redlands, CA, USA). A list summarizing all environmental variables used in this study is given in Table 1.

Table 1. List of all environmental variables and their abbreviations used in this study grouped into climate and topography.

Class	Variable	Abbreviation	Unit	Min. value	Max. value
Climate	Climatic rarity for the Canary Islands	CR_CAN	ha	4	5311
	Climatic rarity for La Palma	CR_LP	ha	2	818
	Elevation	Elev	m a.s.l.	1	2392
	Intra-annual precipitation variability	INTRA_VAR	–	1.18	1.96
	Inter-annual precipitation variability	INTER_VAR	–	0.25	0.48
	Mean annual precipitation	MAP	mm	177	1331
	Mean annual temperature	MAT	°C	8.7	20.0
	Rainfall seasonality index	RSI	–	0.67	0.84
Topography	Annual solar radiation	ASR	Wh/m^2	63650	189110
	Easterness	Easterness	–	0	1
	Geologic age	Age	Ma	0.0005	4
	Macro-aspect	Macro	–	−0.925	0.899
	Northerness	Northerness	–	0	1
	Slope angle	Slope	°	1	55
	Topographic complexity index	TCI	–	1	2.97

Statistical analysis

First, the linear relationship between response and explanatory variables were assessed by bivariate correlations (Spearman's ρ; see Table S2). To undergo a variable reduction for each response variable (species richness, nSIE, nAE, pSIE, pAE), explanatory variables with correlations $-0.1 \leq r \leq 0.1$ were excluded due to weak explanatory power (Fløjgaard *et al.* 2011). In a second step collinearity was addressed by testing correlations for each possible pair of explanatory variables. If $|r| > 0.7$, the explanatory variable performing poorer with the response variable was excluded (S2; see Dormann *et al.* 2013 for a discussion of collinearity). This resulted in a unique set of climatic and topographic variables for each response variable (see Table S3).

Nonlinear relationship between the independent and dependent variables can be accounted for in a regression framework using transformations. To select the appropriate transformation for each response variable, exploratory linear regression models were applied to each explanatory variable. The following transformations were separately tested to find the best model fit: untransformed, logarithmic, square root, quadratic (x^2) and hump-shaped ($x^2 + x$). Transformation selection was based on AICc values (*i.e.* second-order Akaike Information Criterion) of the respective regression models (Grueber *et al.* 2011; see Table S4). The best fitting transformation of each explanatory variable was implemented in a multiple linear regression (MLR). Table S5 displays the resulting MLR for species richness, nSIE, nAE, pSIE and pAE. Model fits of each MLR model were visually checked to ensure compliance with model assumptions.

The residuals of each MLR were tested for spatial autocorrelation using Moran's I to avoid potential parameter estimate bias and inflation of type I errors (Dormann *et al.* 2007). No spatial autocorrelation was detected in the residuals of the MLR for species richness ($I = -0.001$ n.s.) and nSIE ($I = -0.002$ n.s.), whereas nAe ($I = -0.007$, $P < 0.001$), pSIE ($I = -0.004$, $P < 0.001$) and pAE ($I = -0.003$, $P < 0.05$) resulted in significant spatial autocorrelation. To double-check the effect of spatial autocorrelation, simultaneous autoregressive models (SARs) with a spatially dependent error term were implemented based on the same set of explanatory variables as in the MLR. SARs account for spatial autocorrelation by using a neighbourhood matrix (Dormann *et al.* 2007). Results were compared with the respective MLR using Nagelkerke's pseudo-R^2 as correlation coefficient (Nagelkerke 1991, see S3). Performance of MLRs and SARs was comparable, even though Nagelkerke's pseudo-R^2 was consistently higher. For this reason and because the three significant Moran's I values are very close to zero we conclude that spatial autocorrelation is therefore negligible for the following steps and is sufficiently covered by the explanatory variables in the respective MLR.

Variance partitioning using partial linear regression (R package *vegan*) was implemented to assess the overall importance of climate and topography following the guidelines of Legendre (2008). Variance partitioning quantifies the independent and/or joint explanatory power (adjusted R^2 as the goodness-of-fit measure) of different groups of explanatory variables by conducting MLR always between a focal set of explanatory variables and the residuals of a regression between the dependent variable and all other explanatory variables (explained in Legendre 2008). Variance partitioning estimates the proportion of variation the response variable can be attributed exclusively to one set of explanatory variables once the effect of the other explanatory variables has been taken into account. All variables used in this study were pre-classified into being either climatic or topographic drivers (Table 1). The same set of variables as selected for the MLR and the SAR approach and their respective best fitting transformations were used for variance partitioning (see S3). Then, based on this set of variables the independent, overlapping and total contribution of each class (*i.e.* topography or climate) was calculated based on adjusted R^2 values.

Multimodel inference (MMI) is an alternative approach to variance partitioning used to estimate the relative importance of each explanatory variable in relation to each response variable (R package *relaimpo* version 2.2-2). In contrast to traditional null-hypothesis testing, MMI enables inference from more than one model (Johnson & Omland 2004) and has been proposed as a promising method in ecology and biogeography (Millington & Perry 2011). The relative importance and confidence intervals were calculated using bootstrapping (n = 1000) with all possible model combinations. As a result, the explained variation of the MMI for each response variable (indicated as adjusted R^2), and the relative contribution of each explanatory variable (*i.e.* % contribution to the R^2 of the respective MMI) and its confidence interval are given.

In a last step, the spatial interpolation of the response variables was conducted using linear regression kriging (R package *gstat*). Linear regression kriging uses a statistical approach (MLR) to infer the relationship of explanatory variables to the response variable and then interpolates the residuals into space (Garzón-Machado, Otto & del Arco Aguilar 2014). All explanatory variables used in the MLRs and their respective transformations according to each response variable were used as basis of direct spatial interpolation of species richness, endemic richness and endemicity values.

Statistical significance is indicated in the following as '*' for $P < 0.05$, '**' for $P < 0.01$ and '***' for $P < 0.001$. All correlation coefficients in MLRs, MMI and variance partitioning are given as number of variable-corrected adjusted R^2.

Results

Topography vs. climate

The large number of sampled points ensured a comprehensive and spatially dense coverage of the island, including all relevant environmental gradients (Fig. 1a), and a very high spatial density of plots on this scale (1.26 plots per km^2). A total of 79 archipelago endemics were identified (44% of all perennial archipelago endemics of La Palma; Acebes Ginovés *et al.* 2010), 31 of which were SIEs (84% of all La Palma SIEs; Acebes Ginovés *et al.* 2010; see species list Table S1). Species richness ranged from 1 to 57 species per plot, nSIE from 0 to 8 species per plot, nAE from 0 to 24 species per plot, and pSIE as well as pAE from 0% to 100%. The overall explanatory power of MMI was good (species richness, PSIE and pAE) to moderate (nSIE and nAE), that is, a moderately large section of unexplained variance remained (Table 2).

Table 2. Total explained variation and relative importance of each environmental variable in explaining a) species richness, b) endemic richness (nSIE = number of single-island endemics; nAE = number of archipelago endemics) and c) endemicity (pSIE = percentage of single-island endemics; pAE = percentage of archipelago endemics). Total explained variation (given as R^2) results from multimodel inference (bootstrap n = 1000). '% of R^2' gives the relative contribution of the respective environmental variable to the total explained variation. Explanatory variables are ordered by relative contribution and are grouped as either topographic (*italics*) or climatic variable (**bold**). The best fitting transformation is given in 'Transf.'. Tested transformations were: linear, logarithmic, square-root, quadratic and hump-shape ($x^2 + x$). 'Dir.' indicates the direction of correlation (Dir. '+' = positive correlation; '–' = negative correlation; ∩ = hump; ∪ = inverse hump). Abbreviations of variables are given in Table 1. For details see methods section.

a) Species richness				b) Endemic richness								c) Endemicity							
SR				nSIE				nAE				pSIE				pAE			
Total explained variation: $R^2 = 0.50$				Total explained variation: $R^2 = 0.20$				Total explained variation: $R^2 = 0.31$				Total explained variation: $R^2 = 0.44$				Total explained variation: $R^2 = 0.48$			
	% of R^2	Dir.	Transf.		% of R^2	Dir.	Transf.		% of R^2	Dir.	Transf.		% of R^2	Dir.	Transf.		% of R^2	Dir.	Transf.
Elev	27	∩	hump	*TCI*	50	+	sqrt	*TCI*	47	+	sqrt	**Elev**	50	∪	hump	**MAT**	32	∪	hump
ASR	20	∩	hump	*ASR*	22	∩	hump	*ASR*	22	–	sqrt	**RSI**	29	∪	hump	**RSI**	31	∪	hump
RSI	18	∩	hump	*Slope*	16	+	linear	*Slope*	16	+	quad	*ASR*	13	∪	hump	*ASR*	12	∪	hump
TCI	9	+	log	**INTRA_VAR**	5	∪	hump	**CR_LP**	12	+	log	**INTER_VAR**	6	∩	hump	**INTER_VAR**	10	∩	hump
CR_CAN	6	+	log	**CR_LP**	4	+	log	**RSI**	3	∩	hump	*Easterness*	3	–	linear	*Easterness*	6	–	linear
Easterness	6	∪	hump	**INTER_VAR**	4	–	linear									**INTRA_VAR**	6	∩	hump
INTER_VAR	5	∪	hump													*Age*	4	∪	hump
INTRA_VAR	5	∪	hump																
Slope	4	+	quad.																

Variance partitioning revealed that topography and climate both are more or less equally important for species richness (joint explained variation for climate: R^2 = 0.41 and topography: R^2 = 0.34, including a large share of overlapping contribution: R^2 = 0.26; Fig. 2a). However, endemic richness was dominated by the influence of topography for both nSIE (joint explained variation: R^2 = 0.18) and nAE (joint explained variation: R^2 = 0.27), whereas climate had very little influence on endemic richness (joint explained variation: $R^2 \leq 0.06$; Fig. 2b,c). Climate was very important in explaining endemicity for both pSIE (joint explained variation: R^2 = 0.42) and pAE (joint explained variation: R^2 = 0.45; Fig. 2d,e). Moreover, the influence of topography was almost not separable from the explained variation of climate (independent explained variation for pSIE: R^2 = 0.01 and pAE: R^2 = 0.02, overlapping explained variation for pSIE: R^2 = 0.15 and for pAE: R^2 = 0.22). For all values of joint, independent, overlapping and total explained variation of each response variable resulting from variance partitioning, see Table S5.

Environmental components of topography and climate

In the MMI, species richness was mainly explained by elevation, annual solar radiation, the rainfall seasonality index and the topographic complexity index (Fig. 2f, Table 2a), indicating the joint importance of climate (elevation, rainfall seasonality index) and topography (annual solar radiation, topographic complexity index). However, the relative contribution of each variable was relatively moderate (*e.g.* elevation as explanatory variable with the highest relative contribution only reaches 27%). Further climatic (climatic rarity, interannual precipitation variability, intraannual precipitation variability) and topographic variables (easternness and slope) only played a subordinate role (Fig. 2f, Table 2a). Overall explained variation was high (R^2 = 0.5; Table 2a); however, half of the variation remained unexplained.

Endemic richness was best explained by different topographic variables, that is the topographic complexity index, annual solar radiation and slope for both nSIE and nAE (Fig. 2g,h, Table 2b). The topographic complexity index as largest contributor of relative importance reached 50% and 47% for nSIE and nAE, respectively. Climatic variables only had marginal influence of ≤ 5% (*i.e.* intraannual precipitation variability, inter-annual precipitation variability and the rainfall seasonality index), with the slight exception of climatic rarity for La Palma for number of archipelago endemics (12%; Table 2b). Overall explained variation was moderate for both nSIE and nAE and did not surpass R^2 = 0.31 (Table 2b).

Climatic variables had the highest explanatory power for endemicity, whereas topographic variables were of minor importance (Fig 2i-j). For pSIE elevation and the rainfall seasonality index (both climatic variables) as well as annual solar radiation (topographic variable) were the most important variables, ranging from 12% (annual solar radiation) to 50% relative importance (elevation; Table 2c). The climatic variables mean annual temperature (which is strongly linked to elevation; relative importance of 32%) and the rainfall seasonality index (relative importance of 31%) as well as to a certain degree also annual solar radiation (relative importance of 12%) had the highest relative importance for pAE (Table 2c). Other climatic (*i.e.* intraannual precipitation variability and interannual precipitation variability) and topographic variables (*i.e.* geologic age and easternness) only had little additional explanatory power (≤ 6%; Fig. 2i,j, Table 2c). Overall explained variation was high for both percentage of single-island endemics and percentage of archipelago endemics, almost reaching R^2 values of 0.5 for the latter (Table 2c).

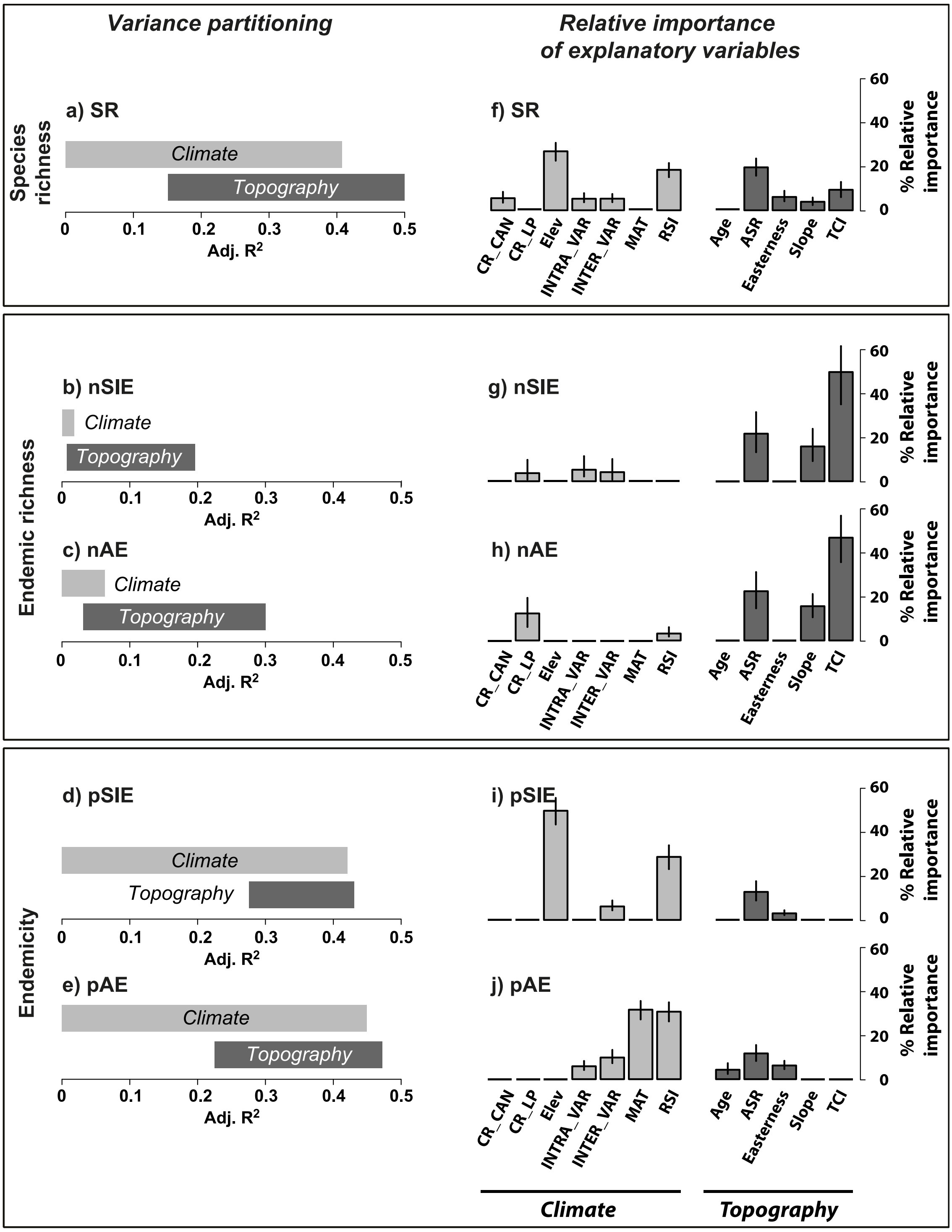

Figure 2. Variance partitioning of the influence of climate and topography (a-d) and relative importance of explanatory variables (f-j) on species richness (SR), endemic richness (nSIE = number of single-island endemics, nAE = number of archipelago endemics) and endemicity (pSIE = percentage of single-island endemics, pAE = percentage of archipelago endemics). Figures a-d show the independent and overlapping explained variation of climate and topography as portion of the total adjusted R^2 values. For values see S5. Figures f-j display the relative importance of each explanatory variable calculated by the multimodel inference approach as per cent contribution to the R^2 for the respective response variable. Gaps indicate that these variables were removed due to collinearity or during the variable reduction process. Both variance partitioning and relative importance result from multiple linear regressions. For details on both methods please see the Material and methods section. Abbreviations of explanatory variables: Age: Geologic age; ASR: Annual solar radiation; CR_CAN: Climatic rarity for the Canary Islands; CR_LP: Climatic rarity for La Palma; Elev: Elevation; INTER_VAR: Inter-annual precipitation variability; INTRA_VAR = Intra-annual precipitation variability; MAT = Mean annual temperature; RSI = Rainfall seasonality index; TCI = Topographic complexity index.

Spatial distribution of species richness, endemic richness and endemicity

A complex pattern of species richness, endemic richness and endemicity is visible for La Palma (Fig. 3). Species richness decreased with elevation and from northeast to southwest (Fig. 3a). The highest values of species richness, however, reached values of around 60 species and were located in the steep barrancos and in the coastal cliffs on the northern part of the island, independent of island aspect. Endemic richness for both nSIE and nAE was highest in the steep barrancos of the north but also reached very high values in the inner cliffs of the Caldera de Taburiente complex (around 8 for nSIE and 24 for nAE; Fig. 3b,c). In general, endemic richness decreased with elevation but without the island aspect-driven asymmetry displayed by species richness. Endemicity for both pSIE and pAE strongly increases with elevation reaching values of 71% and around 100% at the highest elevations for pSIE and pAE, respectively (Fig. 3d,e). While strongly decreasing with elevation in eastern aspects, endemicity can reach quite high values (around 25% for pSIE and 75% for pAE) in barrancos at lower elevations and coastal cliffs of the north, lowland areas of the south and western slopes at mid-elevations (esp. for pAE with values between 50% and 70%).

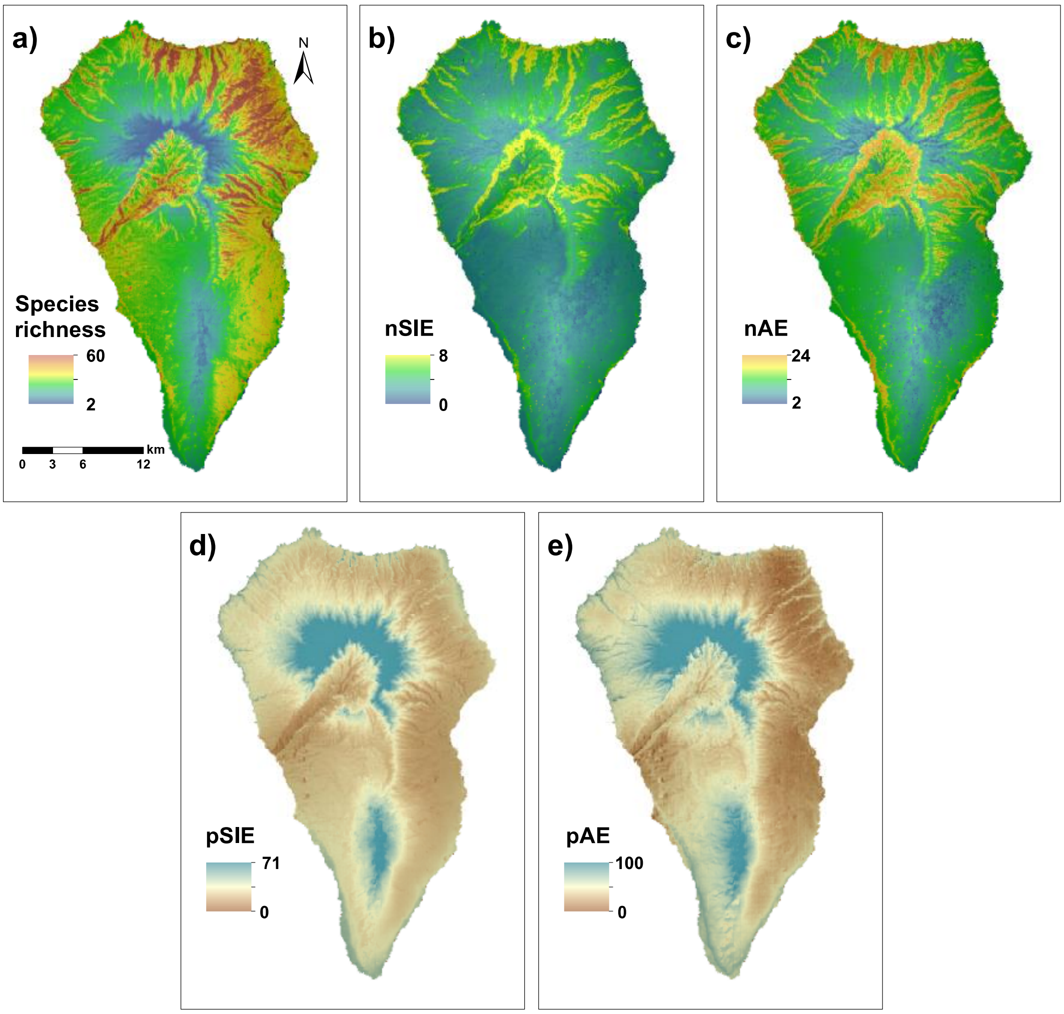

Figure 3. Spatial interpolation maps of a) species richness, b) number of single-island endemics (nSIE), c) number of archipelago endemics (nAE), percentage of single-island endemics (pSIE) and percentage of archipelago endemics (pAE).

Discussion

Effects of topography and climate

The present study shows that topography and climate are important drivers of species richness, endemic richness and endemicity on the landscape scale of the very heterogeneous oceanic island of La Palma. However, we find a shift in the overall importance of either topography or climate in explaining the distribution and spatial patterns of each response variable. The high-resolution spatial patterns of endemic richness and endemicity, which are mainly based on *in situ*-speciated species, allow us to make inferences about the biogeographic drivers important for patterns of evolution and speciation but also have major implications for species conservation on scales rarely targeted. Given the fact that endemic richness and endemicity indicate quite different aspects, we discuss both patterns separately.

The current distribution of endemic richness on La Palma is likely shaped by adaptations to local environmental conditions, and recent human-mediated influence (*i.e.* introduced herbivores, fire and land use), a pattern probably relevant for most regions of the world. We suggest several possible evolutionary processes, which are not mutually exclusive, to be responsible for the strong positive impact of topography on endemic richness, which in our study is mainly influenced by the topographic complexity index.

First, a complex topography such as that found in this study likely renders a high diversity of different habitats and thus a large local niche space (Hortal *et al.* 2013; Steinbauer *et al.* 2013) as well as an increase in area (Leutner *et al.* 2012). Secondly, the high topographic complexity likely offers micro-refugia during periods of climatic fluctuation, thus reducing local extirpation probabilities (Médail & Diadema 2009) and possibly also increasing speciation (Mee & Moore 2014). Thirdly, the low influence of climate on endemic richness might indicate that the endemic species set (as a whole) is adapted to the complete range of climatic conditions on the island, as *in-situ* speciation has happened in all climatic conditions on the island (esp. relevant for SIEs, Beard, Chapman & Gioia 2008). Fourthly, many endemic species are adapted to rupicolous conditions (del Arco Aguilar *et al.* 2010), conditions that are more likely to be present if topographic complexity is high. Finally, the loss of dispersal power is a common syndrome of evolution on islands (Whittaker & Fernández-Palacios 2007). Dispersal limitations within an island (*i.e.* gene-flow barriers) are expected to support speciation processes (Kisel & Barraclough 2010, Gillespie & Roderick 2014).

Like all islands world-wide, La Palma also is, to some degree, affected by anthropogenic influences (Caujapé-Castells *et al.* 2010). Therefore, we suggest that besides the mentioned evolutionary processes, several human-induced influences are present that can potentially explain the identified endemic richness pattern. On the one hand, steep and inaccessible areas might offer 'safe sites' for endemics from introduced herbivores (Irl *et al.* 2014a,b) because endemics are preferentially browsed by introduced herbivores (Garzón-Machado *et al.* 2010; Irl *et al.* 2012) due to lacking defence mechanisms (Bowen & Van Vuren 1997). On the other hand, endemics might experience lower fire frequencies (which have generally increased in recent decades; Climent *et al.* 2004) in topographic complex regions probably due to lower vegetation cover and lower productivity. Finally, human land use cannot occur, at least at high intensities, in areas of highly complex terrain. Thus, the presented pattern might be partially a result of the current land use distribution. However, further studies are needed to disentangle the independent effects of adaptive evolution and range detraction from human influence.

