Developmental instability as an index of adaptation to drought stress in a Mediterranean oak

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Paper type: Research Article.
Abstract

An increase in temperature and water deficits caused by the ongoing climate change might lead to a decline growth rates and threaten the persistence of tree species in drought-prone areas within the Mediterranean Basin. Developmental instability (the error in development caused by stress) may provide an index of the adaptability of woody plants to withstand climatic stressors such as water shortage. This study evaluated the effects of drought stress on growth variables in three stands of a Mediterranean oak (Quercus faginea) exposed to differing climatic conditions (xeric, mesic and cooler) along an altitudinal gradient in northeastern Spain, in two climatically contrasting years (wet and dry years). Two indices of developmental instability, fluctuating and translational asymmetries, which reflect environmental stress, were measured in leaves and current-year shoots respectively. We also measured branch biomass and fractal complexity of branches as indicators of the species’ performance. After a period of drought the individuals’ at the most xeric site presented lower developmental instability and less branch biomass than did the individuals from the mesic and cooler sites. We interpret that difference as an adaptive response to drought which reflects a trade-off between maintenance of homeostasis and growth when water is scarce. The study demonstrated that developmental instability constitutes a useful index to assess the degree of adaptation to stressful environmental conditions. The assessment of developmental instability in sites and years with contrasting climatic conditions provides a means of quantifying the capacity of plants to develop plastic adaptive responses to climatic stress.

Key words: developmental instability; drought stress; fractal dimension; Mediterranean oak; phenotypic plasticity; adaptation.
1. Introduction

Water availability, high temperatures and radiation are among the most important environmental constraints for plant growth and persistence in Mediterranean ecosystems (Chaves et al., 2003; Zunzunegui et al., 2000). Climate models have predicted increases in temperature and frequency of severe drought events in the Mediterranean Basin (Bates et al., 2008; Giorgi and Lionello, 2008; Luterbacher et al., 2004). Furthermore, several studies reported reductions in precipitation in some Mediterranean areas as the Iberian Peninsula (e.g., Rodriguez-Puebla and Nieto, 2010). Such increases in aridity have negative consequences for plant performance in those drought-prone areas (Walther et al., 2002). It is important to understand the responses of plants to drought in those areas in order to predict the possible changes in the natural vegetation in response to global warming. Those responses might include adaptations that involve phenotypic plasticity, which is the capacity of organisms to express alternative phenotypes in response to environmental variation (Schlichting, 1986). Plasticity is one of the most important short-term mechanisms used by plants to cope with rapid environmental change (Ramirez-Valiente et al., 2010; Voesenek and Blom, 1996). A high adaptive phenotypic plasticity might permit populations to persist and adjust to climatic variability (Lindner et al, 2010).

Measurements of developmental instability (DI) can be used to quantify the phenotypic plasticity of plants. Traditionally DI has been used as index of stress (Moller and Swadel, 1997; Polak, 2003), due to being correlated to several biotic and abiotic stressors, including environmental factors such as interspecific competition (Komac and Alados, 2012), drought (Escós et al., 2000; Fair and Breshears, 2005), high temperature (Llorens et al., 2002), elevation (Hagen et al., 2008), radiation (Oleksyk, et al., 2004), herbivory (Moller, 1995; Escós et al., 1997; Puerta-Piñero et al., 2008); and
anthropogenic activities, such as habitat perturbation resulting of military practices, urbanization and pollution (Freeman et al., 2004, Cuevas-Reyes et al., 2013, Velickovic and Savic, 2012, respectively); as well as genetic factors such as mutation, inbreeding and hybridization (Hochwender and Fritz, 1999). DI is based on the hypothesis that as stress increases the ability of the plant developmental program to resist perturbations decreases (Freeman et al., 2004). Under stressful conditions the mechanisms that are intended to insure the correct development are interrupted leading to developmental errors (Freeman et al. 2003). Organisms are never perfectly symmetrical, however, and there is always certain degree of asymmetry, which is caused by developmental noise (DN), the small cumulative random errors in development caused by the stochasticity in cellular processes; DN increases as external stress does (Lens et al., 2002). Organisms have developed mechanisms to buffer against those developmental errors, referred to as developmental stability (DS), an individual ability to produce a predetermined invariant phenotype under particular environmental conditions (Moller and Shykoff, 1999; Polak, 2003). Thus, DS is the internal force which buffers against the errors in development manifested in DN, and DI is the combined result of the balance between the counteracting effects of DN and DS (Lens et al, 2002). Environmental stress can affect development by increasing DN, or by decreasing DS (Lens et al, 2002). If an organism is well adapted to a harsh environment it might have low DI because DS counteracts the increase in DN caused by environmental stress. High DS under stressful environmental conditions reflects that an organism is well adapted to such conditions. The subtle interplay between these three concepts is essential to the sound interpretation of the studies of developmental instability (Supporting information S1). Some studies demonstrated unclear relationship between DI and stress (Auslander et al., 2003; Duda et al., 2003; Fair and Breshears, 2005) or a negative correlation (Hódar, 2002). Those
differences might have occurred because some populations have adapted to certain
degree of stress (Alados et al., 1999; Kaligaric, 2008; Velickovic and Saivc, 2012).
Several authors also suggest that DI might serve as index of adaptation (Graham, 2010;
Jones, 1987). In this study, DI was used as an index of adaptation rather than as an
index of stress. DI in plants can be quantified in several ways, we use fluctuating
asymmetry (bilateral symmetry) and translational asymmetry (based on allometric
relationships). In addition we assessed the fractal complexity of the branches because
fractal dimension can be an efficient indicator if stress in plants (Alados et al., 1998a,

