Genetic differentiation can be predicted from observational data for reproductive but not vegetative traits in a widespread short-lived plant

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Abstract

Phenotypic plasticity can mask population genetic differentiation, reducing the predictability of trait-environment relationships. In short-lived plants, reproductive traits may be more genetically determined due to their direct impact on fitness, whereas vegetative traits may show higher plasticity to buffer short-term perturbations. Combining a multi-treatment greenhouse experiment with global field observations for the short-lived Plantago lanceolata, we 1) disentangled the genetic and plastic responses of functional traits to a set of environmental drivers and 2) assessed the utility of trait-environment relationshisps inferred from observational data for predicting genetic differentiation. Reproductive traits showed distinct genetic differentiation that was highly predictable from observational data, but only when correcting traits for differences in their (labile) biomass component. Vegetative traits showed higher plasticity and contrasting genetic and plastic responses, leading to unpredictable trait patterns. Our study suggests that genetic differentiation may be inferred from observational data only for the traits most closely related with fitness.

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Abstract

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to a set of environmental drivers and 2) assessed the utility of trait-environment relationshisps inferred from observational data for predicting genetic differentiation. Reproductive traits showed distinct genetic differentiation that was highly predictable from observational data, but only when correcting traits for differences in their (labile) biomass component. Vegetative traits showed higher plasticity and contrasting genetic and plastic responses, leading to unpredictable trait patterns. Our study suggests that genetic differentiation may be inferred from observational data only for the traits most closely related with fitness.

Introduction

Functional traits are morphological, physiological or phenological features of organisms that influence the components of fitness, i.e. survival and reproduction (Reich *et al* . 2003, Violle *et al* . 2007, Adler *et al* . 2014). Intraspecific variation in functional traits is widely documented and has important implications for population dynamics (Hughes *et al* . 2008, Villellas & García 2017), evolutionary trajectories (Moran et el. 2016, Caruso *et al.* 2020), community assembly (Violle *et al* . 2012, Des Roches*et al* . 2018), and ecosystem functioning (Crutsinger *et al* . 2006, Breza *et al* . 2012). Disentangling the environmental drivers of functional trait variation is thus of great ecological and evolutionary interest (Liancourt et al. 2013, van de Pol et al 2016, Bruelheide et al 2018), and can improve predictions of species responses to global change (Benito Garzón *et al* . 2011, Violle *et al* . 2014, Moran *et al* . 2016).

The predominant approach to identify the drivers of functional trait variation has relied upon assembling large databases of observed*in situ* trait variation (e.g., Enquist *et al* . 2016, Moran*et al* . 2016, Iversen *et al* . 2017, Kattge *et al* . 2020) and the association of these trait values with candidate environmental drivers. However, interpreting trait-environment relationships inferred from observational field datasets requires understanding the processes underlying trait variation. Intraspecific trait variation observed *in situ* among populations may arise from genetic differentiation and/or phenotypic plasticity (Chevin *et al* . 2010, Merilä & Hendry 2014). Across large environmental gradients, genetic differentiation among populations can result from adaptation to local conditions (but see the role of neutral and historical processes in Keller *et al* . (2009), Santagelo *et al* . (2018)). Genetically determined traits are thus expected to show correlations with the source environment. However, genetic differentiation might be obscured by phenotypic plasticity (which can also be adaptive; see Matesanz *et al* . 2010, Palacio-López *et al.* 2015), reducing the consistency of trait-environment relationships across environmental contexts.

Combining experimental and *in situ* field data enables us to assess the potential uses and misuses of observational trait datasets. A common way to partition trait variation is through a common garden experiment (Clausen *et al.* 1940, MacColl 2011, Franks *et al* . 2014). Specifically, by growing offspring from multiple provenances together in a set of controlled conditions, we can disentangle the effects of source environments (leading to genetic differentiation) from those of exposure environments (driving phenotypic plasticity). Notably, by evaluating the different scenarios involving genetic and plastic effects on traits, we can assess the utility of observational data for predicting genetic differentiation (Fig. 1). For example, a predominance of genetic over plastic effects decreases the relative importance of genotype-by-environment interactions, and increases the predictability of trait values from average environmental conditions of source populations (Fig. 1a,f). In contrast, a high level of plasticity causes traits to be more strongly determined by the exposure environment, decreasing trait predictability from source environment (Fig. 1b,g,h). Source and exposure environments can have similar or opposing effects on traits (Fig. 1c-e), with opposing effects known as countergradient variation (Conover & Schultz 1995, Conover *et al* . 2009). Countergradient variation may lead to an apparent absence of trait variation among populations in the field (Fig. 1d), or even to patterns counter to those of genetic differentiation (Fig. 1e).

The role of genetic differentiation and phenotypic plasticity on intraspecific variation differs among functional traits (Albert *et al* . 2010a, Funk *et al* . 2017, Münzbergová *et al* . 2017). Species may show evolutionary conservation of the traits most directly related to fitness through genetic differentiation (Scheiner 1993, Stearns & Kawecki 1994, Sih 2004), and instead display plasticity in underlying morphological or physiological traits, to buffer environmental perturbations (Sultan 1995, Richards *et al* . 2006). This view is also supported by demographic studies finding that the most influential processes on population growth rate show relatively

low variability (Pfister 1998, Burns *et al* . 2010, Hilde *et al* . 2020; but see McDonald *et al* . 2017). In plants, vegetative traits often show higher plasticity than reproductive traits (Bradshaw 1965, Schlichting & Levin 1984, Frazee & Marquis 1994). For example, both biomass and reproductive investment per unit biomass determine reproduction, but while biomass is expected to show high plasticity due to its influence on several demographic parameters (Harper 1977), reproductive investment per unit biomass may be more conserved. This might be especially true for short-lived taxa, in which reproduction usually has the highest influence on population growth (Silvertown*et al* . 1996, García *et al* . 2008, Shefferson and Roach 2012). Yet reproductive investment may appear to be strongly driven by plasticity if evaluated at the whole plant level, due to the inclusion of a more labile biomass-dependent component (Biere 1995, Weiner*et al* . 2009). It is important therefore to partition reproductive traits into biomass dependent and independent components, to better understand the role of genetic differentiation and plasticity.

