esa

ECOSPHERE

Divergence between phenotypic and genetic variation within populations of a common herb across Europe

JESÚS VILLELLAS,[1,3,]† REGINA BERJANO,[2] ANASS TERRAB,[2] AND MARÍA B. GARCÍA[1]

[1]*Instituto Pirenaico de Ecología (IPE-CSIC), Avda. Montañana 1005, Apdo. 13034, 50080 Zaragoza, Spain*
[2]*Departamento de Biología Vegetal y Ecología, Facultad de Biología, Universidad de Sevilla, Apdo. 1095, 41080 Sevilla, Spain*

Citation: Villellas, J., R. Berjano, A. Terrab, and M. B. Garcia. 2014. Divergence between phenotypic and genetic variation within populations of a common herb across Europe. Ecosphere 5(5):56. http://dx.doi.org/10.1890/ES13-00291.1

Abstract. Analyzing the pattern and causes of phenotypic and genetic variation within and among populations might help to understand life history variability in plants, and to predict their responses to changing environmental conditions. Here we compare phenotypic variation and genetic diversity of the widespread herb *Plantago coronopus* across Europe, and evaluate their relationship with environmental and geographical factors. Genetic diversity was estimated in 18 populations from molecular markers with AFLP. Phenotypic variation was measured in a subset of 11 populations on six life history traits (plant size, plant growth, fecundity, seed mass, mucilage production and ratio between two functionally different seed morphs). To account for ecological and geographical correlates, we estimated variability in local temperature, precipitation and intraspecific competition, and accounted for the central vs. peripheral position of populations. Phenotypic variation and genetic diversity were not significantly correlated within populations throughout the species' range. Phenotypic variation was positively linked to precipitation variability, whereas genetic diversity was correlated with the position of populations, suggesting that both types of variation are shaped by different processes. Precipitation seems to have acted as a selective agent for variation within populations in most life history traits, whereas the species' post-glacial demographic history has likely reduced genetic diversity in northern peripheral populations with respect to central ones. The positive association between precipitation variability and phenotypic variation also suggests that plant populations may have higher adaptive potential in ecologically variable rather than stable environments. Our study offers an additional criterion when predicting the future performance of species under environmental changes.

Key words: adaptive variation; environmental fluctuations; European Atlantic coast; evolutionary potential; genotype; latitudinal gradient; phenotype; *Plantago coronopus*; rainfall; range margin; widespread short-lived perennial.

Received 14 September 2013; revised 25 February 2014; accepted 4 March 2014; final version received 4 April 2014; **published** 15 May 2014. Corresponding Editor: E. Ribbens.

[3] Present address: Department of Ecology and Genetics, Uppsala University, Norbyvägen 18 D, 75236 Uppsala, Sweden.
† **E-mail:** jesus.villellas@ebc.uu.se

INTRODUCTION

The variation in life history traits shown by plant populations constitutes the basis for the evolutionary potential of species (Bradshaw 1991, Bradshaw and McNeilly 1991), and might have a critical role in the face of changing environmental conditions (Reed and Frankham 2001, Dawson et al. 2011). Numerous studies have reported indeed important effects of climate change on the ecology of plant and animal taxa (e.g., Walther et al. 2005, Parmesan 2006), and the existence of a pool of individuals potentially pre-adapted to different environmental scenarios

may be important in the near future (Volis et al. 1998, Jump and Peñuelas 2005). Thus, analyzing intraspecific variation in life history traits and its underlying causes will help to understand the adaptation mechanisms of plants to their current environment, and predict their future performance in new ecological scenarios.

Intuitively, a correlation is expected at the population level between phenotypic and genetic variation. However, genetic diversity based on molecular markers, which has been used to assess the status and evolutionary potential of populations (e.g., Frankham 1995, Haig 1998), has shown no consistent relationship with phenotypic variability (Butlin and Tregenza 1998, Reed and Frankham 2001). In fact, both metrics seem to be affected by different processes. Genetic diversity, usually inferred from neutral loci (Lynch et al. 1999, Holderegger et al. 2006), is mainly affected by the demographic history of species, through processes such as gene flow, genetic drift and founder events (Knapp and Rice 1998, Holderegger et al. 2006, Mitchell-Olds and Schmitt 2006, Lawton-Rauh 2008). Thus, we could expect neutral genetic diversity to be associated with the relative position of populations within species' ranges, since gene flow and population sizes typically decrease towards the periphery, and bottlenecks and founder events are thus more likely there (Lesica and Allendorf 1995, Vucetich and Waite 2003). Analyzing historical (post-glacial) shifts in species distributions and the phylogenetic relationship among populations is thus of high interest in this context (Hewitt 2000). On the other hand, in contrast with genetic variation, phenotypic variation is frequently estimated on fitness-related characters that are likely to be affected by natural selection. Therefore, genetic diversity inferred from neutral markers does not necessarily constitute the best predictor for variation in life history traits (Reed and Frankham 2001).

Phenotypic variation within populations may instead show a closer relationship with environmental conditions. Climate, for example, is a major selective agent in plants at large spatial scales (Joshi et al. 2001, Etterson 2004), and variability in life history traits could be promoted through natural selection by variation in factors such as temperature and precipitation. Environmental variability might also trigger trait variation by means of phenotypic plasticity, which has a genetic basis as well (Schlichting 1986, Pigliucci 2005). However, life history traits may not only present the signal of adaptive but also of random processes such as gene flow or founder events (van Tienderen et al. 2002), although to a lesser extent than neutral genetic variation does (Galloway and Fenster 2000, Joshi et al. 2001). Thus, the effects of local environmental variability and genetic variation should be examined together on phenotypic variation, within both central and peripheral populations. In this way, we can unravel the consequences of the adaptive selection and the demographic history of species. However, very few plant studies have analyzed intra-population variation in different life history traits and its association with genetic diversity across distribution ranges (but see Gömöry et al. 2013), and much research is still needed to understand the role of environmental, geographical and historical factors on such variation.

Widespread plants represent successful examples of life history adaptability to a broad range of local conditions (Waldmann and Andersson 1998, Joshi et al. 2001) and provide a good opportunity to analyze phenotypic variation along large geographical and/or environmental gradients. For that reason we chose as our study case *Plantago coronopus* L., a widespread short-lived herb in Europe, North Africa and Southwest Asia (Hultén and Fries 1986). This taxon presents high variability in vegetative and reproductive traits, as well as in demographic vital rates, both at regional (Waite and Hutchings 1982, Braza et al. 2010) and continental scales (Villellas and García 2013, Villellas et al. 2013*b*). Furthermore, *P. coronopus* produces two types of seeds that differ in size and in the production of a mucilaginous coat that facilitates water absorption (Dowling 1933). Variation among populations of this taxon in traits such as plant size, seed size and mucilage production appears to be highly related to environmental factors such as precipitation, temperature and intraspecific competition (Villellas and García 2013, Villellas et al. 2013*a*). However, it remains to be tested whether environmental variability promotes variation in life history traits also within populations. Alternatively, life history variation could be related with genetic diversity, or with the relative

position of populations within the species' distribution range.