This study evaluated the phenotypic plasticity of a Mediterranean oak *Quercus faginea*
across a climatic gradient in two years that had contrasting climatic conditions. The
spatio-temporal variation in climatic conditions represented by the climatic gradient,
which included a xeric, a mesic and a cold site and the two years of study, provided a
system in which DN might be enhanced by an increase in environmental stress, which
might lead to an increase in DI. If, however, the trees are well adapted to their
environment, the buffering capacity of plants, here assessed as DS, might compensate
for any increase in of developmental error.

Our general objective was to assess the adaptive capacity of *Q. faginea* under
climatically contrasting conditions which would help in predicting the response of this
specie to the warmer and drier conditions forecasted for this region. Specifically we
aimed: (1) to estimate the variation in DI of *Q. faginea* in xeric, mesic and cold
environments in two climatically contrasting years (wet vs. dry conditions), and (2) to
assess the relationship between shoot length and DI. Based on theoretical considerations
we predicted that individuals at the most xeric site were adapted to semi-arid conditions
and, therefore would have less DI after a dry year because they were better adapted to
drought than were the individuals subjected to humid conditions in the most mesic site. We expected to find a trade-off between shoot length and maintenance of DI, as a measure of the ability of the tree to maintain a stable development at expense of biomass production, particularly at the most xeric site.

2. Material and methods

2.1. Study area and species

The study area is located at the province of Huesca, in north-eastern Spain. The sampling sites included three locations along an altitudinal gradient: a xeric site in the Sierra de Alcubierre (Alcubierre site) of the Middle Ebro Basin, and two additional sites in the central Pre-Pyrenees (sites Arguis –mesic site– and Pico del Águila –cold site) which were visited in September and October of 2008 and 2009. The studied altitudinal gradient reflected a marked climatic gradient that was characterized by a decrease in temperature and an increase in precipitation upwards (Table 1 and Supporting information S3; for more information on climatic gradient see Allà and Camarero 2012).

Quercus faginea Lam. is a winter-deciduous Mediterranean oak widely distributed in the Iberian Peninsula in relatively humid areas with basic soils (Castro et al., 2005). The climatic conditions that influence shoot and leaf development are those that occur in the previous year (Chaubert-Pereira et al., 2009; Montserrat-Martí et al. 2009), in our study from August in 2007 until August in 2008 for the sampling year 2008 and from August 2008 until August 2009 for the sampling year 2009, because bud meristems are formed one year before shoot elongation and leaf expansion (Allà et al., 2011). In 2007, annual precipitation in the study area was lower than the mean for the reference period (1960-2006) “, which, for the purposes of our sampling, meant that
2008 was a ‘dry’ year. In 2008, precipitation was slightly higher than the average therefore the sampling year 2009 was a ‘wet’ year (Table 1).

2.2. Field sampling and laboratory methods.

At each of the three sites, ten *Q. faginea* mature individuals that were at least 5 m apart were chosen randomly on each of two transects. The diameter at a height of 1.3 m of the thickest stem of all sampled trees was measured. Shoot and leaf samples were collected from the southward and the upper third of the crown. To quantify translational asymmetry three current-year shoots were collected from each tree, and to measure fluctuating asymmetry three current-year leaves were randomly selected from each of these shoots. In addition, to quantify fractal complexity a five-year-old branch was collected from each tree.

To calculate the translational asymmetry an electronic calliper (resolution 0.01 mm) was used to measure the internode length from the base to the top of each shoot (Fig. 1). To estimate fluctuating asymmetry a 4800-dpi resolution scanner (Epson Perfection 4990 Photo, Seiko Epson Corporation, Japan) was used to take a digital photograph of each leaf, and the symmetry measurements were made using the image analysis software Image Pro-Plus ver. 5.0 (Media Cybernetics, Bethesda, MD). In each leaf we measured the distance from the central vein, here considered as the symmetry axis, to both lateral edges of the leaf at three equidistant points along the axis of symmetry (Fig. 1). To calculate the measurement error measurements were taken twice (Swaddle et al., 1994).