Despite the abundance of studies analysing trait-environment relationships at local or regional scales (e.g., Oleksyn *et al* . 1998, Villellas & García 2013, Preite *et al* . 2015, Münzbergová*et al* . 2017), there is a critical gap in knowledge about the drivers of intraspecific trait variation at global scales (MacColl 2011). Environmental effects may be difficult to detect if drivers are assessed independently from each other, or if studies omit significant parts of a species' environmental niche (Matesanz *et al* . 2010, Hulme & Barrett 2013, Shipley *et al.* 2016). Widespread plants offer a unique opportunity to unravel the multiple drivers of trait variation from local to global scales. While some studies have analysed trait genetic differentiation and plasticity across species' ranges (e.g., Joshi *et al* . 2001, Maron *et al* . 2004, Alexander*et al* . 2012), we lack global assessments of the responses of different types of traits to multiple environmental drivers using the combined power of experimental and observational data.

Here we analyse responses of vegetative vs. reproductive traits of the short-lived herb *Plantago lanceolata* to a set of environmental drivers, both in a common garden and in the field. By growing individuals from multiple populations under several light and water conditions, we tested 1) whether vegetative traits (plant biomass, specific leaf area and root:shoot ratio) showed higher levels of plasticity than reproductive traits (probability of flowering and fecundity), and 2) whether reproductive traits showed more consistent population genetic differentiation across exposure treatments than vegetative traits, and higher consistency between genetic and plastic responses. To account for the potential size-dependency of plant reproductive investment, we examined reproductive traits by both including and excluding plant biomass as a covariate in the analyses. Finally, by comparing experimental results with trait-environment relationships detected from a global-scale observational survey, we evaluated 3) whether observational data provided a better prediction of genetic differentiation for reproductive than vegetative traits.

Material and methods

Study species

Plantago lanceolata L. (Plantaginaceae) is a short-lived perennial herb with a typical lifespan of 2-5 yr (Lacey *et al* . 2003, Roach 2003), although some individuals may exceed 12 yr (Cavers*et al* . 1980). Plants have one or more vegetative rosettes. Inflorescences emerge in late spring or summer; flowers are mostly self-incompatible and both wind- and insect-pollinated, (Clifford 1962, Sagar & Harper 1964). *Plantago lanceolata* is native to Europe, Western Asia and North Africa, although it has been introduced worldwide, mainly during the eighteenth and nineteenth centuries (Hooker 1867, Cavers *et al* . 1980, Meyers & Liston 2008). The species occurs in a range of mostly open habitats, such as grasslands, sand dunes or disturbed sites, showing a wide environmental niche (Fig. 2; Sagar & Harper 1964).

Field sampling of source populations

Populations of *P. lanceolata* included in this study were part of the coordinated project PlantPopNet (Buckley *et al.* 2019). In the growing seasons of 2015 and 2016, we sampled 46 populations across the species' range (29 native and 17 non-native populations; Fig. 2, Table S1, S2 in Supporting Information), spanning a wide range of climatic, management and plant community conditions, and a wide range of genoypes (Smith *et al.* 2020). For each population, we monitored all individual plants within 0.25 m^2 plots along 10 m transects

until we reached a minimum of 100 plants (Buckley *et al* . 2019). We recorded for each plant the number of rosettes and the flowering status (flowering vs. non-flowering). For each rosette, we recorded the number of leaves, the size of the longest leaf, the number of flowering stems if any and the length of the most developed inflorescence. We used these measurements to estimate biomass and total inflorescence length at the whole plant level (see further details on Appendix S1). In a subset of populations and outside the monitoring plots, we collected leaves for the estimation of specific leaf area (SLA; 25 populations) and seeds for the greenhouse experiment (15 populations; Table S1, Appendix S1).

Environmental conditions in source populations

To analyse the effects of environmental conditions of source populations on traits, we collected information on climate, land-use and vegetation for each location (Table S2). Mean annual values and seasonality (coefficient of variation in monthly values) for temperature, precipitation and moisture index were obtained from the BioClim database (Kriticos *et al* . 2012, Fick & Hijmans 2017). We used the highest resolution available for temperature and precipitation (30 s) and for moisture index (10 min). In the field, we recorded whether populations were subject to mowing or not, and estimated the percentage of vegetation cover and bare ground for four random plots per population. In two opposite corners of the plots, we quantified community vegetation height as the height at which a pole was completely obscured by vegetation, looking from a distance of ca. 4 m.

To avoid collinearity in environmental predictor variables (climate, land-use and vegetation data), we performed a Principal Component Analysis (*psych* package in R; R Core Team 2017, Revelle 2018). We performed a second, orthogonal rotation that improved the interpretation of the components (Quinn & Keough 2002). The first three rotated components explained 70.4% of the variance (Fig. S1, Table S1). The first component (hereafter "Aridity") was positively associated with low mean and high seasonality in precipitation and mean moisture index. The second component ("Temperature") was positively associated with high mean and low seasonality in temperature. The third component ("Vegetation cover") was positively associated with high percent vegetation cover, greater height of vegetation and low percent bare ground cover. We used these rotated components and the binary factor Mowing to test the effects of source climate (Aridity, Temperature), vegetation (Vegetation cover) and land-use (Mowing) on trait variation. We used t-tests to analyse differences between native and non-native populations in the rotated components and the underlying variables (effects of native/non-native range on Mowing were tested with a Generalized Linear Model using Binomial errors; *stats* package, R Core Team 2017).