In this study we analyzed both phenotypic variability and genetic diversity in *P. coronopus*. We sampled 18 natural populations spanning the whole latitudinal gradient of the species in Europe, for which we quantified genetic diversity and examined their phylogenetic relationship. Individuals from a subset of 11 populations were monitored in the field to measure phenotypic variation within populations in six key life history traits: plant size, seed mass, ratio between seed morphs, and mucilage production, which are of high ecological importance for plants (Harper and Benton 1966, Westoby et al. 1992, Imbert 2002), and individual annual growth and fecundity, which constitute major drivers of population dynamics for short-lived taxa (Silvertown et al. 1996). Some traits were measured for several years in each population, to obtain unbiased estimates of phenotypic variation. Temporal variability in local climate and intraspecific competition, and the central vs. peripheral location of populations, were also accounted for. Our specific goals were to examine the potential effects of genetic variation, environmental conditions, and range position on the phenotypic variation within populations of *P. coronopus*, and also the effects of environment and range position on the species' genetic variation. With these analyses, we aimed to unravel the pattern and causes of life history variation in a widespread plant across Europe.

Material and Methods

Species and populations studied

Plantago coronopus L. (buck's horn plantain, Plantaginaceae) is a widespread short-lived herb, with a lifespan typically ranging from 1 to 5 yr. It is mainly distributed around the Mediterranean Basin, although it also reaches North Europe through a strip along the Atlantic coast (Hultén and Fries 1986; Fig. 1A). We have worked with the most common subspecies *Plantago coronopus* ssp. *coronopus*, which can be distinguished from the others by the morphology of the bracts (Chater and Cartier 1976). Hereafter we will refer to it as *P. coronopus*.

Individuals of *P. coronopus* show high variability in characters such as leaf shape and size. Plants have one or a few rosettes, producing several spikes with wind-pollinated flowers. The species is gynodioecious and predominantly outcrossing, although it presents high variation in outcrossing rates, from 0.34 to 0.93 (Wolff et al. 1988). Each fruit produces two types of seeds in variable number: up to four large, basal seeds, and one or no small apical seeds. Basal seeds further differentiate morphologically and functionally from apical ones, in possessing a mucilaginous coat that facilitates water absorption (Dowling 1933) and showing faster and higher germination rates (Braza and García 2011). Thus, basal seeds seem to be better adapted to habitats with low water or resources supply. *Plantago coronopus* is present in a wide variety of environmental conditions across its range in terms of climate, soil richness and vegetation cover, but always occurring in open areas, where it may act as a pioneer. In central areas, the species is found in coastal and inland locations (Fig. 1A), in contrasting habitats like sand dunes, cliffs, shrublands or human-disturbed areas. Northern peripheral populations are rather restricted to coastal places (coastal prairies, salt marshes).

To analyze genetic diversity, we have chosen 11 central and seven northern peripheral populations, for a total of 18 populations in six countries, spanning the whole latitudinal and environmental gradient of the species in Europe (Fig. 1A, Table 1). Peripheral populations were located in coastal meadows, and central populations were located in a variety of habitats. For the analysis of phenotypic variability, we have used a representative subset of five central and six peripheral populations, for a total of 11 populations along the Atlantic coast (Fig. 1A, Table 1), all containing thousands of reproductive individuals (J. Villellas and M. B. García, *personal observation*). Considering the large population sizes, the species anemophily and the small distances among conspecifics (typically lower than 20 cm; J. Villellas and M. B. García, *personal observation*), we assumed virtually random mating within populations.

Variability in phenotypic traits

Eleven populations were monitored during up to 8 yr (between 2003 and 2010; Table 1) to quantify within-population variability in six life

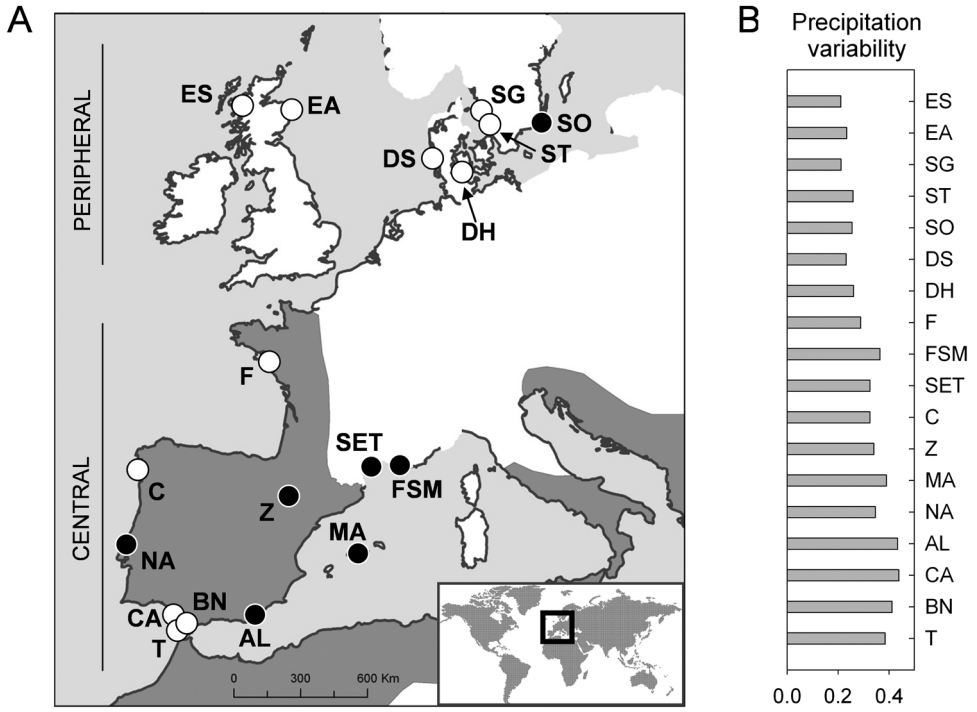

Fig. 1. (A) Location of central and northern peripheral populations of *Plantago coronopus* sampled in this study. Black circles correspond to populations sampled for genetic analyses, and white circles to populations subject both to genetic and phenotypic analyses. In gray, geographic distribution of the species, including some coastal outlines (dark gray) and omitting the southernmost area (simplified from Hultén and Fries 1986). (B) Precipitation variability in populations (see *Material and Methods* for details on estimation), ranked by latitude. Population acronyms are as in Table 1.

history traits (plant size, plant growth, plant fecundity, mass and mucilage production of basal seeds, and ratio between basal and apical seeds). Given the multi-year dataset compiled for some traits (plant size, growth and fecundity), we can assure that our phenotypic measurements are representative of each population, and not influenced by the particular conditions of a given year. On each population, we labelled between 50 and 150 reproductive plants contained in plots randomly distributed within population sites (3–10 plots of 0.25–5 m^2 per population, depending on local plant density), to measure each year the number and length of leaves, and the number and length of inflorescences. We only sampled reproductive plants to use comparable individuals and thus avoid potentially confounding effects. Plant size was estimated for each individual by multiplying the number of leaves and the length of an average leaf. Plant growth rate was calculated as the ratio between plant size in one year and that of the previous year. We estimated fecundity (number of seeds) by multiplying the number of inflorescences, the length of an average inflorescence and the number of seeds per unit of inflorescence length (see Villellas et al. 2013*a* for details). We found in a preliminary analysis that fecundity was correlated with plant size (log-transformed variables; $F_{1,7348} = 3754$, $R^2 = 0.34$, $p < 0.001$; *lm* procedure, package *stats*, R Development Core Team 2011). Since both traits were to be used in the analyses, we calculated fecundity per unit of plant size (hereafter "fecundity") to make the former trait independent from the later.