In contrast to endemic richness, climate has a stronger influence on endemicity than topography on La Palma. Endemicity stands for floristic uniqueness and the relative importance of endemics in a given area (Gillespie, Claridge & Roderick 2008) but is also interpreted as a measure of speciation (Emerson & Kolm 2005; Steinbauer, Irl & Berierkuhnlein 2013). Climate and especially climatic changes directly influence speciation, for example through specific resource availability in terms of energy or water availability or by establishing specific selection pressures (Hua & Wiens 2013). For example, high-elevation ecosystems possess quite harsh conditions for plant growth; thus, selective pressure is very high (Anthelme & Dangles 2012). Idle resources, open niche space and potential isolation effects in high elevation island ecosystems likely stimulate speciation processes (Steinbauer, Irl & Berierkuhnlein 2013). As a result, endemicity potentially indicates evolutionary *in situ* specialization to specific environmental conditions (*i.e.* past adaptive speciation processes), a phenomena which has been proposed to be relevant for all mountainous islands as well as isolated mainland peaks and mountain systems (Steinbauer, Irl & Berierkuhnlein 2013).

Precipitation variability and climatic rarity

Precipitation variability, especially the rainfall seasonality index, contributed substantially in explaining species richness and endemicity but not endemic richness on La Palma. The areas of La Palma with high precipitation variability host generally fewer species, while stable areas are mainly humid regions supporting relatively high species richness. High species richness likely results from high resource availability and low selective pressure for specialization, suitable for common (mesophytic) species communities (Stohlgren *et al.* 2005; Weigelt, Jetz & Kreft 2013). However, in aridity-prone regions, one of the essential resources for plant growth is very limited (*i.e.* water) and/or connected to a high temporal stochasticity, leading to less favourable growing conditions. This results in an increased importance of specialized endemics and thus a higher floristic uniqueness (*i.e.* endemicity) but lower species richness in high-variability conditions (Dewar & Richard 2007). Several studies have shown that centres of endemic richness are connected to long-term climatic stability (Platts *et al.* 2013; Weber *et al.* 2014; Cook, Hardy & Crisp 2015), while endemic richness on La Palma is highest in variable conditions. However, our study used measures of climatic variability on a short-term scale (in the range of several decades), not accounting for stability on geologic timescales. This might indicate that long-term stability and short-term variability are not mutually exclusive; thus times-cales have to be considered for climatic stability analyses.

Although an intriguing concept due to its ability to integrate different climatic factors into a single parameter, climatic rarity only has negligible effects on both species richness and endemic richness. While climatic rarity has been shown to be a good indicator of species' range size and rarity on continental scales (Ohlemüller *et al.* 2008), it seems of only minor importance for richness and endemism on the landscape scale of La Palma. Recent studies have shown that richness and species rarity have a complex relationship (*e.g.* Kreft, Sommer & Barthlott 2006; Hubbell 2013), which might not be as straightforward as previously assumed.

Spatial pattern of species richness, endemic richness and endemicity

The combined negative effect of both the elevation-temperature gradient and annual solar radiation indicates that high species richness is found in steep barrancos and coastal cliffs at low to mid-elevations because low temperatures and high solar radiation are stressful for plant growth (Körner 2003). The main drivers of endemic richness on La Palma are topographic complexity, annual solar radiation and slope. Similar to species richness, the highest values of endemic richness are found in the steep barrancos, coastal cliffs and inner cliffs of the Caldera de Taburiente (Fig. 3b,c), although without the trade wind-induced asymmetry shown for species richness. Earlier studies were not able to identify such small-scale differences in endemic richness on the Canary Islands (*e.g.* Reyes-Betancort *et al.* 2008).

Highest values of endemicity are found at high elevations but also in the barrancos and coastal cliffs at low elevations on the western dry side of the island (Fig. 3d-e). High solar radiation at high elevations might select for adequate adaptation, while impeding the existence of most species that are adapted to conditions of lower radiation (Anthelme & Dangles 2012). This decreases general species richness and facilitates evolutionary adaptation processes, including ecological speciation of specialist endemics via UV-B radiation-induced mutagenesis (Flenley 2011). In addition, high UV-B radiation is responsible for the evolution of characteristic lifeforms in the high-elevation ecosystems. On La Palma, this becomes obvious in the presence of giant rosette plants such as several representatives from the genus *Echium*, and morphological adaptations such as pubescence (*e.g. Genista benehoavensis*, *Echium wildpretii* subsp. *trichosiphon*, *Plantago webbii* or *Teline stenopetala* subsp. *sericea*).

Interestingly, hot spots of species richness, endemic richness and endemicity are spatially decoupled on the landscape scale. In consequence, to conserve the whole spectrum of biological variation it is obviously not enough to only focus on species richness as a measure of biodiversity on the continental scale (Orme *et al.* 2005; Daru, Bank & Davies 2015) or the island scale (Di Virgilio *et al.* 2014). Rather it is important to employ conservation strategies for a wide range of different habitats and various definitions of 'hot spot' to adequately cover and preserve the total breadth of species diversity (Stohlgren *et al.* 2005, Daru, Bank & Davies 2015).

Looking beyond La Palma

While the isolation of oceanic islands has impeded the natural colonization of mammalian herbivores, there is virtually no oceanic island on the global scale without human-introduced mammalian herbivores (Caujapé-Castells *et al.* 2010). Thus, the identified importance of topography for the identification of endemic hot spots may hint for a general importance of 'safe sites' within islands, protecting endemic species from introduced herbivores.

Similar to our results, highest percentages of endemic species on continental mountains are found in topographically complex areas (Verboom *et al.* 2015), often showing a strong increase of endemicity with elevation (*e.g.* Vetaas & Grytnes 2002, Nogué, Rull & Vegas-Vilarrúbia 2013). Thus, our results support a seemingly global pattern found in topographically complex regions, although we focus on the landscape scale of a single island. On continents, larger endemicity in high elevations may, however, not only be driven by enhanced local evolutionary processes (*e.g.* elevation-driven isolation *sensu* Steinbauer *et al.* 2012), but also due to the survival of species widespread during times of colder climatic conditions (Tribsch & Schönswetter 2003). No matter what the underlying cause is, high-elevation areas should, as a consequence, be considered as high-priority sites for nature conservation both on islands and on the continent.

Conclusion

We suggest that future studies (*e.g.* using environmental niche modelling) need to acknowledge the effect of spatio-temporal climate variability but also the equally important effect of topography, especially on the distribution of species richness and endemic richness (Platts *et al.* 2013). On the one hand, the general use of long-term means in modelling of climatic envelops in the face of climate change has recently been criticized (Letten *et al.* 2013). On the other hand, models only based on climate will likely perform poorer than models that also include topographic components, highlighting the importance of using both climate and topography. In addition, we argue that endemics might be more resilient towards ongoing climate change than previously thought because of their preadaptation to variable precipitation conditions. However, this needs to be investigated in future studies explicitly targeting climate change effects on islands (see Harter *et al.* 2015).

In general, high-elevation islands such as La Palma offer the opportunity to study continental-scale environmental gradients with high species turnover and clearly defined zonal ecosystems on the landscape scale of a single island. These so-called climatic *mini-continents* host a large array of endemic species, which mainly evolved *in situ* under island conditions (especially SIEs; Whittaker, Triantis & Ladle 2008). Thus, high elevation islands are optimally suited to provide further insights into the fundamental drivers of diversity and endemism, even possessing the possibility to transfer the gained knowledge to non-island systems.

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Data accessibility

All data used in this article are accessible in the Dryad Digital Repository. For details, please see Irl *et al.* (2015).

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Supporting Information

Supporting information of this manuscript can be found in the electronic version of the publication

Table S1. List of all species identified in this study.

Figure S1 Map of meteorological stations used for interpolating climate data.

Table S2. Bivariate correlation matrix of environmental variables.

Table S3. Table of best fitting transformations for MLR.

Table S4. Table of AICc values of transformations.

Table S5. Table of absolute values of variance partitioning.

Manuscript 5

Spatial and ecological population genetic structures within two island-endemic *Aeonium* species of different niche width

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Abstract

The Crassulacean genus *Aeonium* is a well-known example for plant species radiation on oceanic archipelagos. However, while allopatric speciation among islands is documented for this genus, the role of intra-island speciation due to population divergence by topographical isolation or ecological heterogeneity has not yet been addressed. The aim of this study was to investigate intraspecific genetic structures and to identify spatial and ecological drivers of genetic population differentiation on the island scale. We analysed inter simple sequence repeat (ISSR) variation within two island-endemic *Aeonium* species of La Palma, one widespread generalist that covers a large variety of different habitat types (*Ae. davidbramwellii*), and one narrow ecological specialist (*Ae. nobile*), in order to assess evolutionary potentials on this island.

Gene pool differentiation and genetic diversity patterns were associated to major landscape structures in both species, with phylogeographic implications. However, overall levels of genetic differentiation were low. For the generalist species, outlier loci detection and loci-environment correlation approaches indicated moderate signatures of divergent selection pressures linked to temperature and precipitation variables, while the specialist species missed such patterns.

Our data point to incipient differentiation among populations, emphasising that ecological heterogeneity and topographic structuring within the small scales of an island can foster evolutionary processes. Very likely, such processes have contributed to the radiation of *Aeonium* on the Canary Islands. There is also support for different evolutionary mechanisms between generalist and specialist species.

Keywords

Gene flow barriers, island biogeography, isolation by distance, isolation by ecology, landscape genetics, niche width

Introduction

Species radiations on oceanic archipelagos provide illustrative showcases of evolutionary patterns (see *e.g.* Baldwin *et al.* 1999; Losos and Ricklefs 2009 and references therein), which have been motivating fundamental phylogenetic, biogeographical and ecological research (*e.g.* Lösch 1990; Baldwin and Sanderson 1998; Gillespie 2004; Grant and Grant 2006). However, there are still open questions regarding processes and drivers. It is unclear in many cases if and how often species have diverged in allopatry among islands (isolated evolution after colonisation) or if within-island speciation processes have contributed to such radiations (*e.g.* Whittaker and Fernández-Palacios 2007; Losos and Ricklefs 2009; Thiv *et al.* 2010). While past allopatric speciation among islands is relatively straightforward to infer from phylogenetic and biogeographic patterns, detections of intra-island evolutionary divergence additionally requires the demonstration of evolutionary divergent forces on the comparably small scales of an island (see *e.g.* Savolainen *et al.* 2006; Mallet *et al.* 2014; Papadopulos *et al.* 2014; Suárez *et al.* 2014).

Speciation can be initiated by spatial or ecological isolation among populations. Spatial isolation limits gene flow, so that genetic drift and divergent natural selection can subsequently lead to gene pool divergence (Wright 1943; Slatkin 1993; Hutchison and Templeton 1999). In ecological speciation, strong divergent selection drives populations to differential adaptations, resulting in reciprocal maladaptation and gradual built-up of reproductive isolation by selection against migrants (Nosil 2012; Wang and Bradburd 2014). Further on, interactions between spatial and ecological mechanisms can easily affect the formation of reproductive barriers between populations and subsequent species divergence (Rundell and Price 2009; Nosil 2012; Orsini *et al.* 2013; Wang *et al.* 2013).

Differences in selection regimes among populations due to environmental gradients and heterogeneity are potent and common drivers of evolutionary divergence and speciation (Doebeli and Dieckmann 2003; Nosil 2012; Stein and Kreft 2015). In island biogeographical theory, strong environmental heterogeneity of an island (*e.g.* climatic, geological or edaphic structuring) and also spatial factors such as area and topographical dissection are often postulated to facilitate speciation (Stuessy 2007; Whittaker *et al.* 2008; Losos and Ricklefs 2009; Vitales *et al.* 2014). However, comparably few studies directly assessed and quantified these evolutionary effects on the population level within single islands (but see Mallet *et al.* 2014; Papadopulos *et al.* 2014; Stacy *et al.* 2014; Suárez *et al.* 2014).

The Crassulacean genus *Aeonium* is an iconic example of plant species radiations on islands. On the Canarian archipelago, it comprises about 28 species plus a series of distinct subspecies (Liu 1989; Arechavaleta Hernández *et al.* 2010, numbers vary between authors and taxonomic treatments). The genus was shown to have evolved on the Canaries (Mes *et al.* 1996; Mort *et al.* 2002), with a relatively young phylogenetic origin in the late Miocene (Kim *et al.* 2008) or even later (Thiv *et al.* 2010). A large variety of ecological niches, morphological forms and eco-physiological characteristics (Liu 1989; Lösch 1990; Mort *et al.* 2007) suggest adaptive speciation processes in the history of *Aeonium* (Lems 1960; Jorgensen and Frydenberg 1999; Jorgensen 2002; Thiv *et al.* 2010). However, the spatial scales of these processes, *i.e.* if species divergences occurred within single islands or only between islands, and the evolutionary drivers of differentiation have not been resolved.

Probably, inter-island allopatric speciation has played a major role for the radiation of *Aeonium* (Mes and Hart 1996; Thiv *et al.* 2010). Nevertheless, intra-island events cannot be ignored and are suggested *e.g.* for the closely related Tenerife endemics *Ae. haworthii*, *Ae. urbicum* and *Ae. pseudourbicum* (Liu 1989; Jorgensen 2002; Mort *et al.* 2002), and are also possible for *Ae. canariense*, *Ae. tabuliforme* and *Ae. cuneatum* on the same island (Liu 1989). Nevertheless, the long and vivid geological history of Tenerife that integrated three former distinct islands into one (Carracedo *et al.* 2002) makes it difficult to separate between- and within-island speciation processes on this major island.

In contrast, La Palma is much younger (maximum age: 1.77 Ma) than Tenerife and its three precursors (between 3.9 Ma and ~12 Ma, fusion of the paleo islands ~3.5 Ma) and has a less complex geological history (Carracedo *et al.* 2002). However, equal to Tenerife, La Palma exhibits a high potential for the formation of reproductive isolation between plant populations, due to spatial (topographical heterogeneity, but also Euclidian distance) and ecological factors (strong gradients, *e.g.* elevation/temperature, precipitation). This makes La Palma an ideal system to investigate population genetic patterns and their relation to geographical and ecological landscape factors within species.

Species that cope with a broad range of environmental settings, *i.e.* habitat generalist species, are expected to feature more influence of divergent selection among populations compared to specialised species with populations experiencing less diverse environments (Groot *et al.* 2011). Literature also suggests that the effects of isolated habitats and putatively lower effective population sizes in specialist species lead to higher neutral genetic differentiation among their populations (*e.g.* Groot *et al.* 2011; Gil-López *et al.* 2014; Li *et al.* 2014). However, this over-simplifies population features of specialist *vs.* generalist species, and has rarely been directly evaluated for plant species (but see Gil-López *et al.* 2014). To address the role of ecological amplitudes for genetic structures within *Aeonium* taxa and the potential of within-island evolutionary divergence on La Palma, we investigated range-wide population genetic structures of a wide-spread generalist species (*Ae. davidbramwellii*) and a spatially and ecologically more specialised species (*Ae. nobile*).

The following two hypotheses were tested:

i) The topographic heterogeneity and the related spatial and ecological isolation of *Aeonium* populations is reflected in intraspecific genetic structuring.

ii) The generalist and wide-spread species (*Ae. davidbramwellii*) shows a higher genetic population differentiation compared to the specialist species (*Ae. nobile*).

Material and methods

Study area

La Palma (706 km^2, between 28°27' – 28°51' N and 17°43' – 18°0' W) is the north-westernmost and second youngest island of the Canary archipelago (Carracedo *et al.* 2002). The northern part of La Palma is dominated by an extinct shield volcano (2426 m a.s.l. on the highest peak) with a large central erosional depression (Caldera de Taburiente) that opens to the Southwest (Barranco de las Angustias) and a complex radial network of deep erosion valleys (Barrancos) dissecting its outer flanks (see Fig. 2B). The southern part is geologically younger with a volcanic ridge system starting from the Caldera de Taburiente and running out to the southern tip of the island, where active volcanism still occurs (Carracedo *et al.* 2002). On the western flanks south of the Caldera, the past Cumbre Nueva mega-landslide (~560 ka) created a comparably gently sloping landscape (Carracedo *et al.* 1999; Colmenero *et al.* 2012).

La Palma shows a Mediterranean-subtropical climate of dry summer and more rainy winter seasons. Nevertheless, the high topographical structures generate distinct rain shadow effects with strong differences between the humid north-eastern island sections and dry south-western parts.

Study species and sampling design

The genus *Aeonium* WEBB & BERTHEL. consists of leaf-succulent long-lived perennial herbs or small shrubs with a great diversity of growth forms, ecological niches and physiological attributes (Lems 1960; Liu 1989; Lösch 1990). Large inflorescences with numerous coloured and nectariferous flowers imply entomophily and outcrossing (Esfeld *et al.* 2009). Additionally, *Aeonium* species produce relative small (0.4 – 0.6 mm long) and light (0.02 – 0.04 mg) seeds, suggesting some suitability for wind-dispersal (Liu 1989; Vazačová and Münzbergová 2014). Pollination by insects and wind dispersal of seeds might facilitate common exchange of alleles and genotypes between nearby populations. However, specialised seed traits that would advance anemochory or other potential long-distance dispersal are lacking and dispersal capabilities of *Aeonium* species have been shown only moderate (Vazačová and Münzbergová 2014). Thus, gravity is probably the most important dispersal agent beside more or less occasional wind drift events.

Ae. davidbramwellii H.-Y. LIU (Fig. 1A) is a generalist species, growing on rocks, soil banks and cliffs throughout almost every part of La Palma from 0 – 1000 m a.s.l. (Liu 1989) and up to 1800 m a.s.l. (own observations, see also Fig. 2B). It forms distinct populations but also occurs as scattered individuals and seems to be adapted to a wide range of environmental situations on the island. *Ae. davidbramwellii* is a subshrub, mostly with a polycarpic life cycle and is reported diploid with 2n = 36 (Liu 1989).

Ae. nobile (PRAEGER) PRAEGER (Fig. 1B) realises a far more narrow ecological niche, due to its growth site restrictions to dry slopes, banks and cliffs with high insolation (Liu 1989). It is thus much rarer than *Ae. davidbramwellii* and occurs in distinct and mostly small populations from 0 – 750 m a.s.l. (Liu 1989) and up to 1200 m a.s.l. (own observations). The current range is largely limited to the western sectors of La Palma showing a disjunction into a northern and a southern distribution, separated by the landslide area (see Fig. 4B). However, one population is present in the east of La Palma, possibly representing the remnant of a larger occurrence area in the past (see Voggenreiter 1974; Liu 1989). *Ae. nobile* individuals are monocarpic and show only one large succulent leaf rosette, and have a diploid chromosome set of 2n = 36 (Liu 1989).

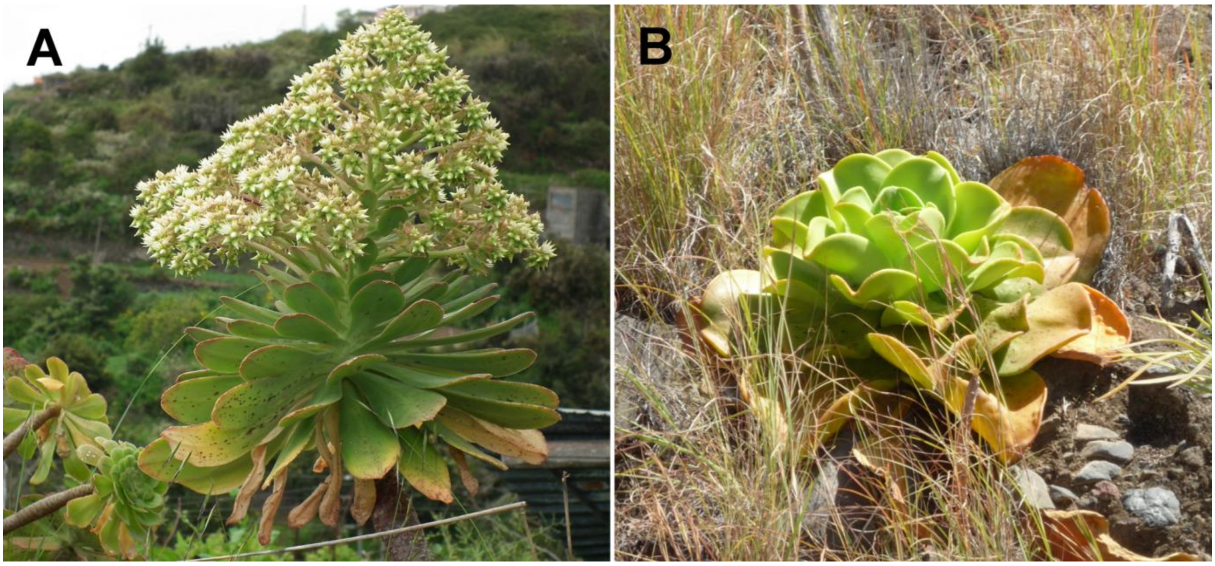

Fig. 1: The two study species *Aeonium davidbramwellii* (A) and *Aeonium nobile* (B). Photographs by Carl Beierkuhnlein and Katharina Staab, respectively.

There is no indication for a sister taxon relationship between the two study species. According to a recent analysis of ITS data (Kondraskov *et al.* 2015), they are separately placed within a clade including six additional species with distinct distributions on four islands of the Canaries. The interspecific relationships are, however, poorly supported. In contrast, Liu's (1989) morphological analyses showed *Ae. nobile* as a rather distant relative of *Ae. davidbramwellii*, more related to *Aeonium* species distributed outside the Canary Islands. It is thus unlikely that the two species derived from each other, but rather that they derived from different ancestors subsequent to independent colonisation events of La Palma.

The sampling aimed to cover the entire distribution of the two species, including potential effects of topographical gene flow barriers and environmental variation within the ranges, respectively. In each population, five distantly growing individuals were chosen arbitrarily to avoid sampling of close relatives and to cover the whole populations' variability. Leaf samples were dried and stored in silica gel. This resulted in 11 populations and 55 individuals sampled for *Ae. davidbramwellii* and ten populations with 50 individuals in total sampled for *Ae. nobile*. The DNA sampling was accompanied by a species mapping project (see Irl *et al.* in press for details), revealing 597 and 70 occurrence points for *Ae. davidbramwellii* and *Ae. nobile* on the entire island, respectively (see Fig 1B and 3B).

DNA extraction and genotyping

Genomic DNA was extracted from leaf tissue using the NucleoMag 96 Plant Kit (Machery-Nagel, Düren, Germany), adapted to the Fastprep tissue homogenizer (FP120, MP Biomedicals Europe, Illkirch, France) and the Kingfisher magnetic particle processor (Thermo Scientific, Langenselbold, Germany): Leaf tissues (ca. 200 mg) were homogenized in 200 µl buffer MC1 for 40 sec. at a speed set of 6 m/s; additional 300 µl Buffer MC1 and 10 µl RNAse A were added to the viscous homogenate and incubated at 56 °C for 30 min. Insoluble tissue debris was pelleted by centrifugation for 5 min. at room temperature and clear supernatant was used to purify genomic DNA as described in Tab. S1.

We applied Inter-Simple Sequence Repeat (ISSR) markers with anchored primers to generate genetic fingerprints, a method that has been proven useful for population genetic analyses of non-model species due its capacity to generate highly polymorphic data with high reproducibility and cost-efficiency (Zietkiewicz *et al.* 1994; Nybom 2004).

In a pre-study, 30 primers were screened in subsets of 20 samples of each study species, respectively, for their usefulness regarding a clear polymorphic and reliable band pattern generation on ethidium bromide-stained agarose gels (see Tab. S2 for primer sequences, annealing temperatures and references). If available, annealing temperatures were taken from the literature; otherwise, gradient PCRs (45 – 60 °C annealing temperatures) were conducted and fingerprint patterns were compared to infer the best conditions for the primers, respectively. The same selection of nine markers turned out to be optimal for both species for the main study (see Tab. S2).

Selected ISSR markers (see Tab. S2) were amplified from genomic DNA in a 12.5 µl reaction volume using the KAPA3G Plant PCR Kit (KAPABIOSYSTEMS, Wilmington, MA, USA): each reaction contained 1x KAPA Plant PCR buffer, 0.5x KAPA Plant PCR Enhancer, 0.3 µM fluorescently-labelled ISSR primer (see Tab. S2), and 0.25 U KAPA3G Plant DNA Polymerase. The PCR profile consisted of an initial denaturation step at 95 °C for 3 min., followed by 40 PCR cycles (95 °C for 30 sec., primer-specific annealing temperatures for 30 sec., 72 °C for 30 sec.), and a final extension step at 72 °C for 5 min.. Amplified ISSR fragments were mixed with the MapMarker size standard (50 – 1200 bp, Bioventures Inc., Murfreesboro, TN, USA) and separated on a capillary electrophoresis system (GenomeLab GeXP Genetic Analysis System; AB Sciex Germany GmbH, Darmstadt, Germany) using a protocol for long DNA fragments as recommended by the manufacturer.

Electropherograms were processed and analysed with GENEMARKER 1.95 (SoftGenetics, State College, PA, USA). The suggested binning was checked and carefully corrected by hand for each preliminary locus, before exporting the peak height tables. These were again processed manually, by specifying thresholds for minimum peak heights for each locus, based on the frequency distributions of its peak heights, respectively, as well as on thresholds for minimum peak number and minimum mean peak height for individual samples. Loci and samples that did not reach the respective thresholds, as well as monomorphic and uninformative loci, were stringently discarded, resulting in very conservative assignments of presences/absences of single ISSR fragments for the final binary matrices of our two study species, respectively.

Data analyses

The two species data sets were analysed equally. AFLP-SURV (Vekemans 2002; Vekemans *et al.* 2002) was used to calculate overall genetic diversity H_t, as well as percentage of polymorphic loci *PLP* and Nei's gene diversity H_e within populations. Additionally, allelic diversity *A* and number of alleles unique to a population (private alleles) were calculated with GENALEX 6.5 (Peakall and Smouse 2012). This program was also used to infer overall population differentiation, applying the Φ_{ST}-statistics with 9999 permutations for significance testing. Furthermore, average frequency-down-weighted marker values (*DW*; see Schönswetter and Tribsch 2005) were calculated using AFLPdat (Ehrich 2006) to measure the amount of rare alleles within populations.

