Received: 1 June 2020 | Revised: 13 May 2021 | Accepted: 19 May 2021

DOI: 10.1111/ele.13858

ECOLOGY LETTERS cnrs WILEY

LETTER

Phenotypic plasticity masks range-wide genetic differentiation for vegetative but not reproductive traits in a short-lived plant

Jesus Villellas[1,2,3] | Johan Ehrlén[4] | Elizabeth E. Crone[5] | Anna Mária Csergő[2,6] | Maria B. Garcia[7] | Anna-Liisa Laine[8,9] | Deborah A. Roach[10] | Roberto Salguero-Gómez[11,12,13] | Glenda M. Wardle[14] | Dylan Z. Childs[15] | Bret D. Elderd[16] | Alain Finn[2] | Sergi Munné-Bosch[17,18] | Benedicte Bachelot[19] | Judit Bódis[20] | Anna Bucharova[21,22] | Christina M. Caruso[23] | Jane A. Catford[24,25] | Matthew Coghill[26] | Aldo Compagnoni[27] | Richard P. Duncan[28] | John M. Dwyer[29,30] | Aryana Ferguson[31] | Lauchlan H. Fraser[26] | Emily Griffoul[32] | Ronny Groenteman[33] | Liv Norunn Hamre[34] | Aveliina Helm[35] | Ruth Kelly[2,36] | Lauri Laanisto[37] | Michele Lonati[38] | Zuzana Münzbergová[39,40] | Paloma Nuche[2] | Siri Lie Olsen[41] | Adrian Oprea[42] | Meelis Pärtel[35] | William K. Petry[43] | Satu Ramula[44] | Pil U. Rasmussen[4,45] | Simone Ravetto Enri[38] | Anna Roeder[46,47] | Christiane Roscher[46,47] | Cheryl Schultz[48] | Olav Skarpaas[49] | Annabel L. Smith[2,50] | Ayco J.M. Tack[4] | Joachim Paul Töpper[41] | Peter A. Vesk[51] | Gregory E. Vose[52] | Elizabeth Wandrag[28,53] | Astrid Wingler[54] | Yvonne M. Buckley[2,13]

[1]Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, Madrid, Spain
[2]School of Natural Sciences, Zoology, Trinity College Dublin, Dublin, Ireland
[3]Facultad de Ciencias de la Salud, Universidad Alfonso X el Sabio, Villanueva de la Cañada, Madrid, Spain
[4]Department of Ecology, Environment and Plant Sciences, Stockholm University, Stockholm, Sweden
[5]Department of Biology, Tufts University, Medford, Massachusetts, USA
[6]Department of Botany and Soroksár Botanical Garden, Szent István University, Budapest, Hungary
[7]Department of Biodiversity Conservation and Ecosystem Restoration, Pyrenean Institute of Ecology (CSIC), Zaragoza, Spain
[8]Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland
[9]Organismal & Evolutionary Biology Research Program, Faculty of Biological & Environmental Sciences, University of Helsinki, Helsinki, Finland
[10]Department of Biology, University of Virginia, Charlottesville, Virginia, USA
[11]Department of Zoology, University of Oxford, Oxford, UK
[12]Max Planck Institute for Demographic Research, Rostock, Germany
[13]School of Biological Sciences, The University of Queensland, St Lucia, Queensland, Australia
[14]School of Life and Environmental Sciences, University of Sydney, Sydney, New South Wales, Australia
[15]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
[16]Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA
[17]Department of Evolutionary Biology, Ecology and Environmental Sciences, Faculty of Biology, University of Barcelona, Barcelona, Spain
[18]Institut de Recerca de la Biodiversitat, Universitat de Barcelona, Barcelona, Spain
[19]Department of Plant Biology, Ecology and Evolution, Oklahoma State University, Stillwater, Oklahoma, USA
[20]Department of Plant Sciences and Biotechnology, Georgikon Faculty, University of Pannonia, Keszthely, Hungary

[Correction added on 9 September 2021, after first online publication: Affiliation 3 has been added to the author Jesus Villellas.]

[21]Biodiversity and Ecosystem Research Group, Institut of Landscape Ecology, University of Münster, Germany
[22]Plant Evolutionary Ecology, Institut of Evolution and Ecology, University of Tübingen, Tübingen, Germany
[23]Department of Integrative Biology, University of Guelph, Guelph, Ontario, Canada
[24]Department of Geography, King's College London, London, UK
[25]Biological Sciences, University of Southampton, Southampton, UK
[26]Department of Natural Resource Sciences, Thompson Rivers University, Kamloops, British Columbia, Canada
[27]Institute of Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany
[28]Institute for Applied Ecology, University of Canberra, Canberra, Australian Capital Territory, Australia
[29]School of Biological Sciences, The University of Queensland, St Lucia, Queensland, Australia
[30]CSIRO Land & Water, EcoSciences Precinct, Dutton Park, Queensland, Australia
[31]Madrona Stewardship, Eugene, Oregon, USA
[32]The Morton Arboretum, Lisle, Illinois, USA
[33]Manaaki Whenua - Landcare Research, Lincoln, New Zealand
[34]Department of Environmental Sciences, Western Norway University of Applied Sciences, Sogndal, Norway
[35]Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia
[36]Agri-Food and Biosciences Institute, Belfast, Northern Ireland, UK
[37]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Tartu, Estonia
[38]Department of Agriculture, Forest and Food Science, University of Torino, Grugliasco, Italy
[39]Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic
[40]Department of Population Ecology, Institute of Botany, Czech Academy of Sciences, Prague, Czech Republic
[41]Norwegian Institute for Nature Research, Oslo, Norway
[42]Botanic Garden "Anastasie Fatu", University "Alexandru Ioan Cuza" Iași, Romania
[43]Department of Plant & Microbial Biology, North Carolina State University, Raleigh, North Carolina, USA
[44]Department of Biology, University of Turku, Turku, Finland
[45]The National Research Centre for the Working Environment, Copenhagen, Denmark
[46]Department of Physiological Diversity, Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany
[47]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
[48]School of Biological Sciences, Washington State University, Vancouver, Washington, USA
[49]Natural History Museum, University of Oslo, Oslo, Norway
[50]School of Agriculture and Food Sciences, University of Queensland, Gatton, Queensland, Australia
[51]School of BioSciences, University of Melbourne, Melbourne, Victoria, Australia
[52]Department of Ecology and Evolutionary Biology, University of California, Irvine, California, USA
[53]Department of Biology, University of York, York, UK
[54]School of Biological, Earth & Environmental Sciences and Environmental Research Institute, University College Cork, Cork, Ireland