Fractal complexity, quantified by information fractal dimension (IFD), was calculated from digitized pictures of each 5-year old branch. The images were captured at a uniform distance and just after the branches were collected. The dry weight of each branch was recorded after it had been oven dried to a constant weight at 60° C.
2.3. Statistical analyses

2.3.1. Fluctuating asymmetry (FA)

The validity of fluctuating asymmetry as an estimate of environmental perturbation requires the absence of directional symmetry and antisymmetry (Palmer and Strobeck, 1986). Fluctuating asymmetry differs from the later two because the values of left minus right sides ($L-R$) follow a normal distribution with a mean of zero. The $L-R$ distribution that differs from ideal fluctuating asymmetry is not a suitable descriptor of developmental instability because some of the asymmetry might have a genetic basis (Palmer and Strobeck, 1992). The distribution of the signed $L-R$ differences was evaluated using a Kolmogorov-Smirnov (K-S) Normality Test. To assess the statistical significance of the fluctuating asymmetry, we used a mixed-model (Two-way ANOVA) that included ‘side’ as a fixed-effect factor, which reflected directional asymmetry, ‘individual’ as a random factor, which reflected the variation among individuals, and a ‘side-by-individuals’ interaction term, which reflected fluctuating asymmetry. (Palmer and Strobeck, 1986). The measurement error was calculated as follows:

$$MSE = \frac{MS_{\text{error}}}{MS_{\text{individual}}} \times 100$$  \hspace{1cm} (1)

where $MS_{\text{error}}$ is the mean square of the error term in the general model of ANOVA and $MS_{\text{individual}}$ is the mean square of individual of type III. In the event that there might be a relationship between the asymmetry measurements and leaf size and therefore a need to normalize $|L-R|$, we performed a correlation between absolute fluctuating asymmetry, $|L-R|$, and the leaf size, $(L+R)$. This correlation was highly significant ($r=0.52, p=0.0001$), therefore the raw data were transformed using
logarithm. Besides, in order to deal with $|L-R|$ half-normal distribution we applied the 
Box-Cox transformation (following the recommendations of Swaddle et al. 1994, Graham et al. 1998 and Freeman et al., 2004). We used FA as a global index of leaf 
responses to stress and it was calculated as the sum of the three measures taken from 
each leaf.

$$FA = \sum_{i=1}^{3} (\ln L_i - \ln R_i + 0.00005)^{0.33}$$  \hspace{1cm} (2)

2.3.2. Translational asymmetry (TA)

Translational asymmetry was measured as the error in the following curve–
fitting equation:

$$L(N) = kN^a e^{-bN}$$  \hspace{1cm} (3)

where $L$ is the internode length, $N$ the internode order (measured from the bottom to the 
top, see Fig. 1), $e$ the natural base and $k$, $a$ and $b$ are the fitted parameters (Alados et al., 
1998b, 2006; Freeman et al., 2004; Tan-Krisanto et al., 2003).

Curve-fitting accuracy and parameter values were obtained after log-log 
linearization and posterior linear regression adjustments for each plant. The coefficient 
of determination, $R^2$, was used as translational asymmetry index (TA), as a measure of 
the degree of developmental instability. A high coefficient of determination, which 
corresponds to a good curve fit, indicates low DI. The parameters $a$, $b$, and $\ln k$ were 
used to quantify the primary growth processes that occur during shoot elongation. The 
$\ln k$ parameter represents the starting conditions of shoot enlargement (length of the first 
internode), $a$ reflects the rate of shoot elongation, and $b$ represents the inhibition process 
of shoot growth.

To test for differences in FA and TA among sites we performed a nested 
ANOVA using the GLM routine in the statistical program SAS (SAS Institute Inc., 
Cary, NC) with the probability of statistical significance set to 0.05 (model III). Site was
a fixed factor and the measurement error was removed from the analysis by including the repeated measure as a random factor in the model (Alados and El Aich, 2008).

2.3.3. Fractal complexity (FC)

Fractal complexity was assessed based on branch fractal dimension, which is a measure of plant's efficiency in occupying the space. The higher the IFD, the more efficient the use of space. The photographs of 5-year old branches used to calculate of fractal complexity were digitized using the software Adobe Photoshop version 8.0.1 (Adobe Systems Incorporated). Photoshop 8.0.1 was used to transform the images into raw data, through a process including transform to grayscale, to flatten, to fit threshold, clean others elements different of target plant with eraser and to transform to uniform dimensions (1024 x 1024 pixels). Using the software DRASME 2009, created by J. Escós and C.L. Alados, we calculated the Information Fractal Dimension (IFD) of each branch (following Alados et al., 1999):

\[ D_f = \lim_{\varepsilon \to 0} \left( \frac{\ln(I(\varepsilon))}{\ln(1/\varepsilon)} \right) \]  

where \[ I(\varepsilon) = -\sum_{i=1}^{N(\varepsilon)} p_i \ln p_i \], and \[ p_i = \frac{x_i}{\sum x} \], \( x_i \) is the number of occupied pixels in each box of size \( \varepsilon \). The process was repeated several times using progressively finer grid sizes. \( I(\varepsilon) \) was plotted against the log of box size and IFD was defined as the slope of the line (Alados and El Aich, 2008).