To analyse population structures, we used two non-hierarchical genetic clustering methods. First, the Bayesian algorithm implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2007) was applied to infer gene pool differentiation and admixture of gene pools within individuals. Numbers of possible gene pools (*K*) ranging from 1 to 11 for *Ae. davidbramwellii* and from 1 to 10 for *Ae. nobile* were tested under the admixture setting. We used the independent allele frequency model to avoid overestimation of gene pool differentiation, but allowed for inclusion of the population origin as prior information in the models (LOCPRIOR; Hubisz *et al.* 2009). For each *K*, 20 runs were performed with 100000 generations after a burn-in period of 50000 runs. The outputs were processed and analysed using STRUCTURE HARVESTER (Earl and vonHoldt 2012) implementing the method of Evanno *et al.* (2005). The results were averaged for a particular *K* using CLUMPP (Jakobsson and Rosenberg 2007) and visualised using DISTRUCT (Rosenberg 2004). Geographical display of STRUCTURE results was done with ARCINFO 10.0 (ESRI Inc., Redlands, CA, USA). Second, DAPC (discriminant analyses of principal components (Jombart *et al.* 2010) were carried out using ADEGENET 1.4-2 (Jombart *et al.* 2014) with population assignments as grouping factor within each species to reveal the genetic relationships among and within populations. Implemented cross-validations were applied to choose the number of principal components in order to get the necessary amount of genetic variation and at the same time prevent overfitting of the discriminant functions.

To test for isolation-by-distance (IBD) patterns, pair-wise differentiation indices (F_{ST}) were calculated with 10000 permutations in AFLP-SURV, using the Bayesian method with non-uniform prior allele frequency distribution and assuming Hardy–Weinberg equilibrium ($F_{IS} = 0$, due to supposed outcrossing in the two study species). Linearised F_{ST}-values were then correlated with logarithmised ($\log_{10}$) pair-wise geographic distances (Rousset 1997) in a Mantel test, performed in GENALEX with 9999 permutations.

Further on, we screened the genetic variation in *Ae. davidbramwellii* and *Ae. nobile* for signatures of divergent selection due to the environmental heterogeneity on La Palma. For this purpose, we applied a combination of two different outlier loci detection methods and two correlative approaches to test potential loci-environment associations:

BAYESCAN (Foll and Gaggiotti 2008) uses a Bayesian framework to estimate F_{ST} coefficients and decompose them into a population-specific component, shared by all loci and a locus-specific component, shared by all populations. Then for every locus a selection model *vs.* a neutrality model is compared, checking whether locus-specific components are necessary to explain the F_{ST}. Analyses were performed with 10*50000 iterations after a burn-in of 100000 iterations and twenty pilot runs with 10000 iterations to infer proposal parameter distributions, respectively. Prior odds for the neutral model were set to 1, assuming an equal likelihood for loci to be under selection *vs.* being not under selection, and a False Discovery Rate (FDR) of 0.15 was used for results processing. MCHEZA (Antao and Beaumont 2011) takes use of the interrelationship of heterozygosity and F_{ST} (deficiency of H_e). The software generates a null sampling distribution of F_{ST} estimates based on neutral expectations and then compares these simulated data with the sampled data. We employed 100000 iterations for our analyses, using the two recommended algorithm extensions "Force mean F_{ST}" and "Neutral mean F_{ST}".

We tested the full sets of loci for possible associations to eight environmental variables, which we assumed to state strong and putatively evolutionary relevant ecological gradients among the populations of our two study species (see headline of Tab. 2 for tested variables). Mean annual temperature and mean annual precipitation were interpolated using data collected from 214 and 288 meteorological stations for the Canary Islands, respectively (see Irl *et al.* in press for sources and processing of raw data). Linear regression kriging (R package gstat; Pebesma and Graeler 2014) was applied to interpolate the variables with a spatial resolution of 100 m × 100 m, with elevation, slope, island, micro- and macro-aspect as co-variables, obtained from a digital elevation model (DEM, resolution 2 m × 2 m). Monthly precipitation data came from 47 meteorological stations (time span: 1969 to 1998) and were interpolated using the same linear regression kriging technique. Rainfall seasonality was computed following the index of Walsh and Lawler (1981). Annual solar radiation (Wh/m^2/a) was calculated with the Area Solar Radiation tool in ARCINFO 10.0, based on the 2 m × 2 m DEM and values were extracted as area averages of 25 m radius circles around population coordinates, respectively.

Loci-environment association tests with LFMM (Latent Factor Mixed Modelling; Frichot *et al.* 2013) use a hierarchical Bayesian mixed model based on a variant of principal component analysis in which residual population structure is introduced via unobserved or latent factors. Analyses were done with 10 runs per environmental variable, each with 100000 iterations, including a burn-in of 50000 iterations. According to the found dominance of one cluster in all individuals in the previous STRUCTURE analyses, we decided to set only one latent factor (one gene pool) to correct for background population structure in the LFMM analyses. Of the 10 runs, the run with the lowest DIC (Deviance Information Criterion) was selected for each variable, respectively. SAMBADA tests logistic regression models to identify possible loci-environment associations (Stucki *et al.* 2014). We ran simple univariate models for each single environmental variable, respectively. Significance of model outputs was assessed taking the implemented log-likelihood ratios (*G*-scores) into account and were provided as *P*-values of their X^2-tests (see also Joost *et al.* 2007).

For loci showing consistent signatures of divergent selection (*i.e.* detection by several methods), the distribution of their allelic variants was geographically displayed in relation to the associated environmental variable suggested by the correlative methods. Additionally, the spatial clustering of their allelic variants was quantified by overall spatial autocorrelation (Moran's *I*) and univariate Local Indicators of Spatial Association (*i.e.* local Moran's *I*; Anselin 1995), both implemented in SAMBADA. We used a weighting scheme based on a Gaussian kernel with 10 km distance for the neighbour comparisons to account for the small scales of our study area and the used sampling design. Creation of LISA maps as well as DAPC analyses were done in R 3.1.0 (R Development Core Team 2014).

Results

Aeonium davidbramwellii

The ISSR genotyping for *Ae. davidbramwellii* resulted in 54 individuals successfully scored for 232 loci. Overall genetic diversity was low ($H_t = 0.116$) and population differentiation was moderate with $\Phi_{ST} = 0.104$ ($P < 0.0001$).

The Bayesian STRUCTURE analysis revealed that $K = 3$ was the most likely number of genetic clusters within *Ae. davidbramwellii*, although $K = 2$ and $K = 4$ also showed high likelihoods, and Evanno's ΔK had its highest value at $K = 2$ (Fig. S1). Genetic structure was dominated by one cluster which determined major parts of the genotypes of all individuals (Fig. 2A, B). However, populations H_d and J_d in the Barranco de las Angustias and its northern edge, as well as C_d in the Barranco de la Madera in the west and A_d in the Barranco Fagundo in the north of La Palma showed considerable assignment proportions to further gene pools. Considering $K = 3$, A_d was differentiated from other populations by having large portions of a cluster that furthermore only contributed to the genetic make-up of population J_d. The same was true for $K = 4$, which also showed a differentiation of H_d (Fig. 2A).

The differentiation of H_d, A_d and J_d was also evident in the DAPC, showing these populations in different outer regions of the ordination plot (Fig. 2C), which reflects the results of the STRUCTURE analysis for $K = 3$ and $K = 4$. However, A_d was more related to I_d than to J_d, which grouped loosely with G_d and F_d which were rather non-differentiated in STRUCTURE.

We found no clear IBD pattern among populations of *Ae. davidbramwellii* on La Palma: The correlation of pairwise spatial distances and pairwise genetic differentiation revealed no significant correlation (Mantel's $R = 0.270$, $P = 0.082$; Fig. S2).

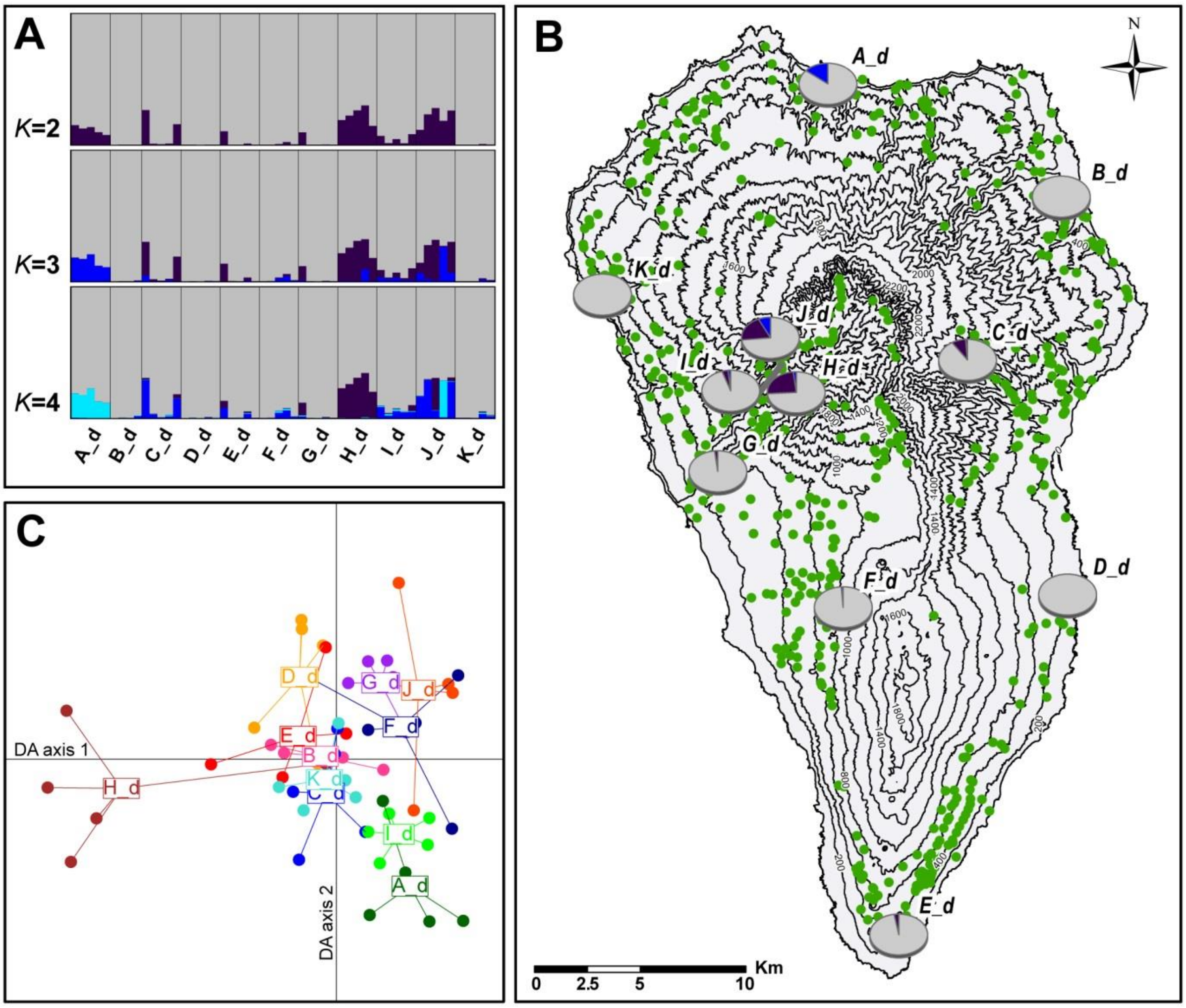

Fig. 2: Genetic clustering results for *Aeonium davidbramwellii*. (A) STRUCTURE results for $K = 2$ to 4. Vertical bars depict colour-coded proportions of genetic cluster assignments for single individuals with populations being separated by vertical black lines. (B) Map of La Palma showing the occurrences of the species (green points) and the genetic structuring from the STRUCTURE results for $K = 3$ as pie charts per analysed population. (C) Discriminant analysis of principal components ordination plot with each point representing one individual, distributed along the two first linear discriminants. Individuals of the same population are connected to its centroid and share the same colouring. Five principal components were retained (representing 31.6% of the total variation) to obtain adequate discrimination of clusters, based on cross validation. Populations are named by their clockwise position around the island, starting from the north.

Highest genetic diversity values for *A*, *PLP* and H_e were found in populations J_d and H_d, both located within or near the Caldera de Taburiente and Barranco de las Angustias, followed by the two differentiated populations outside this region, A_d and C_d, and by I_d, also from the Barranco de las Angustias (Tab. 1). We found comparatively high numbers of private alleles in this region, with seven alleles unique to H_d, the highest value among all populations, and two alleles unique to G_d and I_d, respectively. However, J_d had no private allele at all, and A_d from the northern sector of La Palma had six private alleles. In contrast to the lacking private alleles, J_d had the highest value of overall rarity of alleles (*DW*), followed by H_d, A_d, C_d and I_d. G_d from the lower Barranco de las Angustias revealed low amounts of rare alleles (Tab. 1).

Table 1: Descriptive population genetic parameters for the analysed populations of *Aeonium davidbramwellii*. N: number of scored individuals; *DW*: rarity index; *A*: Allelic diversity; *PLP*: percentage of polymorphic loci; H_e: Nei's Gene diversity.

Population	Longitude UTM	Latitude UTM	Elevation (m a.s.l.)	N	No. of bands	No. of private alleles	*DW*	*A*	*PLP*	H_e
A_d	218814	3192964	80	5	89	6	5.985	0.746	38.4	0.137
B_d	229864	3187626	134	4	51	0	2.661	0.435	22	0.075
C_d	225423	3179899	870	5	88	1	4.878	0.737	37.9	0.134
D_d	230166	3168798	85	5	58	0	2.750	0.474	25	0.087
E_d	222168	3152717	235	5	68	1	3.209	0.582	29.3	0.083
F_d	219540	3168200	893	5	66	0	3.014	0.565	28.4	0.098
G_d	213600	3174629	115	5	61	2	2.881	0.504	26.3	0.091
H_d	217192	3178367	365	5	106	7	7.181	0.905	45.7	0.165
I_d	216611	3179654	1051	5	77	2	4.166	0.642	33.2	0.104
J_d	216095	3180765	1922	5	124	0	7.543	1.047	53.4	0.185
K_d	208142	3182987	271	5	54	1	2.664	0.444	23.3	0.082

The scan for non-neutral genetic variation resulted in indications for divergent selection on at least two loci, although there was inconsistency among the results. BAYESCAN found three candidate loci (Tab. 2, green-coloured cells), which were also suggested by MCHEZA, additional to eight further candidates (Tab. 2, blue-coloured cells). The correlative methods implemented in LFMM and SAMBADA found the most significant ($P < 0.001$) indication of environmental dependence for loci UBC809_292 (mean annual temperature, mean precipitation seasonality) and UBT4_333 (mean precipitation from June to August and from May to October). However, while UBC809_292 was also suggested as outlier by both BAYESCAN ($PP = 0.79$) and MCHEZA ($F_{ST} = 0.25$, $P = 0.96$), UBT4_333 was detected only by MCHEZA ($F_{ST} = 0.32$, $P = 0.99$).

Neither allelic variants in UBC809_292 nor those in UBT4_333 showed significant overall spatial autocorrelation (Moran's $I = 0.007$, $P = 0.110$ and Moran's $I = -0.045$, $P = 0.440$, respectively). However, a clustered pattern of the dominant allele of UBC809_292 in high elevations (*i.e.* low temperature regions) on the western side of La Palma was obvious (Fig. 3A). Nevertheless, the segregation was not entirely consistent, resulting in only partially significant and moderately positive local spatial autocorrelation (local Moran's *I* from 0.1 to 0.5; see Fig. 3A). In UBT4_333, the allelic distribution was broader, covering populations of nearly all island regions (Fig. 3B). The dominant allele showed major contributions to populations in regions of high summer precipitation, but single individuals in populations in regions of medium precipitation, however, also bore this allele, resulting in a mixed spatial pattern and largely missing spatial autocorrelation.

Table. 2: Combined results of outlier loci detection methods for *Aeonium davidbramwellii*. Locus names and overall frequencies of dominant fragments are depicted in the first (left) panel. The second panel depicts results of the F_{ST}-based methods (only indications for divergent selection shown): BAYESCAN (Posterior probabilities, *PP*) and MCHEZA (F_{ST}- and *P*-values). The third and fourth panel show results of the two correlative methods LFMM (for $K = 1$) and SAMBADA, respectively. Outlier loci candidates are highlighted by colouring in the respective columns/panels. Significance of correlations between allelic variation and environmental variables by LFMM and SAMBADA is colour-coded as follows: yellow: $P < 0.05$, orange: $P < 0.01$, red: $P < 0.005$, dark red: $P < 0.001$.

locus	overall frequency	BAYESCAN *PP*	MCHEZA F_{ST}	MCHEZA *P* (simul. F_{ST} < sample F_{ST})	mean annual precipitation	mean precipitation JUN-AUG	mean precipitation DEC-FEB	mean precipitation MAY-OCT	mean precipitation NOV-APR	mean precipitation seasonality	solar radiation	mean annual temperature	mean annual precipitation	mean precipitation JUN-AUG	mean precipitation DEC-FEB	mean precipitation MAY-OCT	mean precipitation NOV-APR	mean precipitation seasonality	solar radiation	mean annual temperature
UBC809_684	0.07	0.52	0.20	0.97	0.893	0.878	0.896	0.727	0.948	0.660	0.846	0.955	0.885	0.688	0.717	0.961	0.891	0.734	0.422	0.546
UBC840_317	0.06	0.51	0.23	0.99	0.556	0.889	0.797	0.713	0.740	0.709	0.541	0.733	0.515	0.749	0.710	0.893	0.740	0.859	0.057	0.543
UBC840_319	0.06	0.51	0.23	0.99	0.527	0.886	0.775	0.713	0.738	0.714	0.544	0.724	0.515	0.749	0.710	0.893	0.740	0.859	0.057	0.543
UBC840_321	0.07	0.51	0.20	0.97	0.389	0.778	0.611	0.418	0.537	0.851	0.735	0.588	0.275	0.991	0.396	0.537	0.430	0.838	0.109	0.356
UBC881_282	0.22	0.49	0.23	0.96	0.243	0.236	0.554	0.392	0.407	0.333	0.534	0.765	0.304	0.432	0.509	0.486	0.507	0.311	0.233	0.724
UBC809_246	0.81	0.58	0.17	0.88	0.975	0.789	0.766	0.688	0.646	0.363	0.760	0.335	0.667	0.226	0.507	0.971	0.417	0.002	0.776	0.034
UBC809_294	0.19	0.58	0.15	0.89	0.824	0.371	0.848	0.535	0.798	0.195	0.136	0.365	0.433	0.006	0.688	0.560	0.664	0.007	0.025	0.061
UBC809_644	0.17	0.49	0.05	0.60	0.185	0.878	0.169	0.205	0.069	0.658	0.924	0.146	0.105	0.859	0.034	0.123	0.034	0.360	0.996	0.006
UBC809_896	0.07	0.48	0.03	0.65	0.582	0.593	0.475	0.957	0.391	0.330	0.693	0.135	0.297	0.061	0.193	0.637	0.211	0.043	0.607	0.010
UBC809_904	0.52	0.65	0.26	0.98	0.875	0.907	0.629	0.861	0.527	0.978	0.878	0.483	0.234	0.529	0.070	0.242	0.061	0.057	0.744	0.006
UBC840_353	0.37	0.68	0.17	0.90	0.967	0.875	0.993	0.490	0.958	0.881	0.190	0.887	0.722	0.415	0.720	0.467	0.723	0.083	0.008	0.500
UBC885_382	0.11	0.48	0.02	0.42	0.237	0.567	0.360	0.771	0.250	0.765	0.846	0.292	0.007	0.212	0.016	0.050	0.020	0.653	0.992	0.010
UBC885_546	0.06	0.47	0.01	0.56	0.691	0.738	0.712	0.731	0.651	0.550	0.397	0.350	0.209	0.005	0.333	0.970	0.341	0.005	0.015	0.009
UBC885_704	0.06	0.47	0.01	0.56	0.202	0.104	0.375	0.261	0.279	0.398	0.770	0.600	0.037	0.009	0.075	0.037	0.074	0.115	0.464	0.348
UBC888_382	0.19	0.54	0.10	0.75	0.910	0.753	0.958	0.504	0.969	0.744	0.498	0.716	0.959	0.005	0.792	0.244	0.813	0.009	0.071	0.137
UBC888_423	0.72	0.54	0.15	0.74	0.683	0.818	0.536	0.975	0.453	0.604	0.830	0.629	0.020	0.949	0.007	0.028	0.009	0.799	0.958	0.016
UBC888_900	0.13	0.47	0.05	0.55	0.973	0.765	0.801	0.659	0.765	0.803	0.558	0.581	0.598	0.010	0.511	0.916	0.523	0.035	0.174	0.048
UBC889_688	0.24	0.48	0.03	0.53	0.458	0.719	0.388	0.872	0.267	0.733	0.871	0.234	0.013	0.551	0.006	0.026	0.006	0.643	0.817	0.002
UBC889_705	0.67	0.55	0.18	0.86	0.957	0.275	0.870	0.997	0.956	0.173	0.494	0.548	0.773	0.018	0.914	0.466	0.940	0.008	0.037	0.143
UBC809_292	0.20	0.79	0.25	0.96	0.403	0.459	0.414	0.874	0.255	0.135	0.157	0.002	0.057	0.002	0.051	0.510	0.040	0.001	0.006	0.000
UBC840_271	0.06	0.48	0.04	0.80	0.124	0.037	0.286	0.107	0.183	0.275	0.312	0.617	0.092	0.009	0.099	0.041	0.103	0.201	0.021	0.587
UBC840_355	0.37	0.94	0.41	1.00	0.881	0.869	0.963	0.483	0.915	0.995	0.274	0.990	0.971	0.380	0.594	0.417	0.569	0.146	0.026	0.804
UBC840_357	0.28	0.83	0.36	1.00	0.755	0.563	0.855	0.353	0.769	0.456	0.146	0.896	0.711	0.130	0.385	0.193	0.390	0.014	0.029	0.647
UBC840_541	0.15	0.50	0.12	0.84	0.116	0.006	0.279	0.056	0.214	0.056	0.134	0.918	0.141	0.003	0.176	0.045	0.184	0.027	0.012	0.987
UBC888_617	0.70	0.70	0.23	0.97	0.385	0.498	0.330	0.486	0.327	0.149	0.580	0.865	0.307	0.457	0.248	0.219	0.314	0.045	0.263	0.770
UBT4_307	0.15	0.58	0.17	0.91	0.010	0.356	0.092	0.358	0.035	0.768	0.976	0.022	0.001	0.045	0.004	0.009	0.005	0.690	0.281	0.004
UBT4_328	0.22	0.58	0.32	0.98	0.015	0.002	0.048	0.001	0.017	0.076	0.260	0.318	0.009	0.000	0.006	0.001	0.007	0.021	0.035	0.313
UBT4_333	0.24	0.63	0.32	0.99	0.010	0.003	0.039	0.000	0.008	0.076	0.229	0.216	0.006	0.000	0.002	0.000	0.002	0.024	0.036	0.186

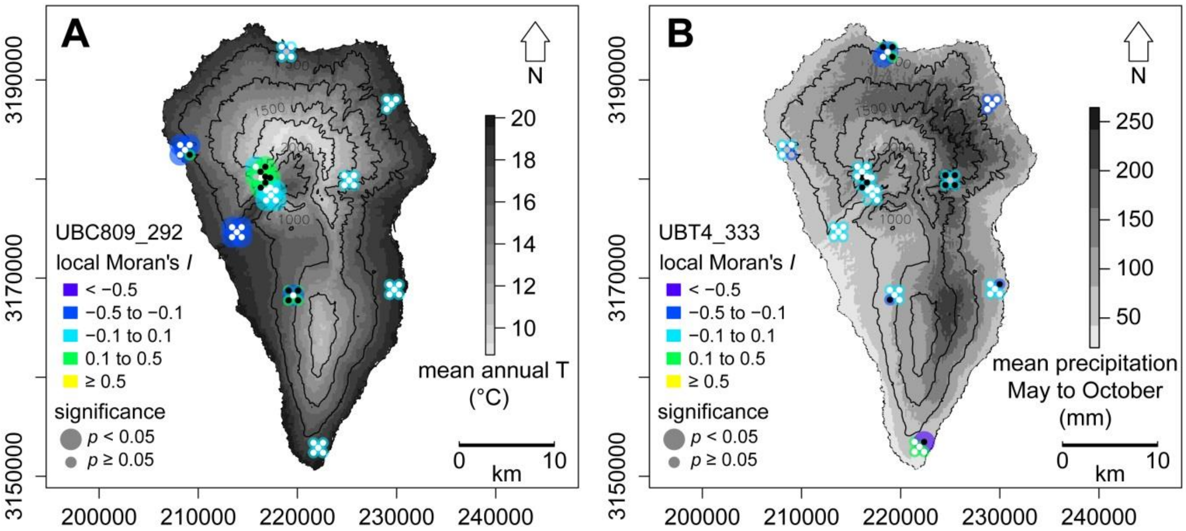

Fig. 3: Potential adaptive genetic differentiation among populations of *Aeonium davidbramwellii* of locus UBC809_292 in relation to mean annual temperature (A) and of locus UBT4_333 in relation to mean precipitation from May to October (B) on La Palma. Black, white and grey dots indicate presence or absence of the respective ISSR-fragment in an individual or missing data, respectively. Local Moran's *I* values › 0.1 indicate local spatial autocorrelation of allelic variation. Single individual coordinates were dispersed around their population centre for mapping purposes.