Greenhouse experiment

We performed a common garden experiment in a greenhouse with a subset of 15 populations (Fig. 2, Table S1). The experiment spanned almost the entire geographical and environmental native range, and included three non-native populations to increase the breadth of source environmental conditions (Appendix S2). We pooled all the collected seeds at the population level. We sowed 2,728 seeds (180-200 per population) and obtained 1485 seedlings in individual pots after 25 days. Seedlings were then exposed to treatments with two levels of water supply crossed with three levels of light availability (one block with six treatment combinations). We used 18 seedlings per treatment combination for each population (except for BG, RO and TW populations with, respectively, 14, 10 and 8 seedlings per treatment combination; Table S1). The treatments were chosen to compare their effects with those of two source environmental drivers: Aridity (related to water availability) and Vegetation cover (related to light availability). These treatments also represent parameters likely affected by climate and land-use change. For the water treatments, half of the plants were watered every three days ("wet" treatment), and the other half every nine days ("dry" treatment), by flooding the supporting trays until soil was soaked with water. Each water treatment level was divided into three light levels: 1) 100% light, 2) 64% light and 3) 33% light (Appendix S2). Watering and light levels were designed to span a wide environmental range, characteristic of cosmopolitan plants.

To collect trait data in the greenhouse, we measured plant leaves, flowering status and inflorescences 2.5 months after the onset of treatments in the same way as in field populations. To account for possible

maternal effects, usually more manifest in early life stages (Roach & Wulff 1987), control leaf measurements were also taken 1 month after the onset of treatments. At the end of the experiment, the longest healthy leaf was collected from each of 10 individuals per population and treatment combination. Leaves were scanned to estimate leaf area, oven-dried (60^{o}C), and weighed to calculate SLA. Root:shoot ratio (RSR) was also calculated in the individuals used for SLA measurements, but only for eight populations (Table S1) and excluding the intermediate light treatment due to logistical constraints. To measure RSR, the remaining leaves and the roots were collected, roots were washed, and both leaves and roots were oven-dried.

Analyses of trait variation in greenhouse and field conditions

We used data from three vegetative and two reproductive traits to analyse the drivers of intraspecific variation in greenhouse and field conditions. Vegetative traits were biomass, SLA and RSR (the latter only measured in greenhouse conditions), and reproductive traits were probability of flowering and fecundity. Biomass was estimated for all greenhouse and field individuals using leaf measurements and an equation obtained for a subset of plants (Appendix S3). Probability of flowering was modelled as a binary variable with data from the flowering vs. non-flowering plant status. Total inflorescence length was used as a proxy for fecundity, as we found a strong correlation between total inflorescence length and seed production (conditional $R^2 = 0.77$; Appendix S3). In a preliminary analysis of field data, we found generally weak correlations among traits (Appendix S3). Thus we did not systematically consider trait covariation when analysing the sources of trait variation. However, the correlation between biomass and fecundity was moderately strong, so reproductive traits were analysed by controlling for biomass. This allowed us to assess size-independent reproductive investment (see below).

To analyse the effects of source and exposure environment on traits in the greenhouse, we applied 1) Linear Mixed Models (LMM) to plant biomass, SLA, fecundity and RSR and 2) Generalized Linear Mixed Models (GLMM) with a binomial error for probability of flowering (see details on Appendix S3). For each trait, we constructed a full model with four source environmental drivers (rotated components for Aridity, Temperature and Vegetation cover, and the binary variable Mowing), Water and Light treatments, interactions between environmental drivers and treatments, and Population as a random effect (Table S3). Full models for biomass, probability of flowering and fecundity included control biomass as a covariate. For a comparison of the role of genetic differentiation vs. plasticity, we assessed whether the effects of two source environmental drivers (Aridity and Vegetation Cover) were higher, similar to, or lower than the effects from their corresponding exposure treatments (Water and Light) and their interactions.

To test for the effects of environmental drivers on traits in field populations, we applied 1) LMMs to biomass, SLA and fecundity, and 2) GLMM with a binomial error distribution for probability of flowering (see details on Appendix S3). We constructed full models including the four source environmental drivers. To account for the possible influence of range (native vs. non-native), the models included the effect of range and its interaction with each environmental driver (Table S4). We added Population and Plot nested within Population as random effects. For probability of flowering and fecundity, we included biomass as a covariate.

Full models of the analyses with either greenhouse or field data were compared with all possible model subsets using the Akaike Information Criterion corrected for finite sample sizes (AIC_c) and the AIC_c weights (Burnham & Anderson 2002, Johnson & Omland 2004). We focused on the best AIC_c models, since they had high support and parameter values were overall consistent across competing models (see Appendix S3, Table S5, S6). Finally, we evaluated the utility of observational datasets to predict genetic differentiation. Genetic differentiation was considered predictable if the presence and direction of source environment effects on traits were the same in greenhouse and field conditions, and unpredictable otherwise. For probability of flowering and fecundity, we also assessed whether excluding the covariate biomass from the original analyses modified our evaluation of the predictability of genetic differentiation.