To evaluate variation in seed traits, the spikes of 25 random individuals were collected on each population in the summers of 2007 or 2008. In the laboratory, we counted the total number of basal and apical seeds in 10 fruits per plant. We then

Table 1. Information on populations of *Plantago coronopus* sampled in this study. N corresponds to the number of individuals used for genetic analyses, Fr_t is the total number of AFLP fragments, Fr_p is the percentage of polymorphic fragments, H_D is mean genetic diversity (± SD) and PV is the number of years of data collection for phenotypic variation.

Population	Coordinates	Habitat	Genetic analyses				PV (yr)
			N	Fr_t	Fr_p (%)	H_D	
Central							
T–Spain	36°02′ N, 05°38′ W	Sand dune	12	315	62.25	0.224 ± 0.11	8
BN–Spain	36°06′ N, 05°32′ W	Forest gaps	12	335	70.71	0.248 ± 0.13	4
CA–Spain	36°25′ N, 06°13′ W	Sand dune	10	285	59.22	0.211 ± 0.11	4
AL–Spain	36°43′ N, 02°11′ W	Sandy cliff	6	239	45.77	0.200 ± 0.11	
NA–Portugal	39°35′ N, 09°04′ W	Sand dune	12	286	56.83	0.203 ± 0.10	
MA–Spain	39°46′ N, 03°45′ E	Sand dune	11	261	52.27	0.194 ± 0.10	
Z–Spain	41°39′ N, 0°50′ W	Riverside	10	276	57.05	0.215 ± 0.11	
C–Spain	42°33′ N, 09°01′ W	Sand dune	11	266	53.14	0.196 ± 0.10	7
SET–France	43°24′ N, 03°39′ E	Lagoon rocks	12	285	59.21	0.212 ± 0.11	
FSM–France	43°27′ N, 04°52′ E	Lagoon rocks	6	214	39.91	0.180 ± 0.10	
F–France	47°18′ N, 02°30′ W	Sand dune	8	221	41.64	0.167 ± 0.09	5
Peripheral							
DH–Denmark	55°08′ N, 09°59′ E	Coastal prairie	11	215	39.91	0.148 ± 0.07	4
DS–Denmark	55°29′ N, 08°15′ E	Coastal prairie	11	268	48.80	0.179 ± 0.09	5
SO–Sweden	56°13′ N, 16°24′ E	Coastal prairie	10	183	34.92	0.136 ± 0.07	
ST–Sweden	56°23′ N, 12°38′ E	Coastal prairie	11	205	33.40	0.124 ± 0.06	5
SG–Sweden	56°55′ N, 12°21′ E	Coastal prairie	9	205	31.88	0.120 ± 0.06	7
EA–Scotland	57°20′ N, 01°55′ W	Coastal prairie	11	208	36.22	0.137 ± 0.07	4
ES–Scotland	57°30′ N, 06°26′ W	Coastal prairie	6	151	27.33	0.125 ± 0.07	4

Note: Some populations were not subject to phenotypic analyses (blank cells in the last column).

calculated the ratio of basal and apical seeds for each individual (hereafter "seed ratio"; not available in population BN) by dividing the total number of basal seeds by that of apical seeds. The production of mucilage and the size of basal seeds were measured in five seeds per individual, in an average of 15 individuals per population. We first soaked the seeds for 1 h in Petri dishes, until mucilage became conspicuous. We then measured the projected seed area, and the total area that contained both the seed and the mucilaginous coat, using the ellipse area formula. Seed mass was estimated from seed area, and mucilage production (hereafter "mucilage ratio") was estimated by substracting the seed area from the total area, and by dividing the result by the seed area. We used a relative measure of mucilage because the area of the mucilaginous coat was positively correlated to seed mass (Villellas and García 2013). For seed mass and mucilage ratio, we calculated for each individual the average across seeds.

For each population, we estimated phenotypic variation from the coefficient of variation (CV) among individuals in each trait: plant size, plant growth, fecundity, seed ratio, seed mass and mucilage ratio. For traits for which we had data from several years we averaged the CV across years.

Environmental variability of populations

In the 11 populations sampled for phenotypic variation, we also estimated the density of *P. coronopus* (*D*) from linear transects (Strong 1966), with the equation $D = \Sigma(1/d) \times (1/T)$, where T is total transect length, and d is the diameter perpendicular to the transect of non-seedling individuals intercepting the transect. We collected data from 3 yr for peripheral populations and from 4 yr for central populations, and the CV in annual density was calculated as a proxy for variation in intraspecific competition.

Meteorological data were obtained for all 18 populations from different databases depending on the country or region: Spanish National Meteorological Agency (populations T, BN, CA, AL, MA and Z), MeteoGalicia (C), MeteoFrance (F), Danish Meteorological Institute (DH and DS), Swedish Meteorological and Hydrological Institute (ST and SG), Met Office (EA and ES) and the website http://www.tutiempo.net (NA, SET, FSM and SO). We obtained annual temperature and annual and monthly precipitation from 10-20 yr within the last four decades (depending on

availability) from the nearest meteorological station to each population. Then, we calculated for each population the CV in annual temperature and three estimates of precipitation variability: (1) the CV in annual precipitation, as a measure of inter-annual variability; (2) the average of the annual Precipitation Concentration Index (PCI; Oliver 1980), which is the ratio between the summatory of the squared monthly precipitation within a year and the squared summatory of monthly precipitation, and reflects intra-annual variability; and (3) the CV of the annual PCI. Finally, since seeds were only collected in 2007 or 2008, we verified that temperature and precipitation in those years were similar to the yearly averages.

Genetic analyses

For all 18 populations, we collected leaf samples of 6–12 individuals per population (Table 1), for a total of 179 individuals. Leaves were collected in situ or from individuals grown in the greenhouse from seeds collected in populations (from different individuals separated by at least 1 m). Plant material was stored in silica gel immediately after collection.