Aeonium nobile

For *Ae. nobile*, 44 individuals were successfully scored for 196 ISSR loci. Overall genetic diversity was on a similarly low level as for the former species ($H_t = 0.115$) and overall population differentiation was a bit lower ($\Phi_{ST} = 0.092$, $P < 0.0001$).

The clustering analyses in STRUCTURE resulted in $K = 3$ as the most likely subdividing genetic structure in *Ae. nobile*, with $K = 2$ and $K = 4$ showed high likelihoods as well and ΔK peaking at $K = 2$ (Fig. S3). All populations and individuals were dominated by one genetic cluster (Fig. 4A). However, populations in the Barranco de las Angustias and its northern edge (E_n, G_n, H_n and F_n), as well as the southern populations (J_n and I_n) and C_n on the western slope of the Taburiente volcano edifice showed some proportions of a second and a third cluster. Especially population H_n in the lowest part of the Barranco de las Angustias showed strong proportions of the second cluster consistently among its individuals. With $K = 4$, the southernmost J_n and I_n were differentiated from the other populations, and E_n in the upper part of the Barranco de las Angustias became more differentiated in the results (Fig. 4 A, B).

The DAPC supported the differentiated state of H_n and put the other populations of the Barranco de las Angustias together in a separate group, too (Fig. 4C). However, J_n and I_n were not differentiated from the northern populations in this analyses and C_n took a very differentiated position as well.

The correlation between pairwise geographic distances and pairwise genetic differentiation between populations revealed the absence of an IBD pattern (Mantel's $R = -0.071$, $P = 0.397$, see Fig. S4).

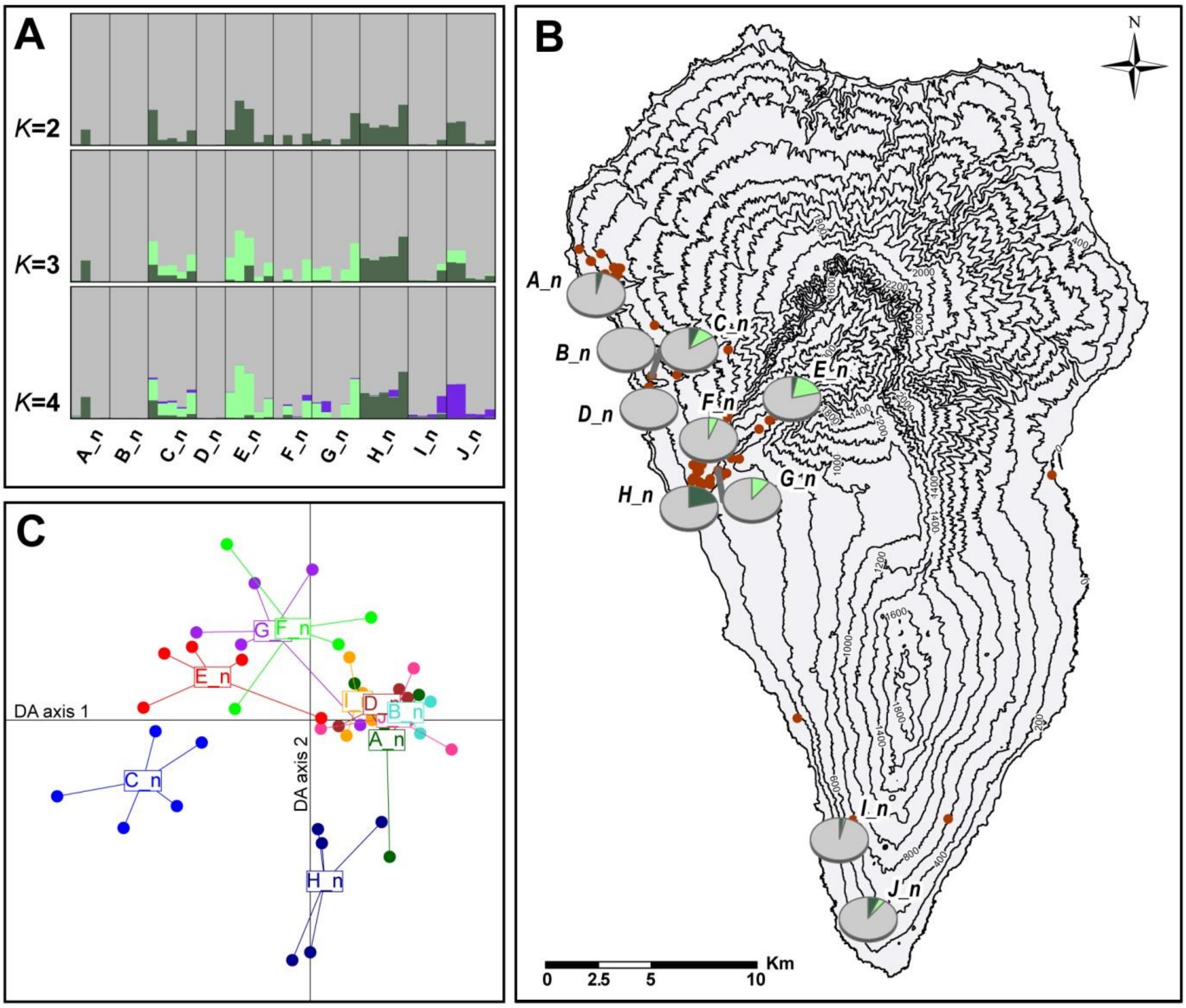

Fig. 4: Genetic clustering results for *Aeonium nobile*. (A) STRUCTURE results for *K* = 2 to 4. Vertical bars depict colour-coded proportions of genetic cluster assignments for single individuals with populations being separated by vertical black lines. (B) Map of La Palma showing the occurrences of the species (brown points) and the genetic structuring from the STRUCTURE results for *K* = 3 as pie charts per analysed population. (C) Discriminant analysis of principal components ordination plot with each point representing one individual, distributed along the two first linear discriminants. Individuals of the same population are connected to its centroid and share the same colouring. Ten principal components were retained (representing 37.7% of the total variation) to obtain adequate discrimination of clusters, based on cross validation. Populations are named by their latitudinal position, starting from the north.

We found the highest genetic diversity values (*A*, *PLP* and H_e) in H_n, followed by the two other populations from within the Barranco de las Angustias (E_n and G_n) and by population C_n (Tab. 3). F_n and the southern range populations J_n and I_n showed intermediate diversity values while the northern populations D_n, B_n and A_n were least diverse (with D_n consisting of only three genotyped individuals, however). H_n had five private alleles, whereas E_n and G_n had no population-specific alleles. The second highest number of three unique alleles was found in the southernmost J_n, and C_n as well as the northernmost A_n had two private alleles. According to *DW*, all populations from the Barranco de las Angustias region showed high values, with H_n and E_n the highest. Other high values were obvious in C_n, J_n and I_n.

Signatures of selective forces on genetic variation in *Ae. nobile* were very weak and inconsistent (Tab. S3). BAYESCAN found no outlier loci under the same settings that were applied for *Ae. davidbramwellii*. MCHEZA suggested 11 loci to show variation caused by divergent selection, which, however were only poorly supported by the two correlative methods used: LFMM, found only few correlations with very low significance (*P*-values between 0.01 and 0.05), and similarly, the correlations indicated by SAMBADA did not reach the significance levels obtained for *Ae. davidbramwellii* and were only partially congruent with the outlier suggestions of MCHEZA. The most significant correlation ($P = 0.001$) was found between the allelic variation in locus UBC809_562 (MCHEZA: $F_{ST} = 0.23$, $P = 0.96$) and mean precipitation from June to August. However, the spatial distribution of this variation was highly inconsistent and did not show a recognisable relationship to the environmental variable (not shown).

Table 3: Descriptive population genetic parameters for the analysed populations of *Aeonium nobile*. N: number of scored individuals; *DW*: rarity index; *A*: Allelic diversity; *PLP*: percentage of polymorphic loci; H_e: Nei's Gene diversity.

Population	**Longitude UTM**	**Latitude UTM**	**Elevation (m a.s.l.)**	**N**	**No. of bands**	**No. of private alleles**	***DW***	***A***	***PLP***	H_e
A_n	207834	3182960	36	4	50	2	3.125	0.500	25.5	0.100
B_n	209258	3180361	293	4	37	1	2.573	0.378	18.9	0.069
C_n	210473	3179117	443	5	79	2	5.202	0.791	40.3	0.143
D_n	210327	3178565	149	3	18	1	1.882	0.179	9.2	0.045
E_n	217096	3178041	363	5	96	0	6.306	0.954	49	0.162
F_n	213028	3175880	720	4	55	1	3.840	0.546	28.1	0.109
G_n	213600	3174629	115	5	80	0	4.442	0.806	40.8	0.123
H_n	212226	3172861	6	5	103	5	6.871	1.041	52.6	0.160
I_n	219344	3157172	275	4	55	1	3.965	0.556	28.1	0.099
J_n	220699	3153422	415	5	60	3	4.448	0.592	30.6	0.108

Discussion

The project aimed to identify population genetic patterns related to geographical and ecological heterogeneity on the western Canary Island of La Palma, to make inferences on the evolutionary potential on the island scale and for potential conservational implications. Our main findings are as follows: 1. Genetic structuring within both species is low although effects of island topography and range patterns are obvious; 2. there are indications of correlations between allelic patterns and environmental heterogeneity in temperature and precipitation variables for *Ae. davidbramwellii*; 3. the differing niche widths of *Ae. davidbramwellii vs. Ae. nobile* did not reflect in differences in the degree of genetic structuring; however, in the generalist species *Ae. davidbramwellii* signatures of selection were more distinct.

Population structures

The weak to moderate overall genetic population structures in *Ae. davidbramwellii* and *Ae. nobile* may be due to the following (non-exclusive) reasons:

First, lineage differentiation might be impeded by extensive gene flow. However, the moderate Φ_{ST}-values but largely missing IBD patterns speak against common gene flow between adjacent populations, but rather suggest random (including long-distance) dispersal events within recent time scales and limited gene flow after the establishment of populations (Slatkin 1993; Hutchison and Templeton 1999). The facts that populations of the analysed species show rather discrete distributions over a topographically highly structured landscape, very variable population sizes (pers. obs.) and only moderate dispersal capacity (Vazačová and Münzbergová 2014) support this interpretation.

Second, recent species spreads and limited lineage differentiation also correspond with a relatively low evolutionary age of the analysed species. Available molecular phylogenies of *Aeonium* place our two study species to the end of very short branches or into unresolved polytomies (Mort *et al.* 2002; Kim *et al.* 2008; Thiv *et al.* 2010; Kondraskov *et al.* 2015), suggesting rather recent species formation without sufficient time to differentiate from their respective ancestor and/or sister species. A recent Bayesian divergence time dating based on ITS sequence data (Kondraskov *et al.* 2015, see also Thiv *et al.*

2010) found a cladogenetic origin in the late Pleistocene or even later for *Ae. davidbramwellii* (mean stem age: 0.5 Ma, 95% highest posterior densities: 0.01 – 1.16 Ma) and a slightly older origin for *Ae. nobile* (mean stem age: 1.19 Ma, 95% highest posterior densities: 0.36 – 2.17 Ma). Many other *Aeonium* species show higher divergence estimates. Geologically young oceanic islands like La Palma generally can be assumed to host more young species and lineages with lower levels of population divergence (see also Kim *et al.* 1999; Bottin *et al.* 2005; Stuessy *et al.* 2014). The low overall genetic diversities compared to other endemic species on older islands of the Canaries support this idea, see *e.g. Atractylis preauxiana* on Gran Canaria (H_t = 0.219) and Tenerife (H_t = 0.229) (Caujapé-Castells *et al.* 2008), *Gnaphalium teydeum* on Tenerife (H_t = 0.173) (González-Pérez *et al.* 2008), both studies done with RAPD analyses; or *Solanum vespertilio* on Tenerife (H_t = 0.205) and *Solanum lidii* on Gran Canaria (H_t = 0.207) observed with AFLP data (Prohens *et al.* 2007).

Third, it is also possible that the low overall genetic diversity and differentiation values are due to catastrophic throwbacks of range expansions and bottleneck events in the past of *Ae. davidbramwellii* and *Ae. nobile*. La Palma has a vivid geological history with different periods of strong volcanism, as well as multiple mega-landslides, especially on the western side of the island (Carracedo *et al.* 1999; Masson *et al.* 2002; Colmenero *et al.* 2012) where the evolutionary origin of our two study species is likely (see below). Such events have been shown in other studies to effectively shape phylogeographic trajectories on the Canary Islands (see *e.g.* Brown *et al.* 2000; Juan *et al.* 2000; Emerson 2003). Violent volcanic events and mega-landslides might have caused severe reductions in population size and interruptions in range extractions after species formation of *Ae. davidbramwellii* and *Ae. nobile*. The observed low levels of population structures can thus be interpreted as representations of initial stages of ongoing differentiation processes after late species evolution and/or delayed (stochastic) spreading over the island, but this is somewhat speculative. DNA sequence-based phylogeographical studies and estimations of demographic histories may help to make more definite statements.

Despite low overall population differentiation within *Ae. davidbramwellii* and *Ae. nobile*, compared to a majority of Canary Island endemics (see Pérez de Paz and Caujapé-Castells 2013), some regional structuring was detectable. In populations located in the lower Caldera de Taburiente and its large erosion valley (Barranco de las Angustias), high portions of exceptional genetic clusters, considerable differentiation, as well as the highest values of genetic diversity indices were observed for both species. The sheltered situation and special topography within the large landscape depression might have provided suitable conditions for species evolution from ancestral colonisers and for population persistence. Gaisberg and Stierstorfer (2005) stated plant speciation centres in steep and rocky regions on El Hierro, the even younger neighbouring island of La Palma, suggesting strong disturbance regimes (*e.g.* erosion, debris fall), low competition and high habitat diversity as reasons for increased evolutionary processes in such habitats.

In *Ae. davidbramwellii*, a high number of private and rare alleles is located in populations within the Barranco de las Angustias and Caldera de Taburiente, also suggesting a possible evolutionary origin of the species in this region. However, the exterior populations C_d and A_d, which were situated in other deep erosion valleys (Barranco de la Madera and Barranco Fagundo, respectively), showed patterns of isolated evolution as well. This indicates a general role of topography in promoting population differentiation, which has already been shown for other plant species on the island scale (*e.g.* Furches *et al.* 2009; Riley *et al.* 2010). Barrier effects due to landscape structures are also a likely explanation for the non-significant correlation between population pairwise genetic differentiation and geographic distance in the IBD test. In general, topographical complexity and its consequences on habitat isolation and environmental heterogeneity is a commonly expected driver of speciation on oceanic islands (see *e.g.* Stuessy 2007; Whittaker *et al.* 2008).

Despite being more restricted in its distribution than *Ae. davidbramwellii*, *Ae. nobile* showed a comparable pattern of genetic differentiation and diversity. Populations in the Barranco de las Angustias were genetically most diverse. Additionally, we found the highest density of populations and largest individual numbers in this region, pointing to a species origin here as well. However, although allele rarity (*DW*) was generally high in the Barranco de las Angustias, only population H_n in the lowest part of the Barranco showed a large number of private alleles. This population additionally was differentiated in the clustering analyses. Populations in the upper parts of the Barranco (G_n and E_n) together with F_n, located slightly behind the northern ridge of the Barranco, made up an own group, pointing to an early separation from H_n. Especially the genetically very poor populations in the north (D_n, B_n and A_n) give rise to the assumption that the northern part of the distribution range is the result of recent colonisation by *Ae. nobile*. However, the highly differentiated position and high diversity values of population C_n suggest a relatively early dispersal event, so that C_n established before other populations in its surrounding and had time to evolve. This supports the idea of rather stochastic dispersal events from the Barranco de las Angustias.

The southernmost populations' slight genetic differentiation reflects the intra-insular range disjunction of *Ae. nobile*, which is congruent with the geomorphological features created by the Cumbre Nueva landslide. Over a large area south of the Barranco de las Angustias, comparably mild and low structured slopes impede the occurrence of *Ae. nobile*. Additionally, the prevailing agriculture, dense settlement and infrastructure probably pose problems for this species here. However, the low differentiation of the southern populations speaks against early vicariance due to the landslide but more for colonisation from the North after this event. The recent volcanic origin of southern areas of La Palma (max. 125 ka Carracedo *et al.* 1999; Colmenero *et al.* 2012) is also more in line with a dispersal scenario. Nevertheless, range disjunctions due to landslide effects may pose an important evolutionary factor for intra-island population divergence if large enough to prevent gene flow (see also Brown *et al.* 2006; Mairal *et al.* 2015).

Unfortunately, we were not able to take samples from the eastern part of La Palma as the only identified natural population was located high on a steep cliff south of Santa Cruz de La Palma, not accessible for sampling, which, however, might have provided additional insight in the history of *Ae. nobile*.

Adaptive variation

While many indications of non-neutral allele distribution in our study were of low significance or were detected only by one or two of the applied approaches, some loci in *Ae. davidbramwellii* showed consistent patterns of selection effects. The strongest indications of selective effects were related to mean annual temperature (MAT) and to mean precipitation (MP) during the summer months (from May to October and from June to August), variables which constitute strong environmental gradients among the analysed populations of this species (MAT: 11.0 – 19.5 °C; $MP_{May-October}$: 35 – 196 mm; $MP_{June-August}$: 0 – 16 mm).

The spatially not entirely consistent association of marker UBT4_333 to summer precipitation all over the island might indicate that its allelic variation was already present in populations during the species' spread over La Palma, and that its actual differentiation potentially represents adaptive allele frequency shifts based on this standing genetic variation. The distinct succulence of *Ae. davidbramwellii* and its CAM-based physiology (Lösch 1990; Mort *et al.* 2007) imply that water limitation and water use efficiency play crucial roles for the fitness and ecological niche of this species. Individuals in mesic conditions thus might profit from pre-existing alleles that modulate water use traits in order to take use of increased resource availability. Selection on standing genetic variation is known as a likely mechanism of fast adaptational processes (Feder *et al.* 2003; Barrett and Schluter 2008). Nevertheless, the more or less island-wide distribution of allelic variants in UBT4_333 can at most represent an early state in a potential continuum of ecological differentiation within *Ae. davidbramwellii* (Nosil 2012).

The clustering of UBC809_292 fragments in populations of low temperature habitats in high elevations only on the western side of La Palma could indicate a newly evolved adaptation. Considering the elevational niche separation among *Aeonium* species, the temperature gradient is a likely driver of evolutionary divergence within this genus (Lösch 1984; Liu 1989). Although the evolutionary potential of the found variation in UBC809_292 remains an open question, it indicates that ecological heterogeneity in combination with topography (in this case the old Taburiente edifice and ridge systems separating eastern and western parts of the island) can foster initial population divergence on the small scale of an island. Such interactions of different habitat conditions and topographical gene flow barriers on plant population divergence on the island scale have recently been shown *e.g.* in *Elaeocarpus photiniifolia* on the Bonin Islands, Japan (Sugai *et al.* 2013) or in *Jumellea rossii* on Réunion, Mascarene Islands (Mallet *et al.* 2014).

Reciprocal ecological disadvantage of migrants and hybrids and built-up of reproductive isolation between divergent populations is needed for further evolutionary separation (Nosil 2012; Savolainen *et al.* 2013). Although gene flow seems to be limited or at least highly variable among populations, genetic patterns in *Ae. davidbramwellii* yet lack signals of such reproductive isolation and show only incipient intraspecific evolutionary differentiation.

Species differences

In the literature, specialist species are expected to have smaller and more isolated populations than related generalist species, thus they would be more affected by genetic drift due to meta-population dynamics, fostering higher variation among populations (*e.g.* Groot *et al.* 2011; Li *et al.* 2014 and references therein). Concordantly, in an allozyme data meta-analysis for Canary Island plant endemics, Pérez de Paz and Caujapé-Castells (2013) found significantly higher differentiation indices (G_{ST} and F_{ST}) and lower genetic diversity in species occurring in small and intermediate population sizes (≤ 500 individuals) compared to species with large populations (> 500 individuals).

Contrastingly, our study revealed no significant differences in overall population structures and total genetic diversity between the generalist and the specialist species. *Ae. davidbramwellii* has a wider and more continuous distribution than *Ae. nobile* and mostly showed larger population sizes (pers. obs.). Thus, one could have expected lower population differentiation from the general model described above. Additionally, it is younger than *Ae. nobile* (Kondraskov *et al.* 2015), thus its populations theoretically had less time to differentiate. However, the wide distribution of *Ae. davidbramwellii* is also characterised by a much stronger environmental heterogeneity and large-scale topographical subdivision than that of *Ae. nobile*. We thus suppose that for the moderate population differentiation within *Ae. davidbramwellii* the effects of a rather continuous distribution, larger population sizes and less evolutionary age were compensated by the effects of differentiating selection pressures and strong landscape structuring. In contrast, the similar differentiation level in *Ae. nobile* might have been dominated by drift effects. In fact, although only marginally significant, a potential IBD-pattern was more discernible in *Ae. davidbramwellii*, pointing to higher gene flow between neighbouring populations and lower importance of drift. Also, consistent signals of differentiating selection were found only in the generalist *Ae. davidbramwellii*, supporting the hypothesised evolutionary effects of strong environmental heterogeneity within its range. In the sampled range of *Ae. nobile*, ecological gradients are limited in strength, missing divergent selection regimes among populations of this species which provides a likely explanation for the low association of allelic patterns and ecological variables. Furthermore, the only potential large-scale landscape barrier for gene flow is the region of the ancient Cumbre Nueva landslide. This emphasises that besides population continuity and population sizes, landscape factors and the strength of environmental heterogeneity should be considered in studies of generalist-specialist-patterns, at least on small spatial scales like single islands.

It also has to be mentioned that we observed considerable regional phenotypic variation in *Ae. davidbramwellii* during our fieldwork on La Palma, which was not true for *Ae. nobile*. Especially in populations in the Caldera de Taburiente and the Barranco de las Angustias, individuals showed exceptionally strong branching, whereas in the southern regions of La Palma, branching in this species was rather uncommon. This is a further indication of divergent selection pressures, although not reflected in our genetic data.

It has been shown that plant speciation can occur by adaptive specialisation of certain populations of generalist species, leading to sympatric pairs of sister species with largely different ecological niche breadths (Grossenbacher *et al.* 2014). This might also be an evolutionary scenario for *Ae. davidbramwellii* and some of its populations, while the specialised *Ae. nobile* actually seems to be in an ‘evolutionary dead end', without great prospects of further intraspecific niche divergence and adaptive differentiation.

However, some of the drawn conclusions might have limitations due to the following two reasons: First, future studies should include more populations and a denser representation of all regions of La Palma. We included many populations from the Barranco de las Angustias region which potentially might have biased our observations of disproportionally high local genetic variation. Further on, larger sampling sizes may allow for more distinct and significant results according to genetic variation estimates within and among populations. Second, although ISSR analyses have proven to be reliable approaches for plant genotyping and population genetic studies (see *e.g.* Nybom 2004) and although we put high effort in genotyping accuracy, this dominant marker system imminently has some potential for genotyping errors due to possible PCR artefacts and homoplasy. Further on, future studies would benefit from the use of haplotype sequence data which might enable a more in-depth inference of phylogeographic and demographic histories. Genomic approaches are necessary to identify evolutionary relevant genes and their effects on phenotypic and environmental variation, as well as potential genomic mechanisms involved in population divergence. Further studies will thus help to understand the processes of adaptive evolution in this genus beyond the level of candidate markers.

Nevertheless, investigations of intraspecific variation and population structures, and therefore of recent eco-evolutionary processes and potential drivers for species divergence in *Aeonium*, have not taken place yet. Although not definite, our study revealed basic patterns within the two analysed species and gives additional insights into species evolution on small oceanic islands.

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Appendix

Table S1: Plate layout and instrument settings for DNA purification via BindIT 3.1 KingFisher software. After step 8, eluted DNA was transferred to plastic cups and stored at -20 °C. Due to increased homogenization volume in comparison to the original protocol, binding of genomic DNA to magnetic beads was carried out in the three initial steps. After the elution step, the magnetic beads were automatically collected and disposed.

Steps		**Step 1 Bind MC2**	**Step 2 Bind MC2**	**Step 3 Bind MC2**	**Step 4 Wash MC3**	**Step 5 Wash MC4**	**Step 6 Wash Ethanol**	**Step 7 Wash MC5**	**Step 8 Elution MC6**
Layout	Row	A	B	C	D	E	F	G	H
	Sample, magnetic beads, buffers	92 µl supernatant 8 µl mag. beads 100 µl MC2	100 µl supernatant 100 µl MC2	100 µl supernatant 100 µl	200 µl MC3	200 µl MC4	200 µl ethanol (80%)	200 µl MC5	20 µl MC6
Beginning	Precollect	no	no	no	no	no	no	no	no
	Release time (mm:ss)	no release	00:30	00:30	00:30	00:30	00:30	no release	00:15
	Release speed	-	fast	fast	fast	fast	fast	-	fast
Mixing	Pause for manual handling	no	no	no	no	no	no	no	no
	Mixing time (mm:ss)	05:00	05:00	05:00	01:00	01:00	01:00	01:00	10:00
	Mixing speed	medium	medium	medium	fast	fast	fast	medium	medium
End	Postmix	no	no	no	no	no	no	no	no
	Collect count	3	3	3	3	3	3	3	6
	Collect time (s)	1.5	1,5	1,5	1.5	1.5	1.5	1.5	1.5

Table S2: Tested primers for the ISSR analyses, their sequences and PCR annealing temperatures. Used labelling (dye) for the capillary electrophoresis and are given only for the markers of the final analyses plus their fragment size spectra and numbers of polymorphic fragments obtained for the two study species, respectively. Annealing temperatures were the same for both species. Sources for primers are indicated by superscript numbers: 1 UBC set 9 (Biotechnology Laboratory, The University of British Columbia, Canada), 2 newly designed for this study, and 3 Wolfe *et al.* 1998, see methods. n.r.: no results.