Correspondence
Jesus Villellas, Departamento de Biodiversidad, Ecología y Evolución, Universidad Complutense de Madrid, 28040 Madrid, Spain; School of Natural Sciences, Zoology, Trinity College Dublin, Dublin 2, Ireland.
Email: jesus.villellas@gmail.com

Funding information
Eesti Teadusagentuur, Grant/Award Number: PRG609 and PUT1409; Academy of Finland; Natural Sciences and Engineering Research Council of Canada; Science Foundation Ireland, Grant/Award Number: 15/ERCD/2803; Spanish Ministry of Science, Innovation and Universities, Grant/Award Number: IJCI-2017-32039; European Regional Development Fund

Editor: Cyrille Violle

Abstract

Genetic differentiation and phenotypic plasticity jointly shape intraspecific trait variation, but their roles differ among traits. In short-lived plants, reproductive traits may be more genetically determined due to their impact on fitness, whereas vegetative traits may show higher plasticity to buffer short-term perturbations. Combining a multi-treatment greenhouse experiment with observational field data throughout the range of a widespread short-lived herb, *Plantago lanceolata*, we (1) disentangled genetic and plastic responses of functional traits to a set of environmental drivers and (2) assessed how genetic differentiation and plasticity shape observational trait–environment relationships. Reproductive traits showed distinct genetic differentiation that largely determined observational patterns, but only when correcting traits for differences in biomass. Vegetative traits showed higher plasticity and opposite genetic and plastic responses, masking the genetic component underlying field-observed trait variation. Our study suggests that genetic differentiation may be inferred from observational data only for the traits most closely related to fitness.

KEYWORDS
biomass, common garden experiment, countergradient variation, fecundity, genotype by environment interaction, intraspecific trait variation, observational datasets, root:shoot ratio, specific leaf area, widespread species

INTRODUCTION

Functional traits are morphological, physiological or phenological features of organisms that influence the components of fitness, that is, survival and reproduction (Adler et al., 2014; Reich et al., 2003; Violle et al., 2007). Intraspecific variation in functional traits is widely documented and has important implications at population, species and community levels (Caruso et al., 2020; Des Roches et al., 2018; Hughes et al., 2008; Villellas & García, 2017; Violle 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 trait databases that are largely observational (e.g., Iversen et al., 2017; Kattge et al., 2020; Maitner et al., 2018) and relating these trait values to candidate environmental drivers. However, we lack evaluation of the potential uses and limitations of trait–environment relationships inferred from observational in situ data. Such assessments are necessary because observed trait variation may result from a combination of underlying processes that operate at different spatiotemporal scales (De Frenne et al., 2013; Kattge et al., 2011) and thus may determine the way species respond to environmental change.

Intraspecific trait variation observed in situ among populations may arise from genetic differentiation and/or phenotypic plasticity (Franks et al., 2014; Merilä & Hendry, 2014; Moran et al., 2016). 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) and Santangelo et al. (2018)). Genetically determined traits are thus expected to show persistent correlations with the source environment. However, plastic phenotypic responses to environmental conditions might obscure trait patterns driven by genetic differentiation (Conover & Schultz, 1995; Gienapp et al., 2008; MacColl, 2011). In addition, genetic differentiation and phenotypic plasticity may play different roles in the current context of rapid environmental change. Genetic differentiation may be indicative of evolutionary potential, which can be necessary in the presence of continued directional environmental change (Franks et al., 2014; Gienapp et al., 2008). In turn, plasticity might allow a faster initial acclimation response under certain conditions (DeWitt et al., 1998; Gienapp et al., 2008). It is thus important to assess the underlying genetic and plastic sources of trait variation, and the effect of plasticity in masking genetic differentiation, especially considering the increasing availability and potential uses of observational data.

A combination of experimental and in situ field data enables us to disentangle the sources of observed trait variation. A standard experimental approach to partition trait variation is the use of a common garden experiment (Clausen et al., 1940; Franks et al., 2014; Merilä & Hendry, 2014). By growing offspring from multiple provenances together in a set of controlled conditions, the effects of persistent source environments (leading to genetic differentiation) can be disentangled from those of short-term exposure environments (driving phenotypic plasticity). Notably, by evaluating different combinations of source and exposure effects on traits, the role of genetic differentiation and plasticity in shaping observational trait–environment relationships can be assessed (Figure 1). The predominance of source over exposure effects (Figure 1a,f) or the presence of source and exposure effects with the same direction (either positive or negative; Figure 1c) indicate a major role of genetic differentiation in shaping observational trait patterns. In contrast, a predominance of exposure effects (Figure 1b), source and exposure effects with opposite direction (Figure 1d,e), or source effects whose direction depends on the exposure environment due to interactions (Figure 1g,h), indicate a stronger role of plasticity and thus lead to inconsistencies between genetically determined and observed trait patterns. For example, opposing source and exposure effects, a phenomenon known as countergradient variation (Conover et al., 2009; Conover & Schultz, 1995), may lead to an apparent absence of trait variation among populations (Figure 1d) or even to patterns counter to those of genetic differentiation due to phenotypic plasticity (Figure 1e). Despite the benefits of combining experimental and observational data (Magnani, 2009; Merilä & Hendry, 2014), such an integrative approach has been rarely implemented in evolutionary ecology across significant parts of species geographic ranges (Oleksyn et al., 2003; Winn & Gross, 1993; Woods et al., 2012). Yet large spatial scales are necessary for spanning the environmental niche of widespread species and for untangling the interrelated effects of different environmental drivers (Hulme & Barrett, 2013; Matesanz et al., 2010; Merilä & Hendry, 2014).