Total genomic DNA was extracted from dry leaves using the unmodified QIAGEN DNeasy Plant Mini Kit protocol. Quality and quantity of extracted DNA were determined electrophoretically after SYBR green staining using a ladder with known amounts of DNA as standards (HyperLadder, Bioline). We performed an amplified fragment length polymorphism (AFLP) analysis following established protocols (Vos et al. 1995). AFLP genotyping allows an accurate assessment of baseline levels of neutral genetic variation across the genome (Roldán-Ruiz et al. 2000, Bonin et al. 2007). An initial screening of selective primers, using 72 primer combinations with three and four selective nucleotides, was performed on a total of eight individuals from eight different populations. The final six primer combinations for the selective PCR were (fluorescent dye in brackets): EcoRI (FAM)-ACT/MseI-CAA, EcoRI (VIC)-AGG/MseI-CTA, EcoRI (NED)-ACC/MseI-CTG, EcoRI (FAM)-ACT/MseI-CTA, EcoRI (VIC)-AAG/MseI-CAT and EcoRI (NED)-AGC/MseI-CAG. MseI primers with four selective nucleotides were chosen for the selective amplification. We replicated 35 individuals (16.6%) to exclude non-reproducible bands and to calculate the genotyping error rate according to Bonin et al. (2004). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis at the "Genomic Unit" (Universidad Complutense, Madrid, Spain), on an automated sequencer (3730 DNA Analyzer, PE Applied Biosystems, Foster City, CA, USA) with an internal size standard (GeneScan¯500 LIZ, Applied Biosystems). Raw data were exported to GeneMarker 1.8 (SoftGenetics, PA, USA) for automatic scoring of fragments, after normalization of the profiles. The peaks were considered present when they were over a scoring fluorescence intensity threshold set at 100 relative fluorescent units. The minimum percent of allele peaks to the highest peak in the lane was set at 1%, and the local region percent, that defines the peak detection threshold based upon the percentage of the highest peak in one locus, was set at 1%. The peaks were confirmed by visual inspection of the electropherograms, and were reproducible between independent replicates. Amplified fragments from 75 to 500 base pairs were scored. The results of the scoring were exported as a presence/absence matrix.

Genetic diversity was estimated for each locus and population using the formula $H_D = 1 - \Sigma(x_i^2)$, where x_i is the population frequency of each phenotype "allele" (1 or 0) at locus i (software Arlequin 3.01, Excoffier et al. 2005; see also Nei 1973). H_D was averaged across all loci for subsequent analyses (Lowe et al. 2004). To check for the influence of uneven sample sizes per population, H_D was also calculated for a standardized population size of six randomly selected individuals using the program HP-Rare (Kalinowski 2005). We calculated two additional metrics for each population with FAMD 1.08 (Schlüter and Harris 2006): the total number of AFLP fragments (Fr_t) and the percentage of polymorphic fragments (Fr_p). A chord distance matrix (single-locus chord distance; Cavalli-Sforza and Edwards 1967) among populations was also constructed with FAMD from allele frequency data, estimated in a Bayesian framework with a non-uniform prior derived from among-locus information (Zhivotovsky 1999). We then constructed a majority rule (50%) consensus neighbor joining tree of 1000 bootstrap replicates.

Analysis of phenotypic variation, genetic diversity and correlates

Previous to the examination of the potential drivers of phenotypic and genetic variation, we performed preliminary analyses. First, we checked for homogeneity in phenotypic variation across sampling plots in a subset of phenotypic traits (plant size, plant growth and fecundity). To do so, we performed for each year of data a linear mixed model, with population as a fixed effect and plot as a random effect, and extracted the components of the variance for the random effect (*lme* and *VarCorr* procedures, package *nlme* in R). Then we calculated the Intraclass Correlation Coefficient (ICC; Sokal and Rohlf 1995), which is the ratio between the trait variance among plots and the sum of variances within and among plots. Second, to test the relationship between the various genetic measures, we performed a Pearson's correlation test (*cor* procedure, package *stats* in R) between H_D and Fr_t, and between H_D and Fr_p. Third, we checked for collinearity among the genetic and environmental explanatory variables (H_D, CV in annual density, CV in annual temperature, CV in annual precipitation, PCI and CV in PCI) with an analysis of variance inflation factor (VIF; *vif* procedure, package *car* in R). The three precipitation variables were similar and showed relatively high VIF values (10, 4.9 and 3.1), which can be problematic (Kleinbaum et al. 1988). Thus, we performed a principal component analysis with the precipitation variables (*prcomp* procedure, package *stats* in R), and the first component explained 80.5% of the total variance. Hence we calculated a new variable from the coefficients of this first component, hereafter referred to as "precipitation variability".

We analyzed the factors affecting overall phenotypic variation with a linear mixed model ($n = 11$ populations; *lme* procedure, package *nlme* in R), including H_D, CV in annual density, CV in annual temperature and precipitation variability as covariates, central vs. peripheral position as a fixed factor and the type of phenotypic trait as a random factor (six levels). To analyze the effect of precipitation variability alone on each phenotypic trait, we performed linear models ($n = 11$ populations; *lm* procedure, package *stats* in R). In these analyses, we corrected *p*-values for multiple testing with the false discovery rate method (Benjamini and Hochberg 1995; *p.adjust* procedure, package *stats* in R), which is appropriate for low sample sizes. Finally, we analyzed the factors that might affect H_D with another linear model ($n = 18$ populations), where CV in annual temperature and precipitation variability were the covariates and position was a fixed factor (we did not include density because we only had data for 11 populations and its effect was non-significant).

Results

General patterns of phenotypic variation and genetic diversity

There were differences among phenotypic traits in the magnitude of within-population variation (Fig. 2A), traits originally measured at the individual level (plant size, growth and fecundity) showing higher variation than seed traits (seed mass, mucilage ratio and seed ratio). The three southernmost populations (T, BN and CA) showed in general higher phenotypic variation, but there were no clear differences between central and peripheral populations. The average ICC across years was 0.16 for plant size, 0.12 for plant growth and 0.11 for fecundity, indicating that only a small part of the phenotypic variance took place among plots.

The six AFLP primer combinations generated in genetic analyses 796 unambiguously scorable fragments, EcoRI (FAM)-ACT/MseI-CAA: 164, EcoRI (VIC)-AGG/MseI-CTA: 135, EcoRI (NED)-ACC/MseI-CTG: 78, EcoRI (FAM)-ACT/MseI-CTA: 184, EcoRI (VIC)-AAG/MseI-CAT: 134, EcoRI (NED)-AGC/MseI-CAG: 101, of which all but one were polymorphic. All 179 investigated individuals had unique AFLP profiles. The genotyping error rate amounted to 2.8%. For subsequent genetic analyses, we selected only the polymorphic bands with a percentage variation higher than the error rate (Bonin et al. 2004), obtaining 461 polymorphic bands.

Mean genetic diversity (H_D) was highly and positively correlated to Fr_t ($t_{16} = 9.52$, $p < 0.001$, $r = 0.92$) and Fr_p ($t_{16} = 14.29$, $p < 0.001$, $r = 0.96$). Northern peripheral populations, located in Denmark, Sweden and Scotland, showed the lowest H_D values, whereas central populations had higher values, especially in South Spain (Table 1, Fig. 2B). The standardized H_D, calculated for a

