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Phenotypic integration may constrain phenotypic plasticity in plants

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Phenotypic plasticity is essential for plant adaptation to changing environments but some factors limit its expression, causing plants to fail in producing the best phenotype for a given environment. Phenotypic integration refers to the pattern and magnitude of character correlations and it might play a role as an internal constraint to phenotypic plasticity. We tested the hypothesis that phenotypic integration – estimated as the number of significant phenotypic correlations between traits – constrains phenotypic plasticity of plants. The rationale is that, for any phenotypic trait, the more linked with other traits it is, the more limited is its range of variation. In the perennial species *Convolvulus chilensis* (Convolvulaceae) and *Lippia alba* (Verbenaceae) we determined the relationship between phenotypic plasticity to relevant environmental factors – shading for *C. chilensis* and drought for *L. alba* – and the magnitude of phenotypic integration of morphological and biomass allocation traits. In *C. chilensis* plants, plasticity to shading of a given trait decreased with the number of significant correlations that it had with the other traits. Likewise, the characters that showed greater plasticity to experimental drought in *L. alba* plants had fewer significant phenotypic correlations with other characters. We report a novel limit to phenotypic plasticity of plants by showing that the phenotypic trait architecture may constrain their plastic, functional responses to the environment.

Phenotypic plasticity is the ability of organisms to modify phenotypic expression in response to changes in the environment (West-Eberhard 2003). Phenotypic plasticity is fundamental for plants to cope successfully with environmental heterogeneity (Donohue et al. 2003, Gianoli and González-Teuber 2005, Ghalambor et al. 2007) and often explains interspecific differences in distribution range (Sultan 2001, Saldaña et al. 2005, Richards et al. 2006). The extent of plant phenotypic plasticity may be limited by both intrinsic and extrinsic factors (reviewed by DeWitt et al. 1998, Givnish 2002, van Kleunen and Fischer 2005, Valladares et al. 2007). Considering that the phenotype expressed by plants in each environmental condition is the result of the integration of their characters (Pigliucci 2003), it has been suggested or implied that, among those limiting factors, phenotypic integration might play a role as an internal constraint to phenotypic plasticity (Schlichting 1986, 1989a, Pigliucci et al. 1995, Gianoli 2001, 2003, Valladares et al. 2007), but empirical evidence is lacking. Phenotypic integration refers to the pattern and magnitude of character correlations (Pigliucci and Preston 2004). Phenotypic integration results from genetic, developmental and/or functional connections among traits (Schlichting and Pigliucci 1998, Pigliucci and Preston 2004), and it is often expressed in terms of the number of significant phenotypic correlations between traits (Schlichting 1989b, Pigliucci and Marlow 2001, Pigliucci 2002). The idea of phenotypic correlation as a constraint to the expression of (beneficial) plasticity of a given trait at the within-generation scale is somewhat analogous with the occurrence of genetic constraints on adaptive trait evolution due to genetic correlations (Agrawal and Stinchcombe 2009).

The ecological significance of patterns of variation of phenotypic integration is not fully understood. For instance, a commonly found result is that phenotypic integration (number of significant correlations among characters) increases with environmental stress (Schlichting 1989b, Waitt and Levin 1993, Kawano and Hara 1995, Gianoli 2004). However, this pattern has not been clearly connected with benefits or limitations for plants. Interestingly, there is evidence that plants show decreased phenotypic responses to a given environmental factor when subjected to intense abiotic stress (Valladares et al. 2005a, 2005b, Sánchez-Gómez et al. 2008) or to significant herbivory (biotic stress) (Quezada and Gianoli 2006, Gianoli et al. 2007, Valladares et al. 2007). These patterns might be at least partly explained by an inverse relationship between phenotypic plasticity and phenotypic integration.

We tested the hypothesis that phenotypic integration constrains phenotypic plasticity of plants. The rationale is that, for a given phenotypic trait, the greater its linkage with other traits, the more limited is its range of variation. Consequently, the expression of functional responses to the environment should be lessened in individual plants with a more integrated phenotype. As model systems we used two perennial plant species, *Convolvulus chilensis* (Convolvulaceae) and *Lippia alba* (Verbenaceae). In these species we determined the relationship between phenotypic plasticity to relevant environmental factors – shading for *C. chilensis* and drought for *L. alba* – and the magnitude of phenotypic integration of morphological and biomass allocation traits.