Results

Effects of source and exposure environment in the greenhouse

In the analyses of drivers of trait variation in the greenhouse, the best models always included effects of at least one source environmental driver and both light and water exposure treatments (see blue lines in Fig. 3; Table S3; Fig. S2-S6), but results differed between vegetative and reproductive traits. For vegetative traits (biomass, SLA and RSR), light or water treatments showed the strongest effect sizes when compared with source drivers (Fig. 4c,f,i). The effect of source drivers on biomass and SLA frequently changed between positive and negative directions depending on the treatment (see interactions in Fig. 3a,b; Fig. 4a,b,e). For biomass, the effects of Aridity, Vegetation cover and Mowing differed among treatments, although effect sizes were low and had 95% confidence intervals (CI) that mostly overlapped with zero (Fig. 3a). For SLA, all source drivers were selected in the best model except for Mowing. SLA showed two contrasting effects between source and exposure environments: 1) SLA was lower in the Dry treatment, but higher in plants from the most arid populations (Fig. 4d); 2) SLA was higher in the treatment with lowest light, but also higher in populations with lowest source vegetation cover and thus highest light availability (this took place in treatment L_{33}; Fig. 4e). RSR increased with source Aridity, although the effect was smaller than the effect of the Dry treatment (Fig. 4g,i).

For reproductive traits (biomass-corrected probability of flowering and fecundity), exposure treatments exerted equivalent or smaller effects than source drivers (Fig. 4l,o) and the effects of source drivers were consistent in direction across treatments (Fig. 3d,e, 4k,m). Probability of flowering was negatively affected by source Vegetation cover and positively affected by Mowing, and exposure treatments changed the magnitude of these source effects but not their sign (Fig. 3d, 4k). Fecundity was positively affected by source Aridity and Temperature, and showed no interactions between source and exposure environments (Fig. 3e, 4m). When biomass was excluded as a covariate from the analyses, 1) source effects decreased in magnitude and exposure effects generally increased for probability of flowering, and 2) source effects were not included in the best model of fecundity (Table S7).

Effects of environmental drivers in field populations

Trait variation for *in situ* field populations was associated with both environmental drivers and biogeographic range (native vs. non-native) in the best models, although their effects did not interact in most cases (see blue lines in Fig. 5; Table S4; Fig. S7-S9). Biomass was positively correlated with Vegetation Cover and Mowing, and was higher in non-native populations (Fig. 5a). SLA did not differ between ranges, although we found an interaction between Temperature and biogeographic range, whereby SLA decreased with increasing Temperature in the native but not the non-native range (Fig. 5b). Biomass-corrected probability of flowering was affected negatively by Vegetation Cover and positively by Mowing, and was lower in non-native populations (Fig. 5c). Biomass-corrected fecundity was positively affected by Aridity and Temperature, and the effect of Aridity was stronger in the native than the non-native range (Fig. 5d). When biomass was excluded as a covariate from the analyses, the best model of probability of flowering lost the effects of Vegetation Cover and Range, and the best model of fecundity incorporated the effects of Mowing and the interaction between Range and several source drivers (Table S8). Non-native populations showed significantly higher temperature and seasonality of moisture index than native populations, and lower values in moisture index (Table S9).

Utility of observational datasets to predict genetic differentiation

Biomass corrected reproductive traits, compared to vegetative traits, showed a better match between observational trait-environment relationships and response to source environmental gradients in the greenhouse (Table 1). For vegetative traits, there were two cases of predictable genetic differentiation out of eight. In both cases, field data correctly predicted not the presence but the absence of genetic differentiation. The low predictability in vegetative traits originated in some cases from interacting (e.g., Fig. 4b) or opposing (Fig. 4d,e) effects of source and exposure environments. In other cases, field patterns did not match with those expected from the combined source and exposure greenhouse effects (Fig. 4a). For biomass-corrected reproductive traits, observational data predicted the presence and direction of all seven source environment effects in the greenhouse. However, when reproductive traits were analysed without biomass as a covariate, observational data only predicted the presence and direction of three out of seven source environment effects

(Table S10).

Discussion

Combining a multi-treatment greenhouse experiment with global-scale field observations, we disentangled the main sources of intraspecific trait variation for the short-lived herb *Plantago lanceolata* . Expression of functional traits along environmental gradients in the field was retained to some extent in the greenhouse, indicative of population genetic differentiation. However, while reproductive traits (biomass-corrected probability of flowering and fecundity) showed similar effects of source environment across exposure treatments, vegetative traits (biomass, specific leaf area and root:shoot ratio) showed more plastic responses and some contrasting effects of source and exposure environments. These results imply a higher predictability of genetic differentiation from field observations in reproductive than vegetative traits.

Effects of source and exposure environment on vegetative and reproductive traits

Vegetative and reproductive traits responded differently to source and exposure environments in the greenhouse, as expected based on the fundamental relationship of each type of trait with overall fitness. Reproductive traits corrected for biomass showed stronger source effects than vegetative traits, and less variability across environmental treatments. According to evolutionary theory, traits with the strongest impact on fitness should show evolutionary conservation (Scheiner 1993, Stearns & Kawecki 1994, Sih 2004). In parallel, the demographic buffering theory predicts that the most influential processes in species life cycles should be maintained relatively constant around local optimal values, to reduce variation in population growth rates (Pfister 1998, Burns *et al* . 2010, Hilde *et al* . 2020). For short-lived plants like *P. lanceolata* , reproduction has been identified as the most influential fitness component (Silvertown*et al* . 1996, García *et al* . 2008, Shefferson and Roach 2012), which may explain the smaller role of plasticity and the higher consistency in genetic differentiation found for biomass corrected reproductive traits.

Stronger genetic differentiation in reproductive investment seemed to be facilitated by a higher plasticity in vegetative traits, buffering short-term environmental perturbations (Scheiner 1993, Alpert & Simms 2002, Sih 2004). This phenomenon, known as fitness homeostasis, has been highlighted before as a mechanism for maintaining high individual performance across a range of environments (Sultan 1995, Richards*et al* . 2006). The adjustment of vegetative traits to environmental conditions was manifest in our greenhouse experiment in several ways, and is best exemplified by SLA patterns. SLA increased in the shade treatment to optimize light capture and decreased in dry conditions to reduce water loss through leaf surface, common plastic responses in herbaceous plants (Poorter *et al* . 2009, Dwyer*et al* . 2014). Remarkably, some effects of exposure treatments on SLA were opposed by source environment effects suggesting countergradient variation (sensu Conover & Schultz 1995), such as the positive effect of source Aridity combined with the negative effect of the dry treatment. This apparent contradiction possibly arises because water scarcity in populations from dry sites is compensated through selection for higher RSR and/or stomatal function.

