ESSAY REVIEW

Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications

FERNANDO VALLADARES, DAVID SANCHEZ-GOMEZ and
MIGUEL A. ZAVALA†

Instituto de Recursos Naturales, Centro de Ciencias Medioambientales, CSIC Serrano 115, Madrid E-28006, Spain, and †Departamento de Ecología, Universidad de Alcalá, Alcalá de Henares, Madrid E-28871, Spain

Summary

1 Global change and emerging concepts in ecology and evolution are leading to a growing interest in phenotypic plasticity (PP), the environmentally contingent trait expression observed in a given genotype. The need to quantify PP in a simple manner in comparative ecological studies has resulted in the prevalence of various indices instead of the classic approaches, i.e. a comparison of slopes in the norms of reactions (trait vs. environment plots).

2 The objectives of this study were: (i) to review the most common methods for quantitative estimation of PP; (ii) to apply them to a specific case study of growth and shoot–root allocation responses to irradiance in seedlings of four woody species grown at 1%, 6%, 20% and 100% full sunlight; and (iii) to propose new methods of estimating PP.

3 The 17 different plasticity indices analysed rendered disparate results, with cross-overs in species PP rankings. Statistical comparisons of PP among species were not possible with most of the indices due to the lack of confidence intervals. The non-linear responses of the traits made the use of the slope of the reaction norm to quantify PP unrealistic, and raised awareness on values derived from studies that consider just two environments.

4 We propose an alternative approach to quantify PP based on phenotypic distances among individuals of a given species exposed to different environments, which is summarized in a relative distance plasticity index (RDPI) that allows for statistical comparisons of PP between species (or populations within species). RDPI was significantly correlated with 12 out of the 17 PP indices analysed. An index including the environmental range leading to the different phenotypes (environmentally standardized plasticity index, ESPI), and thus expressing plasticity per unit of environmental change, is also proposed.

5 The new indexes can statistically segregate and unambiguously rank species according to their PP, which can foster a better understanding of plant ecology and evolution, particularly when common protocols are used by different investigators.

Key-words: Index of phenotypic plasticity, normal distribution, norm of reaction, phenotypic variability, plastic response to environment, *Pinus*, *Quercus*, responsiveness, shoot–root ratio, tree seedlings, variance.

Correspondence: Fernando Valladares, Instituto de Recursos Naturales. Centro de Ciencias Medioambientales. CSIC Serrano 115 dpdo, Madrid E-28006, Spain (tel. +34 917452500 ext. 1204; fax +34 915640800; e-mail valladares@ccma.csic.es).

Introduction

Phenotypic plasticity (PP), or the capacity of a given genotype to render different phenotypic values for a given trait under different environmental conditions, is a basic concept in genetics and evolutionary biology

that has attracted the attention of ecologists for many years (Bradshaw 1965; Bradshaw 2006). The current interest in plasticity results from an urgency to predict species responses to global change (Potvin & Tousignant 1996; Rehfeldt *et al*. 2001) and from the emerging ideas on the importance of plasticity for understanding trait-mediated species interactions (Callaway *et al*. 2003). Distribution shifts triggered by climate change are projected using correlational bioclimate envelope models (see Discussion by Hampe 2004), which can overestimate species losses because key aspects such as plasticity are ignored (Thuiller *et al*. 2005). Analogously, basic models and empirical approaches to community dynamics assume that they are governed by the densities of the interacting species, without considering trait changes that can alter the per capita effect of the reacting species on other species (trait–mediated interactions; Werner & Peacor 2003). Ecological communities are replete with trait–mediated interactions arising from trait plasticity that are often as strong or stronger than density effects (Callaway *et al*. 2003; Werner & Peacor 2003). Thus, the quantification of phenotypic plasticity becomes essential not only for investigators exploring species responses to the environment but also to those aimed at modelling both the effects of global change on species distribution and the outcome of species interactions in community dynamics.

The concept of plasticity is being widely used in an expanding number of disciplines (see Fuller 2003; DeWitt & Scheiner 2004), with an exponential increase of publications in recent decades (Scheiner & DeWitt 2004). For instance, phenotypic plasticity has frequently been reported as the primary mechanism enabling exotics to colonize environmentally diverse areas, a topic explored for more than three decades (e.g. Marshall & Jain 1968) and attracting increasingly more recent attention (Williams *et al*. 1995; Sexton *et al*. 2002; Niinemets *et al*. 2003; Parker *et al*. 2003; Peperkorn *et al*. 2005; Sharma *et al*. 2005). However, even though the literature on phenotypic plasticity is extensive, it fails to provide a clear consensus on the adaptive and evolutionary meaning of plasticity (Via *et al*. 1995; DeWitt & Scheiner 2004). There is agreement on the notion that the degree of phenotypic change across environments differs among species and traits, and that the amount of phenotypic change observed depends on the type of environments considered (Pemac & Tucic 1998; Valladares *et al*. 2002a,b, 2005a; West-Eberhard 2003; Bradshaw 2006). However, there is disagreement regarding its quantification and on the way that natural selection influences reaction norms (trait vs. environment plots; Pigliucci 2001). While those dealing with plasticity accept the working hypothesis that plasticity functions as a way of adapting to variable environments, evolutionary biologists assess plasticity in terms of genetic variation and fitness consequences, plant ecophysiologists translate it in terms of stress tolerance and carbon gain, and developmental biologists in terms of mechanisms by which the environment affects

trait development (Dudley 2004). Plasticity *sensu stricto* has been typically focused on developmental aspects using known genetic lines (e.g. Cheplick 2003; Van Kleunen & Fischer 2003), while plasticity *sensu lato* has been focused on the responses of different species and populations in their ecological context (e.g. Callaway *et al*. 2003; Valladares *et al*. 2005b). The fields of ecology and development are now rapidly developing new insights into plant evolution with plasticity emerging as a key to the understanding of plant development in an ecological context (Farnsworth 2004; Sultan 2005). Ecological development, or 'eco-devo', aims to bridge the gap between the study of developmental mechanisms and the study of ecological and evolutionary diversity (Ackerly & Sultan 2006), the major 'new frontier' in biology (Kafatos & Eisner 2004). Plasticity has become a central focus of this ecological and evolutionary research, bringing new insights into understanding phenotypic variation that shapes ecological interactions and selective change (Ackerly & Sultan 2006).

Research in plasticity has expanded from its initial focus on abiotic factors, such as irradiance or water, to that of biotic factors such as competitors, predators or pollinators (Schlichting 2002; Sultan 2004). A crucial step in ecological approaches to phenotypic plasticity is the quantitative estimation of the phenotypic change induced by the environment, which is of particular relevance in comparative studies of different species and populations (Valladares *et al*. 2000a, 2005a; Balaguer *et al*. 2001). This estimation must be simple, particularly in ecological studies dealing with an ample number of species and traits (e.g. Navas & Garnier 2002; Gratani *et al*. 2003; Castro-Díez *et al*. 2006). In fact, research goals requiring a simplified estimation of plasticity have given rise to a plethora of plasticity indices (e.g. Cheplick 1995; Valladares *et al*. 2000a,b; Richardson *et al*. 2001). Selection of the quantitative estimator of plasticity has an important bearing on both the way plasticity is assessed and the ecological and evolutionary implications that can be extracted. By condensing experimental data, indices can facilitate the presentation and interpretation of complex results, and the use of the same index by different investigators facilitates comparisons of different studies (Weigelt & Jolliffe 2003). However, indices can be flawed and misapplied in different ways, and indices built from similar primary measures can be defined differently, complicating comparisons between studies and the meta-analyses of published data.

In the present study, we first review different approaches undertaken to quantify phenotypic plasticity with special attention to the most common indices used in comparative studies. Secondly, we conducted an experimental case study of plastic responses of woody seedlings to light, to evaluate the degree of coincidence of the various indices in ranking genotypes according to their plasticities. We then introduce a new approach to quantify phenotypic plasticity based on

the phenotypic distances between individuals of a given species exposed to different environments, which is summarized in a relative distance plasticity index (RDPI). RDPI is applied to the study case, and the other indices are regressed against it to determine its utility and consistency. A rescaling of RDPI that includes the environmental range giving rise to each phenotype is also introduced and discussed. Finally, we assess the appropriateness of RDPI and the other indices according to the objectives in each kind of study.