Assesments of the sources of trait variation need also to consider different types of functional traits (Albert

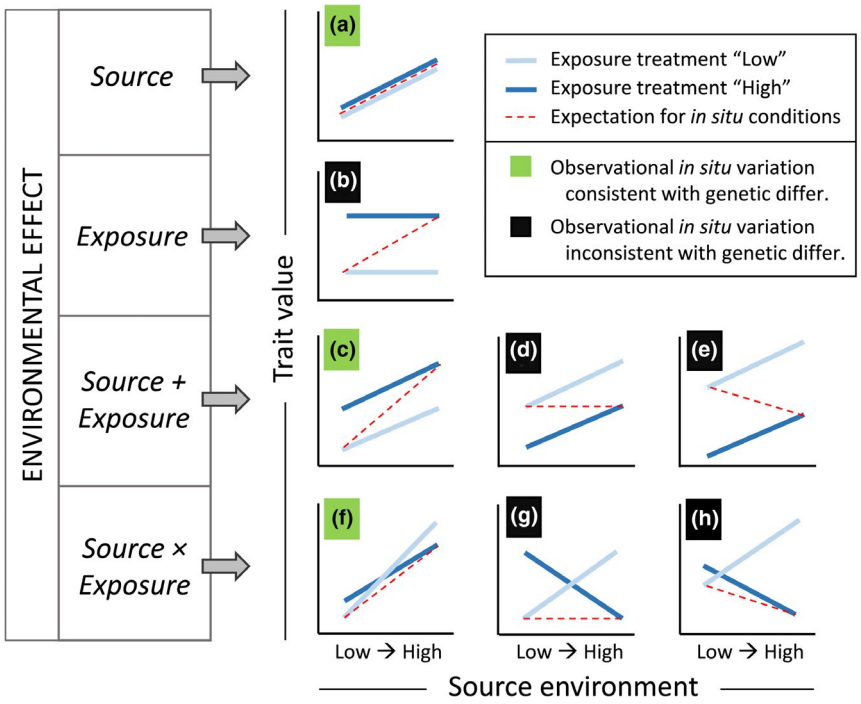

FIGURE 1 Trait variation observed among populations across environmental gradients is shaped by the joint effects of genetic differentiation and phenotypic plasticity. These additive (a–e) or interacting (f–h) effects can be assessed in a common garden experiment, by growing individuals from multiple source environments under controlled conditions in a set of exposure treatments. Population genetic differentiation is identified as trait variation along source environments (blue lines from low to high values along *x*-axes), 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. Note also that the *x*-axes contain source rather than exposure environment, differing thus from classical displays of plasticity in reaction norms. The resulting observational pattern expected across in situ 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). Depending on the joint effects of genetic differentiation and plasticity, observational trait variation across source environments will be consistent (green squares) or inconsistent (black squares) in direction with trait patterns driven by genetic differentiation

et al., 2010a; Funk et al., 2017; Münzbergová et al., 2017). According to evolutionary theory, the traits most directly related to fitness should show stronger selection leading to genetic differentiation (Scheiner, 1993; Sih, 2004; Stearns & Kawecki, 1994). Traits less directly related to fitness would instead display higher plasticity, to buffer short-term environmental perturbations and ultimately maintain fitness homeostasis (Richards et al., 2006; Sultan, 1995). This view is in line with demographic buffering theory, which predicts that the most influential processes in species life cycles should be maintained constant around local optimal values, to reduce the negative consequences of variation in population growth rates (Pfister, 1998; Burns et al., 2010; Hilde et al., 2020; but see McDonald et al., 2017). In plants, reproductive traits often show lower plasticity than vegetative traits (Bradshaw, 1965; Frazee & Marquis, 1994; Schlichting & Levin, 1984). This might be especially true for short-lived taxa, in which reproduction usually has the highest influence on population growth (García et al., 2008; Shefferson & Roach, 2012; Silvertown et al., 1996). Yet reproductive traits may appear to be strongly driven by plasticity if evaluated at the whole plant level rather than per unit biomass, due to the influence of the underlying, more labile biomass component (Biere, 1995; Weiner et al., 2009). For assessment of the roles of genetic differentiation and plasticity on trait variation, it is therefore crucial to consider the expected relationship of traits (and relevant underlying components) to fitness.

Here, we analyse responses of functional traits of the short-lived herb *Plantago lanceolata* to a set of environmental drivers, combining experimental and observational data across large spatial scales in its native and non-native ranges. By growing individuals from multiple populations under several experimental treatments in a common garden, we tested whether genetic differentiation and phenotypic plasticity shape variation in vegetative and reproductive traits in different ways, as predicted by their different relationships with fitness. Our expectation was that:

(1) vegetative traits (plant biomass, specific leaf area (SLA) and root:shoot ratio (RSR)) show a predominance of plastic over genetic responses to environmental drivers, genetic and plastic responses with opposite direction and/or genetic patterns with inconsistent direction among exposure treatments; and.

(2) reproductive traits (probability of flowering and fecundity) show the opposite pattern: a predominance

of genetic over plastic responses, genetic and plastic responses with the same direction and/or genetic patterns with consistent direction among exposure treatments. 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.

Finally, by comparing experimental results with trait–environment relationships detected from a global-scale observational survey, we tested the expectation that:

(3) observational trait patterns show a higher consistency with 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 years (Lacey et al., 2003; Roach, 2003), although some individuals may exceed 12 years (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). *P. lanceolata* is native to Europe, Western Asia and North Africa, although it has been introduced worldwide, mainly during the eighteenth and nineteenth centuries (Cavers et al., 1980; Hooker, 1867; 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 (Figure 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; Figure 2, Tables S1 and S2 in Supporting Information), spanning a wide range of climatic, management and plant community conditions, and a wide range of genotypes (Smith et al., 2020). For each population, we monitored all individual plants within 0.25 m^2 plots along 10 m transects until we