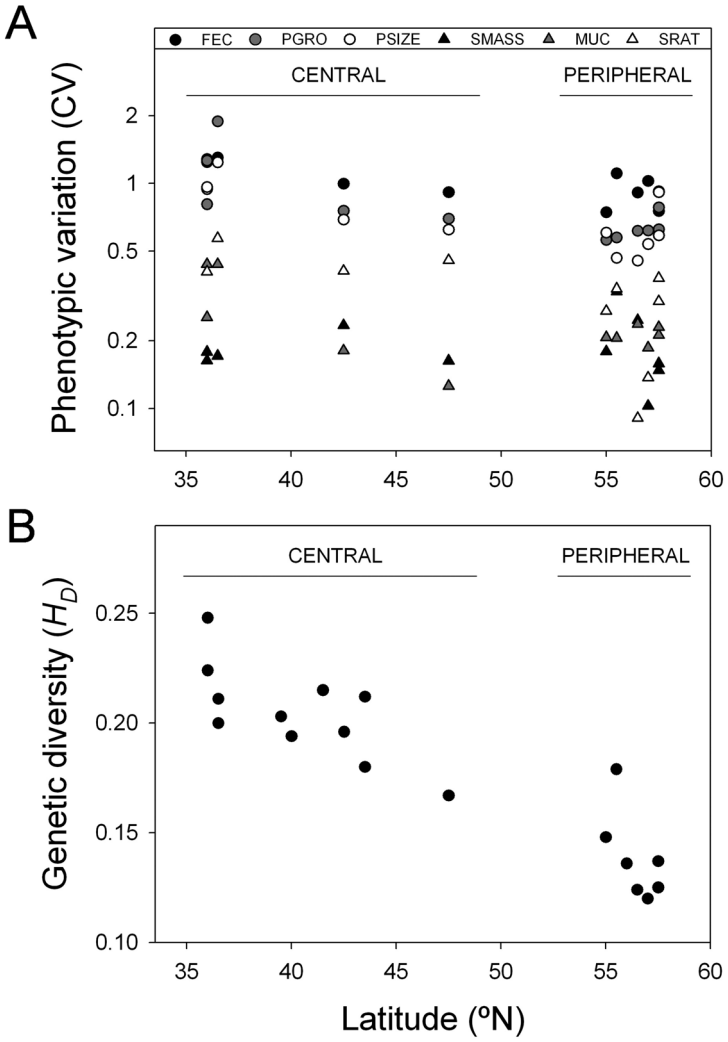

Fig. 2. Phenotypic variation (A), measured with coefficient of variation (CV) in six life history traits, and mean genetic diversity (B), estimated with H_D, in central and northern peripheral populations of *Plantago coronopus* along the latitudinal gradient. In (A), vertical axis is in logarithmic scale for clarity, and abbreviations correspond to traits: fecundity (FEC), plant growth (PGRO), plant size (PSIZE), seed mass (SMASS), mucilage ratio (MUC) and seed ratio (SRAT).

standardized population size of six, showed the same trend, with populations from Denmark, Sweden and Scotland, generally showing the lowest values ($H_D < 1.3$), and central populations showing the highest ones, especially South Spain populations T and BN ($H_D > 1.5$). This indicates that our results were reliable despite low sample sizes for some populations.

The consensus neighbor joining tree (Fig. 3) showed that the northern peripheral populations, located in Denmark, Sweden and Scotland were genetically more similar to one another than to other populations, being clustered with high support (bootstrap support, BS 94%). These populations showed also very high genetic similarities (BS 100%) with the Atlantic populations (F, C and NA), and to a lesser extent (BS 67%) with the French Mediterranean populations (FSM and SET). A clade containing the above mentioned populations formed a basal polytomy with the remaining populations from East and South Spain, grouped in several clades.

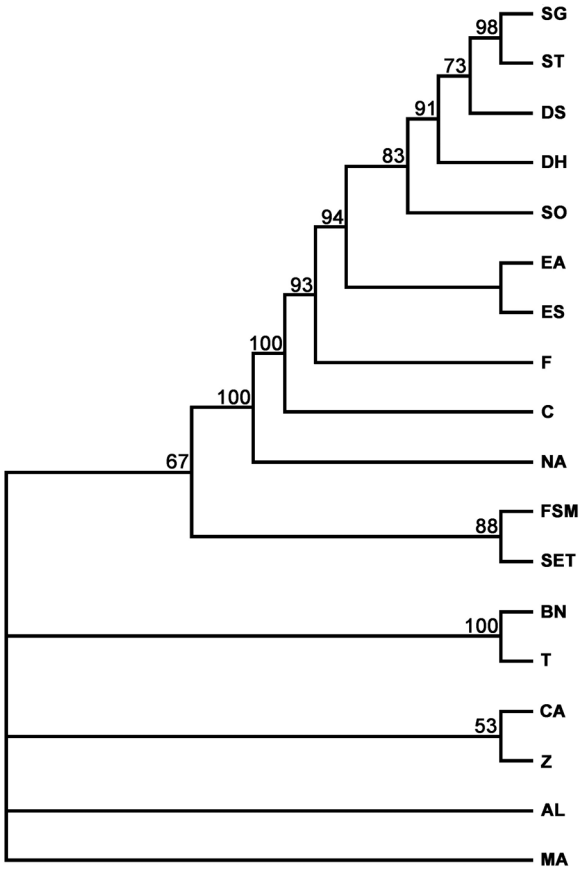

Fig. 3. Neighbor-joining tree showing phylogenetic relationship among populations of *Plantago coronopus*. Values at the nodes are percentages from 1000 bootstrap replicates. Population acronyms are as in Table 1.

Correlates of phenotypic variation and genetic diversity

Precipitation variability showed a gradual decline in the latitudinal gradient, from central to northern peripheral populations (Fig. 1B). Phenotypic variation was significantly correlated to precipitation variability, but density variability, temperature variability, H_D and position showed no significant effects (Table 2). The effect of precipitation variability on phenotypic variation differed to some extent depending on the phenotypic trait (Fig. 4). Precipitation variability was significantly and positively correlated with variation in plant size, fecundity, growth, mucilage ratio and seed ratio, the latter showing the lowest R^2 value. Variation in seed mass was not significantly affected by precipitation variability.

Table 2. Analyses of correlates of phenotypic variation and genetic diversity in *Plantago coronopus*. Fixed effects correspond to precipitation variability (PrVar), coefficient of variation in annual density (CV_{dens}) and in annual temperature (CV_{temp}), mean genetic diversity (H_D) and position (central vs. peripheral).

Fixed effects	Coefficient	t	p
Phenotypic variation			
PrVar	2.22 ± 0.72	3.10_{54}	**0.003**
CV_{dens}	0.05 ± 0.09	0.54_{54}	0.592
CV_{temp}	−1.46 ± 1.07	-1.36_{54}	0.179
H_D	−0.21 ± 1.23	-0.17_{54}	0.867
Position	0.20 ± 0.13	1.49_{54}	0.142
Genetic diversity			
PrVar	0.21 ± 0.13	1.63_{14}	0.126
CV_{temp}	0.11 ± 0.21	0.53_{14}	0.604
Position	−0.05 ± 0.02	-1.91_{14}	**0.076**†

Notes: Significant p-values (<0.05) or those close to significance (<0.08) appear in boldface. The analysis of phenotypic variation included a random effect of type of phenotypic trait (six levels); standard deviation was 0.31 for the random intercept and 0.17 for the error.

† $p < 0.001$ if PrVar and CV_{temp} (non-significant) are removed from the model.

The analysis of genetic diversity showed that position exerted an effect on H_D close to statistical significance, whereas neither precipitation variability nor temperature variability had a significant effect (Table 2). When non-significant covariates were removed from the analysis, the effect of position on H_D became highly significant ($t_{16} = -6.41$, $p < 0.001$).