Methods

LITERATURE REVIEW OF QUANTITATIVE ESTIMATORS OF PLASTICITY

We selected relevant studies published between 1965 and 2005, using print and online versions of Science Citation Index and Biological Abstracts, searching for the terms 'phenotypic plasticity', 'plastic response', 'index', 'quantitative' and 'norm of reaction'. In addition, a comprehensive search of suitable articles was carried out using the reference lists of the selected papers. These searches led to a large number of articles that were then examined for the description or usage of a quantitative estimator of plasticity, primarily in the form of an index. The basic information of each estimator was compiled together with key references (Table 1).

CASE STUDY AND EXPERIMENTAL DESIGN

Our case study compares phenotypic plasticity of four different plant species. The study could equally be applied to those dealing with populations (i.e. geographically or ecologically distinct groups of individuals) or clones (i.e. genetically distinct groups of individuals) within species. Standard protocols for the experiments with tree seedlings were carried out (e.g. Sack 2004; Sánchez-Gómez *et al.* 2006). The phenotypic plasticity of tree seedlings in response to light was estimated using different indices for a key plant trait, shoot–root ratio (Table 1). The indices were applied to a specific data set (see Appendix S1 in Supplementary Material) resulting from a 4×4 factorial design with irradiance and plant species as the two factors. One hundred and fifty seedling of each of the four species (*Quercus robur* L., *Quercus pyrenaica* Willd., *Pinus sylvestris* L. and *Pinus pinaster* Ait.) were grown outdoors from February till November at a commercial nursery (Viveros Barbol, Torremocha del Jarama, Madrid, Spain). The area was located at 40°50′ N, 3°29′ W and at 710 m a.s.l. The climate is continental Mediterranean with hot and dry summers and cold winters. The mean maximum and minimum temperatures were 19 °C and 9.5 °C, respectively, for a 35-year period. Most annual rainfall (350–500 mm) is received during spring and autumn (250–350 mm). Soil substrate (pH 6.5) consisted of 3 : 1 volume mixture of peat Vriezenveen PP1 (Potgrond Vriezenveen BV, Westerhaar, the Netherlands), and washed

river sand. We also added 3 kg m^{-3} of Guanumus Angibaud fertilizer (3/35/2 N P-1 K-1; Angiplant, La Rochelle Cedex, France) and 2 kg m^{-3} of Plantacote mix 4 M fertilizer (15/10/15 N P-1 K-1; Aglukon Spezialdünger GMBH & Co. KG, Dusseldorf, Germany).

Seeds of each species were collected during 2000 from one representative Spanish locality (*Q. robur* from Galicia, *Q. pyrenaica* from Sierra de Guadarrama, Madrid, *P. sylvestris* and *P. pinaster* from Sierra de Gredos, Ávila) and were germinated in February 2001 and transplanted to forest multipot (330 cm^3 each pot) containers in early spring. All seeds of a given species were collected from a single tree, so that seedlings of each species were half-siblings. Local air temperature and available photosynthetic photon flux density (PPFD) were registered every 5 min during the growing season with a data logger (HOBO model H08-006–04; Onset, Pocasset, MA, USA) and external sensors cross-calibrated with a Li-Cor 190SA sensor (Li-Cor, Nebraska, USA). Mean daily PPFD over the summer period was 42 mols m^{-2} d^{-1}. Four irradiance levels (1%, 6%, 20% and 100% of full sunlight) were established by using layers of neutral shade cloth supported by metal frames. This gradient spans over the natural range of light availability found in Iberian forest understoreys, 20% being the most common shade under Mediterranean forest canopies and 6% of full sunlight being relatively frequent in humid and sub-humid temperate forests (Gómez *et al.* 2004; Valladares & Guzmán 2006). A shade of 1% of full sunlight is typically found in habitats such as tropical and moist, temperate forests (Canham *et al.* 1990), and also in Mediterranean forests, although less frequently (Gratani 1997; Valladares & Guzmán 2006). This low light level was included to explore seedling responses across a complete irradiance gradient. Air mean temperature during the experiment was similar (± 1 °C) across different irradiance environments. Plants were watered regularly to soil capacity and water availability was monitored by estimation of soil volumetric water content with an Aquaterr Moisture meter (model EC-200, Aquaterr Instruments, Fremont, CA), a capacitance probe that measures the dielectric constant of the soil–air–water combination. Watering was adjusted to obtain similar water availabilities across the different irradiance regimes. Seedlings were arranged along six blocks randomly distributed within each irradiance level. A total number of 44 seedlings were randomly selected for each combination of irradiance and species, except for pines under deep shade, where only 5–34 seedlings survived by the end of the experiment. Each plant was separated into leaves, stem plus branches and roots, and each fraction was dried in an oven at 68 °C ± 2 °C for a minimum of 72 h to obtain dry mass values.

ESTIMATORS OF PHENOTYPIC PLASTICITY AND STATISTICAL COMPARISONS

One-way ANOVA was used to test for species differences in growth (estimated by final biomass) and shoot–root

Table 1 Quantitative estimators of phenotypic plasticity and ranking of the four species studied according to their plastic shoot–root ratio response to light after each estimator. An estimator providing statistical power for comparison is that which provides an estimation of variance that can be used for testing the significance of the differences. Abbreviations of species taken from their scientific name; a letter code within parenthesis indicates different rankings. Rationale for plasticity of different species also applies to populations within species and to clones or genetic lines within populations. In addition, estimators can be applied to comparisons of plasticities for different traits within a given species. Note that data of different individuals within each environment are pooled together for all estimators except for those indicated with an asterisk

Estimator of plasticity	Calculation	Source or examples	Complexity	Weak points	Comments	Ranking of species of the present study
Coefficient of variation-total (CV_t)	Standard deviation/mean *(for the whole data set of each genotype)* (*)	Abundant	Easy	Mixes variability within and between environments. Requires normality. Statistical limitations for comparisons of species	Useful for exploring phenotypic variability in general, including developmental instability. Not a proper estimator of plasticity	Pp > Ps > Qp > Qr (a)
Slope of norm of reaction	Slope of regression of dependent variable vs. environment	Pemac & Tucic, 1998; Schlichting & Pigliucci, 1998	Intermediate	Frequent non-linear responses lead to unreliable or arbitrary values. Statistical limitations for comparisons of species	Not very useful when more than two environments are considered	Pp > Ps > Qr > Qp (b)
Scope of plastic response (D)	Mean at high resource availability-mean at low resource availability	Stearns, 1992	Easy	Not standardized, so different traits cannot be compared. Statistical limitations for comparisons of species	Applicable only when two environments are considered	Pp > Ps > Qr > Qp (b)
Response Coefficient (RC)	Ratio of mean values at high and low resource availability	Larcher, 1995; Poorter & Nagel, 2000	Easy	Not standardized, so different traits cannot be compared. Statistical limitations for comparisons of species	Applicable only when two environments are considered	Pp > Ps > Qr > Qp (b) *Note: a value of 1 indicates no response*
Coefficient of variation over the environments, based on means (CV_m)	Standard deviation of means/mean of means	Schlichting, 1986; Schlichting & Levin, 1984; Valladares *et al.*, 2002a	Easy	Assumes normality. Statistical limitations for comparisons of species		Pp > Ps > Qp > Qr (a)
Coefficient of variation over the environments, based on medians (CV_{md})	Standard deviation of medians/mean of medians	Present study	Easy	Statistical limitations for comparisons of species		Pp > Ps > Qp > Qr (a)
Grand plasticity (P_t)	Standard deviation of means/mean of adjusted means using biomass as a covariate	Navas & Garnier, 2002	Easy	Assumes normality. Statistical limitations for comparisons of species	For exploration of plasticity when some covariate is expected to influence the target variable or trait	Pp > Ps > Qp > Qr (a)
Phenotypic Plasticity Index, based on least square means (PP_F)	$100 \times$ ((least square mean in one environment – least square mean in the other)/ least square mean in the first environment)	Cheplick, 1995	Intermediate	Assumes normality. Requires measurement of a covariate (e.g. seed weight, plant size, time of germination) and adjustment of means for this covariate before any statistics is applied. Statistical limitations for comparisons of species	For exploration of plasticity when some covariate is expected to influence the target variable or trait	Pp > Ps > Qp > Qr (a)
Phenotypic Plasticity Index, based on maximum and minimum means (PI_v)	(Maximum mean-minimum mean)/maximum mean	Valladares *et al.*, 2000a,b, 2002a,b, 2005a,b; Balaguer *et al.*, 2001; Gratani *et al.*, 2003	Easy	Assumes normality. Statistical limitations for comparisons of species	If a covariate is expected to influence the target variable, least square means can be used as in PP_F. A robust, simple and widely used index	Pp > Ps > Qp > Qr (a)
Phenotypic Plasticity Index, based on maximum and minimum medians (PI_{md})	(Maximum median-minimum median)/maximum median	Present study	Easy	Statistical limitations for comparisons of species	Useful when data depart from normality	Pp > Ps > Qp > Qr (a)