The complex interplay between plasticity and genetic differentiation, and the trait-specific nature of environmental effects found in our study highlight the variety of strategies for plant response to local conditions (see also Albert *et al* . 2010b, Le Bagousse-Pinguet*et al* . 2015, Roybal & Butterfield 2019), but also the difficulty of assessing the mechanisms and drivers of trait variation. The trait patterns found in *P. lanceolata* , including countergradient variation, could be partly explained by the influence of additional drivers not considered in the analyses, such as nutrient availability or biotic interactions (Chevin & Lande 2015). Additionally, further research could be undertaken to disentangle genetic differentiation from unaccounted maternal environment effects.

Accounting for size effects and life history to refine analyses of trait variation

The effect of plasticity on reproductive traits increased in our greenhouse experiment when plant biomass was not accounted for in the models. We thus show here that variance in reproductive effort at the individual scale has a "biomass" component that is strongly driven by plasticity, and an "investment per unit biomass" component that is more genetically determined. Our results emphasize the importance of dissec-

ting reproduction into size dependent and independent components. These dependencies among traits have implications for the expectations of demographic buffering and may explain some of the cases contradicting this theory (see McDonald *et al* . 2017, Hilde *et al* . 2020), e.g. when reproductive traits are strongly driven by underlying individual biomass.

Our study organism is a short-lived plant, with reproduction having a strong influence on population performance. However, in species with different life histories, other demographic rates and their underlying traits might exert the largest effects on fitness. For example, longer-lived taxa usually depend more on survival rates for population persistence (Silvertown *et al* . 1996, Morris & Doak 2005), and may display low variance in survival-related traits. In fact, Preite*et al* . (2015) found stronger genetic differentiation for survival than reproduction in a long-lived herb. Environmental drivers of trait variation for various taxa with different life histories and ecological strategies should be analysed in order to better generalise the results presented here.

Plant size or biomass is likely to structure the most relevant demographic rates (Harper 1977, Easterling *et al* . 2000, Biere 1995), and decomposition of trait variability into size dependent and independent components will also help to shed light on the drivers of trait variability, as shown in our study. Accounting for biomass dependency in trait variation across different life histories may refine previous findings of stronger local adaptation in reproduction than in survival across plant and animal species (Hereford 2009), of higher levels of plasticity vs. local adaptation in reproductive traits of invasive plants (Liao *et al* . 2016), and of an absent relationship between trait plasticity and its proximity to fitness (Acasuso-Rivero*et al* . 2019). The detection of more common genotype-by-environment interactions in short-lived than long-lived plants (Matesanz and Ramírez-Valiente 2019) could also be evaluated for different trait categories separately. These additional interpretations from functional and demographic perspectives may advance our understanding of trait-environment relationships and improve our predictions of species responses to climate change.

Observational datasets and their utility for predicting genetic differentiation

Our global observational dataset revealed that different combinations of biotic and abiotic factors drove variation on each trait. This trait-specificity would have remained hidden had the environmental and geographical scale of the study been smaller, since we could have not analysed together such a variety of environmental conditions and drivers. In addition, the combination of large-scale field and experimental studies, rarely implemented in evolutionary ecology (but see, e.g., Winn & Gross 1993, Woods *et al* . 2012), allowed us to assess the potential uses and misuses of observational datasets. In particular, trait-environment relationships inferred from *in situ*populations correctly predicted genetic differentiation for reproductive but not vegetative traits. For vegetative traits, the predictability diminished as the presence of plasticity led to interacting or opposing effects of source and exposure environments, as initially forecasted (Fig. 1). The predictability of genetic differentiation was also low for reproductive traits when analysed without accounting for their size-dependence. Therefore, observational data may reliably inform about the current drivers of selection and the adaptive capacity of species only for the traits most closely related with fitness. This might be important for species- and community-level predictive models that rely on trait-environment relationships, and for conservation programs focusing on intraspecific genetic diversity.

Evaluating trait-environment relationships can also be useful for predicting plant performance in populations introduced outside native ranges (Alexander *et al* . 2012, Hulme & Barrett 2013). In*P. lanceolata* , traits showed broadly similar correlations with environmental factors in both native and non-native ranges, in agreement with previous work in other taxa (Maron *et al* . 2004, Montague*et al* . 2008, Rosche *et al* . 2019; but see Keller *et al* . 2009). Notably, the similarities in trait patterns between ranges held despite the location of non-native populations in warmer and more arid conditions. This suggests that the trait-environment correlations largely persist for some species even if they occupy more extreme areas of environmental space, facilitating ecological predictions in a context of global change. Yet some trait-environment correlations observed in*P. lanceolata* were weaker in the non-native range (see also Alexander *et al* . 2012). This finding highlights that genetic differentiation may be less predictable for non-native populations and that a total equivalence in trait patterns between ranges cannot be taken for granted due to potential evolutionary divergence. The presence of weaker trait-environment relationships in non-native populations may be due

to a higher role of plasticity (although the latter is not clearly supported by a recent meta-analyses across species; see Palacio-López & Gianoli 2011), or may instead result from repeated introductions in the non-native range (Smith *et al* . 2020). Further sudies on widespread species might help to clarify the processes and patterns resulting from ecological and evolutionary divergence at large spatial scales. In particular, our observational network can form the basis for future experimental work.