To tests for differences in branch IFD and biomass among sites, we performed a nested ANOVA. Site was a fixed factor and the other nesting levels were included as random factors. To evaluate the differences in translational asymmetry between the two years we used a repeated measures test because the samples were not independent. TA and shoot length were included in this analysis. We also tested the statistical significance of the interaction term site x year. To assess the statistical significance of
the differences among sites in mean values of each variable (FA, TA, a, b, k, IFD and branch biomass) we used a Tukey test.

3. Results

3.1. Fluctuating asymmetry

At the study sites in northeastern Spain, *Q. faginea* leaves did not exhibit directional symmetry, DS, ($F=0.04, p=0.838$) or antisymmetry, AS, because although the $L$-$R$ distribution was non-normal (K-S test, $p=0.004$) the distribution was leptokurtic (skewnes $(g1)=0.141$, $t(g1)=1.906$; kurtosis $(g2)=1.141$, $t(g2)=8.335$; significance threshold at $\alpha=0.05$ is $t=1.96$) (Supporting Information, Fig. S2). Thus, the leaf asymmetry was due to true fluctuating asymmetry, FA ($F=28.27, p=0.0001$).

In 2008 leaf FA differed significantly among sites, and the trees at the mesic site (Arguis) had the highest FA (Table 2, Fig. 2). Leaf development was more stable, showing the lowest mean values of the index of leaf response to stress (FA) in the xeric site (Alcubierre) than it was at the mesic and cold sites.

3.2. Translational asymmetry (TA).

The repeated measures analyses of TA, a index for developmental instability, showed that the interaction “year by location” was significant ($F=8.13, p<0.001$) (Fig. 3). The same analyses for the variable shoot length showed statistical differences between years and also the interaction “site x year” was significant ($F=20.61$ and $F=20.72$ respectively, $p<0.001$) (Fig. 3). TA values significantly differed among the three sites in both years of the study, but differences were pronounced in 2008 than they were in 2009 (Table 2). Differences in the climatic conditions across the gradient at the three sites influenced DI in *Q. faginea*. In 2008 the curve-fitting was best at the xeric
site (Alcubierre), and worst at the mesic site (Arguis) (Fig. 3). In 2009, however, the best curve fitting was at the cold site (Pico del Águila) and the worst at the xeric site (Fig. 3). In 2009, the Tukey test did not reveal statistically significant differences in TA values among sites.

Shoot length and TA were significant negatively correlated (Kendall correlation \( \tau \)) in 2008 (\( \tau = -0.22, p=0.0001 \)), but not in 2009 (\( \tau = -0.06, p=0.112 \)). That is, growth and DI was negatively correlated in 2008 but not in 2009. In the models of shoot growth the xeric site (Alcubierre) exhibited the highest \( a \), \( b \), and \( \ln k \) for in the two years (Fig. 4, Table 3), which indicates that \( Q. faginea \) at the xeric site had the highest internode elongation rate and the fastest decline at the shoot top. In 2008 all of the fitted parameters except \( \ln k \) differed significantly between the three sites; again, at Alcubierre, the values differed significantly from the values at the other two sites (Tables 2 and 3). In 2009, \( b \) was the only the parameter that differed significantly among sites (Tables 2 and 3). Thus, shoot growth was more similar among sites in 2009 than in 2008 (Table 2). Evidently, climatic stressors such as drought can influence the rate of shoot growth in \( Q. faginea \).

3.3 Fractal complexity (FC).

IFD and branch biomass differed significantly among sites (Table 2); the highest values occurred at the mesic site (Arguis) and the lowest in the xeric site (Alcubierre) for both variables (Table 4). The branch IFD and biomass were strongly correlated and the correlation in the log-transformed data was linear, i.e. data fit a power law function (Fig. 5).

4. Discussion
Fluctuating asymmetry and translational asymmetry indices revealed that *Q. faginea* trees from the xeric site (Alcubierre) were developmentally more stable after a dry period than were the trees in the other two populations that occurred in more humid and cold areas, which suggest an adaptive response to drought by *Q. faginea*. The shrub *Anthyllis cytisoides* exhibited a similar response (Alados *et al.*, 2001). At the semi-arid drought-prone site (Alcubierre), *Q. faginea* trees might have greater resistance to drought stress after the dry period in 2008 than did the individuals in the mesic (Arguis) and cold (Pico del Águila) sites, which are accustomed to having more water available.