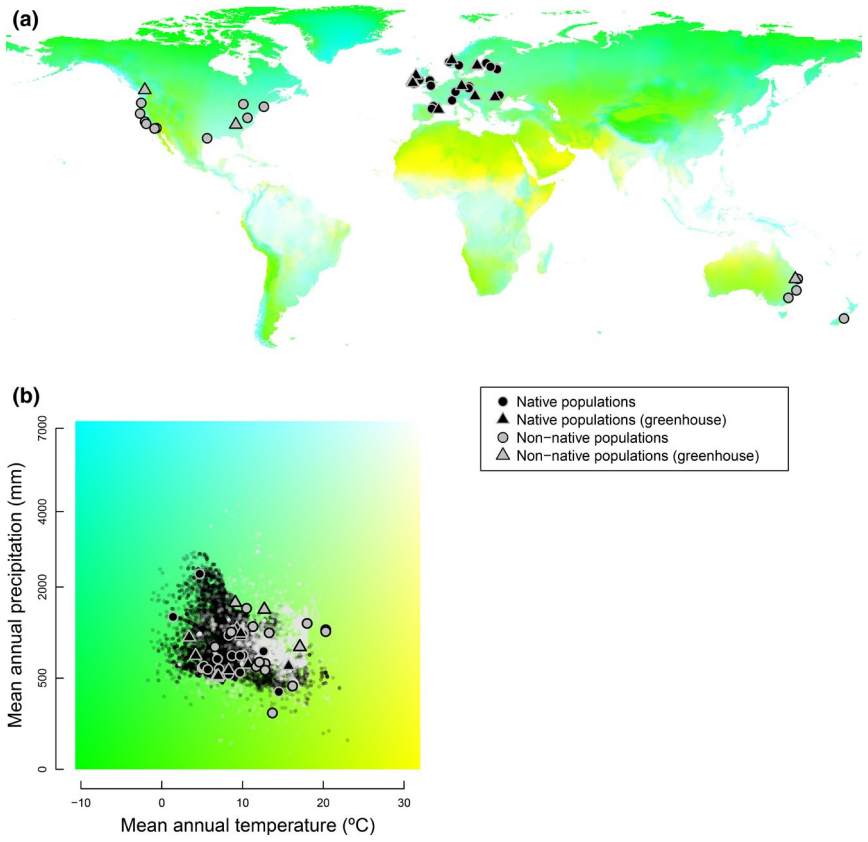

FIGURE 2 Location of native (black) and non-native (grey) study populations of *Plantago lanceolata* in geographical (a) and environmental (b) space. Circles indicate populations studied in the field; triangles indicate populations studied in the field and included in the greenhouse experiment. Colours 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 ranges, respectively, according to occurrence data from GBIF and BIEN databases (GBIF.org, 2014; Maitner et al., 2018)

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 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 (Fick & Hijmans, 2017; Kriticos et al., 2012). 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 (Figure 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 and 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 generalised 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 (Figure 2, Table S1). This subset of populations spanned almost the entire geographical and environmental native range and contained three non-native sites to increase the breadth of source environmental conditions (Appendix S2). We pooled all the collected seeds at the population level. We sowed 2728 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 3 days ("wet" treatment), and the other half every 9 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 (60ºC) and weighed to calculate SLA. 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). The same approach was used to estimate initial control biomas from leaf measurements taken at the beginning of the greenhouse experiment. Probability of flowering was modelled as a binary variable with data from the flowering versus 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 (LMMs) to plant biomass, SLA, fecundity and RSR and (2) generalised linear mixed models (GLMMs) 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.

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. nonnative), 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, because they had high support and parameter values were overall consistent across competing models (Appendix S3; Tables S5 and S6). Finally, we qualitatively evaluated the consistency between observational trait–environment relationships and genetic differentiation. Observational and genetic patterns were considered consistent if the presence and direction of source environment effects on traits were the same in greenhouse and field conditions, and inconsistent otherwise. We also assessed whether considerations on the consistency in trait patterns changed when biomass was excluded as a covariate from the analyses of probability of flowering and fecundity.

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 Figure 3; Table S3; Figures S2–S6), but results differed between vegetative and reproductive traits. Vegetative traits showed source and exposure effects that interacted or opposed to each other, or a high prevalence of exposure relative to source effects (Figure 4a–l). Biomass and SLA showed complex effects of source environmental drivers, which frequently varied in direction among exposure treatments due to interacting effects (Figure 4a–g). SLA showed also two cases of opposing source and exposure effects: (1) SLA was on average lower in the dry treatment but higher in plants from the most arid populations (Figure 4d) and (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}; Figure 4e). RSR responded strongly to exposure treatments, whereas it was only moderately affected by source Aridity (Figure 4i).

For reproductive traits corrected for biomass, the effects of source drivers were consistent in direction across treatments (Figure 4o–r), despite the existence of some source by exposure interactions (Figure 3d). 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 (Figure 4o,p). Fecundity was positively affected by source Aridity and Temperature and showed no interactions between source and exposure environments (Figure 4q,r). When biomass was excluded as a covariate from the analyses of probability of flowering, source effects also showed the same direction irrespective of the exposure environment, although they decreased in magnitude relative to exposure effects (Table S7). When biomass was excluded from the analyses of fecundity, source effects were not included in the best model (Table S7).

Effects of environmental drivers in field populations

Trait variation for in situ field populations was associated with both environmental drivers and biogeographic