Conclusions

Our study improves the understanding of intraspecific trait variation along environmental gradients, showing that the underlying ecological and evolutionary mechanisms differ between reproductive and vegetative traits of *P. lanceolata* . The environmental structuring of variation in biomass-corrected reproductive traits was retained in common greenhouse conditions, indicative of genetic differentiation. In contrast, vegetative traits showed strong plastic responses to buffer short-term environmental variation, sometimes in opposition to genetic differentiation. Differences between vegetative and reproductive traits seem to arise due to the different relationship between each type of trait and overall fitness. These results provide a crucial insight into the potential uses and limitations of observational data, which is readily available for a considerable number of species and traits, but which may provide more uncertain information than common-garden experiments. While genetic differentiation was accurately predicted from observational trait-environment relationships for biomass-corrected reproductive traits, it was sometimes masked for vegetative traits by phenotypic plasticity and countergradient variation. Thus, evaluating evolutionary responses to environment from observational data may lead, in the case of vegetative traits or traits not closely related with fitness, to underestimate the capacity of plants to adapt to new environmental conditions. We also advocate for considering biomass dependency in trait variation analyses, as well as the implications of species life histories on trait-fitness relationships. In view of the general call for including intraspecific trait variation in ecological models (Moran *et al* . 2016, Funk *et al* . 2017), these considerations are important for a more informed prediction of species responses to global change.

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Supporting information

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).

Tables

Table 1. Assessment of the utility of observational field data to predict genetic differentiation in *Plantago lanceolata* . Vegetative traits are biomass and specific leaf area (SLA), and biomass corrected reproductive traits are probability of flowering (Flw Prob) and fecundity. The effects of environmental drivers (Aridity, Temperature, Vegetation Cover and Mowing) on each trait are compared for greenhouse (Grh) vs. field (Fld) conditions, based on effects from best models shown in Figures 3 and 5. The comparison (Comp) determines genetic differentiation to be 1) predictable if effects in greenhouse and field conditions share presence or absence, and direction if present (green) or 2) unpredictable otherwise (black; cf. Fig. 1). Signs of effects are "+" (positive), "-" (negative), "~" (inconsistent direction due to interactions; note some interactions do not lead to inconsistent direction), "abs" (absent) and "na" (not analysed; no comparison is made).

Trait	**Aridity**	**Aridity**	**Aridity**	**Temperature**	**Temperature**	**Temperature**	**Cover**	**Cover**	**C**
	Grh	Fld	**Comp**	Grh	Fld	**Comp**	Grh	Fld	**C**
(Vegetative)									
Biomass	~	abs		abs	abs		~	+	
SLA	+	abs		~	~		~	abs	
(Reproductive)									
Flw Prob	abs	abs		abs	abs		-	-	
Fecundity	+	+		+	+		abs	abs	

Figure legends

Figure 1. Predictability of trait genetic differentiation among populations after comparison of experimental data and observational field data. In a common garden experiment with individuals from multiple provenances ("source" environments) growing in a set of treatments ("exposure" environments), one can partition the independent (a-e) or interacting (f-h) effects of genetic differentiation and phenotypic plasticity. Population genetic differentiation is identified as trait variation along source environments (blue lines), and plasticity is detected by comparing trait values between low (light blue) and high (dark blue) levels of the exposure environment. Note that source and exposure environments are driven here by the same underlying environmental factor. The resulting pattern expected to be observed across field populations is shown with red dashed lines, linking two extreme populations along the environmental gradient in the treatment closest to their corresponding source conditions (from low to high treatments). Observational field data will provide a reliable prediction of genetic differentiation (green squares) in the presence of (a) only source effects, (c) source and exposure effects with the same direction, or (f) source effects with consistent direction but inconsistent slope across exposure environments. In contrast, genetic differentiation will not be predictable from field patterns (black squares) in the presence of (b) only exposure effects, (d,e) source and exposure effects with opposite directions ("countergradient variation"), or (g,h) source effects with inconsistent direction across exposure environments. Note that the x-axis contains source rather than exposure environment (this differs thus from classical displays of plasticity in reaction norms, as the exposure environment is best envisaged as a set of treatments with observations over a continuous environmental gradient comprising the source environments).

Figure 2. Location of native (black) and non-native (grey) study populations of *Plantago lanceolata* in geographical (a) and environmental (b) space. Circles indicate populations studied in the field; triangles indicate populations studied in the field and included in the greenhouse experiment. Colours filling the world map in a) correspond to mean annual temperature and precipitation as shown in b). In b), small black and grey background points correspond to the environmental niche occupied by the species in the native and non-native range, respectively, according to occurrence data from GBIF and BIEN databases.

Fig. 3. Effects from the best model (blue) and competing models (grey; [?]$AIC_c < 2$) for each trait of*Plantago lanceolata* in the greenhouse, with 95% confidence intervals. The effects correspond to source environmental drivers (A = Aridity; T = Temperature; C = Vegetation Cover; M = Mowing), experimental treatments of Water (W_d = dry) and Light (L_{64} and L_{33}), and the interactions between them. Vegetative traits (a-c) are biomass, specific leaf area (SLA) and root:shoot ratio (RSR), and reproductive traits (d-e) are probability of flowering ("Flw Prob") and fecundity ("Fecund"). For simplicity, we omit the effects of control biomass. The effects of L_{64} treatment and Mowing were not tested in RSR and fecundity, respectively (absent labels; see *Material and methods*for details).