The individuals at the xeric site exhibited the most rapid internode elongation, probably because of a rapid growth during the short growing season in early spring (Montserrat-Martí *et al.*, 2009). In contrast, the trees at the more mesic sites where more water is available can develop their shoots over a longer period than can those from xeric sites (Alla *et al.*, 2011). Other studies also observed differences in growth rates along aridity gradients (Matesanz *et al.*, 2009; Schlichting, 1986). As expected, after a wet year the shoots of trees at the xeric site behave similarly to those at the sites that had more humid climates resulting in longer internodes and shoots than following a dry periods.

The correlation between translational asymmetry and shoot length in *Q. faginea* suggests that there is a trade-off between biomass production (shoot length and branch biomass) and developmental stability when water is scarce. After the dry year in 2008 shoot length and TA were negatively correlated; that is, the shorter the shoots, the lower the developmental instability; however, the correlation was not significant after the humid year. Thus, when precipitation is scarce a trade-off between tree growth and maintenance of homeostasis can occur.
After a drought, individuals that were most accustomed to dry conditions shortened their growing period, produced shorter shoots and produced less branch biomass and had lower IFD than did the individuals that were not used to severe and frequent water shortages. That integrative response keeps growth rates relatively low so that developmental stability and homeostasis are maintained. Trees at the mesic sites produced longer shoots, bigger branches and had higher IFD than did the trees at the xeric site, at the expense of higher developmental instability and a change in the allometric relationship between branch biomass and its fractal dimension. After a humid year trees from the xeric site invested their resources in production rather than into maintaining of homeostasis.

Branch fractal dimension is a measure of plant’s efficiency in occupying the space, which might reflect how plants are in contact with the environment, as the efficiency in the capture of light and, plausibly, in the diffusion of CO₂ to the atmosphere at the expense of a higher transpiration rate (Foroutan-pour et al., 1999, Foroutan-pour et al., 2001). Conversely a low IFD might reflect a low transpiration rate and a reduced water loss (Alados et al., 2008) at the expense of a reduction in efficient light interception (Horn, 1971). In addition, water stress reduced lateral bud bursting in *Q. faginea*, which affect crown development (Alla et al., 2011; Sanz-Pérez and Castro-Díez, 2010). Apical buds may be favored in order to increase water uptake because they maximize the difference in water potential between the crown and the roots (Lortie and Aarssen, 1997). Thus, trees at the mesic sites, but not those at xeric sites, can maintain a high IFD.

Water availability and temperature during bud organogenesis influence primary growth dynamics of Mediterranean *Quercus* species (Alla et al. 2012). The complex responses in primary growth to climate are the basis for its plasticity and the capacity of
Mediterranean oaks to produce more than one growth unit within a single growth period and to produce viable buds of different ages (Barthélémy and Caraglio, 2007). Rainfall can have an immediate effect on shoot elongation depending on whether it occurs during bud organogenesis or shoot elongation (Chaubert-Pereira et al., 2009). The climatic conditions that affect bud formation influence shoot asymmetry, but annual shoot length can be influenced also by the climate of bud development (Chaubert-Pereira et al., 2009). In *Q. faginea* bud enlargement occurs in August-September of the year before shoot elongation (Alla et al., 2012) and typically bud bursting occurs from March to April (Montserrat-Martí et al., 2009). In humid locations like Arguis, however, it can occur slightly later (Sanz-Pérez, 2010), which may be why in Arguis shoots were longer in 2008 than in 2009. In Arguis bud enlargement period in 2009 was drier and colder than the average, which probably shortened the spring growing season when shoots elongate (see appendix for climate data). Furthermore, Alla et al. (2011) reported similar shoot lengths in the same years at the same study sites.

Water uptake is critical for primary growth in drought-prone areas. Turgor pressure limits cell enlargement and consequently cell division (Hsiao et al., 1976). In addition, drought limits photosynthesis and carbon uptake through stomatal closure and a reduction in ribulose biphosphate carboxylase/oxygen activity (Flexas and Medrano, 2002), which might be why the shortest shoots found in the *Q. faginea* trees from the xeric Alcubierre site. Furthermore, stressed plants tend to show decreased growth because it reduces the demand for water and nutrients (Grime, 1977). Changes in plant size involve shifting priorities among growth types (e.g., shoot elongation vs. shoot thickening) and changing the allocation priority of resources within the plant (water, nutrients, carbohydrates) (Tilman, 1988). A reduction in growth might drive more resources into assimilating organs (leaves) and fewer into supporting tissues (wood).
which increases the likelihood of survival in harsh xeric environments (Chapin, 1991). In addition, the phenotypic expression of traits that are functionally important to the organism, such as the allometric relationships between organs or leaf symmetry, influence plant fitness (Alados et al., 2001).