Table 1 *Continued*

Estimator of plasticity	Calculation	Source or examples	Complexity	Weak points	Comments	Ranking of species of the present study
Phenotypic Plasticity Index, based on maximum and minimum least square means (PI$_{LSM}$)	(Maximum least square mean-minimum least square mean)/maximum least square mean	Present study	Intermediate	Assumes normality. Requires measurement of a covariate (e.g. seed weight, plant size, time of germination) and adjustment of means for this covariate before any statistics is applied. Statistical limitations for comparisons of species	For exploration of plasticity when some covariate is expected to influence the target variable or trait	Pp > Ps > Qp > Qr (a)
Relative Trait Range (RTR)	(Mean in one end of environmental gradient-mean in the opposite end)/absolute maximum value	Richardson *et al.*, 2001	Easy	Assumes normality. Very sensitive to outliers. Statistical limitations for comparisons of species		Pp > Ps > Qr > Qp (b)
Phenotypic Plasticity Index (PI$_R$)	(Maximum mean-minimum mean)/mean at which maximum growth rate is achieved	Robinson, 1989	Intermediate	Assumes normality. Requires knowledge of RGR. Statistical limitations for comparisons of species		Pp > Ps > Qp > Qr (a)
Phenotypic Inertia (PIN)	(Σ(Survival$_i$∗performance$_i$))/(n∗SD) Calculated for each (i) of the n environments	Milberg *et al.*, 1999	Complex	Assumes normality. Requires knowledge of survival. Statistical limitations for comparisons of species	For exploration of plasticity, assuming that mortality is the ultimate expression of lack of plasticity	Ps > Pp > Qr > Qp (c) *Note: the inverse of PIN is used for this ranking*
Relative Distances Plasticity Index (RDPI)	Absolute phenotypic distances between individuals of same genotype and different environments, divided by one of the two phenotypic values (*)	Present study	Intermediate	Complex computing when the number of replicates or environments generates too long arrays of data (distances)	For exploration of plasticity with strong statistical power to test for differences in plasticity between genotypes. If distribution of the distances cannot be normalized, medians should be used instead of means.	Pp > Ps > Qr = Qp (d)
Simplified Relative Distances Plasticity Index (RDPI$_s$)	Absolute phenotypic distances between means of same genotype and different environments, divided by one of the two mean phenotypic values	Present study	Easy	Assumes normality. If there is not an ample number of environments compared (≥ 3), there is no advantage of using this index instead of previous ones (e.g. P$_I$, PI$_v$ PI$_R$)	For exploration of plasticity with statistical power for testing for differences in plasticity between genotypes	Pp > Ps > Qr = Qp (d)
Environmentally Standardized Plasticity Index (ESPI)	(Maximum mean-minimum mean)/absolute distance between environmental values at maximum and minimum (*)	Present study	Easy	Assumes normality. Choice of appropriate environmental range is crucial (see Fig. 3). Maintains the units of the variable, so comparisons of plasticity for different traits are not possible	For exploration of the effect of environment on target trait with statistical power. If response to environment is linear or well-known, results from data sets using different environmental ranges can be compared	Pp > Ps > Qr = Qp (d)
Environmentally Standardized Plasticity Index for individual distances (ESPI$_{ID}$)	Absolute phenotypic distances between individuals of same genotype and different environments, divided by absolute distance between environmental values (*)	Present study	Complex	Determination of environmental distances among individuals is complex and time consuming	Useful when environment is taken as a continuous variable and values for each individual are known	Not calculated (not appropriate environmental data)

ratio, and to test for species–treatment interactions using Statistica version 6.0 (StatSoft Inc., Tulsa, OK, USA). Shoot–root ratio was transformed ($x' = x^{-0.1}$) to obtain a normal distribution before running the ANOVA. Seventeen different estimators of phenotypic plasticity of shoot–root ratio in response to light were calculated in seedlings of the four species. The original data set is presented in Appendix S1. Each index is presented in Table 1 together with a brief description, relevant citations and additional comments. The ranking of the four species studied according to their plasticity for shoot–root ratio was calculated for each estimator. Next we describe alternative approaches for phenotypic plasticity quantification based on pairwise comparisons across individuals of each species grown under different environments (RDPI), as well as an environmentally standardized plasticity index (ESPI). The rationale followed for interspecific comparisons of phenotypic plasticity can equally be applied to populations or clones within species. Calculations of all indices of plasticity (including RDPI and ESPI) must be done within a given species and a given trait, and, whenever possible, within a given genotype. In our case, all replicates within species were half-siblings and we did not distinguish genotypes within species. The relationships between phenotypic plasticity in response to light of shoot–root ratio of each species estimated with different indices was determined by linear regression analyses using Statistica version 6.0.

RELATIVE DISTANCES PLASTICITY INDEX AND ENVIRONMENTALLY STANDARDIZED PLASTICITY INDEX

For a single species and trait we can consider our data set as an array or rectangular matrix X_{ij} where i (rows) represents a given level of the environmental treatment, and j (column) refers to the individual number identification along a given row (environmental treatment). In our case study there are four irradiance levels ($i = 1, 2 \ldots 4$) and the total number of treatments (I) equals four. The symbol j is the seedling number. If we denote as N_i the sample size or number of seedlings included in each irradiance level (i) ($N_i = 44$), then for each species j ranges $j = 1, 2 \ldots 44$. We can refer as x_{ij} the trait value of a given individual j ($j = 1, \ldots, N_i$), subjected to light treatment i ($i = 1 \ldots I$).

We can relate phenotypic plasticity for a given trait (x) and species with respect to environmental variable L, to the difference in x among two individuals of the same species grown in different environments. Phenotypic plasticity can then be defined as a random variable, each realization being described by the absolute distance between two randomly selected individuals (j and j') of the same species belonging to different environments (i and i', where i is always different from i', as individuals were grown in different environments). We can extend this approach to our whole data set and compute pairwise distances across all individuals and environments.

Specifically, we define the distance among trait values $d_{ij \to i'j'}$ for all pairs of individuals for which i is different from i' (the two individuals were grown under different light environments) as the absolute value of the difference $x_{i'j'} - x_{ij}$ when $i \neq i'$, and obtain relative distances by dividing this difference by the sum ($x_{i'j'} + x_{ij}$). Therefore, relative distances $\text{rd}_{ij \to i'j'}$ are defined as $d_{ij \to i'j'}/(x_{i'j'} + x_{ij})$ for all pairs of individuals of a given species grown in different environments. This set of distances is thus taken as a random variable that describes phenotypic distance for a given trait among individuals grown in different environments for a given species. If we compute these distances for all species under consideration, the resulting statistical distribution of relative distances for each species can be subjected to hypothesis testing to test for differences among the dependent (phenotypic distances) and the independent variable (species). The error term in these distributions would primarily account in each species for uncontrolled intraspecific genetic differences, undetected fine grain environmental heterogeneity, error measurements, and developmental instability. A relative distance plasticity index (RDPI) ranging from 0 (no plasticity) to 1 (maximal plasticity) can be obtained for each species as

$$\text{RDPI} = \sum (d_{ij} \to i'j'/(x_{i'j'} + x_{ij}))/n$$

where n is the total number of distances. When the number of replicates, species and environments excessively complicates the calculations, the index can be simplified (RDPI_S) by calculating the distances among mean phenotypic values for each species–environment combination. RDPI_S is the result of more than one distance only when more than two environments are used for a given species and trait. In our case study, the number of distances for RDPI_S was six for each species and trait, from which we calculated the mean and the variance for the statistical comparisons of species.