Figure 4. Effects of two source environmental drivers (A = Aridity; C = Vegetation Cover) and their corresponding exposure treatments (Water and Light) on *Plantago lanceolata* traits in the greenhouse. Vegetative traits are biomass (a-c), specific leaf area (SLA; d-f) and root:shoot ratio (RSR; g-i), and reproductive traits are probability of flowering (Flw Prob; j-l) and fecundity (Fecund; m-o). Results are presented with 95% confidence intervals (CI), and correspond to the best model according to Akaike Information Criterion

(empty subpanels or bars indicate no effect in the best model). In the left and middle columns, trait values are shown for wet (W_w) and dry (W_d) water treatments, and for L_{100}, L_{64} and L_{33}light treatments. All traits are mean centred and scaled by the standard deviation, except for probability of flowering (Y-axis in logit scale). Source drivers are mean centred and scaled by two times the standard deviation (see Appendix S3). The distribution of populations along source environment values is shown by rug marks on the inside of the x axis. In the right column, the effects of source environment (genetic differentiation; yellow), exposure environment (plasticity; orange) and their interaction (red) are compared. Note that effect sizes are given as absolute values for comparison, and only the CI upper limit is shown.

Fig. 5. Effects from the best model (blue) and competing models (grey; [?]$AIC_c < 2$) for each trait of*Plantago lanceolata* in the field, with 95% confidence intervals. The effects correspond to environmental factors (A = Aridity; T = Temperature; C = Vegetation Cover; M = Mowing), non-native range (R_{nnat}), and the interactions between them. Vegetative traits (a-b) are biomass and specific leaf area (SLA), and reproductive traits (c-d) are probability of flowering ("Flw Prob") and fecundity ("Fecund"). The effects of environmental factors alone correspond to native populations; the effects of environmental factors on non-native populations can be deduced by summing environmental effects alone and the effects of range × environment interactions. For simplicity, we omit the effect of biomass in models of probability of flowering and fecundity.