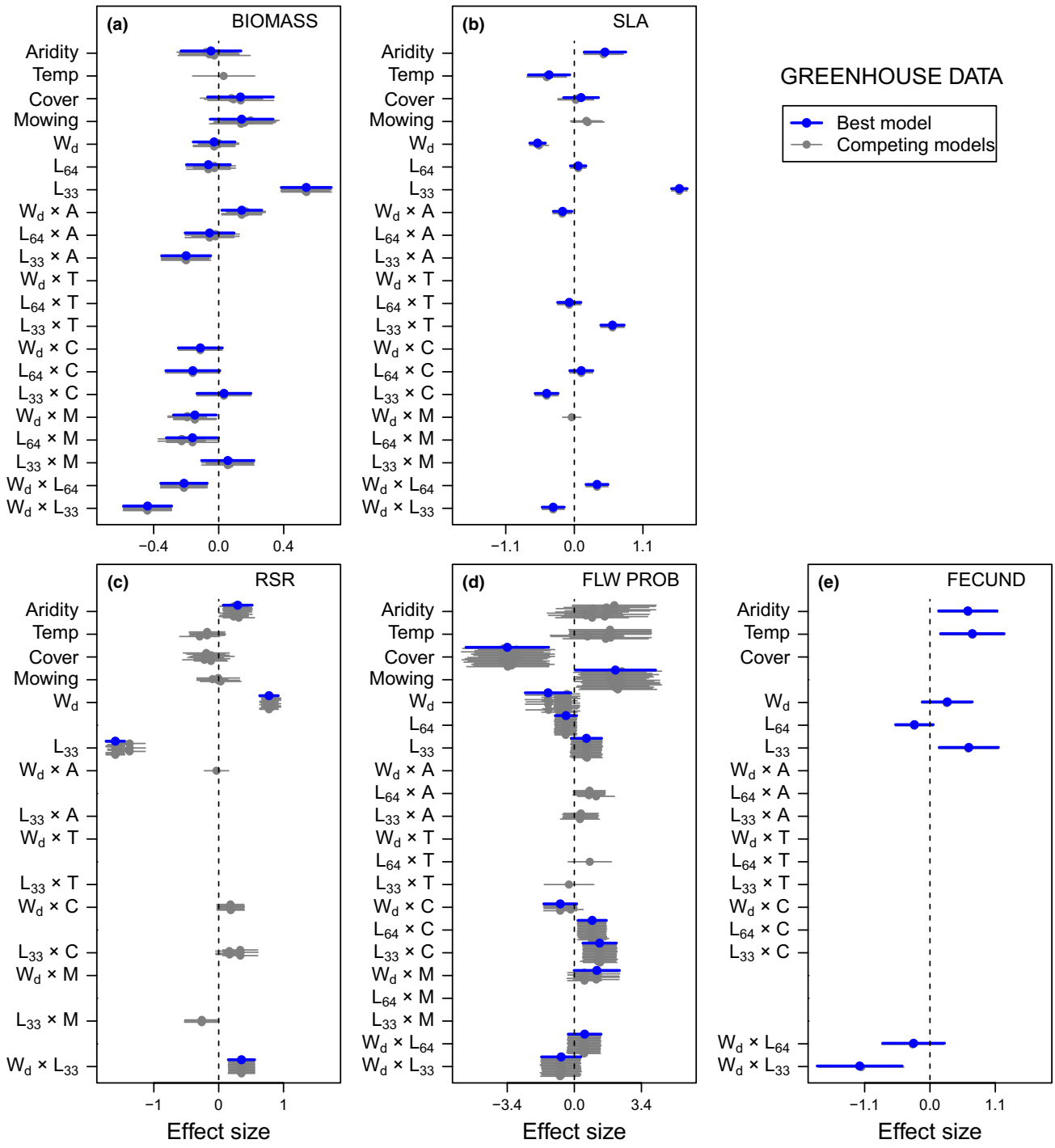

FIGURE 3 Effects from the best model (blue) and competing models (grey; ΔAIC_c <2) for each trait of *Plantago lanceolata* in the greenhouse, with 95% confidence intervals. The effects correspond to source environmental drivers (A = Aridity; T = Temperature; C = Vegetation Cover; and M = Mowing), experimental 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"). The effects of source environmental drivers alone correspond to wet and L_{100} treatments; the effects of source environment under the remaining water and light treatments can be deduced by summing source environmental effects alone and the effects of source × exposure environment interactions. 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)

range (native vs. non-native) in the best models, although their effects did not interact in most cases (see blue lines in Figure 5; Table S4; Figures S7–S9). Biomass was positively correlated with Vegetation Cover and Mowing and was higher in non-native populations (Figure 5a). SLA showed an interaction between Temperature and biogeographic range, whereby SLA decreased with Temperature in the native but not in the non-native