In our case study, differences between species in RDPI and RDPI_S for the variable shoot–root ratio were evaluated with one-way ANOVA and *post hoc* Tukey mean comparison test using Statistica version 6.0, considering 'species' as a factor.

Finally, it may be of interest to standardize plasticity for a given environmental change. Thus, if X and x are the maximum and minimum mean phenotypic values of a given species across different environments, respectively, and E and e are the mean environmental values at which X and x were achieved, we can refer to an environmentally standardized plasticity index (ESPI) as:

$$\text{ESPI} = (X - x)/|E - e|.$$

By combining RDPI and ESPI, an environmentally standardized plasticity index for individual distances (ESPI_{ID}) can be calculated as the mean of absolute phenotypic distances between individuals of the same species but exposed to different environments, divided by absolute distance between the environmental values
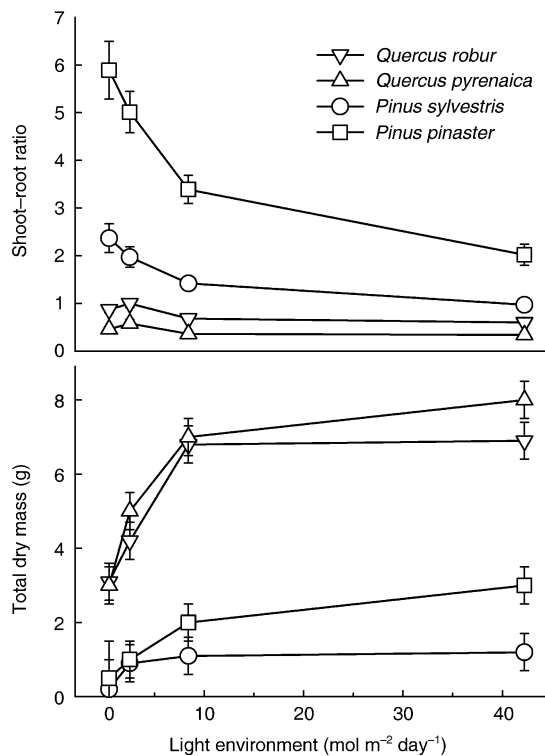
Fig. 1 Shoot–root ratio and plant biomass plastic responses to light in seedlings of four tree species (norms of reaction of the data set of Appendix S1). Light environment is defined by the mean photosynthetically active radiation available in each treatment integrated over the day for a 15-day period in July 2001; the four treatments corresponded approximately to 1%, 6%, 20% and 100% full sunlight. Data points are means + SD (unless eclipsed by the symbol); $n = 36$, except for *Pinus sylvestris* at 5% ($n = 26$) and *P. pinaster* at 5% ($n = 5$).

Results

Plant biomass increased and shoot–root ratio decreased with light availability (Fig. 1). Both responses significantly differed among seedlings of the four species studied, but in all cases the responses were non-linear with only minor phenotypic changes from moderate shade (8 mol photon m^{-2} day^{-1}, equivalent to 20% full sunlight) to full sunlight (42 m^{-2} day^{-1}). Two-way ANOVA of shoot–root ratio revealed significant differences between species and light treatments (Table 2). The species–treatment interaction was significant when plant biomass was taken as a covariate, revealing significant species differences in their plastic phenotypic response to light, that were not simply due to differences in plant size.

Four different rankings of species according to their phenotypic plasticity of shoot–root ratio in response to light were obtained using the different indices (Table 1). All the indices indicated higher phenotypic plasticity in pines than in oaks, but the relative order of each pine and oak species differed. The significance of the differences in phenotypic plasticity among species could only be obtained for RDPI and ESPI. Both revealed that the plasticity of the two oak species was not significantly different ($P > 0.05$, Table 1).

All indices of phenotypic plasticity were correlated (data not shown), and all regressions of the different indices with RDPI were significant (Fig. 2). Only the coefficient of variation for all data (not the coefficient calculated over the environments) failed to exhibit any relationship with RDPI. The regression slope was positive except for phenotypic inertia (PIN), scope of the plastic response (D), phenotypic plasticity index using biomass as a covariate (PPF), and response coefficient (RC) (Fig. 2).

The ESPI calculated for all the different environmental distances between light treatments revealed that the largest mean response in shoot–root ratio was obtained when differences in light availability were large (39 mol m^{-2} day^{-1}) while the largest difference in plant growth (estimated by total biomass at the end of

experienced by each individual. Other statistics such as the median can be used when the resulting distribution is not normal. $ESPI_{ID}$ can only be obtained when environment is taken as a continuous variable and environmental values for each individual are known. Thus, $ESPI_{ID}$ minimizes the variability introduced by the environmental heterogeneity within each environment.

Table 2 Results of the ANOVA of the shoot–root ratio of seedlings of *Quercus robur*, *Q. pyrenaica*, *Pinus sylvestris* and *P. pinaster* in response to four different light treatments both with and without total plant biomass as a covariate. Shoot–root ratio was transformed ($x' = x^{-1}$) to obtain a normal distribution. Original data set in Appendix S1

| Source of variance	Degrees of freedom	Sum of squares	Mean squares	F	P
(a) No covariate					
Species	3	2.300	0.767	474.31	< 0.0001
Light treatment	3	0.303	0.101	62.58	< 0.0001
Interaction (species × treatment)	9	0.028	0.003	1.91	0.048
Residual	519	0.839	0.002		
(b) Using biomass as covariate					
Species	3	1.614	0.538	344.76	< 0.0001
Light treatment	3	0.272	0.091	58.18	< 0.0001
Interaction (species × treatment)	9	0.039	0.004	2.78	0.0035
Covariate (biomass)	1	< 0.0001	< 0.0001	0.29	0.591
Residual	519	0.891	0.002		
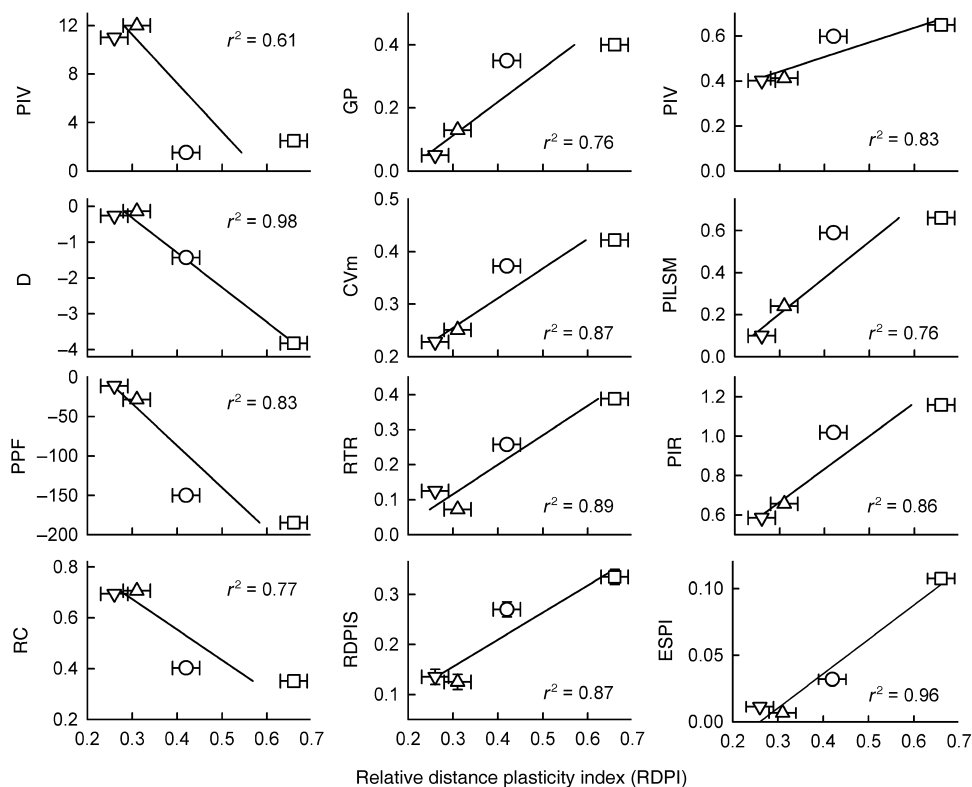
Fig. 2 Linear regression analysis of different indices of phenotypic plasticity vs. relative distances plasticity index (RDPI, this study). Indices abbreviations are: PIN, phenotypic inertia (Milberg *et al.* 1999); D, scope of plastic response (Stearns 1992); PPF, phenotypic plasticity index of Cheplick (1995) based on least square means; RC, response coefficient (Poorter & Nagel 2000); GP, grand plasticity (Navas & Garnier 2002); CVm, coefficient of variance over environments (Schlichting 1986); RTR, relative trait range (Richardson *et al.* 2001); PIR, plasticity index of Robinson (1989); CVM, coefficient of variation using means for each environment; PIV, plasticity index of Valladares *et al.* (2000b); PILSM, plasticity index of Valladares *et al.* (2000b) but using least square means with biomass as a covariate; RDPI$_S$, simplified RDPI; and ESPI, environmentally standardized plasticity index (see Table 1 for more details). 95% confidence interval for the mean (ANOVA, Tukey test) for RDPI and RDPI$_S$ is shown unless eclipsed by the symbol. Coefficient of determination (r^2) is indicated in each case; all regressions were significant ($P < 0.001$). Legend for species in Fig. 1.