Discussion

Understanding species' life history variability requires the identification of the main evolutionary and demographic processes operating on populations (Lynch et al. 1999, Reed and Frankham 2001, Mitchell-Olds and Schmitt 2006). Our analyses of a widespread plant across its latitudinal gradient in Europe showed that phenotypic variation measured on fitness-related traits and genetic diversity inferred from molecular markers were unrelated, and seemed to be influenced by different processes. Phenotypic variation within populations was mainly shaped by precipitation variability, suggesting adaptive variation, whereas genetic diversity was correlated with the central vs. peripheral position of populations, invoking the demographic history

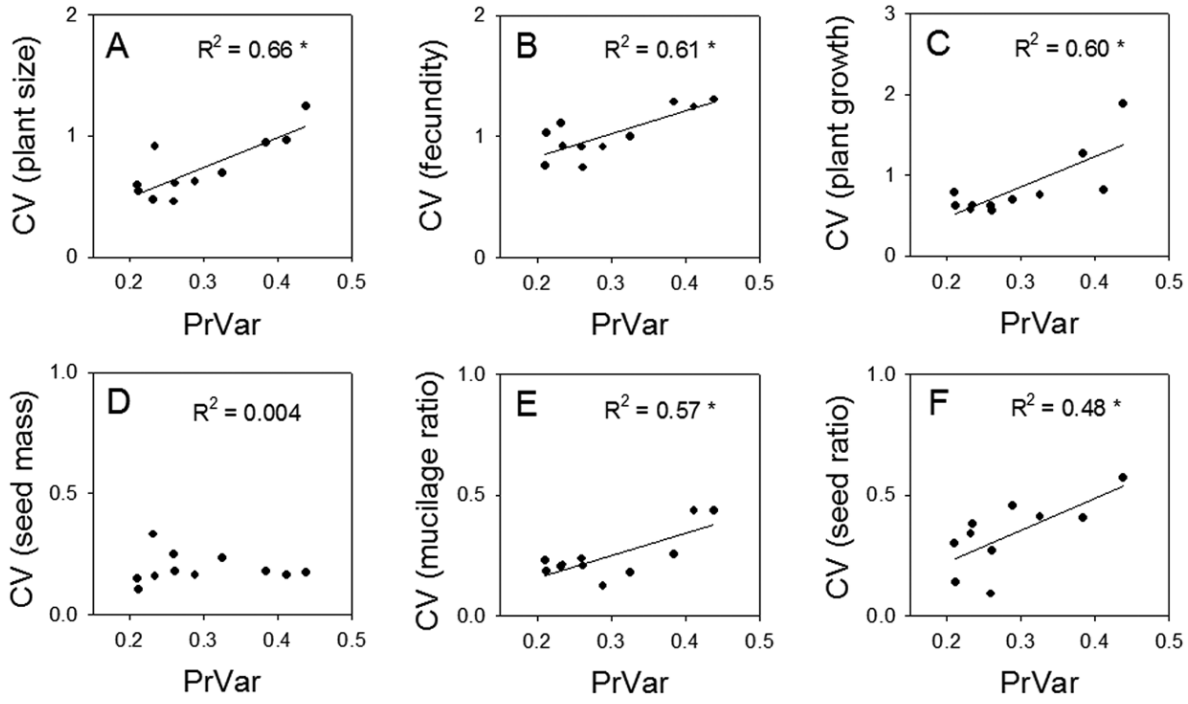

Fig. 4. Relationship between phenotypic variation in life history traits within populations of *Plantago coronopus*, measured as coefficient of variation (CV) among individuals, and precipitation variability (PrVar; see *Material and Methods* for details on its estimation). Traits are (A) plant size, (B) fecundity, (C) plant growth, (D) seed mass, (E) mucilage ratio and (F) seed ratio. R^2 values are given for each regression analysis, and the statistical significance is represented by asterisks: $^*p < 0.05$ (corrected by the false discovery rate method).

of the species. Several studies have shown a similar lack of correspondence between genetic diversity and variation in life history traits in plants (e.g., Waldmann and Andersson 1998, McKay et al. 2001), and Reed and Frankham (2001) concluded that molecular measures of genetic diversity constituted poor predictors of adaptive genetic variability. However, Gömöry et al. (2013) found a significant correlation between phenotypic and genetic intra-population variation throughout the distribution range of beech in Europe, highlighting the need for more large-scale studies across different plant life forms.

Phenotypic variation in *P. coronopus* was highly correlated to temporal fluctuations in local precipitation, suggesting that selective forces have promoted life history variability within populations. These results indicate that variability in certain environmental parameters, which has already been proposed as a surrogate for trait divergence among populations (Knapp and Rice 1998, Bekessy et al. 2003), may also be used to infer evolutionary potential within populations. Evolutionary potential confer populations better means to face local and global changes, and its use might be very informative when predicting future population performance, in particular with conservation tools such as species distribution models (Botkin et al. 2007). On the other hand, the reliability of environmental variation as an indicator for evolutionary potential must not always be taken for granted, because stochasticity could sometimes result in a lower demographic performance of populations (Lewontin and Cohen 1969) that would hamper the development of their evolutionary potential. However, populations of *P. coronopus* with higher environmental (and phenotypic) variation show similar growth rates than those subject to lower environmental variation (Villellas et al. 2013*b*), suggesting no demographic limitations for evolutionary potential.

Our analyses highlight the importance of precipitation over other environmental factors

in shaping life history variability in *P. coronopus*, which applies well to a short-lived, frequently pioneer herb. In addition, this species has a largely Mediterranean distribution, and one of the most distinctive factors of Mediterranean ecosystems is precipitation unpredictability. Seed-related traits, whose variation across populations is mediated by a trade-off between fecundity and the resources allocated to seed tolerance to stress (Villellas and García 2013), seem to be particularly sensitive to precipitation regime. Seed mass was the only trait in our study that remained virtually unaffected by environmental variability, but considering the seed dimorphism of *P. coronopus*, variation in average seed mass may also be regulated in practice through the ratio between big basal and small apical seeds. The correlation between precipitation and variation in plant size and growth, in turn, may take place through different demands on resource acquisition, or indirectly through the close association between plant size and seed production (Villellas and García 2013). Overall, phenotypic variability was positively affected by environmental variability, although some differences found among life history traits suggest the importance of encompassing different parts of species' life cycles to obtain reliable results. Fitness-related traits are usually of more interest than purely morphological characters (Reed and Frankham 2001).

Phenotypic variation was estimated in this study in natural populations, and thus it may include the effect of phenotypic plasticity besides that of adaptive genetic variation. Indeed, both sources of variation seem to be present in *P. coronopus*: Wolff (1991*a*, 1991*b*) reported significant levels of genetic variation within populations, but also found evidences of plasticity (see also Waite and Hutchings 1982, Smekens and van Tienderen 2001). Yet phenotypic plasticity itself can also be considered a trait where selection acts (Schlichting 1986, Pigliucci 2005), so we expect both genetic variation and plasticity to increase under selective forces such as environmental variability (Lande 2009, Dawson et al. 2011). In this context, analyses of heritability across the range of *P. coronopus* would help to quantify both phenomena separately, although the influence of phenotypic plasticity was minimized on purpose in our study by using mean yearly values of variation in life history traits.