The behavior of *Q. faginea* at the xeric site in northeastern Spain is consistent with Levitt (1972) concept of “resistance adaptation” as an explanation for how plants adapt to a high intensity stress event after having been subjected to the same stress, previously, but a lower intensity (“capacity adaptation”). There is a climatic threshold at which “resistance adaptation” is triggered in those individuals best adapted to changes in climatic conditions. Several studies have demonstrated that responses thresholds to environmental changes exist in plants (Bielorai, 1973; Razzahi, 2011), and that a minimum threshold of a climatic factor is needed to trigger a growth response in trees (Deslauriers et al., 2008; Levitt, 1972). Even gradual changes in environmental conditions can induce sharp responses in trees, e.g., the way they use water in semi-arid ecosystems (Williams and Ehleringer, 2000). The drought in Alcubierre triggered a conservative strategy in *Q. faginea*, there was a point within the continuous variation in environmental conditions through the time when *Q. faginea* developed an adaptive response.

In Alcubierre, how did *Q. faginea* adapt to the variability and uncertainty in water availability? Morphological and physiological tradeoffs prevent plants from being optimally adapted to both dry and wet conditions (Schwinning and Ehleringer 2001). Adaptive response thresholds might be common in species that depend on fluctuating resource supply, as soil water in semi-arid areas, because a threshold response involves adaptations to minimize the cost-to-benefit ratio of resource use (Schwinning and Sala,
2004). To our knowledge our study is one of the few that have demonstrated an adaptive response threshold in trees.

In our study, the interaction between ‘year’ and ‘location’ on TA and shoot length demonstrate that trees from the xeric site are able to tolerate the environmental stress imposed by a severe water shortage. The flexibility of that trait in response to the environmental change reflects the adaptive phenotypic plasticity of *Q. faginea*. Other studies have shown that phenotypic plasticity in woody plants such as shrubs can be an adaptive response to a local climatic constraint (Bedetti et al, 2011).

An understanding of the adaptive phenotypic plastic responses to changes in environmental conditions is important because inter-annual variability in weather is expected to increase as a result of climate change which means that severe droughts might become more frequent in the Mediterranean Basin (Giorgi and Lionello 2008). Long-lived sessile organisms such as trees might experience rapid climate change along one or two generations and may do not have enough time to evolve responses to rapidly changing conditions (Fallour-Rubio et al., 2009; Lindner et al. 2010).

We conclude that *Quercus faginea* can generate an adaptive response to drought in xeric environments. If the pace of climate change is faster than the individuals’ ability to adapt, trees will not be able to produce a plastic response and will exhibit developmental errors in the shape of their crown. Developmental instability can be used as an indicator of stress, and it can indicate a degree of adaptiveness of the species to specific environmental conditions. Fluctuating asymmetry reflects the degree of adaptation of a population to site conditions (Graham et al., 2010). As an index of adaptation developmental instability can be used to estimate the viability of a tree population, to detect adaptive changes or shifts in organisms, and to identify the environmental conditions that lead to adaptive responses. Developmental instability can
be used as an indicator of the adaptive ability of a tree species to specific environmental conditions and as an estimator of threshold adaptive responses by measuring several growth characters during years that have contrasting climatic conditions.

Acknowledgements

We gratefully acknowledge the support of the Spanish Economy and Competition Ministry (PN-MICINN) (CGL2008-00655/BOS and CGL2011-27259). We thank the AEMET for providing meteorological information. We thank G. Montserrat-Martí for providing relevant information of the oak trees populations. We also thank Bruce MacWhirter and two anonymous referees for critically reading and providing helpful suggestions on the manuscript.

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Table 1. Characteristics of the studied sites, years and *Q. faginea* trees in northeastern Spain. Climate data were obtained from nearby meteorological stations collected in the period 1960–2006 (see Alla et al. 2011 and Alla and Camarero 2012).

Table 2. Statistical parameters derived from nested ANOVAs of stress indicators (FA, $R^2$, $a$, $b$, $\ln k$, IFD, branch biomass) in *Q. faginea* among study sites and years (2008, 2009) ($F$ values and, in brackets, the degrees of freedom). Significance levels: **$p$<0.0001, *$p$<0.05.

Table 3. Values (mean ± SE) of the growth parameters ($a$, $b$, $\ln k$) in *Q. faginea* for the three study sites and the two study years (2008, 2009). Different letters show significant ($P$<0.05) differences among sites in each year of study based on Tukey tests.

Table 4. Values (mean ± SE) of *Information fractal dimension* (IFD) and branch biomass of 5-year old branches of *Q. faginea* in 2008 at the three study sites, Pico del Águila (cool site), Arguis (mesic site) and Alcubierre (xeric site).
Figure 1. Leaf and a current-year shoot of *Q. faginea*. In the leaf, A-B represents the central axis, C-G, E-I and D-K and C-F, E-H and D-J represent right and left measures, respectively, used to calculate fluctuating asymmetry. In the shoot S1, S2, etc. represent the lengths of each internode (distance between successive non-apical or lateral buds) used to calculate translational asymmetry.