the investigation) was obtained for relatively minor differences in light availability (8 mol m^{-2} day^{-1}) (Fig. 3). A small increase of light availability in the shaded end of the light gradient significantly enhanced growth with no changes in allocation, while allocation was affected by large differences in light availability that had proportionally small effects on total plant mass due to the non-linear growth response to light, which saturates from moderate to high light.

Discussion

DISSECTION OF THE PHENOTYPIC VARIATION

Phenotypic variation is a primary requisite for plant evolution by natural or artificial selection but its understanding is far from trivial (Schmid 1992; Briggs & Walters 1997; Valladares *et al.* 2002a). Phenotypic plasticity is part of this variability but it is not necessarily linked to higher phenotypic variability, as was found in our case study by a lack of relationship between the coefficient of variation and any estimator of plasticity. A comparative survey of a wide range of

ecological studies has revealed that ecologists are roughly explaining half of the variation in the variables of interest (Peek *et al.* 2003), significantly more than previously estimated (Moller & Jennions 2002) but still far from the 100% target. The amount of variance explained by a given factor (e.g. plastic response to the environment) depends on the extent to which confounding variables are controlled for, either experimentally or statistically, although certain unexplained variance is always inevitable. Data scatter, however, can hide biologically meaningful information as argued by Sultan (1992), and even a minute size effect (e.g. a small fraction of phenotypic variation explained by an environmental change) may be biologically important. This is potentially the case of most evolutionary issues because small effects can be greatly magnified when a persistent pattern occurs across many generations. We argue that a complete understanding of plant responses to the environment requires the dissection of phenotypic variation into as many components (e.g. phenotypic plasticity and developmental instability) and ultimate causes (e.g. genetic variability, measurement accuracy, environmental heterogeneity and ontogenetic effects) as possible (Fig. 4).
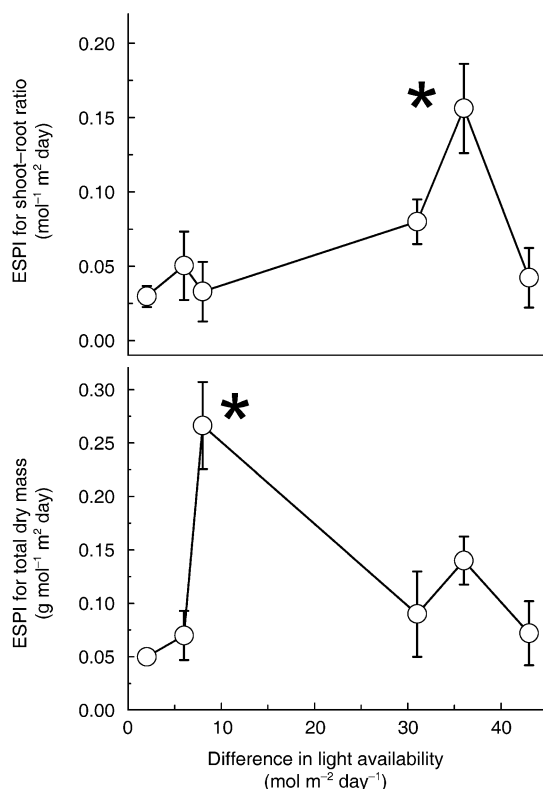
Fig. 3 Environmentally standardized plasticity index (ESPI) for shoot–root ratio (above) and total dry mass (below) for the six intervals of light availability resulting from the four light treatments. Values are the mean + SE for the four species studied, and asterisks indicate the value significantly different from the others (ANOVA, Tukey test, $P < 0.05$).

THE REACTION NORM AND THE CHOICE OF THE ESTIMATOR OF PLASTICITY

The norm of reaction is the most immediate way of exploring phenotypic plasticity (Schlichting & Pigliucci 1998; Stelzer 2002) and many investigators commonly use reaction norms to analyse the microevolution and plasticity of life-history traits (Stearns 1992). Assuming

linear changes, the reaction norm is usually represented by the regression line of the plot of trait expression against environment, and for comparative purposes, the magnitude of phenotypic plasticity can be evaluated as the slope of the reaction norm of the trait (Gianoli & Gonzalez-Teuber 2005). However, because plastic responses to environment are generally complex and not linear, as in the case for the traits explored here, the general validity of this approach is unclear. Only when the response of a given genotype to the whole environmental range is well known or when species or populations typically segregate in two contrasting environments can the study be based on two-environment reaction norms (Table 3, Fig. 4).

Undesired variance in a reaction norm can be reduced by measuring the environmental conditions experienced by each individual plant instead of taking the mean environmental value experienced by all the individuals within a given experimental or natural environment. Thus, the environmental variable is taken as continuous and the reaction norm becomes probabilistic, translating a distribution of environments into a distribution of phenotypes (Thompson 1991; Heino *et al.* 2002). This in turn, alleviates problems of pseudoreplication (Hurlbert 1984). The environmentally standardized plasticity index calculated with phenotypic distances ($ESPI_{ID}$) expresses phenotypic change per unit of environmental change and allows for statistical comparisons across species and populations when values for the environment of each individual are available.

Many studies have used phenotypic plasticity indices to summarize the environmentally contingent trait expression of a given species, set of species or populations within a given species (Cheplick 1995; Poorter & Nagel 2000; Valladares *et al.* 2000a, 2002b, 2005a; Balaguer *et al.* 2001; Gratani *et al.* 2003) (see Table 1). Simple plasticity indices based on the mean phenotypes observed in each environment (e.g. the coefficient of variation over environments, Schlichting 1986; or
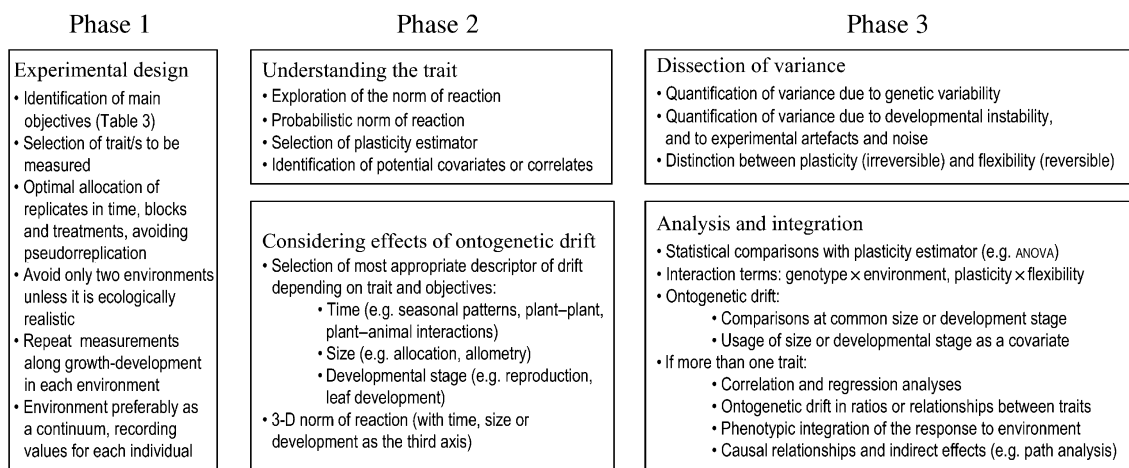