				Aeonium davidbramwellii		*Aeonium nobile*	
Primer	**Primer sequence**	**Fluorescent label for capillary electrophoresis**	**Annealing temperature (° C)**	**Fragment size range (bp)**	**No. polymorphic fragments**	**Fragment size range (bp)**	**No. polymorphic fragments**
UBC807[1]	$(AG)_8$-T	BMN-6	51	282–703	15	234–863	18
UBC809[1]	$(AG)_8$-G	BMN-5	51	56–930	49	280–982	25
UBC840[1]	$(GA)_8$-YT	BMN-5	48	271–909	54	115–1060	47
UBC848[1]	$(CA)_6$-RG	DY-751	51	280–928	11	215–848	7
UBC881[1]	$(GGGT)_3$-G	BMN-6	51	152–941	9	85–935	29
UBC885[1]	BHB-$(GA)_7$	BMN-6	45	232–870	25	265–859	19
UBC888[1]	BDB-$(CA)_7$	BMN-5	51	249–907	38	263–897	30
UBC889[1]	DBD-$(AC)_7$	DY-751	51	443–948	22	425–748	5
UBT4[2]	$(GA)_8$-YA	DY-751	45	307–792	9	271–1089	16
UBC812[1]	$(GA)_8$-A	-	45	-	-	-	-
UBC822[1]	$(TC)_8$-A	-	51	-	-	-	-
UBC841[1]	$(GA)_8$-YC	-	51	-	-	-	-
UBC842[1]	$(GA)_8$-YG	-	56	-	-	-	-
UBC845[1]	$(CT)_8$-RG	-	45	-	-	-	-
UBC850[1]	$(GT)_8$-YC	-	45	-	-	-	-
UBC857[1]	$(AC)_8$-YG	-	45	-	-	-	-
UBC859[1]	$(TG)_8$-RC	-	45	-	-	-	-
UBC861[1]	$(ACC)_6$	-	58	-	-	-	-
UBC890[1]	VHV-$(GT)_7$	-	56	-	-	-	-
UBC891[1]	HVH-$(TG)_7$	-	51	-	-	-	-
UBT1[2]	$(AC)_8$-CH	-	45	-	-	-	-
UBT2[2]	$(AG)_8$-CR	-	51	-	-	-	-
UBT3[2]	$(CG)_8$-RC	-	n.r.	-	-	-	-
UBT5[2]	$(GC)_8$-YG	-	n.r.	-	-	-	-
UBT6[2]	$(GT)_6$-AG	-	45	-	-	-	-
UBT7[2]	$(GT)_8$-YT	-	45	-	-	-	-
UBT8[2]	$(TG)_8$-CR	-	45	-	-	-	-
UBT9[2]	$(TG)_8$-RG	-	45	-	-	-	-
17901[3]	$(GT)_6$-YR	-	45	-	-	-	-
17902[3]	$(GT)_6$-AY	-	45	-	-	-	-

Code for degenerated 5'- or 3'-anchor bases: Y = C or T, R = A or G, B = C, G or T, D = A, G or T, H = A, C or T, and V = A, C or G.

Wolfe, Andrea D.; Xiang, Qiu-Yun; Kephart, Susan R. (1998): Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeat (ISSR) bands. *Molecular Ecology* 7, pp. 1107–1125.

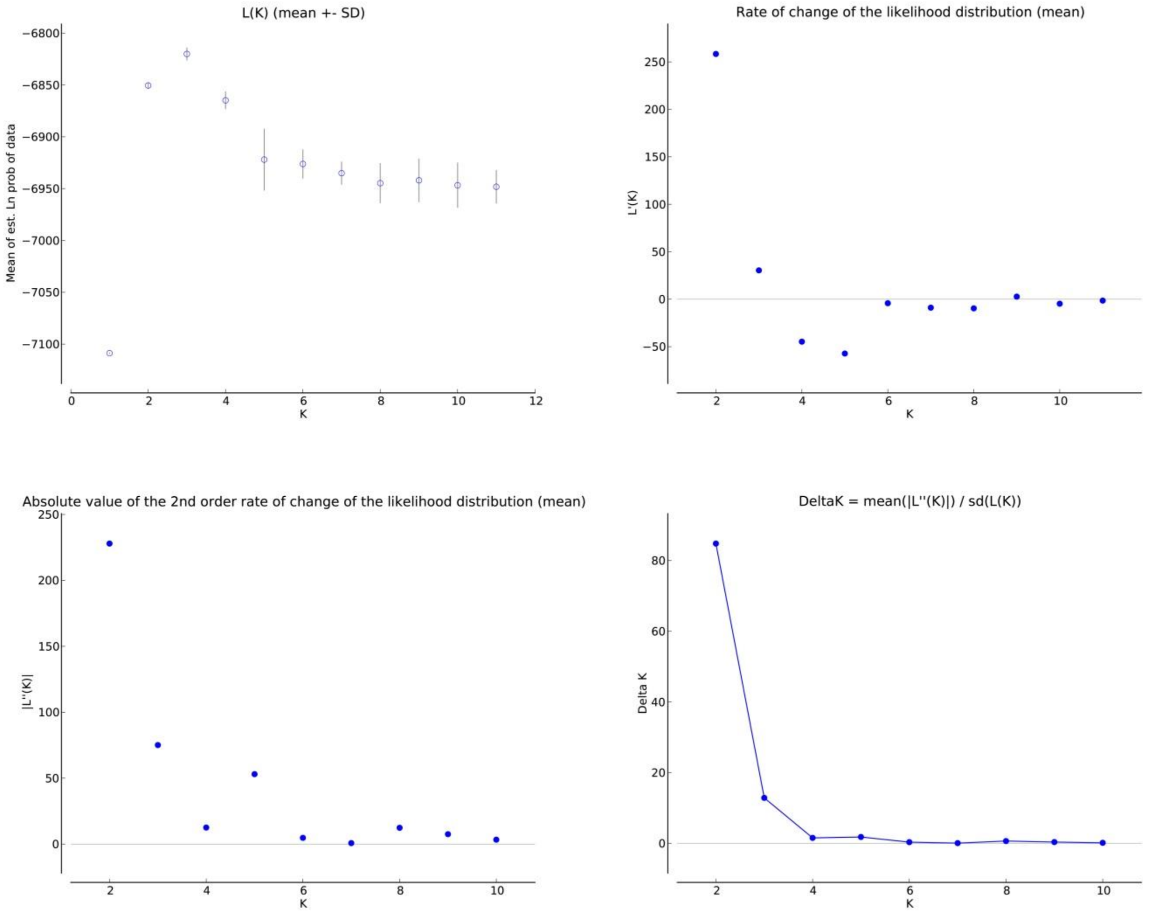

Fig. S1: Assessment of the most likely number of clusters of STRUCTURE runs using the method of Evanno *et al.* (2005) for *Aeonium davidbramwellii*. Shown are means of (top left) the log probability of the data mean L(K), (top right) the rate of change of the likelihood distribution mean L'(K), (bottom left) the absolute values of the second order rate of change L''(K) and (bottom right) ΔK, over 20 runs for each K value, respectively. Plots created with STRUCTURE HARVESTER (Earl and vonHoldt 2012).

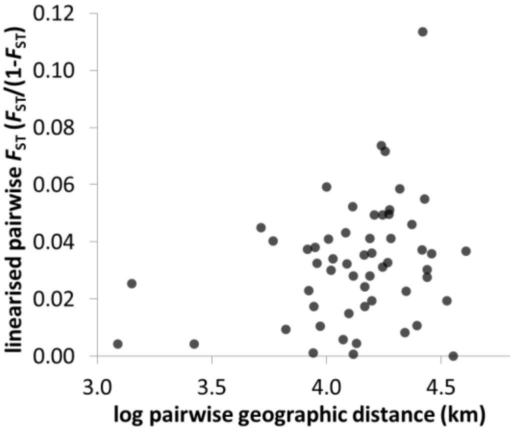

Fig. S2: Correlation of pair-wise geographic distance and pair-wise linearised F_{ST} between populations of *Aeonium davidbramwellii*: Mantel's R = 0.270, P = 0.082.

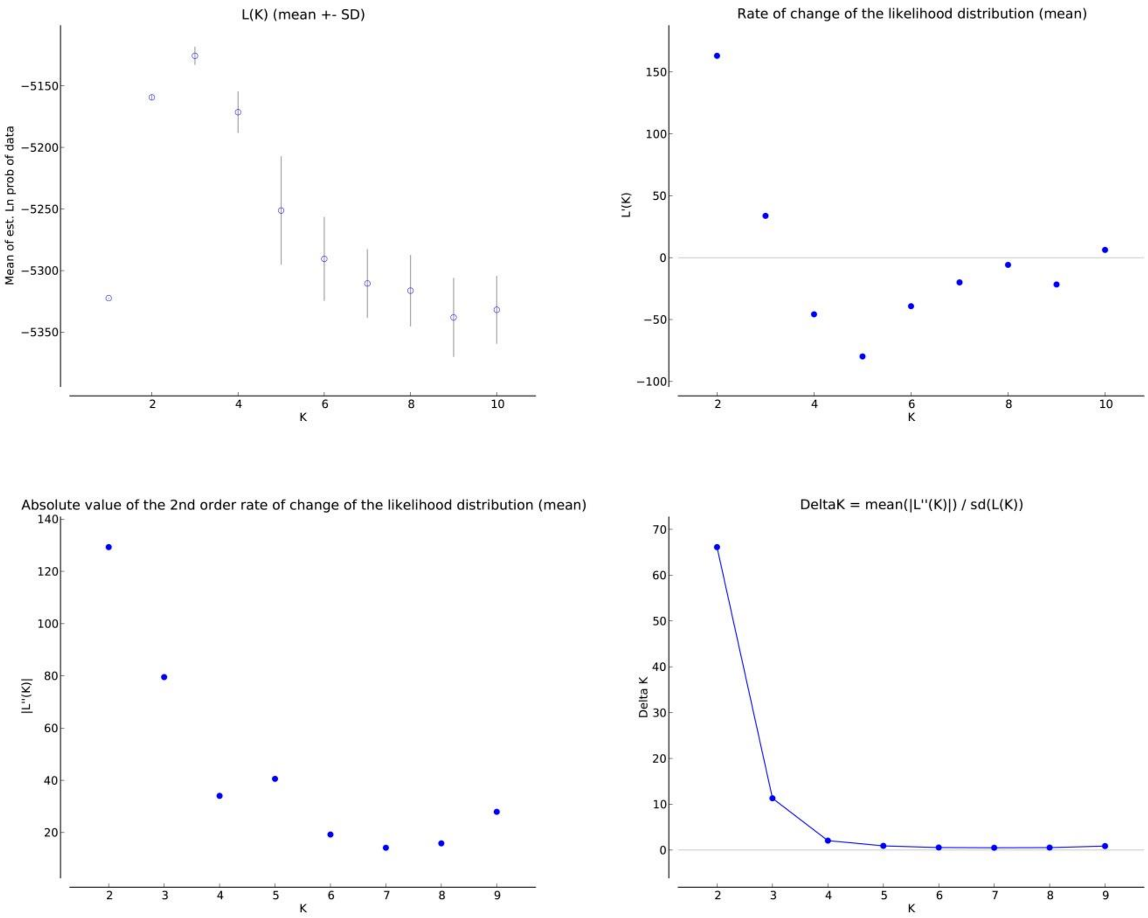

Fig. S3: Assessment of the most likely number of clusters of STRUCTURE runs using the method of Evanno *et al.* (2005) for *Aeonium nobile*. Shown are means of (top left) the log probability of the data mean L(K), (top right) the rate of change of the likelihood distribution mean L'(K), (bottom left) the absolute values of the second order rate of change L''(K) and (bottom right) ΔK, over 20 runs for each K value, respectively. Plots created with STRUCTURE HARVESTER (Earl and vonHoldt 2012).

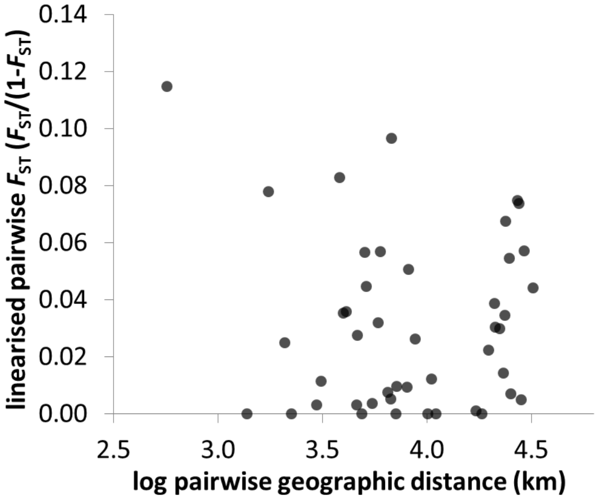

Fig. S4: Correlation of pair-wise geographic distance and pair-wise linearised F_{ST} between populations of *Aeonium nobile*: Mantel's $R = -0.071$, $P = 0.397$.

Table S3: Combined results of outlier loci detection methods for *Aeonium nobile*. Locus names and overall frequencies of dominant fragments are depicted in the first (left) panel. The second panel depicts results of the F_{ST}-based methods (only indications for divergent selection shown): BAYESCAN (Posterior probabilities, *PP*) and MCHEZA (F_{ST}- and *P*-values). The third and fourth panel show results of the two correlative methods LFMM (for $K = 1$) and SAMBADA, respectively. Outlier loci candidates are highlighted by colouring in the respective columns/panels. Significance of correlations between allelic variation and environmental variables by LFMM and SAMBADA is colour-coded as follows: yellow: $P < 0.05$, orange: $P < 0.01$, red: $P < 0.005$, dark red: $P < 0.001$.

locus	overall frequency	BAYESCAN *PP*	MCHEZA F_{ST}	MCHEZA *P*(simul. F_{ST} < sample F_{ST})	mean annual precipitation	mean precipitation JUN - AUG	mean precipitation DEC - FEB	mean precipitation MAY - OCT	mean precipitation NOV - APR	mean precipitation seasonality	solar radiation	mean annual temperature	mean annual precipitation	mean precipitation JUN - AUG	mean precipitation DEC - FEB	mean precipitation MAY - OCT	mean precipitation NOV - APR	mean precipitation seasonality	solar radiation	mean annual temperature
UBC840_404	0.09	0.48	0.00	0.53	0.979	0.934	0.996	0.701	0.887	0.538	0.491	0.865	0.950	0.858	0.690	0.515	0.814	0.429	0.005	0.425
UBC840_406	0.16	0.49	0.04	0.68	0.584	0.869	0.796	0.973	0.849	0.847	0.336	0.506	0.121	0.494	0.548	0.703	0.405	0.559	0.008	0.029
UBC840_721	0.07	0.47	0.04	0.88	0.328	0.731	0.633	0.648	0.585	0.762	0.834	0.427	0.004	0.319	0.038	0.111	0.036	0.210	0.439	0.022
UBC848_848	0.11	0.50	0.07	0.85	0.304	0.622	0.420	0.375	0.346	0.629	0.709	0.267	0.086	0.753	0.006	0.009	0.007	0.331	0.314	0.051
UBC881_487	0.07	0.47	0.00	0.64	0.652	0.410	0.925	0.947	0.923	0.743	0.334	0.661	0.336	0.082	0.788	0.993	0.925	0.250	0.002	0.384
UBC881_935	0.14	0.47	0.00	0.53	0.832	0.495	0.607	0.548	0.596	0.828	0.132	0.780	0.761	0.246	0.322	0.355	0.415	0.568	0.007	0.700
UBC888_790	0.05	0.48	0.05	0.95	0.223	0.861	0.217	0.173	0.130	0.987	0.559	0.120	0.026	0.492	0.009	0.014	0.009	0.910	0.690	0.010
UBC889_594	0.07	0.47	0.00	0.51	0.778	0.891	0.850	0.587	0.736	0.411	0.300	0.941	0.642	0.953	0.532	0.456	0.629	0.283	0.002	0.769
UBC807_379	0.82	0.62	0.22	0.96	0.196	0.542	0.532	0.338	0.320	0.131	0.528	0.239	0.009	0.056	0.033	0.075	0.047	0.016	0.218	0.021
UBC807_615	0.11	0.49	0.16	0.96	0.995	0.195	0.862	0.748	0.664	0.969	0.565	0.859	0.780	0.005	0.319	0.699	0.381	0.208	0.025	0.932
UBC807_654	0.52	0.70	0.21	0.95	0.960	0.920	0.923	0.849	0.911	0.695	0.921	0.962	0.891	0.607	0.879	0.953	0.949	0.963	0.765	0.902
UBC881_467	0.16	0.60	0.20	0.97	0.804	0.823	0.974	0.829	0.842	0.522	0.851	0.710	0.454	0.212	0.791	0.923	0.730	0.428	0.937	0.391
UBC881_476	0.07	0.50	0.12	0.97	0.298	0.543	0.630	0.595	0.551	0.200	0.417	0.276	0.006	0.077	0.076	0.149	0.054	NA	0.093	NA
UBC881_581	0.05	0.48	0.16	1.00	0.898	0.810	0.956	0.808	0.897	0.692	0.512	0.901	0.731	0.397	0.855	0.709	0.859	0.764	0.043	0.878
UBC888_470	0.39	0.51	0.20	0.95	0.855	0.670	0.978	0.858	0.800	0.677	0.560	0.673	0.580	0.082	0.701	0.959	0.643	0.937	0.183	0.307
UBC807_342	0.09	0.51	0.20	0.98	0.118	0.653	0.110	0.077	0.049	0.980	0.276	0.041	0.007	0.910	0.004	0.006	0.004	0.583	0.010	0.003
UBC809_493	0.14	0.51	0.15	0.94	0.118	0.552	0.096	0.070	0.042	0.616	0.439	0.083	0.115	0.544	0.036	0.059	0.036	0.551	0.356	0.047
UBC809_562	0.34	0.56	0.23	0.96	0.213	0.053	0.983	0.774	0.949	0.080	0.670	0.834	0.196	0.001	0.830	0.779	0.835	0.003	0.750	0.782
UBC881_320	0.16	0.50	0.13	0.91	0.696	0.042	0.889	0.906	0.966	0.919	0.535	0.805	0.426	0.003	0.857	0.627	0.843	0.643	0.165	0.563
UBC885_524	0.23	0.50	0.08	0.76	0.131	0.800	0.091	0.044	0.034	0.525	0.994	0.134	0.075	0.585	0.020	0.031	0.023	0.524	0.674	0.081
UBC885_537	0.14	0.50	0.08	0.84	0.844	0.992	0.982	0.783	0.861	0.313	0.907	0.957	0.597	0.627	0.713	0.785	0.823	0.050	0.112	0.900
UBC885_859	0.34	0.60	0.26	0.98	0.031	0.426	0.151	0.062	0.070	0.020	0.890	0.077	0.016	0.428	0.007	0.014	0.012	0.003	0.784	0.042
UBC888_485	0.48	0.75	0.32	1.00	0.663	0.623	0.612	0.372	0.378	0.507	0.918	0.626	0.125	0.027	0.004	0.015	0.007	0.403	0.172	0.146
UBT4_271	0.07	0.48	0.00	0.64	0.804	0.911	0.960	0.700	0.806	0.304	0.744	0.990	0.463	0.789	0.534	0.487	0.646	0.038	0.115	0.889
UBT4_321	0.09	0.49	0.03	0.84	0.962	0.978	0.947	0.878	0.970	0.364	0.839	0.864	0.915	0.805	0.917	0.958	0.928	0.112	0.281	0.498
UBT4_674	0.16	0.50	0.06	0.78	0.501	0.979	0.682	0.446	0.458	0.243	0.953	0.537	0.054	0.720	0.093	0.109	0.104	0.023	0.660	0.127

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Holocene re-colonisation, central-marginal-distribution and habitat specialisation shape population genetic patterns within an Atlantic European grass species

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Abstract

Corynephorus canescens (L.) P.Beauv. is an outbreeding, short-lived and wind-dispersed grass species, highly specialised on scattered and disturbance-dependent habitats of open sandy sites. Its distribution ranges from the Iberian Peninsula over Atlantic regions of Western and Central Europe, but excludes the two other classical European glacial refuge regions on the Appenine and Balkan Peninsulas.

To investigate genetic patterns of this uncommon combination of ecological and biogeographical species characteristics, we analysed AFLP variation among 49 populations throughout the European distribution range, expecting i) patterns of SW-European glacial refugia and post-glacial expansion to the NE, ii) decreasing genetic diversity from central to marginal populations, and iii) interacting effects of high gene flow and disturbance-driven genetic drift.

Decreasing genetic diversity from SW to NE and distinct gene pool clustering imply refugia on the Iberian Peninsula and in western France from where range expansion originated towards the NE. High genetic diversity within and moderate genetic differentiation among populations, and a significant pattern of isolation-by-distance indicate a gene flow-drift-equilibrium within *C. canescens*, probably due to its restriction to scattered and dynamic habitats and limited dispersal distances. These features as well as the re-colonisation history were found to affect genetic diversity gradients from central to marginal populations.

Our study emphasizes the need for including the specific ecology into analyses of species (re-)colonisation histories and range centre-margin analyses. To account for discontinuous distributions, new indices of marginality were tested for their suitability for studies of centre-periphery gradients.

Keywords

Abundance-centre hypothesis, DAPC, degree of peripherality, metapopulation dynamics, phylogeography, Poaceae, psammophyte pioneer species

Introduction

Past and current species distribution ranges and their genetic consequences in Europe are strongly shaped by the Pleistocene climate oscillations and re-colonisation processes from the last glacial refugia (Hewitt, 1999, 2000; Taberlet *et al.*, 1998). Repeated climatic oscillations alternately led to distribution range contractions during glacials, when most temperate species were forced into one or more southern refugia for thousands of years, and to (re-)colonisations during interstadials, interglacials or the Postglacial (Cheddadi *et al.*, 2005; Hewitt, 2000, 2004). Refugial regions for European species predominantly existed on the Iberian, Appenine and Balkan peninsulas, but also in areas of Southeast Europe and in Northern Turkey (Comes & Kadereit, 1998; Hewitt, 1999, 2004; Taberlet *et al.*, 1998). Additionally, more recent evidence from phylogeographic and palynological data suggest that also some smaller refugia beyond these regions existed, allowing for some species to persist in more northern locations (Bhagwat & Willis, 2008; Kelly *et al.*, 2010; Magri *et al.*, 2006; Stewart *et al.*, 2010).

When a species survived in several isolated refugia, this vicariance often caused isolated intraspecific microevolution and led to genetic differentiation between refugia (*e.g.* Hewitt, 1996; Magri *et al.*, 2006; Naydenov *et al.*, 2007). Thus, subsequent re-colonisations can result in intraspecific hybrid zones in regions of secondary contact, with significant genetic mixture of individuals and admixture within individuals (*e.g.* Hewitt, 2004; Petit *et al.*, 2003; Taberlet *et al.*, 1998). Additionally, along routes of range expansion, genetic diversity is in general expected to decrease from refugial areas to the colonisation front due to sequential founder events of leading edge populations (Comps *et al.*, 2001; Hewitt, 1996; Ibrahim *et al.*, 1996).

A number of theoretical and empirical studies have shown that from the centre to the margin of a species range decreasing environmental suitability can lead to reduced individual fitness and population density (*e.g.* Brown, 1984; Hengeveld & Haeck, 1982; Lawton, 1993; Vucetich & Waite, 2003 and references therein). In turn, this can decrease gene flow, increase effects of genetic drift and thus limit the accumulation of genetic diversity but enhance genetic differentiation (Eckert *et al.*, 2008; Holt *et al.*, 2005; Lira-Noriega & Manthey, 2014; Sexton *et al.*, 2009). Additionally, environmental and demographic stochasticity increase local extinction and re-colonisation (Eckert *et al.*, 2008; Gerst *et al.*, 2011). Potential founder effects should therefore be more recent in marginal populations. As a consequence, genetic variation within populations is expected to decrease towards the range periphery (Eckert *et al.*, 2008). However, assuming dynamic species ranges, leading edge areas can be assumed to have been colonised later than persistent rear edge areas (Hampe & Petit, 2005). The latter likely have acted as re-colonisation sources and their current peripheral status is in Europe typically the result of past unidirectional northward rather than centrifugal range expansion. Such differences between range periphery regions due to the biogeographic history are rarely considered in existing central-peripheral analyses. Additionally, often only broad categories of "central" and "peripheral" are used rather than assessing explicitly the degree of marginality.