Figure 2. Values of the index of stress (FA) for each study site. Different letters show significant (*P*<0.05) differences between sites (Tukey test). Means ± SE (n=1080).

Figure 3. Mean $R^2$ (a) and mean shoot length (b) of *Q. faginea* in two years at three sites in northeastern Spain. Different letters show significant (*P*<0.05) differences between sites based on Tukey tests.

Figure 4. Estimated internode lengths of *Q. faginea* shoots in two years at three sites in northeastern Spain that differed in climate as a function of node order derived from the equation, $L(N) = kN^2e^{-bN}$.

Figure 5. *Information Fractal Dimension* (IFD) and branch biomass of *Q. faginea* branches at the three study sites. The fitted regression to log-transformed values of both variables was highly significant ($R^2 = 0.92$, *p*<0.001).
Supporting Information

Figure S1. Relevant concepts in the study and in Developmental Instability theory, their abbreviation, definition, goal and references.

Figure S2. Histogram of the distribution of left minus right (L–R) sides of *Q. faginea* leaves.

Figure S3. Ombrothermic diagram of temperature (°C) and precipitation (mm) in Alcubierre and Argis in the years that influenced the shoots development, 2007, 2008 and 2009, and the reference period.
Figure 2

Figure 3a.

Figure 3b.
The graphs show the length (mm) against node order for 2008 and 2009. The data points are represented for three different samples: Pico del Águila (solid line), Arguis (dashed line), and Alcabierre (dotted line).
<table>
<thead>
<tr>
<th>Site (type)</th>
<th>Altitude (m)</th>
<th>Diameter at 1.3 m (cm)</th>
<th>Mean temperature (°C)</th>
<th>Total precipitation (mm)</th>
<th>Reference period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2007/ August- July **</td>
<td>2008/ August-July **</td>
<td></td>
</tr>
<tr>
<td>Pico del Águila (cold site)</td>
<td>1490</td>
<td>9.2 ± 0.8</td>
<td>7.03/ 7.40</td>
<td>933/1271</td>
<td>1215</td>
</tr>
<tr>
<td>Arguis (mesic site)</td>
<td>1140</td>
<td>15.9 ± 1.0</td>
<td>8.83/ 9.30</td>
<td>646/880</td>
<td>849</td>
</tr>
<tr>
<td>Alcubierre (xeric site)</td>
<td>650</td>
<td>12.0 ± 1.5</td>
<td>11.43/ 12.04</td>
<td>350/477</td>
<td>540</td>
</tr>
</tbody>
</table>