Plantago coronopus showed values of genetic diversity similar to other widespread short-lived perennials, and higher than plants with similar life forms but narrower ranges (Hamrick and Godt 1996). Genetic diversity within populations was negatively correlated with peripherality, populations showing a decline in H_D from the range centre in the Mediterranean region to the edge in countries of North Europe. Such decline in genetic diversity in peripheral populations is a frequent pattern in comparative analyses across species' distributions (see Eckert et al. 2008 for review). Differences in genetic diversity along geographical gradients are commonly associated with processes such as genetic drift, reduced gene flow and founder effects (Lesica and Allendorf 1995, Vucetich and Waite 2003), which could have eroded the genetic pool in the northern range margin of *P. coronopus* during post-glacial migrations (Hewitt 2000). The existence of a distinct phylogenetic clade containing all the northern populations analyzed in this study supports this hypothesis. It is interesting to note, though, that in contrast with past demographic events, northern peripheral populations of *P. coronopus* show at present higher densities and comparable population sizes and growth rates than central ones (Villellas et al. 2013*a*, Villellas et al. 2013*b*). Similar divergences between present demographic and genetic patterns have been indeed reported for other perennial herbs, such as *Lychnis viscaria* (Lammi et al. 1999) and *Cirsium heterophyllum* (Jump et al. 2003), and call for caution when using information from one component of species' biology to infer patterns in other components.

The breeding system may have a significant role in the genetic structure of populations (Loveless and Hamrick 1984), and could also help to explain the lower genetic diversity found in northern periphery of *P. coronopus*. Preliminary data from greenhouse bagging experiments suggest higher probability of selfing in northern populations (M. B. García, *personal observation*), which could have contributed to decrease genetic variability. However, the species' anemophily could mask the effects of these differences across the range, and comprehensive studies of geographic variation in selfing rates in *P. coronopus* would be needed to confirm and further interpret

the importance of breeding system in the current genetic pattern.

The present study included central and northern peripheral populations of *P. coronopus*. Northernmost populations of European taxa are frequently of high evolutionary interest, owing to their role in leading species' range expansions after last glaciations (Hewitt 2000). However, other peripheral populations such as those in the rear edge may also be very important (Hampe and Petit 2005), and it remains an open question whether the results of this study would be similar after the inclusion of southern peripheral populations in the analyses. This seems likely, since reduced genetic diversity towards range margins is a consistent result in the literature irrespective of which periphery is analyzed (Eckert et al. 2008).

The combination of ecological, phenotypic and genetic information is crucial for analyzing the pattern and causes of trait variation within taxa, and for evaluating their future adaptive potential (Crandall et al. 2000, Bekessy et al. 2003, Narbona et al. 2010). Our study of a widespread plant at a continental scale showed that phenotypic variation within populations in life history traits was neither correlated with genetic diversity inferred from molecular markers, nor with the position of populations within the species' range. Instead, phenotypic variation was strongly associated to precipitation variability, suggesting that populations may have a higher adaptive potential in variable rather than stable environments. The use of environmental variability as a proxy for evolutionary potential could thus be considered in tools such as niche-models, to improve the management of biodiversity in an environmentally changing world.

Acknowledgments

This study was funded by the Spanish Ministry of Science and Innovation by means of National Projects to M.B.G. (CGL2006-08507; CGL2010-21642) and A.T. (CGL2009-08713), and a FPU scholarship to J.V. We are grateful to A. Adsuar, A. Barcos, R. Braza, R. Castillo, M.L. Dehesa, R. Corrià, F. Domínguez, R. Forrest, M.C. García, E. López, J. Martínez, E. Morán, C. Niklasson, F. Ojeda, J.M. Olesen, S. Palacio, I. Pardo, A. Pérez, A. Primilla, C. Pérez, P. Sánchez, A. Taboada, M. Talavera and A. Vale for their valuable help in field and laboratory work through years. We thank R. Braza, V. Simón, J. Thompson and A. Traveset for plant material for genetic analyses, R. Braza for phenotypic data from population F and BN, and M. Pazos for her assessment in statistical analyses. X. Picó, J. Arroyo, T. Iriondo, P. Vargas and three anonymous referees provided very helpful comments on the manuscript.

Literature Cited

Bekessy, S. A., R. A. Ennos, M. A. Burgman, A. C. Newton, and P. K. Ades. 2003. Neutral DNA markers fail to detect genetic divergence in an ecologically important trait. Biological Conservation 110:267–275.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society B 57:289–300.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetics studies. Molecular Ecology 13:3261–3273.

Bonin, A., D. Ehrich, and S. Manel. 2007. Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. Molecular Ecology 16:3737–3758.

Botkin, D. B., et al. 2007. Forecasting the effects of global warming on biodiversity. BioScience 57:227–236.

Bradshaw, A. D. 1991. The Croonian Lecture—1991. Genostasis and the limits to evolution. Philosophical Transactions of the Royal Society B 333:289–305.

Bradshaw, A. D., and T. McNeilly. 1991. Evolutionary response to global climatic change. Annals of Botany 67:5–14.

Braza, R., J. Arroyo, and M. B. García. 2010. Natural variation of fecundity components in a widespread plant with dimorphic seeds. Acta Oecologica-International Journal of Ecology 36:471–476.

Braza, R., and M. B. García. 2011. Spreading recruitment over time to cope with environmental variability. Plant Ecology 212:283–292.

Butlin, R. K., and T. Tregenza. 1998. Levels of genetic polymorphism: marker loci versus quantitative traits. Philosophical Transactions of the Royal Society B 353:187–198.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. American Journal of Human Genetics 19:233–257.

Chater, A. O., and D. Cartier. 1976. *Plantago* L. Pages 38–44 *in* T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb, editors. Flora Europaea. Cambridge University Press, Cambridge, UK.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace,

and R. K. Wayne. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology and Evolution 15:290–295.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond predictions: biodiversity conservation in a changing climate. Science 332:53–58.

Dowling, R. E. 1933. The reproduction of *Plantago coronopus*: an example of morphological and biological seed dimorphism. Annals of Botany 47:861–872.

Eckert, C. G., K. E. Samis, and S. C. Lougheed. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. Molecular Ecology 17:1170–1188.

Etterson, J. R. 2004. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. 1. Clinal patterns of selection along an environmental gradient in the great plains. Evolution 58:1446–1458.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1:47–50.

Frankham, R. 1995. Conservation genetics. Annual Review of Genetics 29:305–327.

Galloway, L. F., and C. B. Fenster. 2000. Population differentiation in an annual legume: local adaptation. Evolution 54:1173–1181.

Gömöry, D., B. Comps, L. Paule, and G. Von Wühlisch. 2013. Allozyme and phenotypic variation in beech (*Fagus sylvatica* L.): Are there any links? Plant Biosystems 147:265–271.

Haig, S. M. 1998. Molecular contributions to conservation. Ecology 79:413–425.

Hampe, A., and R. J. Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Ecology Letters 8:461–467.

Hamrick, J. L., and M. J. W. Godt. 1996. Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society B 351:1291–1298.