Here, we analyse intraspecific genetic patterns of grey hair-grass, *Corynephorus canescens* (L.) P.Beauv. (Poaceae), a disturbance-dependent habitat specialist. This species is widespread throughout large parts of western and Central Europe, being an element of the Atlantic floristic region with additional areas in boreal and sub-continental Central European regions. Among the potential Mediterranean refugial regions only the Iberian Peninsula is currently populated (Fig. 1), making *C. canescens* a model for post-glacial biogeographical history of Atlantic floristic elements. The distribution is discontinuous, including large exclaves and outposts, resulting in large proportions of marginal areas. Additionally, the distribution of *C. canescens* is scattered, due to its strong association with habitats of dry, acidic and nutrient-poor open sandy soils (Jentsch & Beyschlag, 2003; Quinger & Meyer, 1995; Rychnovská, 1963). It depends on substrate dynamics and frequent soil disturbance, *e.g.* on open sand dunes and drought- and animal-driven vegetation gaps or anthropogenic sand mines and military training areas (Jentsch *et al.*, 2002; Jentsch *et al.*, 2009; Marshall, 1965; Tschöpe & Tielbörger, 2010). When disturbance ceases such habitats are prone to succession and concomitant soil formation which suppresses the poorly competitive *C. canescens* (Ellenberg & Leuschner, 2010; Jentsch & Beyschlag, 2003; Ujházy *et al.*, 2011). Thus, the existence of populations is often temporally limited. This fact, together with its limited dispersal capacity (Böger, 2007), potentially promotes genetic drift and population differentiation. In contrast, reproduction is by seeds resulting from wind-pollination and predominant outcrossing (Böger, 2007), potentially allowing for high gene flow within and between populations.

We focus on the following three hypotheses: i) *C. canescens* survived the last glacial maximum in refugia in south-western Europe from which it re-colonised central and Eastern Europe to its recent distribution. This would be reflected in differentiated gene pools, private alleles in potential refugial areas and decreasing genetic diversity from SW to NE. ii) marginal NE populations in areas of re-colonisation underwent founder events and genetic drift and thus are genetically less diverse. iii) as an outcrossing, wind-pollinated but short-lived and disturbance-dependent species, both gene flow and genetic drift can play a significant role in structuring genetic variation among populations. We therefore expect high genetic variation within populations, moderate population differentiation and a pattern of isolation-by-distance (IBD).

Methods

Study species

Corynephorus canescens is a tussock-building pioneer grass species, with life span of two to six years (Böger, 2007; Marshall, 1967). It is diploid (2n = 14; Albers, 1980), wind-pollinated and predominantly outcrossing (Böger, 2007). Reproduction is almost exclusively by seed (Marshall, 1967), although clonal growth has occasionally been reported (Frey & Hensen, 1995; Schulz, 1950). Although seed production is high, seed viability and dispersal capacity of *C. canescens* are limited, as typical diaspore dispersal distances lie within only a few meters (Böger, 2007; Marshall, 1968). The habitat of *C. canescens* has been widespread during the last centuries, because forest logging and livestock grazing maintained dry acidic grasslands on coastal and inland dunes (Jentsch & Beyschlag, 2003; Quinger & Meyer, 1995). However, land use change, in particular afforestation, land consumption, and atmospheric nitrogen deposition have led to habitat loss and fragmentation (Jentsch & Beyschlag, 2003; Quinger & Meyer, 1995; Riecken *et al.*, 2006).

Plant sampling and DNA extraction

Forty-nine populations were sampled for the current study, covering most parts of the distribution range of *C. canescens* (Fig. 1, Tab. 1, Tab. S1). In each population eight vital and healthy tussocks were arbitrarily chosen, resulting in 384 sampled individuals in total. Leaf samples were dried and stored in silica gel and genomic DNA was extracted applying the *DNeasy® 96 Plant Kit* following the protocol of the manufacturer (QIAGEN, 2002).

Amplified fragment length polymorphism (AFLP) analyses and genotyping

We used Amplified Fragment Length Polymorphisms (AFLP; Vos *et al.*, 1995) following the protocol of Kloss *et al.* (2011). Three primer combinations were used: $\mathrm{EcoRI}_{\mathrm{FAM}}$-ACT – CTA-MseI, $\mathrm{EcoRI}_{\mathrm{JOE}}$-AGG – CAG-MseI, $\mathrm{EcoRI}_{\mathrm{TAMRA}}$-ACC – CAG-MseI. Genotyping was done with *GeneMapper* software (Applied Biosystems) by manual binning to produce preliminary peak height matrices. These were processed manually, specifying a threshold for minimum individual peak height based on the individual frequency distributions of peak heights, as well as thresholds for minimum peak number and minimum mean peak height for individual samples. Loci and samples that did not reach the respective thresholds were stringently discarded, resulting in a final binary presence/absence matrix of 326 samples and 137 distinct and polymorphic AFLP loci.

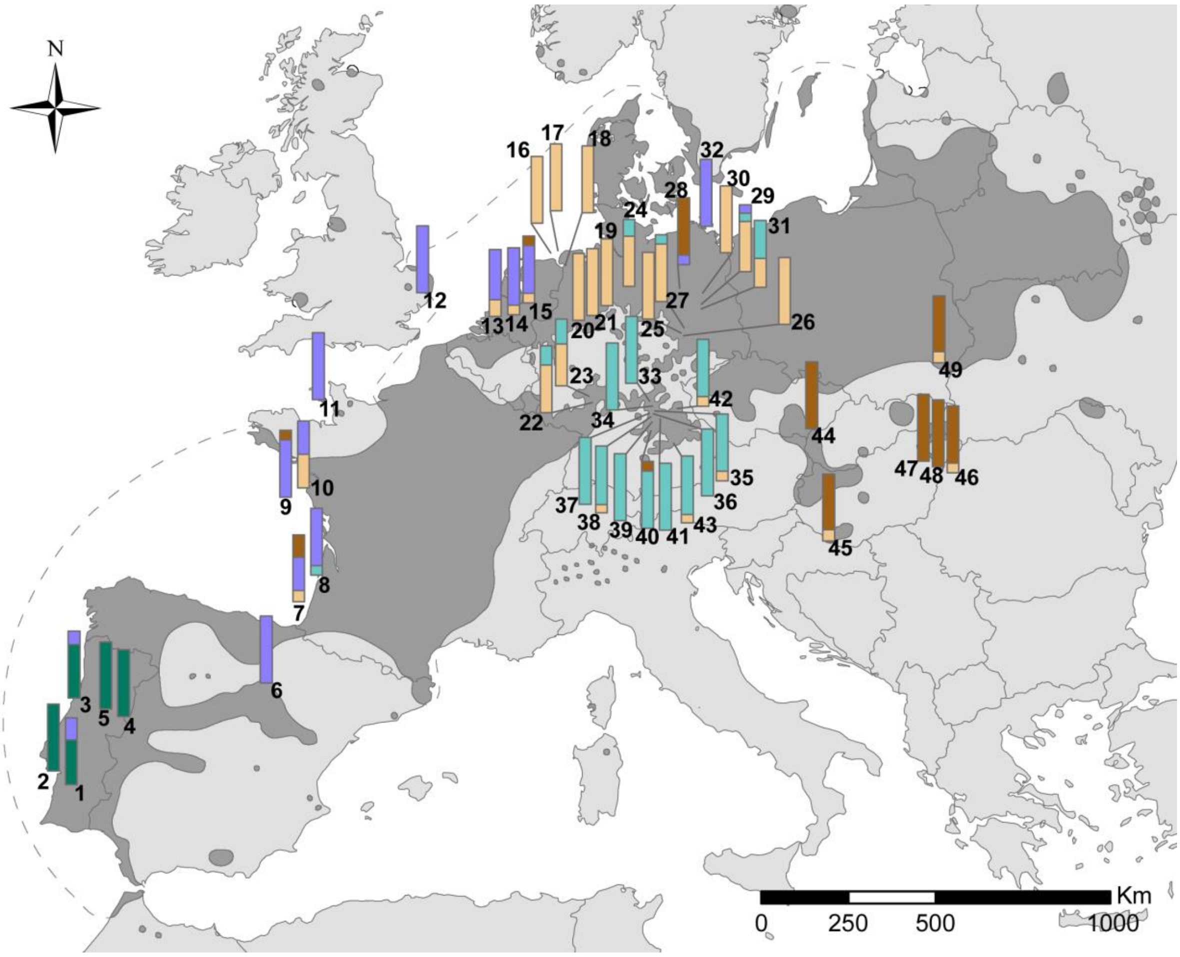

Fig. 1. Distribution range of *C. canescens* across Europe (dark grey shading) and sampled populations. Bars represent the portions of individual assignments to the five clusters found in the *k*-means cluster analysis implemented in the DAPC. Colours of the clusters correspond with colouring of points in the DAPC ordination plots (Fig. 2) and with the other cluster analyses (Fig. S2-S4). Dashed lines represent the assumed climatic borders off the coast following Hegi & Conert (1998).

Data analysis

To analyse gene pool differentiation we applied three methods of non-hierarchical genetic cluster analysis. First, we used the Bayesian clustering approach implemented in *Structure* v. 2.3.3 (Falush *et al.*, 2003, 2007; Pritchard *et al.*, 2000), which accounts for admixture of gene pools within individuals and dominant markers. Accordingly, the admixture model with correlated allele frequencies was calculated with *K* ranging from 1 to 20. For each *K* twenty runs were performed with 100000 replicates after a burn-in period of 100000 runs. The outputs were processed and analysed using *Structure Harvester* (Earl & vonHoldt, 2012) implementing the method of Evanno *et al.* (2005). The results were averaged for a particular *K* using *Clumpp* (Jakobsson & Rosenberg, 2007) and visualised using *Distruct* (Rosenberg, 2004).

Second, we conducted a Discriminant Analysis of Principal Components (DAPC; Jombart *et al.*, 2010) implemented in the *R*-package *adegenet* (v. 1.3-5; Jombart, 2008) which allows to assess the genetic relationships among gene pools. DAPC uses a *k*-means clustering approach to identify individual assignments to genetic clusters which are then analysed in a two-step way. First the genetic data is transformed and summarised in a principal component analysis (PCA), and then a discriminant analysis (DA) uses the PCA-variables to maximise group differentiation based on *k*-means clustering. Cluster numbers from 1 to 50 were tested with the *k*-means analysis and the most likely number of clusters was inferred by comparing the Bayesian Information Criterion. The optimal number of principal components (n = 13) was detected using the *a*-score optimisation (5000 iterations) to avoid over-fitted discrimination of clusters.

Third, we applied a Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in *Geneland* v. 4.0.3 (Guillot *et al.*, 2005a; Guillot *et al.*, 2005b; Guillot *et al.*, 2012) which takes also the spatial origin into account to assign individuals to different genetic clusters. Twenty independent MCMC runs were made using 10 million iterations with a thinning of 1000 to save 10000 iterations under the spatial model with uncorrelated allele frequencies among populations. The number of genetic clusters was set to unknown but the maximal possible number of clusters was limited to twelve to offer a large-enough search space for the MCMC algorithm. As no individual spatial data were available we set an uncertainty level of 50 m to account for realistic extents of sampling sites and to allow for individual mixture within populations. MCMC post-processing was done with a burn-in of 2000 iterations and the average posterior probability was used to select the best suited run.

Genetic diversity was assessed as band richness (*Br*) and the percentage of polymorphic loci without alleles of frequencies lower than 5 % ($PLP_{5\%}$) using a rarefaction method implied in *AFLPdiv* v. 1.1 (Coart *et al.*, 2005) to account for different sample sizes. Analyses of Nei's gene diversity (H_j) and global and pairwise differentiation indices (F_{ST}) were performed with *AFLP-surv* v. 1.0 (Vekemans, 2002; Vekemans *et al.*, 2002), using the Bayesian method with non-uniform prior allele frequencies distribution and assuming Hardy-Weinberg equilibrium ($F_{IS} = 0$) to account for negligible selfing in *C. canescens* (Böger, 2007). Significance of the global F_{ST} was tested with 10000 permutations. Finally, numbers of private bands were determined with *GenAlEx* v. 6.4 (Peakall & Smouse, 2006). To test for isolation-by-distance patterns a Mantel test was performed between matrices of geographic distance and linearised F_{ST} (Rousset, 1997) using *GenAlEx* v. 6.4 (Peakall & Smouse, 2006) with 9999 permutations. The same software served for an analysis of molecular variance (AMOVA), which was done based on Φ-statistics, an Euclidian distance measure (Huff *et al.*, 1993) and 9999 permutations.

We tested whether genetic variation is related to geographic location, marginality and continentality. For the analysis of marginality we digitized the distribution range of *C. canescens* from Hegi & Conert (1998) and FloraWeb (Bundesamt für Naturschutz, 2006) and displayed it with *ArcInfo* v. 10.0 (ESRI Inc.). The placement of distribution borders is not trivial for *C. canescens* as the current range extends to the Atlantic coastlines. However, recent range maps place the distribution borders off the coast following assumed bioclimatic limitations (Hegi & Conert, 1998; Marshall, 1968, see Fig. 1). For the calculations of marginality we therefore took these sea areas into account. The discontinuous distribution range of *C. canescens* precluded the use of centre-based centrality-marginality indices, *e.g.* distance from an assumed centre approximated by average latitude/longitude-coordinates (Dixon *et al.*, 2013). Instead, we calculated three new indices of the marginality of populations within the species' distribution range.

First, we calculated Areal Marginality (M_r) as the percentage area outside the distribution range of circular buffers constructed around each location:

$$M_r = \frac{A_{out}}{\pi r^2} \cdot 100$$

where A_{out} is the proportion of area outside the distribution range, and r is the buffer radius. We used three radii (50, 100 and 200 km) resulting in three measures of Areal Marginality (M_{r50}, M_{r100}, M_{r200}).

Second, we calculated Minimum Distance Marginality (M_{min}) as the minimum log distance of a location to the range boundary. Normalised values (0 – 1) were obtained by dividing absolute distances by the maximum possible minimum distance within the distribution range:

$$M_{min} = 1 - \frac{\log d_{min}}{\log d_{maxmin}}$$

with d_{min} being the minimum distance of a location to the distribution range boundary, and d_{maxmin} being the maximum possible distance within the distribution range to the nearest boundary (352 km, referring to a location in northern Poland: 4856047 E, 3395747 N in ETRS89).

Third, we calculated Average Distance Marginality (M_{av}) as the average log distance of a location to the range boundary in multiple geographic directions, normalised to the maximum possible average distance within the distribution range:

$$M_{av} = 1 - \log \frac{\sum_{i=1}^{n} d_{(i \cdot 360°/n)}}{n} / \log d_{maxav}$$

where n is the desired number of directions, d is the distance of the sampling point of interest to the distribution range boundary at a specified angle, and d_{maxav} is the maximum possible average distance of any given location within the distribution range (597 km, referring to location 4623018 E, 3398409 N in north-western Poland). We calculated M_{av} using $n = 16$ geographic directions, *i.e.* 22.5° increments starting from 0° (North).

The degree of continentality was calculated for every location following (Gorczyński, 1922) as

$$c = \frac{1.7 \cdot A}{\sin \theta} - 20.4$$

where A is the annual temperature range and θ the latitude, using temperature data (1950 – 2000) from WorldClim (Hijmans *et al.*, 2005). Calculations of M_r, M_{min} and c were done with *ArcInfo* v. 10.0 (ESRI Inc.), and the calculations of M_{av} were carried out with the statistical software environment *R* v. 2.15.2 (R Core Team, 2012) with packages *sp* (Pebesma *et al.*, 2013) and *rgeos* (Bivand *et al.*, 2013).

We also used *R* to test for differences in genetic diversity between gene pools performing an analysis of variance (ANOVA) and Tukey's post hoc test for pairwise group differences, as well as for all linear regression analyses throughout the study. Normal distribution of residuals was tested and model fits of regression analyses were verified visually in each analysis. To test the marginality indices for their consistency a correlation matrix (*R*-package *fAssets*, Würtz, 2012) was analysed. Additionally, regression models with genetic diversity (*Br*) were calculated and compared.

Results

Large scale differentiation patterns

The three types of cluster analyses yielded largely congruent results indicating the existence of four or five gene pools. The Bayesian *Structure* analysis revealed $K = 2$ gene pools (Fig. S1) with all populations of Central Europe, except two populations from NE Germany (#28, #32), forming one cluster and all others the second one (Fig. S2). However, local maxima of L'(*K*) and Δ*K* (Fig. S1) suggested the presence of four gene pools ($K = 4$) which had high prevalence in Iberian populations, in Southern Germany, in Northern Germany and in Eastern Europe, respectively (Fig. S3). A high level of individual admixture was detected, especially in populations from the Atlantic coast and some northern German populations, where no distinct assignment to a regional cluster was obvious (Fig. S2, S3).

The *k*-means analysis resulted in five clusters which best described genetic structure of *C. canescens* (Fig. 1). The clusters resemble those inferred by *Structure*, however, an additional cluster was identified along the Atlantic coast. In the DAPC analysis the thirteen retained PCs accounted for 37.4 % conserved variation out of the AFLP dataset. A clear association between individuals of the Northern German and the Southern German populations was obvious in the scatterplots, as well as between individuals from Eastern European and Atlantic coast populations (Fig. 2). The individuals of the Portuguese cluster were the most differentiated with only slight association to the Atlantic coast individuals.

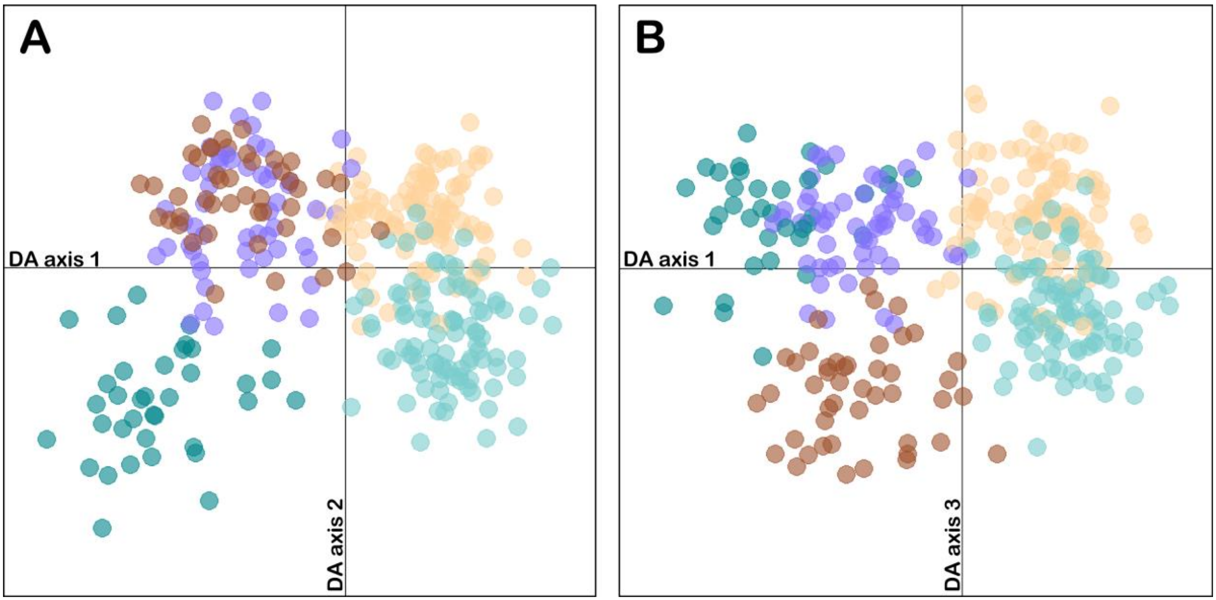

Fig. 2. Ordination plots from the Discriminant Analysis of Principal Components (DAPC) corresponding to Fig. 1. The first two (A) and the first and third (B) axes of discriminant functions are shown. Each point represents one individual distributed along the two linear discriminants, respectively. 13 principal components were retained to get adequate discrimination of clusters, based on *a*-score optimisation.

The *Geneland* analyses revealed mean posterior probabilities for the 20 runs from $P = -27113$ to $P = -28078$. In the most likely solution, the posterior density distribution indicated five clusters (Fig. S4) which resembled the results of the *Structure* and *k*-means approaches. Again, the Portuguese populations formed cluster IP – Iberian Peninsula, the populations along the Atlantic coast region plus the population from Spain made up cluster CA – Coastal Areas, the northern German (cluster NG) and southern German (cluster SG) populations were clearly separated and finally the eastern populations formed cluster EE – Eastern Europe. In contrast to previous analyses, populations 28 and 32 from Northern Germany were included in the CA cluster.

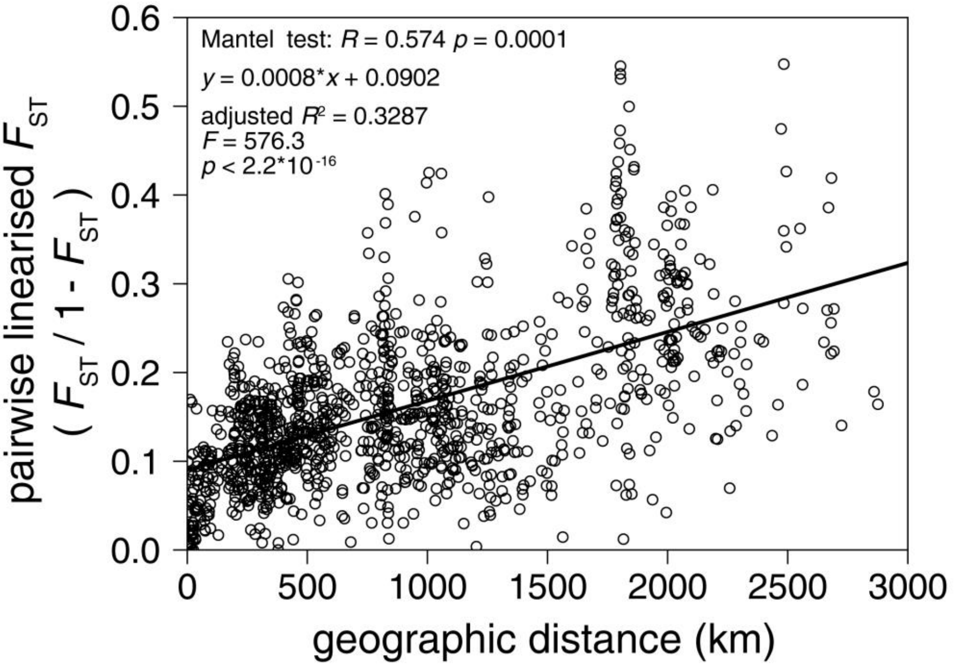

Fig. 3. Correlation of pairwise geographic distance and pairwise linearised F_{ST} between populations.

Genetic differentiation was moderate with $F_{ST} = 0.136$ ($p < 0.0001$) among populations and $F_{ST} = 0.105$ ($p < 0.0001$) among the five gene pools revealed in the *Geneland* analysis. Population differentiation among populations was highest within cluster IP, intermediate in NG and EE, and lowest in CA and SG (Tab. 1). Hierarchical partitioning of molecular variance (AMOVA) revealed the highest proportion of variance within populations (75.2%, $p < 0.0001$) and lower proportions among populations (11.8%, $p < 0.0001$) or among gene pools (13.0%, $p = < 0.0001$; see Tab. S2). Pairwise genetic differentiation was significantly correlated to geographic distance as revealed in the Mantel test for all sites ($R = 0.574$, $p = 0.0001$; Fig. 3), demonstrating a large scale isolation-by-distance pattern among *C. canescens* populations.

Genetic variation and marginality

Measures of genetic diversity on the population level are given in Tab. S1. Nei's gene diversity (H_j) ranged from 0.182 (population #47, Martinka, Hungary) to 0.305 (#40, Mandlesmühle, southern Germany). However, even though the two highest values of H_j were found in southern German populations, the majority of higher values were found in populations of the western parts of the sampling range. A similar geographic trend was observed for Band richness (*Br*), which ranged from 1.234 (#47, Martinka, Hungary) to 1.470 (#7, Contis Plage, France) and was negatively correlated both to longitude and latitude (Fig. 4). Likewise, percentage of polymorphic loci was lowest in a Hungarian population (#47, Martinka: $PLP_{5\%} = 0.307$) and highest in a French population (#10, Ile de Normoutier: 0.620). The only populations with private alleles were Mira (#3) and Figueira da Foz (#4) in Portugal.

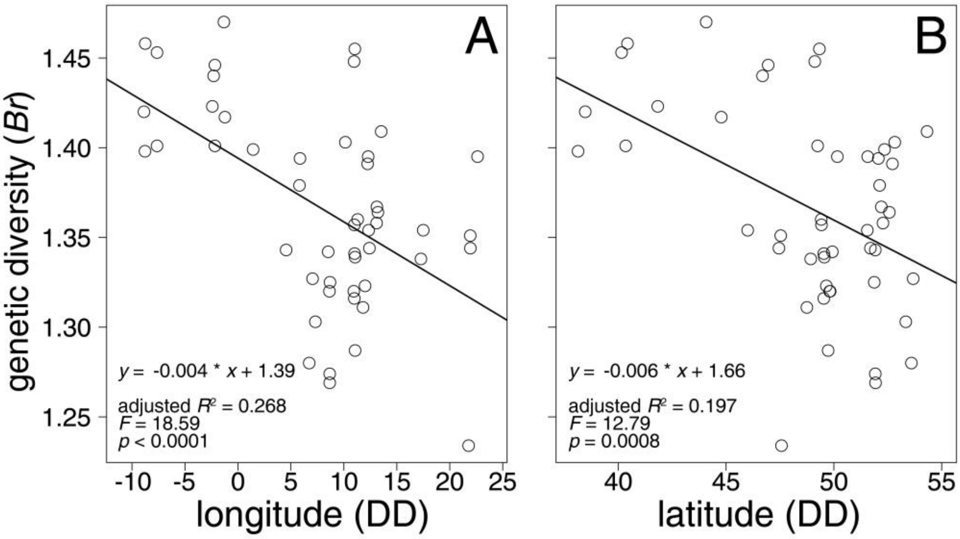

Fig. 4. Relationship between genetic diversity of populations measured as band richness (*Br*) and their (A) longitudinal and (B) latitudinal coordinates shown in decimal degrees (DD).