**Period including part of bud preformation and primary growth from August 2007 up to July 2008, and from August 2008 up to July 2009, respectively. Note that the reference period was calculated considering Julian years.**
<table>
<thead>
<tr>
<th></th>
<th>Site</th>
<th>Transect (Site)</th>
<th>Individual (Transect)</th>
<th>Shoot (Individual)</th>
<th>Leaf (Shoot)</th>
<th>Repet (Leaf/Shoot)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluctuating asymmetry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FA</td>
<td>56.57** (1023,2)</td>
<td>8.29* (1023,2)</td>
<td>1.18 (1023,9)</td>
<td>1.37 (1023,18)</td>
<td>1.29 (1023,4)</td>
<td>1.17 (1023,3)</td>
</tr>
<tr>
<td><strong>Translational asymmetry</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TA</td>
<td>12.29** (313,2)</td>
<td>0.24 (313,2)</td>
<td>2.87* (313,9)</td>
<td>1.55 (313,18)</td>
<td>---</td>
<td>1.12 (313,3)</td>
</tr>
<tr>
<td>a</td>
<td>7.29* (313,2)</td>
<td>0.58 (313,2)</td>
<td>1.24 (313,9)</td>
<td>1.00 (313,18)</td>
<td>---</td>
<td>1.00 (313,3)</td>
</tr>
<tr>
<td>b</td>
<td>25.76** (313,2)</td>
<td>0.17 (313,2)</td>
<td>1.45 (313,9)</td>
<td>1.66 (313,18)</td>
<td>---</td>
<td>0.07 (313,3)</td>
</tr>
<tr>
<td>ln k</td>
<td>0.46 (313,2)</td>
<td>3.94* (313,2)</td>
<td>0.76 (313,9)</td>
<td>2.11* (313,18)</td>
<td>---</td>
<td>5.79** (313,3)</td>
</tr>
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<td>2009</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>3.14* (311,2)</td>
<td>3.01 (311,2)</td>
<td>2.50* (311,9)</td>
<td>1.64* (311,18)</td>
<td>---</td>
<td>0.20 (311,3)</td>
</tr>
<tr>
<td>a</td>
<td>2.33 (311,2)</td>
<td>0.22 (311,2)</td>
<td>2.51* (311,9)</td>
<td>1.00 (311,18)</td>
<td>---</td>
<td>0.12 (311,3)</td>
</tr>
<tr>
<td>b</td>
<td>5.24* (311,2)</td>
<td>0.39 (311,2)</td>
<td>2.23* (311,9)</td>
<td>1.11 (311,18)</td>
<td>---</td>
<td>0.09 (311,3)</td>
</tr>
<tr>
<td>ln k</td>
<td>0.21 (311,2)</td>
<td>1.57 (311,2)</td>
<td>2.03* (311,9)</td>
<td>1.29 (311,18)</td>
<td>---</td>
<td>0.42 (311,3)</td>
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<tr>
<td><strong>Fractal complexity</strong></td>
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<tr>
<td>IFD</td>
<td>49.41** (36,2)</td>
<td>0.91 (36,2)</td>
<td>0.88(36,18)</td>
<td>---</td>
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<tr>
<td>Biomass</td>
<td>29.91** (36,2)</td>
<td>3.93* (36,2)</td>
<td>1.04(36,18)</td>
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<td>Growth parameters</td>
<td>Pico del Águila (cold site)</td>
<td>Arguis (mesic site)</td>
<td>Alcubierre (xeric site)</td>
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<tr>
<td><strong>2008</strong></td>
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<tr>
<td>$a$</td>
<td>$2.324 \pm 0.594^b$</td>
<td>$2.517 \pm 0.677^{ab}$</td>
<td>$2.646 \pm 0.747^a$</td>
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<tr>
<td>$b$</td>
<td>$0.327 \pm 0.147^b$</td>
<td>$0.335 \pm 0.170^b$</td>
<td>$0.498 \pm 0.295^a$</td>
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</tr>
<tr>
<td>ln $k$</td>
<td>$0.065 \pm 0.378$</td>
<td>$0.012 \pm 0.584$</td>
<td>$0.006 \pm 0.614$</td>
<td></td>
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<tr>
<td><strong>2009</strong></td>
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<tr>
<td>$a$</td>
<td>$2.981 \pm 0.644$</td>
<td>$2.994 \pm 0.961$</td>
<td>$3.210 \pm 1.163$</td>
<td></td>
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<tr>
<td>$b$</td>
<td>$0.455 \pm 0.160^c$</td>
<td>$0.492 \pm 0.264^{ab}$</td>
<td>$0.565 \pm 0.407^a$</td>
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<tr>
<td>ln $k$</td>
<td>$0.271 \pm 0.436$</td>
<td>$0.163 \pm 0.472$</td>
<td>$0.309 \pm 0.662$</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Pico del Aguila</td>
<td>Arguis</td>
<td>Alcubierre</td>
<td></td>
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<tr>
<td>IFD</td>
<td>1.611 ± 0.081</td>
<td>1.664 ± 0.061</td>
<td>1.44 ± 0.071</td>
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<td>Biomass (g)</td>
<td>44.68 ± 18.83</td>
<td>76.09 ± 47.96</td>
<td>7.55 ± 3.37</td>
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<tr>
<td>Concept</td>
<td>Abbr.</td>
<td>Definition</td>
<td>Goal</td>
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<td>-------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------</td>
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<tr>
<td>Developmental instability</td>
<td>DI</td>
<td>The error in the development of organism due to genetic or environmental stress.</td>
<td>Traditionally used as index of stress</td>
<td>• Møller &amp; Swaddle. 1997. Asymmetry, Developmental Stability and Evolution. OUP.</td>
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<td></td>
<td></td>
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<td>• Polak. 2003. Developmental instability. OUP.</td>
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<td>• Polak. 2003. Developmental instability. OUP.</td>
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<tr>
<td>Fluctuating asymmetry</td>
<td>FA</td>
<td>The bilateral asymmetry. The distribution of the L–R is normal with mean 0.</td>
<td>DI measure</td>
<td>• Palmer &amp; Strobeck. 1986. Annu. Rev. Ecol. Syst. 17, 391-421.</td>
<td></td>
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<tr>
<td>Directional symmetry</td>
<td>DS</td>
<td>Presented in organisms that are genetically determined to be asymmetric. All the individuals have one side greater than the other. The distribution of L-R is normal but the mean is not 0.</td>
<td>DI methodology</td>
<td></td>
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<tr>
<td>Antisymmetry</td>
<td>AS</td>
<td>Presented in organisms that are genetically determined to be asymmetric. Some organisms have one side greater and others organisms the other. The L-R distribution is platikurtic bimodal.</td>
<td>DI methodology</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$N = 1080$
Mean  $0.0054$
Median $0.0023$
Standard deviation $0.5939$