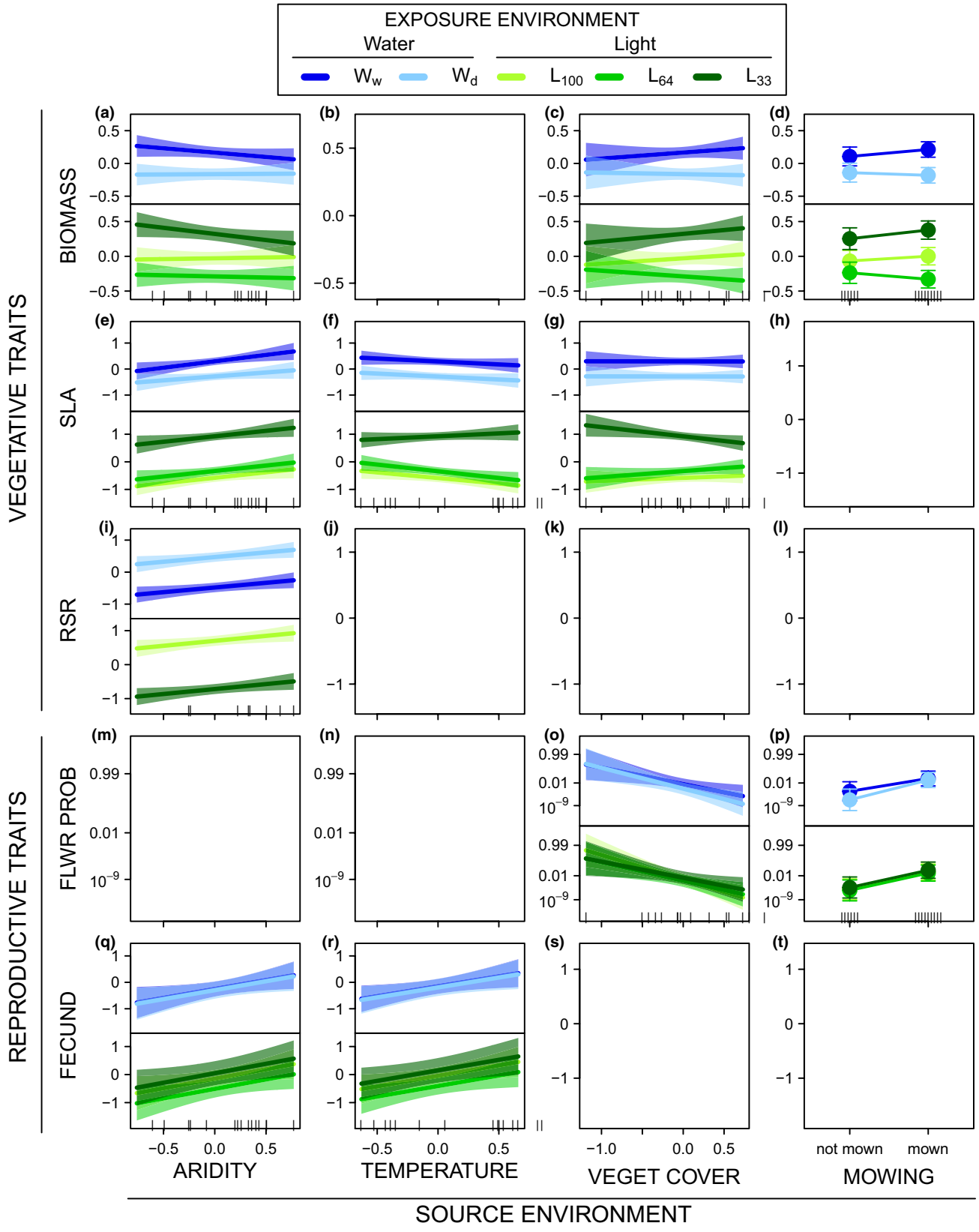

FIGURE 4 Effects of source environmental drivers (Aridity, Temperature, Vegetation Cover and Mowing) and exposure treatments (water and light) on *Plantago lanceolata* traits in the greenhouse. Vegetative traits are biomass (a–d), specific leaf area (SLA; e–h) and root:shoot ratio (RSR; i–l). Reproductive traits are probability of flowering (Flwr Prob; m–p) and fecundity (Fecund; q–t) and are corrected for biomass. Results are presented with 95% confidence intervals and correspond to the best model according to Akaike Information Criterion (empty subpanels indicate no effect in the best model). All traits are mean centred and scaled by the standard deviation, except for probability of flowering (*y*-axis in logit scale). Continuous source environmental drivers are mean centred and scaled by two times the standard deviation (Appendix S3). The distribution of populations along source environment values is shown by rug marks on the inside of the *x*-axis

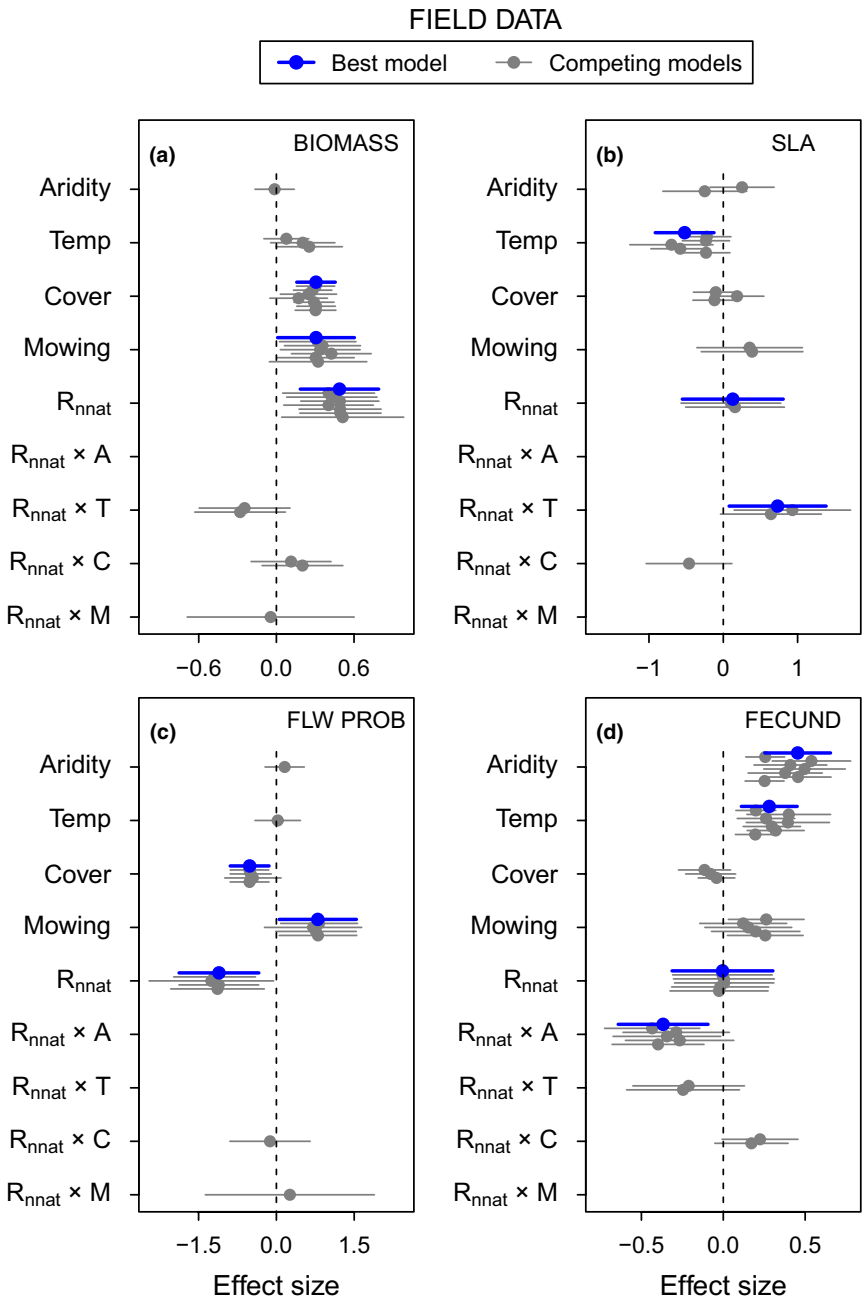

FIGURE 5 Effects from the best model (blue) and competing models (grey; ΔAIC_c <2) for each trait of *Plantago lanceolata* in the field, with 95% confidence intervals. The effects correspond to environmental factors (A = Aridity; T = Temperature; C = Vegetation Cover; and M = Mowing), non-native range (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

range (Figure 5b). Biomass-corrected probability of flowering was affected negatively by Vegetation Cover and positively by Mowing and was lower in non-native populations (Figure 5c). Biomass-corrected fecundity was positively affected by Aridity and Temperature, and the effect of Aridity was stronger in the native than in the non-native range (Figure 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).