When populations were grouped by clusters, genetic diversity was significantly higher in the western gene pools (IP and CA) than in the more eastern ones (NG, SG and EE), which was more obvious for *Br* than for H_j and not true for $PLP_{5\%}$ (Tab. 1).

Table 1. List of inferred gene pools from *Geneland* results (according to Fig. S4) with mean values ± standard deviation of descriptive parameters over assigned populations and population differentiation (F_{ST}) within gene pools, respectively. Population numbers are according to Tab. S1 and Fig. 1. H_j = Nei's gene diversity; *Br* = Band richness, $PLP_{5\%}$ = Proportion of polymorphic loci without alleles of frequencies lower than 5 %; *Br* and $PLP_{5\%}$ were standardised for different sample sizes of populations by rarefaction to n = 4. Equal lower case letters behind mean values for H_j and *Br* indicate homogeneous groups as detected by Tukey's post hoc test subsequent to significant results of an ANOVA (H_j: $F = 6.544$, $p = 0.0003$, *Br*: $F = 8.214$, $p = 0.0005$) based on gene pools as grouping factors. ANOVA on $PLP_{5\%}$ revealed no significant differences.

Inferred gene pool	Abbreviation	Populations assigned (No.)	H_j	Br(4)	$PLP_{5\%}$ (4)	No. private alleles	Marginality M_{min}	F_{ST} within gene pool	*p*-value of F_{ST}
Iberian Peninsula	IP	1 – 5	0.278 **a** ± 0.017	1.426 **a** ± 0.028	0.480 ± 0.058	0.4 ± 0.5	0.14 ± 0.04	0.1128	< 0.0001
Coastal Areas	CA	6 – 15, 28, 32	0.279 **a** ± 0.012	1.409 **a** ± 0.033	0.486 ± 0.077	0 ± 0	0.33 ± 0.23	0.0693	< 0.0001
Northern Germany	NG	16 – 21, 24 – 27, 29 – 31	0.241 **b** ± 0.022	1.336 **b** ± 0.044	0.453 ± 0.054	0 ± 0	0.33 ± 0.19	0.0808	< 0.0001
Southern Germany	SG	22 – 23, 33 – 43	0.249 **ab** ± 0.028	1.348 **b** ± 0.050	0.456 ± 0.068	0 ± 0	0.55 ± 0.06	0.0644	< 0.0001
Eastern Europe	EE	44 – 49	0.240 **ab** ± 0.032	1.336 **b** ± 0.054	0.424 ± 0.059	0 ± 0	0.65 ± 0.09	0.0745	< 0.0001

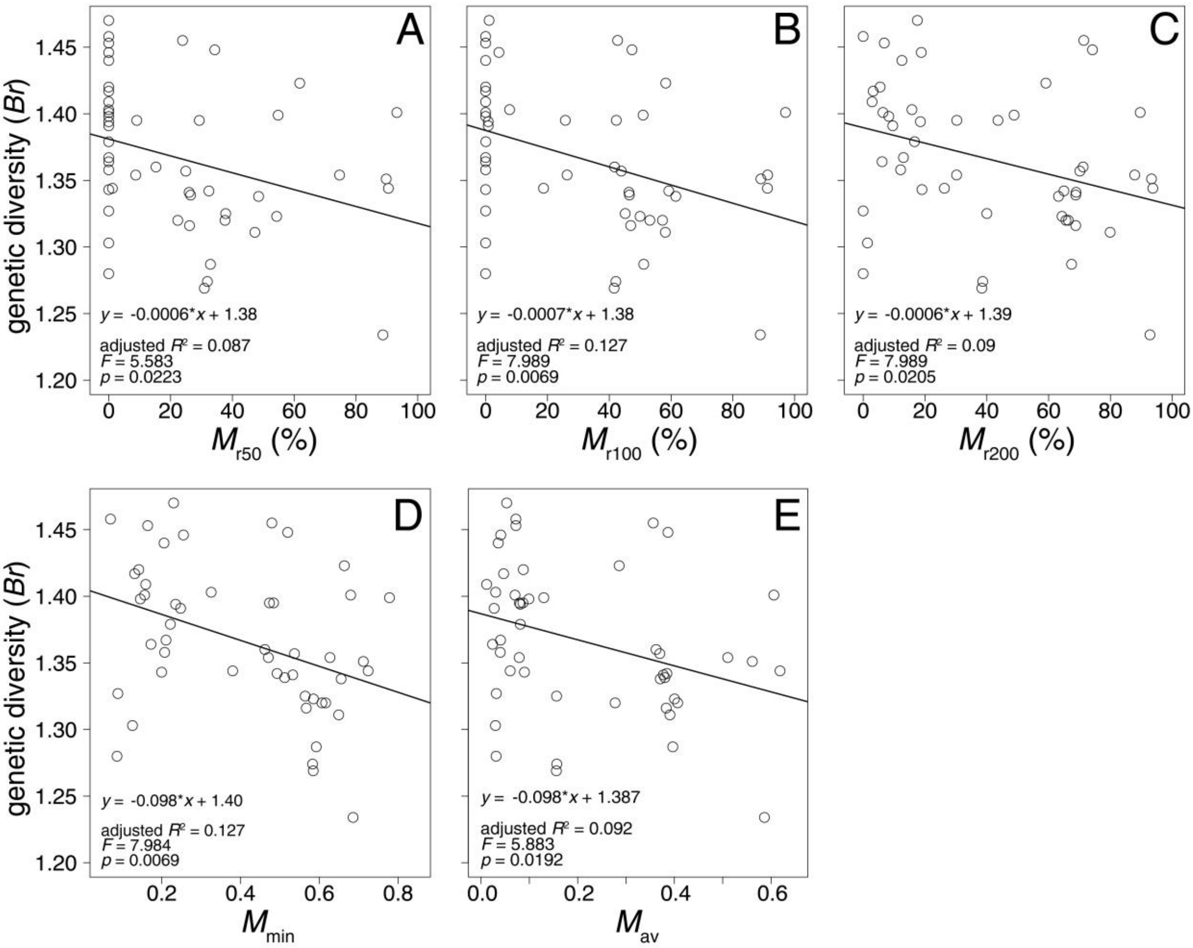

Fig. 5. Relationship between genetic diversity of populations measured as band richness (*Br*) and different marginality indices: Areal Marginality M_r for radii (A) 50 km, (B) 100 km, and (C) 200 km; (D) Minimum Distance Marginality M_{min}; (E) Average Distance Marginality M_{av}.

The indices of marginality were highly correlated to each other (Pearson's $r \geq 0.8$, $p < 0.001$, Fig. S5) and all of them were significantly negatively correlated to genetic diversity *Br* ($r \geq -0.33$, $p < 0.05$) with M_{r100} and M_{min} showing the highest correlations (both: $r = 0.381$ and $p < 0.01$). Genetic variation was not related to continentality (adj. $R^2 = -0.006$, $F = 0.74$, $p = 0.395$).

In simple linear regression analyses, genetic diversity *Br* was weakly related to marginality indices M_{r50} (adj. $R^2 = 0.087$, $p = 0.022$), M_{r200} (adj. $R^2 = 0.090$, $p = 0.021$) and M_{av} (adj. $R^2 = 0.092$, $p = 0.019$) and more strongly to M_{r100} (adj. $R^2 = 0.127$, $p = 0.0069$) and M_{min} (adj. $R^2 = 0.127$, $p = 0.0069$, see Fig. 5). A multiple linear regression including longitude, latitude and marginality M_{min} and their interactions as predictors of genetic diversity *Br* yielded a significant correlation (adj. $R^2 = 0.424$, $F = 6.898$, $p < 0.0001$) with all variables and interactions significant (Tab. S3). In particular, marginality (M_{min}) had a negative effect on genetic diversity after accounting for longitude and latitude. In similar multiple regressions analyses additionally including the other marginality indices, none of the latter were included in the respective final models after accounting for longitude and latitude (data not shown).

Discussion

Re-colonisation patterns

We found a moderate but clear genetic differentiation between gene pools of the Iberian Peninsula, French Atlantic Coast, Northern Germany, Southern Germany, and Eastern Europe. Genetic diversity was highest in populations on the Iberian Peninsula and along the Atlantic coast and showed a significant decrease from the SW to the NE of Europe. This genetic structure suggests that *C. canescens* persisted in SW Europe during the last glacial maximum and that re-colonisation took place from these refugia into eastern and north-eastern regions of Europe during Holocene climate warming, supporting our first hypothesis. Similar genetic structure and post-glacial re-colonisations from the Iberian Peninsula towards Central and Northern Europe were also suggested for other plant species with Atlantic distributions (*Festuca pratensis* Fjellheim *et al.*, 2006, *Corylus avellana* Palmé & Vendramin, 2002, *Hedera* sp. Grivet & Petit, 2002, *Ilex aquifolius* Rendell & Ennos, 2003, *Ceratocapnos claviculata* Voss *et al.*, 2012).

Three genetic patterns typically arise in such refugium-re-colonisation systems. First, diversity and population differentiation was highest in the putative refugial Iberian gene pool. This indicates accumulation of genetic population divergence among different micro-refugia over long periods of local persistence, which can be assumed for glacial refugia (Hewitt, 1996; Petit *et al.*, 2003; Schmitt, 2007). The Iberian Peninsula as a glacial refugium has been shown for other plant species as facilitative for local population differentiation (*e.g.* Hampe *et al.*, 2003; Jiménez-Mejías *et al.*, 2012). However, for *C. canescens* it is not clear whether the Iberian Peninsula represents the only refugium. The French Atlantic coast CA gene pool was clearly separated in some analyses, and showed similar or higher genetic diversity than the more southern IP gene pool. This potentially indicates that the French Atlantic coast served as refugium and as source for re-colonisation, similar to *Himantoglossum hircinum* (Pfeifer *et al.*, 2009). The presence and local spread of the CA gene pool during Holocene climate warming might have prevented a northward expansion of Iberian populations. Additionally, individuals of southern Germany were differentiated from all but the northern German gene pool and showed disproportionally high local genetic diversity. This may point to a discontinuous pattern of gene pool relatedness from the SW to the NE of Europe and contradicts the proposed unidirectional re-colonisation and thus might indicate an additional refugium in southern Germany. Palynological evidence from Central Europe suggests long-term persistence of herbaceous temperate species between northern and alpine ice-sheets throughout the last glaciation (*e.g.* Bos *et al.*, 2001; Müller *et al.*, 2003). However, definite inferences on the origin of the southern German gene pool cannot be made due to lacking information from southern and central France which also might have acted as source regions for central European populations.

Second, decreasing genetic diversity towards areas of re-colonisation is a common pattern of stepping stone-like or leptokurtic range expansions (Ibrahim *et al.*, 1996) characteristic for species of narrow habitat requirements (like *C. canescens*) and for species capable of long distance dispersal (Schmitt, 2007). Decreasing genetic diversity along colonisation routes was accordingly found in plant species like *Fagus sylvatica* (Comps *et al.*, 2001), *Fraxinus excelsior* (Heuertz *et al.*, 2004), *Corrigiola litoralis* (Durka, 1999) and *Ceratocapnos claviculata* (Voss *et al.*, 2012).

Third, the differentiation into regional gene pools found in *C. canescens* potentially reflects sequential founder effects and genetic drift along the re-colonisation routes (Hewitt, 1996; Ibrahim *et al.*, 1996). The range of *C. canescens* is structured into larger areas due to physical barriers (e.g. Pyrenean mountains) and geological conditions like the absences of suitable sandy habitats. Thus, gene flow will likely be restricted across such barriers, facilitating the build-up of regional gene pools. Potentially also mutations arising in the leading edge of advancing populations may contribute to the generation of new lineages during range expansions by effects of gene surfing, due to their preferential contribution in subsequently colonised areas (Excoffier *et al.*, 2009; Hallatschek & Nelson, 2008; Klopfstein *et al.*, 2005). In addition to neutral processes, different selection pressures in different biogeographic and phylogeographic areas are expected to lead to regional genetic adaptation which eventually will be detectable using molecular markers (Frei *et al.*, 2012). Although gene pools form rather coherent areas across the range of *C. canescens*, some populations, notably in Northern Germany (#28, #32) are assigned to geographically more distant clusters which might indicate possible long distance dispersal.

Marginality

Populations of *C. canescens* in marginal areas showed reduced genetic diversity (*Br*), generally confirming our hypothesis of genetic drift and founder effects in marginal populations. This result is in accordance with the large majority of other studied species (see Eckert *et al.*, 2008). However, although a consensus emerged that continuous measures of marginality are preferable to categorical definitions (Abeli *et al.*, 2014; Eckert *et al.*, 2008), various parameters have been used. In many studies, marginality is measured as distance to the range centre (*e.g.* Dixon *et al.*, 2013; Holliday *et al.*, 2012; Lira-Noriega & Manthey, 2014; Pfeifer *et al.*, 2009). However, for species with discontinuous and scattered ranges, like *C. canescens*, centrality-related measures may be misleading. We used three marginality indices taking into account distance to, or area outside, the range border. The marginality indices were highly correlated with each other and with genetic diversity, suggesting that they are useful for marginality analyses in discontinuous range distributions. Nevertheless, the different marginality measures may fit to specific cases depending on the shape and size of the distributional range. Distance to next range border (equivalent to M_{min}) has also been used in other studies (*e.g.* Hoban *et al.*, 2010; Schwartz *et al.*, 2003) and was preferred here due to its simple calculation without any assumptions. However, M_{min} may overestimate marginality when small unoccupied enclaves or narrow range gaps in the proximity of a study site represent unsuitable azonal or extrazonal environments rather than large scale, *i.e.* mainly climatic range limits. In such cases the unoccupied area in the vicinity of a site (M_r) may represent a suitable estimate of marginality for discontinuous distributions. However, there is no straightforward value of the buffer radius around a sampling site used to calculate M_r without prior analyses of specific gene flow distances. Lastly we employed the standardised mean distance to the range edges in multiple geographical directions (M_{av}) to avoid potential biases of the other marginality measures. It includes unbiased distances without any pre-assumptions and should account well for complex range shapes. Nevertheless, M_{av} showed a weaker association to genetic diversity than M_{min} and M_{r100}. This may be caused by a dominating effect of the size of the hinterland resulting in different M_{av} values for sites at the same distance to the edge.

Marginality, however, had only a moderate effect on genetic diversity in univariate analyses compared to a model including marginality, longitude, latitude and their interactions. This model accounts for both, marginality and the direction and distance of range expansion. Including historic, *e.g.* post-glacial colonisation patterns into studies of genetic diversity patterns along marginality gradients has been suggested previously (*e.g.* Eckert *et al.*, 2008; Guo, 2012; Hoban *et al.*, 2010; Pfeifer *et al.*, 2009). Thus, a more realistic model of genetic diversity along the core-periphery-gradient is obtained by integrating effects of large scale, long-term re-colonisation and of locally acting processes among spatially structured populations.

The proposed effect of continentality on genetic diversity within populations of *C. canescens* was not found. Probably, large scale colonisation histories and local availability of open sandy substrate override potential gradual effects of continentality on environmental suitability.

Gene flow and disturbance

We found moderate genetic differentiation and a significant pattern of isolation-by-distance among populations of *C. canescens*, which reflects a gene flow-drift-equilibrium. This is in contrast to other outcrossing grass species with more common and continuous habitat types, in which isolation-by-distance is weak (*e.g. Elymus athericus* Bockelmann *et al.*, 2003, *Arrhenaterum elatius* Michalski *et al.*, 2010) or lacking (*e.g. Festuca arundinacea* subsp. *arundinacea* Sharifi Tehrani *et al.*, 2009, *Alopecurus myosuroides* Menchari *et al.*, 2007; but see also Larson *et al.*, 2004). Gene flow is expected to be high within and among populations of *C. canescens* as it is wind-pollinated and outcrossing with non-specialised seed dispersal. However, its life history and habitat requirements make it susceptible to genetic drift. First, its populations are spatially isolated from each other due to scattered distribution of sandy habitat. This limits gene flow and facilitates differentiation between populations as has been found for other habitat specialist grasses in Central Europe (Durka *et al.*, 2013; Wagner *et al.*, 2011). Second, due to the dynamic character of its habitats, *C. canescens* may undergo metapopulation dynamics with disturbance-driven opening of sandy substrate sites, colonisation by founder propagules, followed by vegetation succession, competition-driven declines and population bottlenecks or local extinctions (Jentsch *et al.*, 2002; Tschöpe & Tielbörger, 2010) resulting in drift effects (Banks *et al.*, 2013). Moreover, the species is a short-lived perennial having a short-lived soil seed bank (Jentsch, 2001) both contributing to high temporal population dynamics potentially resulting in reduction of effective population size. However, any such drift effects had only minor effects as despite a West-East decline of genetic variation high genetic variation was maintained in at least some populations throughout the species range.

A few shortcomings of our analysis must not be neglected. First, the geographic sample did not include Central and southern France and parts of NE Europe. While additional sampling would not fundamentally change the overall pattern, such additions would *e.g.* allow drawing a more detailed picture of the relationships of the south German gene pool to France. Second, we used AFLP as marker system, which typically have error rates of about 3% (Bonin *et al.*, 2004). Genotyping errors partly may account for both, observed levels of genetic variation within and between populations. Third, homoplasy cannot be totally excluded in AFLP (Mechanda *et al.*, 2004) which likely would lead to an underestimation of genetic differentiation between populations. Thus, additional analyses with alternative marker systems like chloroplast DNA and/or co-dominant nuclear SNPs (McCormack *et al.*, 2013; Senn *et al.*, 2014) will improve our understanding of the phylogeography of *Corynephorus canescens*.

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Supplement

Table S1. List of analysed populations with sampling information and descriptive parameters of genetic variation. Gene pools were inferred from *Geneland* results, according to Fig. S5. n = sample size; H_j = Nei's gene diversity; *Br* = Band richness, $PLP_{5\%}$ = Proportion of polymorphic loci without alleles of frequencies lower than 5 %; *Br* and $PLP_{5\%}$ are standardised for different sample sizes by rarefaction to n = 4.

Population No.	Country	Population	Latitude	Longitude	Marginality M_{min}	n	Inferred gene pool	H_j	*Br* (4)	$PLP_{5\%}$ (4)	No. private alleles
1	Portugal	Melides	38.13	−8.78	0.15	6	IP	0.269	1.398	0.474	0
2	Portugal	Troia	38.47	−8.88	0.14	8	IP	0.263	1.420	0.547	0
3	Portugal	Mira	40.43	−8.75	0.07	5	IP	0.294	1.458	0.526	1
4	Portugal	Figueira da Foz	40.15	−7.63	0.16	4	IP	0.299	1.453	0.453	1
5	Portugal	Serra de Estrela	40.35	−7.63	0.16	4	IP	0.267	1.401	0.401	0
6	Spain	Buitrago	41.83	−2.42	0.66	4	CA	0.282	1.423	0.423	0
7	France	Contis Plage	44.08	−1.32	0.23	6	CA	0.303	1.470	0.569	0
8	France	Cap Ferret	44.78	−1.22	0.13	7	CA	0.274	1.417	0.526	0
9	France	Ile d'Yeu	46.70	−2.29	0.21	7	CA	0.282	1.440	0.555	0
10	France	Ile de Normoutier	46.95	−2.18	0.26	8	CA	0.299	1.446	0.620	0
11	France	La Falaise	49.25	−2.16	0.68	5	CA	0.283	1.401	0.453	0
12	Great Britain	Great Yarmouth	52.35	1.43	0.78	5	CA	0.276	1.399	0.453	0
13	Netherlands	Amsterdam	51.92	4.55	0.2	4	CA	0.261	1.343	0.343	0
14	Netherlands	Deelense Zand	52.06	5.85	0.24	7	CA	0.276	1.394	0.504	0
15	Netherlands	Otterlose Zand	52.12	5.81	0.22	7	CA	0.263	1.379	0.467	0
16	Germany	Borkum	53.60	6.73	0.09	8	NG	0.210	1.280	0.387	0
17	Germany	Juist	53.68	7.05	0.09	8	NG	0.235	1.327	0.467	0
18	Germany	Emden	53.33	7.30	0.13	7	NG	0.233	1.303	0.387	0
19	Germany	Hassler	51.93	8.67	0.58	8	NG	0.211	1.269	0.372	0
20	Germany	Oerlinghausen	51.92	8.67	0.58	8	NG	0.205	1.274	0.409	0
21	Germany	Augustdorf	51.87	8.68	0.56	8	NG	0.240	1.325	0.482	0
22	Germany	Eberstadt	49.81	8.65	0.62	7	SG	0.238	1.320	0.416	0
23	Germany	Gräfenhausen	49.93	8.53	0.49	8	SG	0.245	1.342	0.489	0
24	Germany	Misselhorn	52.82	10.13	0.33	8	NG	0.275	1.403	0.540	0
25	Germany	Petersroda	51.57	12.30	0.47	7	NG	0.265	1.395	0.526	0
26	Germany	Schwedenschanze	51.55	12.33	0.47	6	NG	0.257	1.354	0.438	0
27	Germany	Tagebau Gröbern	51.70	12.42	0.38	7	NG	0.240	1.344	0.438	0
28	Germany	Gülpe/Havel	52.72	12.25	0.25	7	CA	0.277	1.391	0.511	0
29	Germany	Ziebchenberg	52.28	13.10	0.21	8	NG	0.248	1.358	0.489	0
30	Germany	Baumberge	52.58	13.22	0.17	6	NG	0.262	1.364	0.453	0
31	Germany	Schönhagen	52.20	13.12	0.21	7	NG	0.257	1.367	0.496	0
32	Germany	Vilm	54.32	13.53	0.16	4	CA	0.274	1.409	0.409	0
33	Germany	NSG Pettstadter Sande	49.83	10.93	0.61	5	SG	0.231	1.320	0.358	0
34	Germany	Büg	49.73	11.05	0.59	7	SG	0.210	1.287	0.365	0
35	Germany	NSG Tennenloher Forst	49.55	11.05	0.51	7	SG	0.247	1.339	0.445	0
36	Germany	NSG Exerzierplatz	49.53	11.02	0.53	7	SG	0.252	1.341	0.453	0
37	Germany	Eltersdorf	49.53	10.98	0.57	8	SG	0.229	1.316	0.460	0
38	Germany	NSG Hainberg	49.42	10.98	0.54	8	SG	0.251	1.357	0.496	0
39	Germany	Schwabach	49.33	11.03	0.48	6	SG	0.304	1.455	0.577	0
40	Germany	Mandlesmühle	49.12	10.97	0.52	7	SG	0.305	1.448	0.577	0
41	Germany	Altdorf	49.41	11.31	0.46	7	SG	0.258	1.360	0.460	0
42	Germany	Weiden Opf.	49.65	12.00	0.59	7	SG	0.241	1.323	0.401	0
43	Germany	Abensberger Sande	48.75	11.82	0.65	8	SG	0.224	1.311	0.431	0
44	Czech Republic	Bzenec	48.92	17.27	0.66	8	EE	0.239	1.338	0.460	0
45	Hungary	Darány	46.00	17.50	0.63	6	EE	0.254	1.354	0.431	0
46	Hungary	Bagamér	47.45	21.95	0.72	7	EE	0.241	1.344	0.445	0
47	Hungary	Martinka	47.57	21.78	0.69	7	EE	0.182	1.234	0.307	0
48	Hungary	Vámospércs	47.53	21.93	0.71	6	EE	0.244	1.351	0.431	0
49	Poland	Sieniawa	50.16	22.62	0.48	6	EE	0.278	1.395	0.467	0

Table S2. Hierarchical partitioning of molecular variance (AMOVA) with gene pool assignments based on the *Geneland* clustering results. df: degrees of freedom, SS: sums of squares, MS: mean sums of squares, est. var.: estimated variance of the hierarchical level, %: variance portion found at the hierarchical level, *p*-value for %: *p*-value for the Null-hypotheses based on *Φ*-statistics.

	df	SS	MS	est. var.	%	*p*-value for %
among gene pools	4	694.6	173.6	2.3	12.99%	< 0.0001
among populations	44	1218.8	27.7	2.1	11.77%	< 0.0001
within populations	277	3760.2	13.6	13.6	75.24%	< 0.0001
total	325	5673.6		18.0	100%	

Table S3. Result table of the regression analysis (linear model) for the dependence of genetic diversity (*Br*) on the population location's latitude, longitude and areal marginality as well as their interactions, done with the statistical software *R* (R Core Team, 2012).

```
lm(formula = Br ~ Longitude + Latitude + Mmin + Longitude:Latitude +
    Longitude:Mmin + Latitude:Mmin, data = data)

Residuals:
     Min       1Q   Median       3Q      Max
-0.07595 -0.02872 -0.01257  0.03581  0.07463

Coefficients:
                    Estimate   Std. Error    t value    Pr(>|t|)
(Intercept)         2.4957191  0.3066920     8.138      3.6e-10     ***
Longitude           0.0507421  0.0200097     2.536      0.015025    *
Latitude           -0.0226241  0.0063413    -3.568      0.000916    ***
Mmin               -1.3252161  0.6046623    -2.192      0.034000    *
Longitude:Latitude -0.0007863  0.0003643    -2.159      0.036646    *
Longitude:Mmin     -0.0244937  0.0070117    -3.493      0.001138    **
Latitude:Mmin       0.0268121  0.0122987     2.180      0.034907    *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.04181 on 42 degrees of freedom
  (34 observations deleted due to missingness)
Multiple R-squared: 0.4963,    Adjusted R-squared: 0.4244
F-statistic: 6.898 on 6 and 42 DF, p-value: 3.798e-05
```

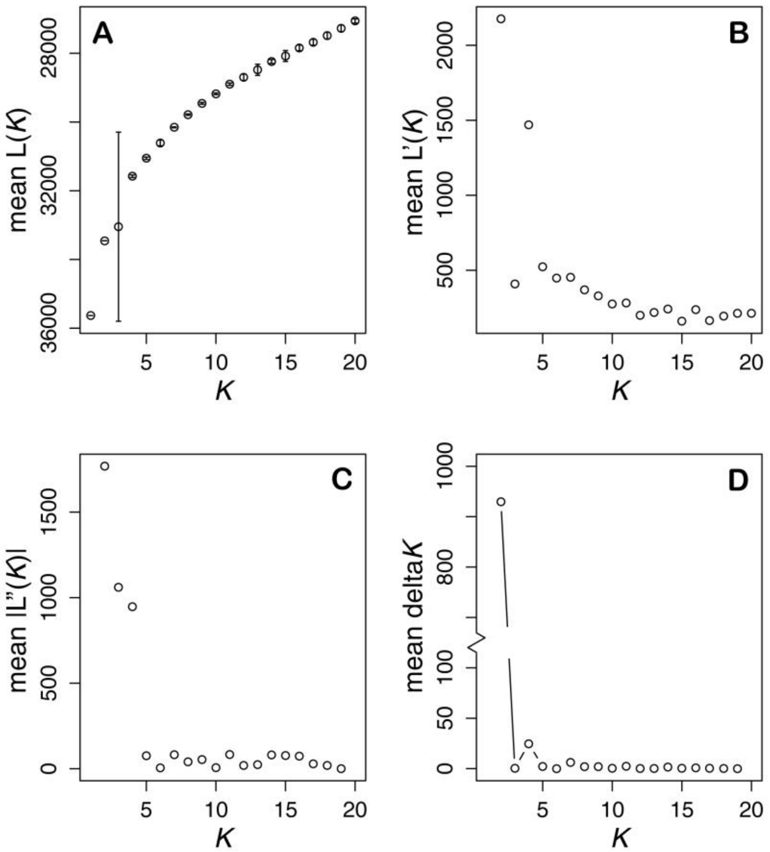

Figure S1. Assessment of the most likely number of clusters of Structure runs using the method of Evanno *et al.* (2005). Shown are means of (A) the log probability of the data mean L(K), (B) the rate of change of the likelihood distribution mean L'(K), (C) the absolute values of the second order rate of change L''(K) and (D) ΔK, over 20 runs for each K value, respectively.