Methods

Species

The study species are perennial plants that are easily reared under greenhouse conditions. *Convolvulus chilensis* (Convolvulaceae) is a trailing or twining self-compatible herb endemic to north–central Chile (O′Donell 1957, Suárez et al. 2004). *Convolvolus chilensis* has shown remarkable phenotypic plasticity to shading (González and Gianoli 2004, González-Teuber and Gianoli 2007), which may be crucial for plant fitness (González-Teuber and Gianoli 2008). *Lippia alba* (Verbenaceae) is an aromatic small shrub distributed in tropical and sub-tropical America (Burkart 1979) with a wide ecological breadth (Pezo and Gonzáles 1998), being able to establish populations on humid as well as on dry habitats (Vit et al. 2002). *Lippia alba* has shown phenotypic plasticity to soil moisture (Palacio-López and Rodríguez-López 2008). Plants of *C. chilensis* were obtained from seeds that were collected in a population close to El Quisco, central Chile (33°24′S, 71°41′W). Plants of *Lippia alba* were grown from branch cuttings obtained from several mother plants collected in a population close to Guatiguará, northeastern Colombia (06°59′N, 73°02′W).

Data

Data on phenotypic plasticity were obtained after a re-analysis of data that were published earlier. González and Gianoli (2004) evaluated the phenotypic plasticity of three *Convolvulus* species to shading. We chose data of *C. chilensis* for the sake of statistical power because this species had the greatest final sample size. Palacio-López and Rodríguez-López (2008) evaluated the phenotypic plasticity of *L. alba* to water shortage under low and high light conditions (33% and 100% of full daylight). We chose the latter data because of their greater ecological realism. Data on phenotypic integration are original and were calculated from data sets of those two studies. In both papers phenotypic plasticity evaluations considered the ‘genotype’ at the population level.

Experiments

The experiment with *C. chilensis* (included in González and Gianoli 2004) was carried out in a greenhouse in central Chile. Collected seeds were first subjected to acid scarification to improve germination. From an initial pool of 120 seedlings, 48 seedlings at the two-leaf stage were chosen because of their healthy and homogeneous appearance. These seedlings were randomly assigned to three shading treatments (100%, 20% and 5% of full daylight), with $n = 16$ seedlings per treatment. A single-layer and double-layer black cloth hung 2 m above plants provided the 20% and 5% shading treatments, respectively. Light availability was recorded with a light meter. Full daylight at noon on clear days was 1700–2000 μmol m^{-2} s^{-1} PAR. To maintain comparable soil moisture due to differential transpiration, plants were watered every two, four and seven days in the 100%, 20% and 5% treatments, respectively. Plants were grown individually on 5–l pots filled with commercial potting soil. After two and a half months of shading treatment, the following traits were measured in each plant: internodes length (average of three internodes at mid shoot), petiole length (average of three measures at mid shoot), main stem diameter, main stem length, number of stems, number of leaves, leaf mass (average of three leaves oven-dried at 80°C for 48 h), leaf area, leaf shape (perimeter area^{-1}) and specific leaf area (cm^2 mg^{-1}). Morphological measurements were made with a digital calliper (resolution 0.01 mm). Leaf area and perimeter were estimated using SigmaScan software.

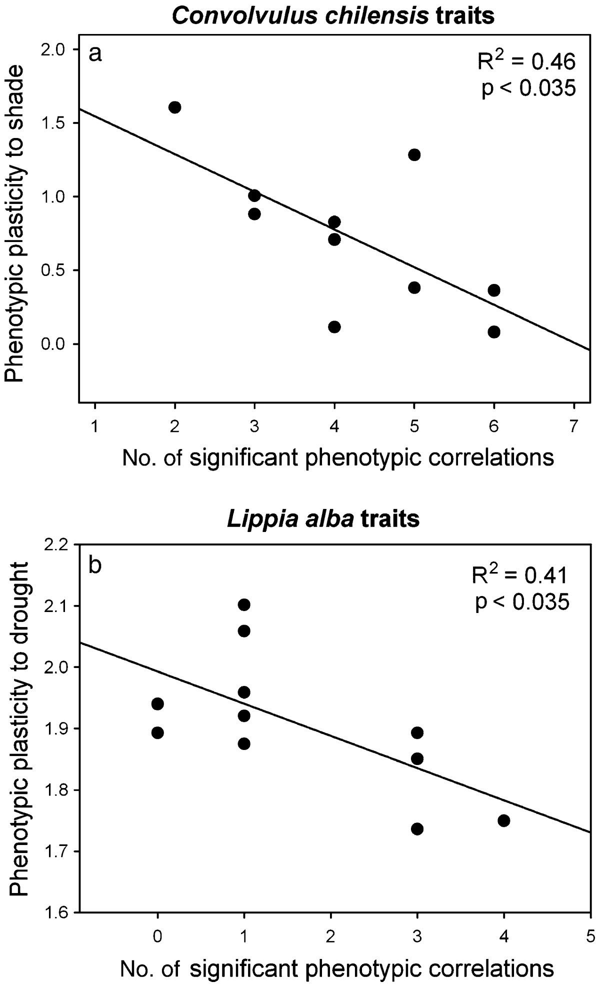
The experiment with *L. alba* (included in Palacio-López and Rodríguez-López 2008) was carried out in a greenhouse in northeastern Colombia. Forty plants developed from branch cuttings from mother plants in the field were randomly assigned to control (80% soil water saturation) and drought treatment (40% soil water saturation), with $n = 20$ plants per treatment. Relative soil moisture was daily recorded using a soil moisture sensor and water was added when necessary. Plants were grown individually on 5-l pots filled with commercial potting soil. After three months of contrasting watering regimes, when approximately 80% of the plants in each treatment had attained the flowering stage, plants were harvested. Plants were then separated into fractions and oven-dried at 80°C for 72 h. Several morphological and biomass allocation traits were determined: specific leaf area, branch length, root length, plant height, number of leaves, number of flowers, branch mass ratio (branch mass plant mass^{-1}), root mass ratio (root mass plant mass^{-1}), leaf mass ratio (leaf mass plant mass^{-1}), flower mass ratio (flower mass plant mass^{-1}) and total biomass.