TABLE 1 Assessment of consistency between observational trait–environment relationships across in situ populations (Obs) and genetic differentiation detected under experimental conditions (Exp) in *Plantago lanceolata*

	Aridity			Temperature			Cover			Mowing		
Trait	**Obs**	**Exp**	**Comp**	**Obs**	**Exp**	**Comp**	**Obs**	**Exp**	**Comp**	**Obs**	**Exp**	**Comp**
Vegetative												
Biomass	abs	~	■	abs	abs	■	+	~	■	+	~	■
SLA	abs	+	■	~	~	■	abs	~	■	abs	abs	■
Reproductive												
Flw Prob	abs	abs	■	abs	abs	■	−	−	■	+	+	■
Fecundity	+	+	■	+	+	■	abs	abs	■	abs	na	

Vegetative traits are biomass and specific leaf area (SLA), and reproductive traits corrected for biomass are probability of flowering (Flw Prob) and fecundity. The effects of environmental drivers (Aridity, Temperature, Vegetation Cover and Mowing) on traits correspond to best models shown in Figures 3 and 5. In the comparison (Comp), observational trait patterns are (1) consistent with genetic differentiation if environmental effects detected for in situ and experimental conditions share presence or absence, and direction if present (green) or (2) inconsistent with genetic differentiation otherwise (black; cf. Figure 1). Signs of effects are "+" (positive direction), "−" (negative direction), "~" (changing direction due to interactions; note some interactions may change the slope but not the direction), "abs" (absent) and "na" (not analysed; no comparison is made).

The role of genetic differentiation and plasticity in shaping observational trait patterns

Biomass-corrected reproductive traits, compared with vegetative traits, showed a higher consistency between observational trait–environment relationships and genetic differentiation (Table 1). For vegetative traits, two cases out of eight of observational patterns were consistent with genetic differentiation detected in the greenhouse. In both cases, the consistency was not in the presence but in the absence of environmental effect. The observational–genetic mismatch in vegetative traits originated in some cases from the existence of source effects that varied in direction among exposure treatments and which were low in magnitude relative to exposure effects (Figure 4a,c,d,f). In other cases, the mismatch was due to opposing effects of source and exposure environments (Figure 4e,g). For biomass-corrected reproductive traits, observational and genetic patterns were consistent in all seven trait–environment relationships (Table 1) due to a minor role of phenotypic plasticity in shaping trait variation (Figure 4o–r). However, when reproductive traits were analysed without biomass as a covariate, such consistency was observed in only three out of seven cases (Table S10).

DISCUSSION

By combining a multi-treatment common garden experiment with a global-scale observational survey, we disentangled the main sources of functional trait variation among populations of the widespread short-lived herb *P. lanceolata*. Trait expression along environmental gradients in the field was retained to some extent in the common garden, 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, SLA and RSR) showed stronger plastic responses and interacting or opposing effects of source and exposure environments. These findings imply a higher consistency between observational and genetic patterns in reproductive traits, as expected from their closer relationship with fitness relative to vegetative traits. We call for further studies to test the generality of this result for other species with different life histories and environmental contexts.

Genetic differentiation and phenotypic plasticity in vegetative and reproductive traits

The roles of genetic differentiation and phenotypic plasticity on *P. lanceolata* trait variation differed depending on the fundamental relationship of each type of trait with overall fitness, as initially expected. Reproductive traits corrected for biomass showed distinct genetic differentiation patterns along source environmental gradients, which were consistent in direction irrespective of exposure treatments. In contrast, vegetative traits showed stronger plastic responses to exposure environment and interacting effects of source and exposure environments. According to evolutionary theory, genetic differentiation should be more common in the traits with the strongest impact on fitness (Scheiner, 1993; Sih, 2004; Stearns & Kawecki, 1994). In parallel, the demographic buffering theory predicts that processes that most influence population growth rate should show relatively low variability (Burns et al., 2010; Hilde et al., 2020; Pfister,

1998). Thus, the smaller role of plasticity and the higher consistency in genetic differentiation found for reproductive traits is likely explained by the strong influence that reproduction has on fitness in short-lived plants like *P. lanceolata* (García et al., 2008; Shefferson & Roach, 2012; Silvertown et al., 1996).

The stronger genetic differentiation in reproductive investment may be facilitated precisely by the higher plasticity found in vegetative traits, buffering short-term environmental perturbations (Alpert & Simms, 2002; Scheiner, 1993; 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 (Richards et al., 2006; Sultan, 1995). The plastic adjustment of vegetative traits to environmental conditions was manifested in our greenhouse experiment in several ways and is best exemplified by SLA patterns. SLA increased in the shade treatment to optimise light capture and decreased in dry conditions to reduce water loss through leaf surface, common plastic responses in herbaceous plants (Dwyer et al., 2014; Poorter et al., 2009). 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 could arise if water scarcity in populations from dry sites was compensated through selection for higher RSR (Figure 4i) or higher stomatal function.

The role of genetic differentiation and plasticity in shaping observational trait data

Our global observational dataset revealed that different combinations of biotic and abiotic factors drove variation of each trait. Such trait specificity would have remained hidden had we conducted the study at smaller environmental and geographical scales. 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 role of genetic differentiation and plasticity in shaping observational trait–environment relationships. For reproductive traits, observational patterns were largely determined by genetic differentiation. For vegetative traits, observational patterns were inconsistent with genetic differentiation because the latter was masked by the interacting or opposing effects of plasticity, as initially forecasted (Figure 1). The consistency between observational and genetic patterns was also low for reproductive traits when analysed without accounting for the confounding effect of biomass. Therefore, our study suggests that observational data may reliably inform about the species evolutionary potential and drivers of selection only for the traits most closely related to fitness. The availability of observational trait datasets is rapidly growing worldwide (Iversen et al., 2017; Kattge et al., 2020; Maitner et al., 2018), and guidelines on the interpretation of their sources of variation may be crucial if data are to be used in predictive models based on trait–environment relationships, or in conservation programs involving the translocation of species to new suitable habitats.