Fig. 4 Main phases in the process of quantifying phenotypic plasticity

Table 3 Estimators of phenotypic plasticity recommended for each kind of study. Estimators marked with asterisks should only be used in studies of only two environments. Ontogenetic effects must be taken into account particularly for objectives 2, 3 and 4. Rationale of comparative studies of plasticity across species also applies to populations within species and to clones or genetic lines within populations. Abbreviations and descriptions of indices in Table 1

Objective of the study	Estimator
1. Description of the phenotypic response to the environment	Norm of reaction, developmental reaction norm (DRN, Pigliucci *et al.*, 1996; Cheplick, 2003)
2. Detection of differences in plasticity among species	ANOVA (environment × genotype interaction). Individuals of similar size or developmental stage must be compared
3. Ranking species according to their plasticity	RDPI, RDPI$_S$. Statistics with other indices are problematic. Individuals of similar size or developmental stage must be compared
4. Exploration of relative plasticity of two (or more) functionally related traits	Ontogenetic drift must be first studied in each trait independently using developmental reaction norms (Coleman *et al.*, 1994; Cheplick, 2003). Then, regression and general statistical analyses with PI$_V$, PI$_{md}$, PI$_{LSM}$, RTR*, CV$_m$, CV$_{md}$, P$_i$, PP$_F$, PI$_R$, Slope of norm of reaction*, RDPI, RDPI$_S$
5. Comparison of plasticity for different traits, e.g. physiological vs. morphological traits	PI$_V$, PI$_{md}$, PI$_{LSM}$, RTR*, CV$_m$, CV$_{md}$, P$_i$, PP$_F$, PI$_R$, Slope of norm of reaction*, RDPI, RDPI$_S$
6. Quantification of the phenotypic change for a given environmental change	ESPI, ESPI$_{ID}$
7. Exploration of changes in flexibility (acclimation capacity, i.e. reversible phenotypic change) with development	ANOVA (plasticity × flexibility interaction; Piersma & Drent, 2003)

the plasticity index of Valladares *et al.* 2000b) are quick estimates that have been used relatively often but are weak for statistical comparisons (e.g. Castro-Díez *et al.* 2006). In this investigation, we have analysed the potential for comparing genotypes of a new quantitative approach to phenotypic plasticity based on phenotypic distances among individuals from different environments, which can be summarized in an index (RDPI). RDPI has the advantage of not assuming any particular distribution of the data and of significantly increasing the power of the statistical analyses, a highly critical issue in factorial experiments with low number of replicates. Its calculation is simple, but contrary to other indices (PIV, RTR and PPF), RDPI is sensitive to the number of environments studied and whether they are balanced according to the norm of reaction of each genotype. For example, if more than one light environment that is well-above saturation for photosynthesis is considered, photosynthetic plasticity would be underestimated by RDPI. The same applies to indices where a wide range of environments is considered (slope of norm of reaction and coefficient of variation). When the level of response is not equal across different environments (e.g. higher phenotypic values towards one end of the environmental gradient), indices using maximum and minimum values (PIV, PIR) are better estimators of overall plasticity. A correction for this underestimation of RDPI could be obtained by calculating pairwise distances among a subset of the environments, which can be selected after exploration of the norm of reaction. The estimation of plasticity using phenotypic values at one end of the environmental gradient vs. those at the other end (e.g. RTR, D and PPF) can also lead to bias because maximum and minimum phenotypic values are frequently observed at intermediate environments.

The consideration of only extreme environments plus the usage of the maximum absolute value, which is quite sensitive to outliers, makes RTR (Richardson *et al.* 2001) not a very reliable index. Taking into account the complexity and the requirements of each index (Table 1) together with the different objectives of each study, different indices can be recommended in each case (Table 3).

Because there is no estimator of dispersion in most plasticity indices, statistical comparisons of plasticity among genotypes or variables are not possible. A way of overcoming this lack of replicates is to compare the phenotypic plasticity obtained as the mean of the index values for several traits, as has been done elsewhere (Valladares *et al.* 2002b; Gratani *et al.* 2003; Castro-Díez *et al.* 2006), but pooling together different variables can be problematic due to their different mathematical properties and biological meanings.

Quantitative estimation of phenotypic plasticity is, however, more than the appropriate choice of an index. It is as an integrated process that involves a robust experimental design, a correct choice and understanding of the trait, a detailed dissection of all sources of phenotypic variation, and a correct incorporation of developmental drift (Fig. 4).

ONTOGENETIC EFFECTS AND OTHER METHODOLOGICAL CONSIDERATIONS

Consideration of genetic and environmental effects on microevolution may not suffice if genotype by environment interactions change over developmental time (Cheplick 2003). There are many traits that change during ontogeny and this developmental trajectory must be taken into account when quantifying plasticity (Watson *et al.* 1995). A case in point is light capture

efficiency, which decreases with plant age (Lusk 2004; Pearcy *et al*. 2005). Because plants in the shade both grow more slowly and minimize self-shading, a careful examination of self-shading evolution during growth must be carried out to disentangle both processes. If the trait cannot be measured several times throughout the development of the plant to obtain a 3-D norm of reaction (Pigliucci & Schlichting 1995), it must be measured earlier in the most productive or favourable environment than in the least productive environment. The so-called developmental reaction norm represents the set of ontogenetic trajectories that can be produced by a genotype exposed to different environmental conditions (Cheplick 2003), and the plastochron index can be used to establish the developmental stage of each plant (Yamashita *et al*. 2002). The problems arising from not considering ontogeny in studies of plasticity are exemplified by a comparative study of the response of 27 plant species to nutrient availability (Müller *et al*. 2000): different biomass ratios under different nutrient conditions represented points on simple allometric trajectories, i.e. most of the allocation changes were largely a consequence of plant size.

Common surrogates of plant development are plant size and biomass, which can be used as covariates for certain indices (Table 1). The usage of plant biomass as a covariate when exploring allometric traits is recommended by many investigators (Cheplick 1995; Pemac & Tucic 1998; McConnaughay & Coleman 1999; Ryser & Eek 2000). However, it only marginally affected the results of the ANOVA in our case study, making the species–light treatment interaction more significant (Table 2). It must be noted that the values of the indices did change when plant biomass was used as a covariate for ANOVA and least square means were used instead of plain means. For example PIV ranged from 0.4 to 0.6 and it ranged from 0.1 to 0.6 with plant biomass as the covariate (PI$_{LSM}$). Coleman *et al*. (1994) suggested that the standards for comparing phenotypic traits depend on the objectives of the study (see Fig. 4 for examples). In the case of studies addressing phenotypic responses of two or more interrelated phenotypic characters such as shoot–root ratio (the current study) or reproductive–vegetative biomass, the interpretation of these ratios depends on the amount of ontogenetic drift exhibited by each individual trait (Coleman & McConnaughay & Ackerly 1994; Stearns *et al*. 1991).

Theoretically, phenotypic plasticity should be estimated in genetically identical individuals exposed to different environments. However, this is not possible in many ecological studies, where plasticity can only be taken in a general sense (e.g. Callaway *et al*. 2003; Berg *et al*. 2005; Griffith & Sultan 2005; Peperkorn *et al*. 2005). Mean plasticity across similar but not identical genotypes of a species can be referred to as population phenotypic plasticity, as suggested elsewhere (Valladares 2003; Einhorn 2005), but an adequate sampling of the population is required for the unbiased estimation of this mean overall plasticity. In order to minimize

problems in comparisons across species due to uncontrolled genetic variability, all individuals of a given species should have the same *a priori* variability. In our case study, as is frequently the case for studies dealing with woody plants, this was achieved by using half-siblings.

RANKING OF GENOTYPES ACCORDING TO THEIR PLASTICITY

Even though most studies of phenotypic plasticity have been focused on a single species or population, there is increasing interest in both demonstrating and understanding why plasticity differs substantially even in closely related species or proximal populations (Balaguer *et al*. 2001; Gianoli & Gonzalez-Teuber 2005; Griffith & Sultan 2005; Valladares *et al*. 2005a,b). The question of why plasticity is not universally widespread in plants (Van Kleunen & Fischer 2005) remains unsolved and comparative studies that rank species according to their plasticity are essential to this important question (Valladares *et al*. 2000a; Bradshaw 2006). Despite the general correlation found here among most indices, four different rankings of species were obtained in our case study (Table 1). This emphasizes the need for careful selection of the best method and index to be used for the quantitative estimation of plasticity in comparative studies. By statistically segregating species according to their plasticity, studies using indices can provide fundamental knowledge for further research into the mechanisms and evolutionary implications of these differences.