Harper, J. L., and R. A. Benton. 1966. Behaviour of seeds in soil II. The germination of seeds on the surface of a water supplying substrate. Journal of Ecology 54:151–166.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Holderegger, R., U. Kamm, and F. Gugerli. 2006. Adaptive vs. neutral genetic diversity: implications for landscape genetics. Landscape Ecology 21:797–807.

Hultén, E., and M. Fries. 1986. Atlas of North European vascular plants. North of the Tropic of Cancer. Maps 1–996. Koeltz Scientific Books, Konigstein, Germany.

Imbert, E. 2002. Ecological consequences and ontogeny of seed heteromorphism. Perspectives in Plant Ecology Evolution and Systematics 5:13–36.

Joshi, J. et al. 2001. Local adaptation enhances performance of common plant species. Ecology Letters 4:536–544.

Jump, A. S., and J. Peñuelas. 2005. Running to stand still: adaptation and the response of plants to rapid climate change. Ecology Letters 8:1010–1020.

Jump, A. S., F. I. Woodward, and T. Burke. 2003. *Cirsium* species show disparity in patterns of genetic variation at their range-edge, despite similar patterns of reproduction and isolation. New Phytologist 160:359–370.

Kalinowski, S. T. 2005. HP-Rare: A computer program for performing rarefaction on measures of allelic diversity. Molecular Ecology Notes 5:187–189.

Kleinbaum, D. G., L. L. Kupper, and K. E. Muller. 1988. Applied regression analysis and other multivariables methods. PWS, Boston, Massachusetts, USA.

Knapp, E. E., and K. J. Rice. 1998. Comparison of isozymes and quantitative traits for evaluating patterns of genetic variation in purple needlegrass (*Nassella pulchra*). Conservation Biology 12:1031–1041.

Lammi, A., P. Siikamaki, and K. Mustajarvi. 1999. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. Conservation Biology 13:1069–1078.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. Journal of Evolutionary Biology 22:1435–1446.

Lawton-Rauh, A. 2008. Demographic processes shaping genetic variation. Current Opinion in Plant Biology 11:103–109.

Lesica, P., and F. W. Allendorf. 1995. When are peripheral-populations valuable for conservation. Conservation Biology 9:753–760.

Lewontin, R. C. and D. Cohen. 1969. On population growth in a randomly varying environment. Proceedings of the National Academy of Sciences USA 62:1056–1060.

Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15:65–95.

Lowe, A., S. Harris, and P. Ashton. 2004. Ecological genetics: design, analysis, and application. Blackwell, Oxford, UK.

Lynch, M., M. Pfrender, K. Spitze, N. Lehman, J. Hicks, D. Allen, L. Latta, M. Ottene, F. Bogue, and J. Colbourne. 1999. The quantitative and molecular genetic architecture of a subdivided species. Evolution 53:100–110.

McKay, J. K., J. G. Bishop, J. Z. Lin, J. H. Richards, A. Sala, and T. Mitchell-Olds. 2001. Local adaptation

across a climatic gradient despite small effective population size in the rare sapphire rockcress. Proceedings of the Royal Society B 268:1715–1721.

Mitchell-Olds, T., and J. Schmitt. 2006. Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. Nature 441:947–952.

Narbona, E., B. Guzman, J. Arroyo, and P. Vargas. 2010. Why are fruit traits of *Cistus ladanifer* (Cistaceae) so variable: A multi-level study across the western Mediterranean region. Perspectives in Plant Ecology Evolution and Systematics 12:305–315.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences USA 70:3321–3.

Oliver, J. E. 1980. Monthly precipitation distribution: a comparative index. Professional Geographer 32:300–309.

Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology Evolution and Systematics 37:637–669.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: where are we going now? Trends in Ecology and Evolution 20:481–486.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.

Roldán-Ruiz, I., J. Dendauw, E. Van Bockstaele, A. Depicker, and M. De Loose. 2000. AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding 6:125–134.

Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. Annual Review of Ecology and Systematics 17:667–693.

Schlüter, P. M., and S. A. Harris. 2006. Analysis of multilocus fingerprinting data sets containing missing data. Molecular Ecology Notes 6:569–572.

Silvertown, J., M. Franco, and E. Menges. 1996. Interpretation of elasticity matrices as an aid to the management of plant populations for conservation. Conservation Biology 10:591–597.

Smekens, M. J., and P. H. van Tienderen. 2001. Genetic variation and plasticity of *Plantago coronopus* under saline conditions. Acta Oecologica 22:187–200.

Sokal, R., and F. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. Third edition. W. H. Freeman, New York, New York, USA.

Strong, C. W. 1966. An improved method of obtaining density from line-transect data. Ecology 47:311–313.

van Tienderen, P. H., A. A. de Haan, C. G. van der Linden, and B. Vosman. 2002. Biodiversity assessment using markers for ecologically important traits. Trends in Ecology and Evolution 17:577–582.

Villellas, J., J. Ehrlén, J. M. Olesen, R. Braza, and M. B. García. 2013*a*. Plant performance in central and northern peripheral populations of the widespread *Plantago coronopus*. Ecography 36:136–145.

Villellas, J., and M. B. García. 2013. The role of the tolerance-fecundity trade-off in maintaining intraspecific seed trait variation in a widespread dimorphic herb. Plant Biology 15:899–909.

Villellas, J., W. F. Morris, and M. B. García. 2013*b*. Variation in stochastic demography between and within central and peripheral regions in a widespread short-lived herb. Ecology 94:1378–1388.

Volis, S., S. Mendlinger, L. Olsvig-Whittaker, U. N. Safriel, and N. Orlovsky. 1998. Phenotypic variation and stress resistance in core and peripheral populations of *Hordeum spontaneum*. Biodiversity and Conservation 7:799–813.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

Vucetich, J. A., and T. A. Waite. 2003. Spatial patterns of demography and genetic processes across the species' range: Null hypotheses for landscape conservation genetics. Conservation Genetics 4:639–645.

Waite, S., and M. J. Hutchings. 1982. Plastic energy allocation patterns in *Plantago coronopus*. Oikos 38:333–342.

Waldmann, P., and S. Andersson. 1998. Comparison of quantitative genetic variation and allozyme diversity within and between populations of *Scabiosa canescens* and *S. columbaria*. Heredity 81:79–86.

Walther, G. R., L. Hughes, P. Vitousek, and N. C. Stenseth. 2005. Consensus on climate change. Trends in Ecology and Evolution 20:648–649.

Westoby, M., E. Jurado, and M. Leishman. 1992. Comparative evolutionary ecology of seed size. Trends in Ecology and Evolution 7:368–372.

Wolff, K. 1991*a*. Analysis of allozyme variability in three *Plantago* species and a comparison to morphological variability. Theoretical and Applied Genetics 81:119–126.

Wolff, K. 1991*b*. Genetic analysis of morphological variability in three *Plantago* species with different mating systems. Theoretical and Applied Genetics 81:111–118.

Wolff, K., B. Friso, and J. M. M. Vandamme. 1988. Outcrossing rates and male-sterility in natural populations of *Plantago coronopus*. Theoretical and Applied Genetics 76:190–196.

Zhivotovsky, L. A. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Molecular Ecology 8:907–913.