Combining observational and experimental data can also be useful for assessing plant performance 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 the 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 meta-analysis across species; see Palacio-López & Gianoli, 2011), or result from repeated introductions in the non-native range leading to high population genetic diversity and a breakdown of environmentally determined population differentiation (Smith et al., 2020). Further studies 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 research.

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

Some nuances and limitations of our approach must be considered for a more realistic interpretation of results. In our greenhouse experiment, the role of plasticity on reproductive trait variation increased when plant biomass was not accounted for in the models. We thus show that 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 emphasise the importance of dissecting reproduction

into size-dependent and size-independent components. These dependencies among traits have implications for the expectations of demographic buffering and may explain some of the cases contradicting this theory (Hilde et al., 2020; McDonald et al., 2017), for example, 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 (Morris & Doak, 2005; Silvertown et al., 1996). 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. Accounting for these life history differences as well as for a potential biomass dependency in trait variation may refine previous findings of stronger local adaptation in reproduction than in survival rates (Hereford, 2009), of higher levels of plasticity than 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 & 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 global change.

Additional confounding factors may be controlled in the future to refine analyses of the sources of trait variation. Complementary research could be undertaken to disentangle genetic differentiation from the maternal environment effects unaccounted in our study. In addition, establishing common garden experiments in different locations would help to discard biases associated to specific experimental setting conditions, especially in the presence of genotype by environment interactions (Merilä & Hendry, 2014). However, the strong consistency between observational and genetic patterns found for all the environmental drivers of reproductive traits suggests that the lack of common garden replicates in our study has not been very influential in our results. Finally, the trait patterns found in *P. lanceolata*, including countergradient variation, could be partly explained by additional drivers not considered in the analyses, such as nutrient availability or biotic interactions (Chevin & Lande, 2015). Overall, the complex interplay between plasticity and genetic differentiation found in our study, and the trait-specific nature of environmental effects, highlights 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 correctly assessing the mechanisms and drivers of trait variation.

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 from the different relationships between each type of trait and overall fitness. These results provide a crucial insight into the potential uses and limitations of observational trait data, whose availability is rapidly growing, but which may provide more uncertain information than common-garden experiments. In particular, the masking effect of phenotypic plasticity and countergradient variation in *P. lanceolata* vegetative traits has resulted in inconsistencies between observational trait–environment relationships and genetic differentiation. Thus, inferring evolutionary responses to environment from observational data may lead, in the case of 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 (Funk et al., 2017; Moran et al., 2016), these considerations are important for a more informed prediction of species responses to global change.

ACKNOWLEDGEMENTS

This publication emanated from research conducted with the financial support of Science Foundation Ireland (SFI) under Grant Number 15/ERCD/2803 (YMB). Financial support was also provided by the Spanish Ministry of Science, Innovation and Universities to JV (IJCI-2017-32039), by the Natural Sciences and Engineering Research Council of Canada to LHF, by the Academy of Finland to SR, by the Estonian Research Council (grants PRG609 to MP and PUT1409 to LL) and by the European Regional Development Fund (funding for the Centre of Excellence EcolChange to MP). S. P. Blomberg and J. van Groenendael helped design the PlantPopNet

network. We thank A. Ehrlén and A. Pettersson for their help in the greenhouse experiment and S. Coutts for help with Figure 2. We are also grateful to the Archipelago Research Institute and to numerous field assistants and landowners of sampled sites. B. Pujol and one anonymous reviewer helped to improve the manuscript with their suggestions.

AUTHORSHIP

YMB coordinated the PlantPopNet network. The founding steering committee (YMB, EEC, AMC, JE, MBG, A-LL, DAR, RS-G and GMW) designed the PlantPopNet network and wrote the field protocol. JV, JE and YMB designed the current study. All the authors (except for A-LL, EG, GMW and PN) collected trait data on field populations. AJMT, ALS, AMC, AR, BE, CR, JB, JD, JE, LHF, LNH, MC, PUR, RK, RS-G, SM-B and YMB provided seeds for the common garden experiment. JV carried out the common garden experiment with help from PN and JE and the statistical analyses with help from JE and YMB. JV wrote the first draft of the manuscript with help from JE and YMB, and all authors contributed substantially to revisions.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/ele.13858.

DATA AVAILABILITY STATEMENT

The dataset and code used for the analyses and figures are archived in Figshare (https://doi.org/10.6084/m9.figshare.15029115.v1).

ORCID

Jesus Villellas https://orcid.org/0000-0001-7805-5683
Elizabeth E. Crone https://orcid.org/0000-0002-5287-221X
Roberto Salguero-Gómez https://orcid.org/0000-0002-6085-4433
Sergi Munné-Bosch https://orcid.org/0000-0001-6523-6848
Benedicte Bachelot https://orcid.org/0000-0003-3348-9757
Jane A. Catford https://orcid.org/0000-0003-0582-5960
Richard P. Duncan https://orcid.org/0000-0003-2295-449X
John M. Dwyer https://orcid.org/0000-0001-7389-5528
Ruth Kelly https://orcid.org/0000-0001-7982-5993
Lauri Laanisto https://orcid.org/0000-0003-2215-7298
William K. Petry https://orcid.org/0000-0002-5230-5987
Pil U. Rasmussen https://orcid.org/0000-0003-0607-4230
Peter A. Vesk https://orcid.org/0000-0003-2008-7062
Yvonne M. Buckley https://orcid.org/0000-0001-7599-3201

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Villellas, J., Ehrlén, J., Crone, E.E., Csergő, A.M., Garcia, M.B., Laine, A.-L., et al (2021) Phenotypic plasticity masks range-wide genetic differentiation for vegetative but not reproductive traits in a short-lived plant. *Ecology Letters*, 24, 2378–2393. https://doi.org/10.1111/ele.13858