After many decades of studies on the phenotypic plasticity of plants, we know more of its potential ecological and evolutionary implications than of its real extent in plants from different habitats, of different growth forms and from different phylogenetic groups (Grime & Mackey 2002; Gratani *et al*. 2003; Maron *et al*. 2004; Scheiner & DeWitt 2004). Common, standard protocols and reliable quantitative estimators of plasticity should be adopted to fill this gap. Here, we attempt to raise awareness of the need for an integrated experimental design and a careful selection of the quantitative estimator of plasticity. The scientific reward for such integrated studies of phenotypic plasticity, particularly in the light of rapidly changing environmental conditions, is likely to be large.

© 2006 The Authors
Journal compilation
© 2006 British
Ecological Society,
Journal of Ecology,
94, 1103–1116

evolutionary concepts involved. The manuscript has also benefited from thoughtful comments of two anonymous referees. Financial support was provided by two grants of the Spanish Ministry of Education and Science (RASINV, CGL2004-04884-C02-02/BOS, and PLASTOFOR, AGL2004-00536/FOR). Cooperative data analysis was made possible by the Spanish thematic network GLOBIMED (www.globimed.net).

References

Ackerly, D. & Sultan, S. (2006) Mind the gap: the emerging synthesis of plant 'ecodevo'. *New Phytologist*, **170**, 648–653.

Balaguer, L., Martínez-Ferri, E., Valladares, F., Pérez-Corona, M.E., Baquedano, F.J., Castillo, F.J. & Manrique, E. (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology*, **15**, 124–135.

Berg, H., Becker, U. & Matthies, D. (2005) Phenotypic plasticity in *Carlina vulgaris*: effects of geographical origin, population size, and population isolation. *Oecologia*, **143**, 220–231.

Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.

Bradshaw, A.D. (2006) Unravelling phenotypic plasticity – why should we bother? *New Phytologist*, **170**, 644–648.

Briggs, D. & Walters, S.M. (1997) *Plant Variation and Evolution*. Cambridge University Press, Cambridge.

Callaway, R., Pennings, S.C. & Richards, C.L. (2003) Phenotypic plasticity and interactions among plants. *Ecology*, **84**, 115–1128.

Canham, C.D., Denslow, J.S., Platt, W.J., Runkle, J.R., Spies, T.A. & White, P.S. (1990) Light regimes beneath closed canopies and tree-fall gaps in temperate and tropical forests. *Canadian Journal of Forestry Research*, **20**, 620–631.

Castro-Díez, P., Navarro, J., Pintado, A., Sancho, L.G. & Maestro, M. (2006) Interactive effects of shade and irrigation on the performance of seedlings of three Mediterranean *Quercus* species. *Tree Physiology*, **26**, 389–400.

Cheplick, G.P. (1995) Genotypic variation and plasticity of clonal growth in relation to nutrient availability in *Amphibromus scabrivalvis*. *Journal of Ecology*, **83**, 459–468.

Cheplick, G.P. (2003) Evolutionary significance of genotypic variation in developmental reaction norms for a perennial grass in competition. *Evolutionary Ecology*, **17**, 175–196.

Coleman, J.S. & McConnaughay & Ackerly, D.D. (1994) Interpreting phenotypic variation in plants. *Trends in Ecology and Evolution*, **9**, 187–191.

DeWitt, T.J. & Scheiner, S.M., eds (2004) Phenotypic plasticity. *Functional and Conceptual Approaches*. Oxford University Press, Oxford.

Dudley, S.A. (2004) The functional ecology of phenotypic plasticity in plants. *Phenotypic Plasticity Functional and Conceptual Approaches* (eds T.J. De Witt & S.M. Scheiner). Oxford University Press, Oxford.

Einhorn, K.S. (2005) Growth and physiology of ash (*Fraxinus excelsior*) and beech (*Fagus sylvatica*) seedlings in response to a light gradient following natural gap formation *Ecological Bulletins*, **52**, 1–53.

Farnsworth, E. (2004) Hormones and shifting ecology throughout plant development. *Ecology*, **85**, 5–15.

Fuller, T. (2003) The integrative biology of phenotypic plasticity. *Biology and Philosophy*, **18**, 381–389.

Gianoli, E. & Gonzalez-Teuber, M. (2005) Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). *Evolutionary Ecology*, **19**, 603–613.

Gómez, J.M., Valladares, F. & Puerta-Piñero, C. (2004) Differences between structural and functional heterogeneity caused by seed dispersal. *Functional Ecology*, **18**, 787–792.

Gratani, L. (1997) Canopy structure, vertical radiation profile and photosynthetic function in a *Quercus ilex* evergreen forest. *Photosynthetica*, **33**, 139–149.

Gratani, L., Meneghini, M., Pesoli, P. & Crescente, M.F. (2003) Structural and functional plasticity of *Quercus ilex* seedlings of different provenances in Italy. *Oecologia*, **17**, 515–521.

Griffith, T.M. & Sultan, S.E. (2005) Shade tolerance plasticity in response to neutral vs green shade cues in *Polygonum* species of contrasting ecological breadth. *New Phytologist*, **166**, 141–148.

Grime, J.P. & Mackey, J.M.L. (2002) The role of plasticity in resource capture by plants. *Evolutionary Ecology*, **16**, 299–307.

Hampe, A. (2004) Bioclimate envelope models: what they detect and what they hide. *Global Ecology and Biogeography Letters*, **13**, 469–476.

Heino, M., Dieckmann, U. & Godo, O.R. (2002) Measuring probabilistic reaction norms for age and size at maturation. *Evolution*, **56**, 69–678.

Hurlbert, S.H. (1984) Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, **54**, 187–211.

Kafatos, F. & Eisner, T. (2004) Unification in the century of biology. *Science*, **303**, 1257.

Larcher, W. (1995) Physiological plant ecology. *Ecophysiology and Stress Physiology of Functional Groups*. Springer-Verlag, Berlin-Heidelberg.

Lusk, C.H. (2004) Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Functional Ecology*, **18**, 820–828.

Maron, J.L., Vila, M., Bommarco, R., Elmendorf, S. & Beardsley, P. (2004) Rapid evolution of an invasive plant. *Ecological Monographs*, **74**, 261–280.

Marshall, D.R. & Jain, S.K. (1968) Phenotypic plasticity of *Avena fatua* and *A. barbata*. *American Naturalist*, **102**, 457–467.

McConnaughay, K.D.M. & Coleman, J.S. (1999) Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. *Ecology*, **80**, 2581–2593.

Milberg, P., Lamont, B.B. & Pérez-Fernández, M.A. (1999) Survival and growth of native and exotic composites in response to a nutrient gradient. *Plant Ecology*, **145**, 125–132.

Moller, A.P. & Jennions, M.D. (2002) How much variance can be explained by ecologists and evolutionary biologists? *Oecologia*, **10**, 1007–1020.

Müller, I., Schmid, B. & Weiner, J. (2000) The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 115–127.

Navas, M.L. & Garnier, E. (2002) Plasticity of whole plant and leaf traits in *Rubia peregrina* in response to light, nutrient and water availability. *Acta Oecologica*, **23**, 375–383.

Niinemets, U., Valladares, F. & Ceulemans, R. (2003) Leaf-level phenotypic variability and plasticity of invasive *Rhododendron ponticum* and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites. *Plant Cell and Environment*, **26**, 941–956.

Parker, I.M., Rodriguez, J. & Loik, M.E. (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology*, **17**, 1–15.

Pearcy, R.W., Muraoka, H. & Valladares, F. (2005) Crown architecture in sun and shade environments: assessing function and tradeoffs with a 3-D simulation model. *New Phytologist*, **166**, 791–800.

Peek, M.S., Leffler, A.J., Flint, S.D. & Ryel, R.J. (2003) How much variance is explained by ecologists? Additional perspectives. *Oecologia*, **137**, 161–170.