Figures

Fig. 1

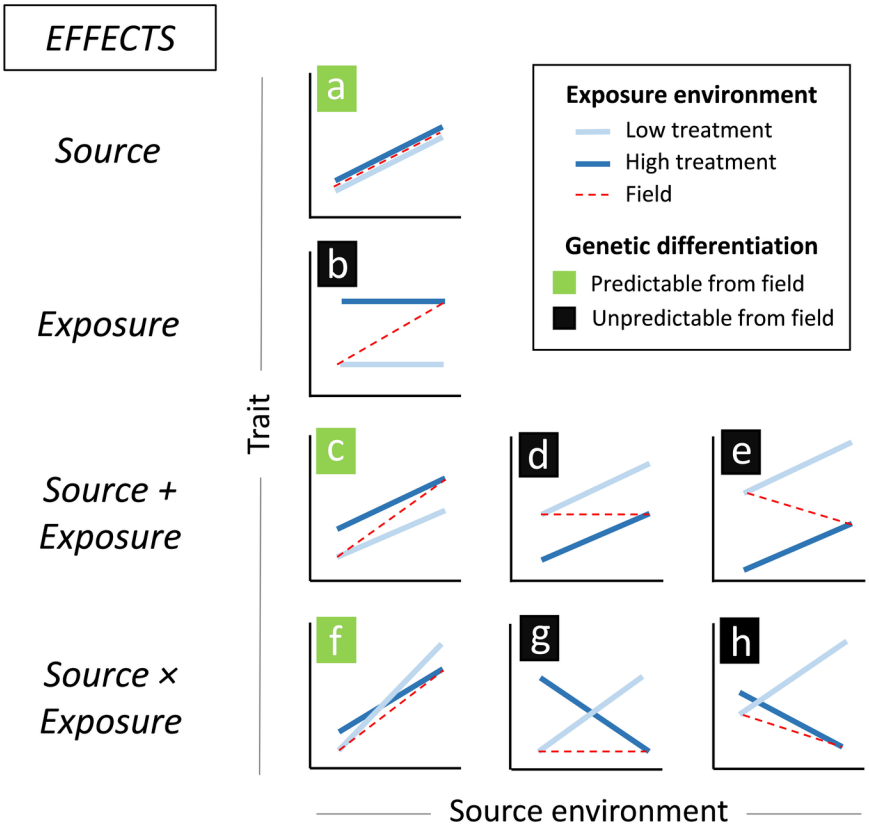

Fig. 2

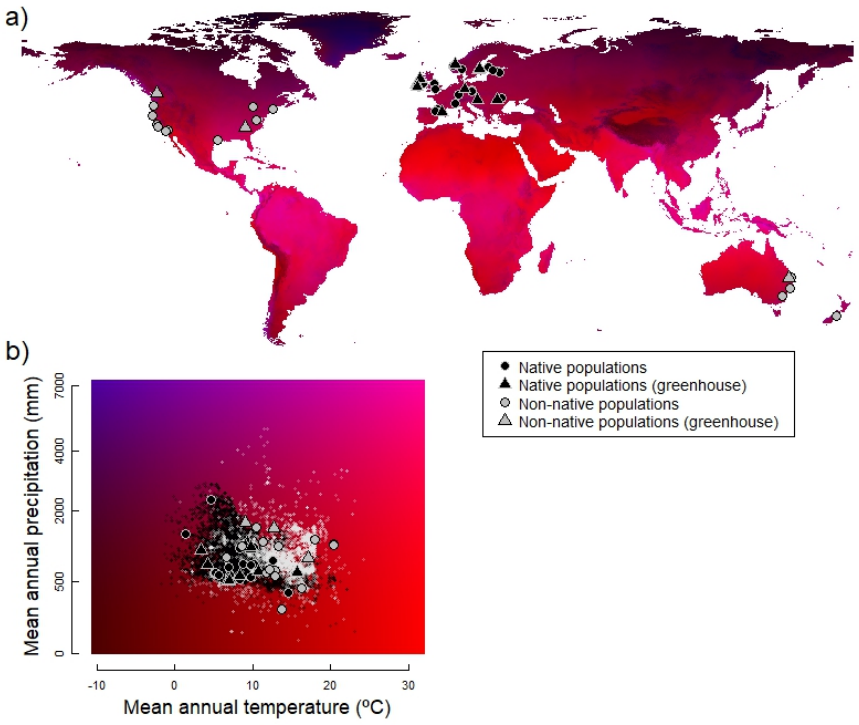

Fig. 3

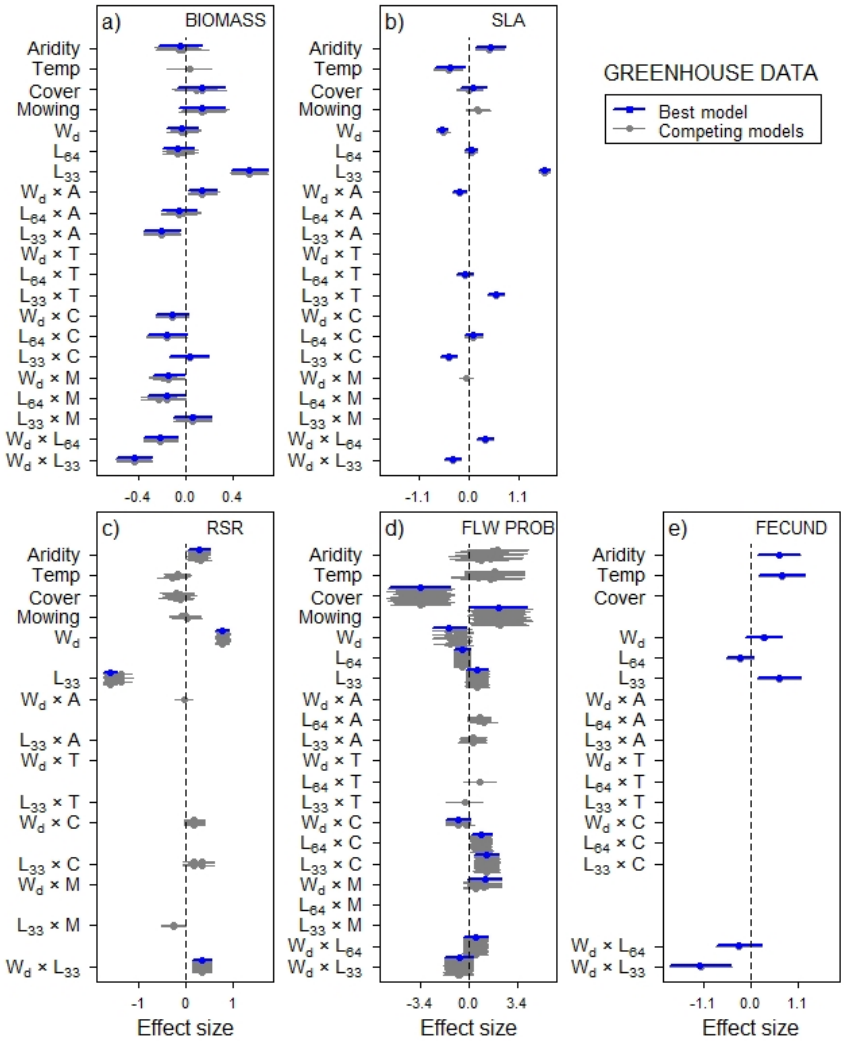

Fig. 4

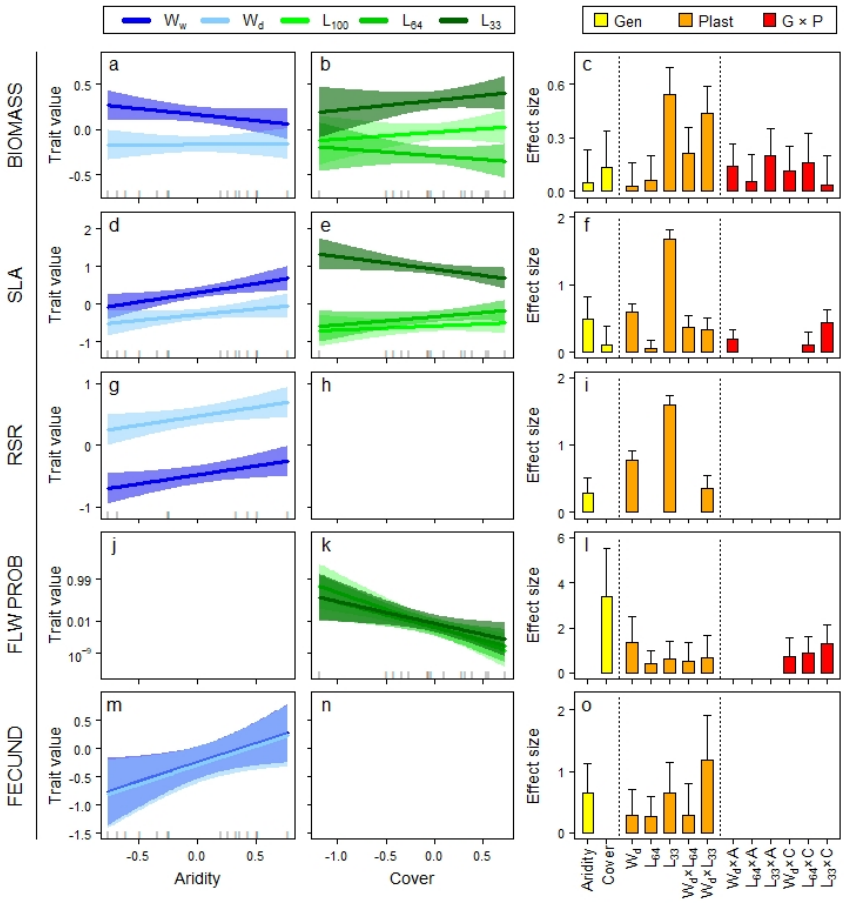

Fig. 5