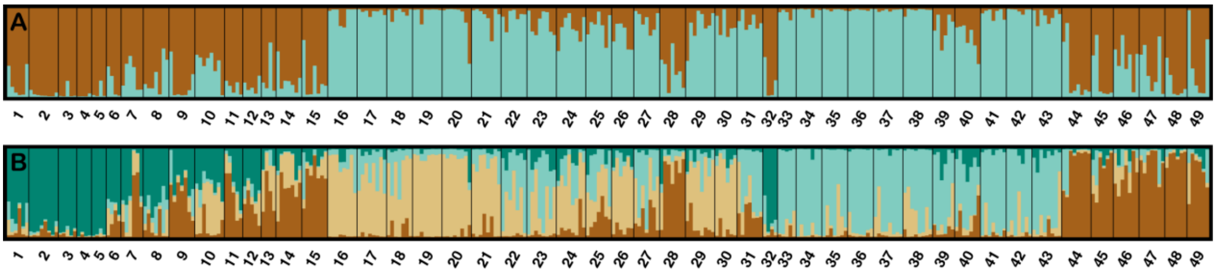

Figure S2. Results of the Structure analyses depicted by the average cluster assignment probability Q over 20 runs with (A) $K = 2$ and (B) $K = 4$. Each vertical bar represents one individual with coloured segment length proportional to each of the inferred clusters; populations are separated by black lines. Population numbering corresponds to Fig. 1 and Tab. S1.

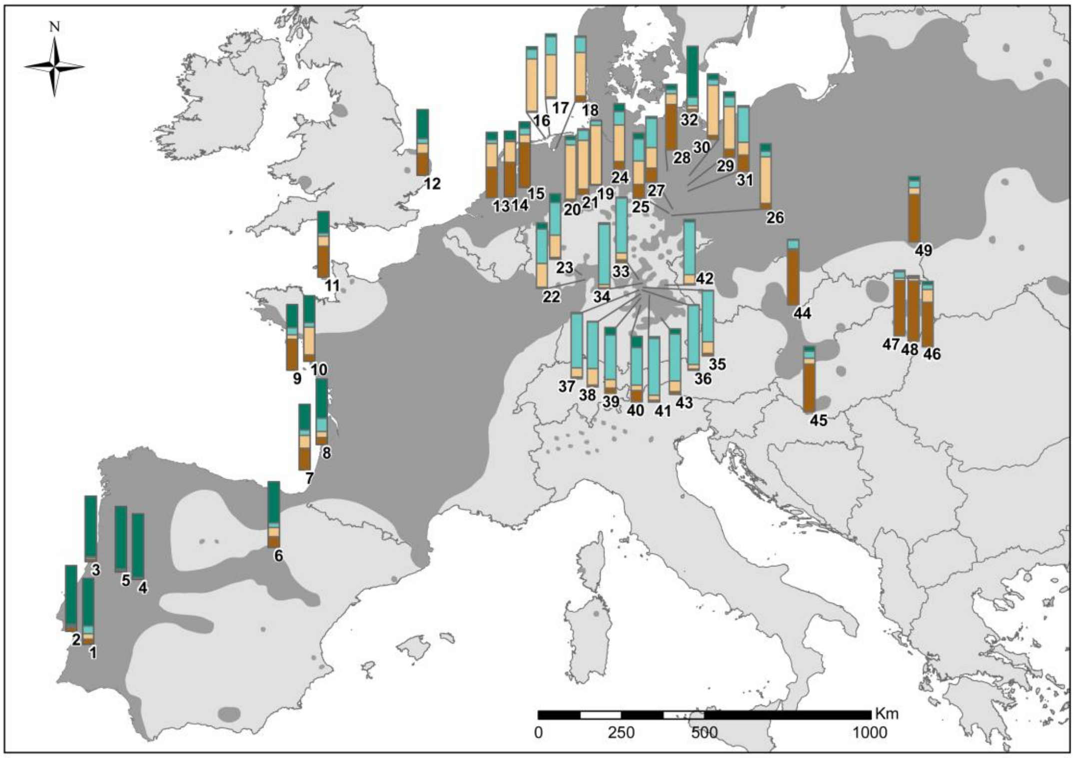

Figure S3. Geographical map showing the results of the Structure analysis. The populations are represented by vertical bars depicting the average cluster assignment probabilities *Q* over 20 runs with *K* = 4, respectively. Colouring of the cluster assignments corresponds to Fig. 1 and 2, Fig. S2 and Fig. S4.

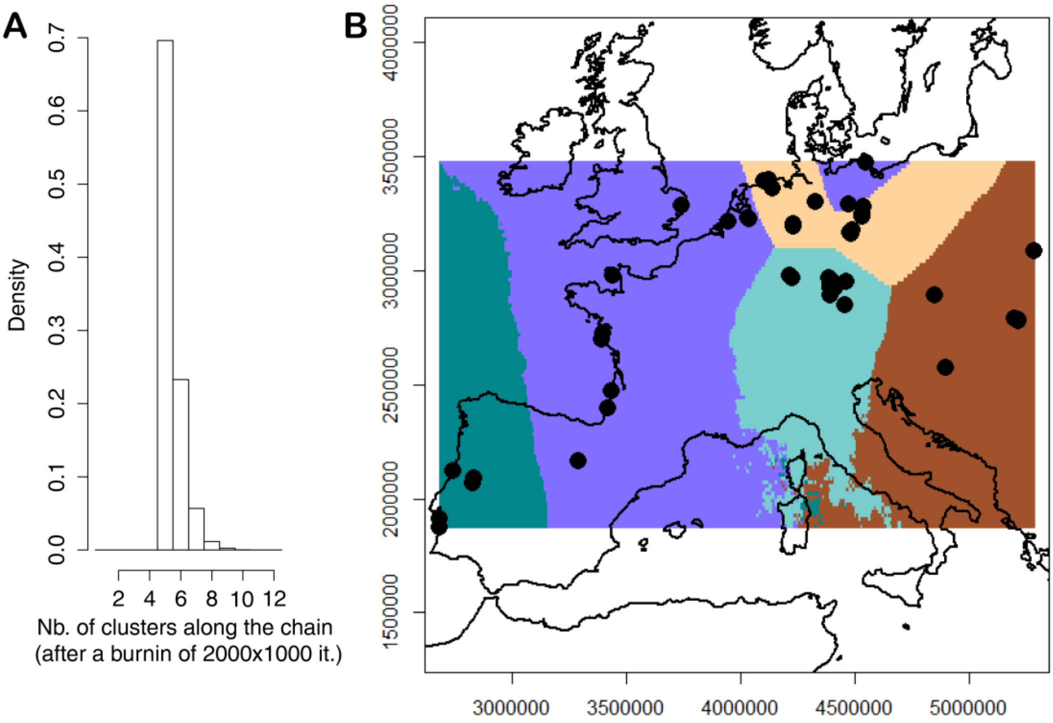

Figure S4. Results of the Geneland analysis. (A) Histogram showing the posterior density distribution of the number of clusters estimated using the spatial model. (B) The estimated cluster memberships of the populations according to the five clusters. Colours correspond to those of Fig. 1 and 2, and Fig. S2 and S3.

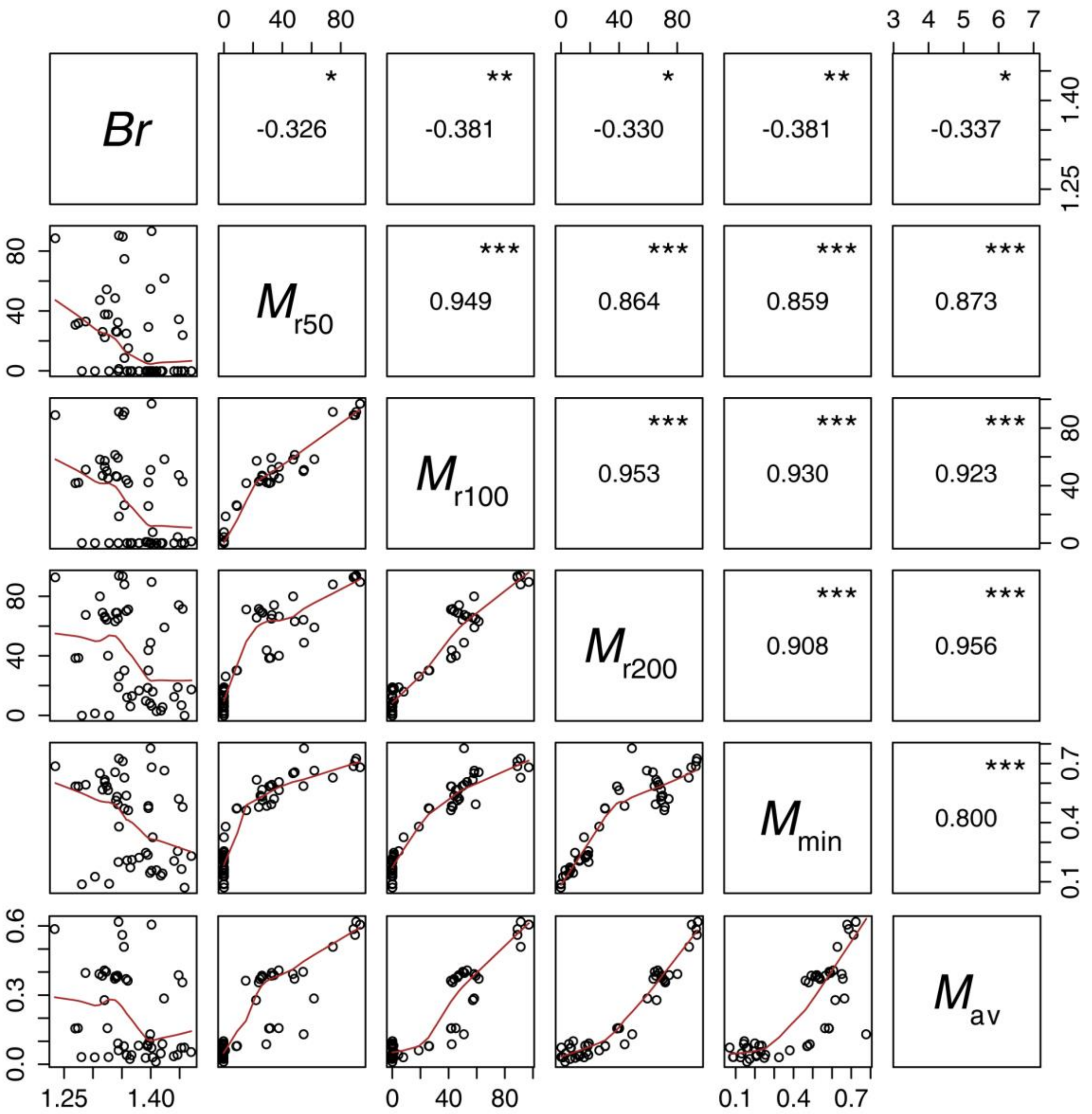

Figure S5. Correlation matrix for marginality measures and Band richness. Above the diagonal correlation coefficients (Pearson's *r*) and significance levels (* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001) are given.

Summary

Spatial and temporal variation of climate determines ecological and evolutionary trajectories of plants. This can be seen *e.g.* in arrangements of plant species and functional traits along climate gradients, in palynological records, phylogeographic patterns or in plant responses to contemporary climate change. Current and expected future climatic alterations are special in their potential to influence plant life, due to particularly high rates of change but also to co-occurring and synergistic anthropogenic stressors to species and ecosystems.

This thesis aims to enhance the understanding of past, present and future response capacities of plants. It is based on six manuscripts that study different aspects of plant responses to climatic variation in space and time and combines conceptual literature reviewing, experimental studies, population and landscape genetic analyses, phylogeographic approaches, as well as field studies and biogeographic pattern analyses. Studies include continental systems on different scales, as well as oceanic islands on a global perspective and in case studies from La Palma, Canary Islands.

Oceanic islands are important contributors to global biodiversity and have played pivotal roles in biogeographical, ecological and evolutionary research. However, knowledge about the outcomes of climate change on oceanic islands is very scarce. Within this thesis, a conceptual overview depicts the wide range of impacts and reveals a large threat potential of anthropogenic climate change to island floras. Research needs and potentials are identified.

On La Palma, population structures in two species of the evolutionarily significant genus *Aeonium*, as well as patterns of endemic species distributions are shown to be determined by climatic and topographic landscape heterogeneity. This highlights the importance of climatic variation in shaping evolutionary pathways and generating biodiversity patterns. Heterogeneous selection and divergent adaptation interact with various effects of landscape structures. These range from sheltering local species and gene pools from climatic variability or human-induced influences (introduced herbivores, fire, land use) in (micro-)refugia over provisioning of habitat diversity to acting as gene flow barriers and increasing reproductive isolation between divergent populations.

Phenotypic plasticity is a direct and rapid response of organisms to environmental change and is known to vary intraspecifically in many species. Potential links of such variation to the history of populations and their resultant genetic make-up are experimentally studied and discussed. In *Fagus sylvatica*, genetically diverse populations from regions that acted as glacial refugia tend to show higher plasticity towards extreme weather conditions than populations from re-colonised regions. This hints to the importance of phylogeographical histories for the capacity of populations to respond to climate change and to a role of genetic diversity besides promoting adaptability.

In a study on *Corynephorus canescens*, phylogeography-dependent patterns of genetic diversity were shown to be modified by range centre-periphery gradients. Additionally, the species' uncommon ecology affected the population genetic structure. To allow for adequate models of genetic diversity, *e.g.* for assessing eco-evolutionary response capacities, those factors need to be integrated.

Furthermore, a consideration of transgenerational effects of extreme weather events on perennial species is given. It shows changes in germination timing and success, but also modifications in growth and leaf stoichiometry in offspring plants of *Genista tinctoria* and *Calluna vulgaris* after their mother plants were exposed to drought and heavy rain treatments. Perennial species rely on plastic responses to environmental changes due to long generation times and low rates of possible genetic adaptation and/or range shifts, making (adaptive) transgenerational plasticity an important mechanism for population persistence. It can increase individual fitness from the seedling stage and thereby provide new variants for selection to act on and potentially induce evolutionary change.

To sum up, the thesis elaborates a conceptual overview over plant responses to climatic variation on different spatial and temporal scales. It mainly focusses on processes and their drivers, and how past and current biogeographic, evolutionary, natural or anthropogenic factors can influence current and future response capabilities, thereby facilitating the mechanistic understanding of climate change influences on plants. Resulting from this thesis, new research questions arise in climate change ecology and island biology that particularly address the value and driving forces of plasticity within and among plant populations in changing environments, landscape effects on evolutionary processes on the scale of single islands, and the impacts of climate change on oceanic islands.

Zusammenfassung

Klimaänderungen in Raum und Zeit bestimmen die ökologische und evolutionäre Entwicklung von Pflanzen. Dies ist z.B. im Gefüge von Arten und ihrer funktionellen Eigenschaften entlang ökologischer Gradienten, in palynologischen Zeitreihen, phylogeographischen Mustern oder in Reaktionen von Pflanzen auf den derzeitigen Klimawandel erkennbar. Auf Grund der Geschwindigkeit, aber auch durch synergistisch wirkende anthropogene Stressoren sind die aktuellen und prognostizierten Klimaveränderungen in ihren Auswirkungen auf Arten und Ökosysteme außergewöhnlich.

Diese Dissertation zielt auf eine Verbesserung des Verständnisses der Reaktionsvermögen von Pflanzen ab. Sie basiert auf sechs Manuskripten, die verschiedene Aspekte pflanzlicher Reaktionen auf Klimavariation untersuchen. Konzeptionelle, experimentelle, populations- und landschaftsgenetische Studien, phylogeographische Ansätze, genauso wie Feldarbeiten und biogeographische Musteranalysen werden kombiniert. Kontinentale Systeme auf verschiedenen Skalen, ozeanische Inseln aus einer globalen Perspektive und Fallstudien auf La Palma, Kanarische Inseln, werden bearbeitet.

Ozeanische Inseln tragen stark zur globalen Biodiversität bei und spielen bis heute wichtige Rollen in der Biogeographie, Ökologie und Evolutionsforschung. Die Auswirkungen des Klimawandels auf diesen Inseln sind jedoch bisher kaum erforscht. Ein konzeptioneller Überblick in dieser Dissertation zeigt die vielen möglichen Folgen des anthropogen bedingten Klimawandels und deckt ein großes Bedrohungspotential auf ozeanische Inselfloren auf. Weiterhin werden Forschungslücken und –potentiale aufgezeigt.

Klimatische und topographische Heterogenität innerhalb La Palmas wird als Treiber für Populationsstrukturen zweier Arten der evolutionär bedeutsamen Gattung *Aeonium* und für Verteilungen von Endemiten belegt. Dies betont die Bedeutung klimatischer Variation für Evolutionsvorgänge und die Erzeugung biogeographischer Muster. Selektionsheterogenität und divergente Anpassungen interagieren mit Effekten von Landschaftsstrukturen. Diese reichen vom Schutz lokaler Arten- und Genpools vor klimatischer Variabilität oder menschlichen Einflüssen in (Mikro-)refugien über Habitatvielfalt bis hin zur Erhöhung reproduktiver Isolation zwischen divergierenden Populationen.

Phänotypische Plastizität ist eine direkte Reaktion auf Umweltänderungen und kann intraspezifisch variieren. Potentielle Verbindungen solcher Variation zur Historie von Populationen und deren resultierenden genetischen Aufstellung wird experimentell untersucht und diskutiert. Unter Extremwetterbedingungen tendierten genetisch diverse Populationen von *Fagus sylvatica* aus Regionen eiszeitlicher Refugien zu höherer Plastizität als Populationen aus wiederbesiedelten Regionen. Dies spricht für einen Einfluss phylogeographischer Historien von Populationen auf ihre Reaktionsfähigkeit gegenüber

klimatischem Wandel und für eine Rolle von genetischer Diversität neben der Förderung genetischer Anpassung.

In einer Studie zu *Corynephorus canescens* wird gezeigt dass phylogeographisch bedingte Muster genetischer Diversität durch Peripherie-Effekte im Verbreitungsgebiet modifiziert werden. Zusätzlich beeinflusst die ungewöhnliche Ökologie der Art ihre genetischen Strukturen. In Modellen genetischer Diversität müssen diese Faktoren bedacht werden, z.B. um öko-evolutionäre Reaktionsvermögen abzuschätzen.

Weiterhin werden transgenerationale Effekte von Extremwetterereignissen auf perennierende Arten betrachtet. Veränderungen in Keimungszeiten und -erfolgen und Modifikationen im Wachstum und der Blatt-Stöchiometrie von Nachkommen Dürre- und Starkregen-exponierter *Genista tinctoria*- und *Calluna vulgaris*-Mutterpflanzen werden gezeigt. Perennierende Arten sind im Klimawandel auf Grund langer Generationszeiten und geringer Raten möglicher genetischer Anpassung und/oder Ausbreitung auf plastische Reaktionen angewiesen. Dies macht (adaptive) transgenerationelle Plastizität zu einem wichtigen Mechanismus für den Populations-Fortbestand, indem sie individuelle Fitness schon ab dem Sämlingsstadium erhöhen kann und neue selektionsrelevante Varianten erschaffen und evolutiven Wandel einleiten kann.

Diese Dissertation erarbeitet einen konzeptionellen Überblick über pflanzliche Reaktionen auf klimatische Änderungen auf verschiedenen räumlichen und zeitlichen Skalen. Sie konzentriert sich auf Prozesse und deren Treiber und die Einflüsse biogeographischer, evolutionärer, natürlicher und menschlicher Faktoren auf heutige und zukünftige Reaktionsvermögen und erhöht das mechanistische Verständnis von Klimawandelauswirkungen auf Pflanzen. Aus dieser Arbeit resultieren neue wissenschaftliche Fragen in der Klimawandelökologie und Inselbiologie, insbesondere zur Bedeutung und treibenden Kraft von Plastizität in Populationen und Arten in einer sich ändernden Umwelt, zu Landschaftseffekten auf evolutive Prozesse innerhalb einzelner Inseln und zu Klimawandelauswirkungen auf ozeanische Inseln.

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Declarations

(Eidesstattliche) Versicherungen und Erklärungen

(§ 5 Nr. 4 PromO)

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

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Hiermit erkläre ich mich damit einverstanden, dass die elektronische Fassung meiner Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung hinsichtlich der eigenständigen Anfertigung der Dissertation unterzogen werden kann.

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Hiermit erkläre ich eidesstattlich, dass ich die Dissertation selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

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Ich habe die Dissertation nicht bereits zur Erlangung eines akademischen Grades anderweitig eingereicht und habe auch nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden.

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………………………………………………………………………………

Ort, Datum, Unterschrift

Appendix

Appendix 1: Manuscripts not included in this thesis.

Beierkuhnlein, C.; Gargallo-Garriga, A.; **Harter, D.E.V.**; Sardans, J.; Pérez-Trujillo, M.; Oravec, M.; Urban, O.; Jentsch, A.; Parella, T.; Wohlwend, M.; Irl, S.D.H.; Peñuelas, J.: Short-distance divergence in functional phenotypes within an endemic species (*Aeonium canariense*) on a heterogenous oceanic island (La Palma). In preparation for submission to New Phytologist.

Irl, S.D.H.; Schweiger, A.H.; **Harter, D.E.V.**; Steinbauer, M.; Jentsch, A.; Medina, F.M.; Fernández-Palacios, J.M.; Beierkuhnlein, C.: An island view of endemic rarity – environmental drivers and consequences for nature conservation. In review at Diversity and Distributions.

Steinbauer, M.; Beierkuhnlein, C.; Arfin Khan, M.A.S.; **Harter, D.E.V.**; Irl, S.D.H.; Jentsch, A.; Schweiger, A.H.; Svenning, J.-C.; Dengler, J.: How to differentiate facilitation and environmentally driven coexistence. In revision for re-submission to Journal of Vegetation Science.

Steinbauer, M.; Field, R.; Grytnes, J.A.; Trigas, P.; Ah-Peng, C.; Attorre, F.; Birks, H.J.B.; Borges, P.A.V.; Cardoso, P.; Chou, C.-H.; De Sanctis, M.; de Sequeira, M.M.; Duarte, M.C.; Elias, R.B.; Fernández-Palacios, J.M.; Gabriel, R.; Gereau, R.E.; Gillespie, R.G.; Greimler, J.; **Harter, D.E.V.**; Huang, T.J.; Irl, S.D.H.; Jeanmonod, D.; Jentsch, A.; Jump, A.S.; Kueffer, C.; Nogué, S.; Otto, R.; Price, J.; Romeiras, M.M.; Strasberg, D.; Stuessy, T.; Vetaas, O.R.; Beierkuhnlein, C.: Topography-driven isolation, speciation and a globally consistent pattern of endemism. Accepted with minor revisions in Global Ecology and Biogeography.

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