Pemac, D. & Tucic, B. (1998) Reaction norms of juvenile traits to light intensity in *Iris pumila* (Iridaceae): a comparison of populations from exposed and shaded habitats. *Plant Systematics and Evolution*, **209**, 159–176.

Peperkorn, R., Werner, C. & Beyschlag, W. (2005) Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. *Functional Plant Biology*, **32**, 12.

Piersma, T. & Drent, J. (2003) Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution*, **18**, 228–233.

Pigliucci, M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture John*. Hopkins University Press, Baltimore.

Pigliucci, M. & Schlichting, C.D. (1995) Ontogenetic reaction norms in *Lobelia siphilitica* (Lobeliaceae): reponse to shading. *Ecology*, **76**, 2134–2144.

Pigliucci, M., Schlichting, C.D., Jones, C.S. & Schwenk, K. (1996) Developmental reaction norms: the interactions among allometry, ontogeny and plasticity. *Plant Species Biology*, **11**, 69–85.

Poorter, H. & Nagel, O. (2000) The role of biomass allocation in the growth response of plants to different levels of light, CO_2, nutrients and water: a quantitative review. *Australian Journal of Plant Physiology*, **27**, 595–607.

Potvin, C. & Tousignant, D. (1996) Evolutionary consequences of simulated global change: genetic adaptation or adaptive phenotypic plasticity. *Oecologia*, **108**, 683–693.

Rehfeldt, G., Wykoff, W.R. & Ying, C.C. (2001) Physiological plasticity, evolution, and impacts of a changing climate on *Pinus contorta*. *Climatic Change*, **50**, 355–376.

Richardson, A.D., Ashton, P.M.S., Berlyn, G.P., McGroddy, M.E. & Cameron, I.R. (2001) Within-crown foliar plasticity of western hemlock, *Tsuga heterophylla*, in relation to stand age. *Annals of Botany*, **88**, 1007–1015.

Robinson, D. (1989) Phenotypic plasticity in roots and root systems: constraint, compensations and compromises. *Aspects of Applied Biology*, **22**, 49–55.

Ryser, P. & Eek, L. (2000) Consequences of phenotypic plasticity vs. interspecific differences in leaf and root traits for acquisition of aboveground and belowground resources. *American Journal of Botany*, **87**, 402–411.

Sack, L. (2004) Responses of temperate woody seedlings to shade and drought: do trade-offs limit potential niche differentiation? *Oikos*, **107**, 107–127.

Sánchez-Gómez, D., Valladares, F. & Zavala, M.A. (2006) Functional traits and plasticity underlying shade tolerance in seedlings of four Iberian forest tree species. *Tree Physiology*, **26**, in press.

Scheiner, S.M. & DeWitt, T.J. (2004) Future research directions. *Phenotypic Plasticity Functional and Conceptual Approaches* (eds T.J. De Witt & S.M. Scheiner). Oxford University Press, Oxford.

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

Schlichting, C.D. (2002) Phenotypic plasticity in plants. *Plant Species Biology*, **17**, 85–88.

Schlichting, C.D. & Levin, D.A. (1984) Phenotypic plasticity of annual *Phlox*: tests of some hypotheses. *American Journal of Botany*, **71**, 252–260.

Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution. A Reaction Norm Perspective*. Sinauer Associates Inc, Sunderland.

Schmid, B. (1992) Phenotypic variation in plants. *Evolutionary Trends in Plants*, **6**, 45–60.

Sexton, J.P., McKay, J.K. & Sala, A. (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications*, **12**, 1652–1660.

Sharma, G.P., Singh, J.S. & Raghubanshi, A.S. (2005) Plant invasions: emerging trends and future implications. *Current Science*, **88**, 726–734.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, New York.

Stearns, S.C., De Long, G. & Newman, B. (1991) The effects of phenotypic plasticity on genetic correlations. *Trends in Ecology and Evolution*, **6**, 122–126.

Stelzer, C.P. (2002) Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: mechanisms and adaptive significance. *Functional Ecology*, **16**, 835–841.

Sultan, S.E. (1992) What has survived of Darwin's theory? Phenotypic plasticity and the Neo-Darwinian legacy. *Evolutionary Trends in Plants*, **6**, 61–71.

Sultan, S.E. (2004) Promising directions in plant phenotypic plasticity. *Perspectives in Plant Ecology, Evolution and Systematics*, **6**, 227–233.

Sultan, S.E. (2005) An emerging focus on plant ecological development. *New Phytologist*, **166**, 1–5.

Thompson, J.D. (1991) Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution*, **6**, 246–249.

Thuiller, W., Lavorel, S., Araujo, M.B., Sykes, M.T. & Prentice, I.C. (2005) Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Science*, **102**, 8245–8250.

Valladares, F. (2003) Light heterogeneity and plants: from ecophysiology to species coexistence and biodiversity. *Progress in Botany* (eds K. Esser, U. Lüttge, W. Beyschlag & F. Hellwig), Vol. 64, pp. 439–471. Springer Verlag, Heidelberg.

Valladares, F., Arrieta, S., Aranda, I., Lorenzo, D., Tena, D., Sánchez-Gómez, D., Suarez, F. & Pardos, J.A. (2005a) Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Ilex aquifolium* in continental-Mediterranean sites. *Tree Physiology*, **25**, 1041–1052.

Valladares, F., Balaguer, L., Martinez-Ferri, E., Perez-Corona, E. & Manrique, E. (2002a) Plasticity, instability and canalization: is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytologist*, **156**, 457–467.

Valladares, F., Chico, J.M., Aranda, I., Balaguer, L., Dizengremel, P., Manrique, E. & Dreyer, E. (2002b) Greater high light seedling tolerance of *Quercus robur* over *Fagus sylvatica* is linked to a greater physiological plasticity. *Trees, Structure and Function*, **16**, 395–403.

Valladares, F., Dobarro, I., Sánchez-Gómez, D. & Pearcy, R.W. (2005b) Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. *Journal of Experimental Botany*, **56**, 483–494.

Valladares, F. & Guzmán, B. (2006) Canopy structure and spatial heterogeneity of understory light in abandoned Holm oak woodlands. *Annals of Forest Science*, **63**, 1–13.

Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E. & Manrique, E. (2000a) Low leaf-level response to light and nutrients in Mediterranean evergreeen oaks: a conservative resource-use strategy? *New Phytologist*, **148**, 79–91.

Valladares, F., Wright, S.J., Lasso, E., Kitajima, K. & Pearcy, R.W. (2000b) Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology*, **81**, 1925–1936.

Van Kleunen, M. & Fischer, M. (2003) Effects of four generations of density-dependent selection on life history traits and their plasticity in a clonally propagated plant. *Journal of Evolutionary Biology*, **16**, 474–484.

Van Kleunen, M. & Fischer, M. (2005) Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist*, **166**, 49–60.

Via, S., Gomulkiewicz, R., Dejong, G., Scheiner, S.M., Schlichting, C.D. & Vantienderen, P.H. (1995) Adaptive phenotypic plasticity – consensus and controversy. *Trends in Ecology and Evolution*, **10**, 212–217.

Watson, M.A., Geber, M.A. & Jones, C.S. (1995) Ontogenetic contingency and the expression of plant plasticity. *Trends in Ecology and Evolution*, **10**, 474–475.

Weigelt, A. & Jolliffe, P. (2003) Indices of plant competition. *Journal of Ecology*, **91**, 707–720.

Werner, E.E. & Peacor, S.D. (2003) Review of trait–mediated indirect interactions in ecological communities. *Ecology*, **84**, 1083–1100.

West-Eberhard, M.J. (2003) *Developmental Plasticity and Evolution*. Oxford University Press, New York.

Williams, D.G., Mack, R.N. & Black, R.A. (1995) Ecophysiology of introduced *Pennisetum setaceum* on Hawaii: the role of phenotypic plasticity. *Ecology*, **76**, 1569–1580.

Yamashita, N., Koike, N. & Ishida, A. (2002) Leaf ontogenetic dependence of light acclimation in invasive and native subtropical trees of different successional status. *Plant, Cell and Environment*, **25**, 1341–1353.

Supplementary material

The following supplementary material is available for this article:

Appendix S1 Shoot–root ratios for the 535 seedlings of the four species studied

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2745.2006.01176.x (This link will take you to the article abstract). Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.