Data analysis

Regression analysis was used to determine whether phenotypic plasticity (dependent variable) and phenotypic integration of traits (independent variable) are inversely related in *C. chilensis* and *L. alba*. In the case of *C. chilensis*, for each trait, phenotypic plasticity was estimated as the slope of the reaction norm across the three light environments. The slope was obtained after applying a linear regression. In the case of *L. alba*, for each trait, phenotypic plasticity was estimated as the percentage of change in trait expression in the drought treatment compared to the control treatment. Phenotypic integration was estimated as the number of significant correlations ($p < 0.05$; Pearson product–moment correlation) of each trait with the other traits in the stressful environment (shade for *C. chilensis* and drought for *L. alba*). Consequently, the number of points included in the regression corresponds to the number of traits measured (10 for *C. chilensis* and 11 for *L. alba*). We decided to use phenotypic integration data from the stressful environments because it is in these environments where the target, functional phenotype is – or is not – attained. Mean values of phenotypic plasticity were log-transformed before analysis (log[100x + 1] and log[x] for *C. chilensis* and *L. alba*, respectively).

Results

In the case of *C. chilensis* plants, plasticity to shading of a given trait decreased with the number of significant correlations that it had with the other traits ($n = 10$, $R = -0.68$, $R^2 = 0.46$, $F_{1,8} = 6.75$, $p = 0.0318$; regression analysis; Fig. 1a). Likewise, the characters that showed greater plasticity to experimental drought in *L. alba* plants had fewer significant phenotypic correlations with other characters ($n = 11$, $R = -0.64$, $R^2 = 0.41$, $F_{1,9} = 6.36$, $p = 0.0326$; regression analysis; Fig. 1b). Thus, for both *C. chilensis* and *L. alba* there was a negative relationship between phenotypic plasticity and phenotypic integration of their traits.


Convolvulus chilensis traits

Lippia alba traits

Figure 1. The relationship between phenotypic plasticity of a given trait and the number of significant correlations that it has with other traits (phenotypic integration). (a) *Convolvulus chilensis* traits. For each trait, phenotypic plasticity was calculated as the slope of the reaction norm (m) of mean values across three light treatments (100%, 20%, and 5% daylight). Values of log(100m + 1) are shown. (b) *Lippia alba* traits. For each trait, phenotypic plasticity was calculated as the % change in mean trait expression in plants growing under two treatments of soil moisture (80% and 40% soil water saturation). Values of log(% change) are shown. Each point in the regression analysis corresponds to a single trait.

Discussion

Phenotypic plasticity was associated negatively with phenotypic integration in the two study species. These relationships were significant despite the small sample size (= number of traits) included in the analysis: 10 for *C. chilensis* and 11 for *L. alba*. This evidence suggests that phenotypic integration may constrain phenotypic plasticity of plants. For a putative constraint to be ecologically meaningful it has to affect a potentially adaptive trait or response. Although we lack data on the fitness consequences in natural populations of the evaluated phenotypic responses of these species (González and Gianoli 2004, Palacio-López and Rodríguez-López 2008), they all fit with theoretical expectations from ecophysiological models of resource allocation or exploitation (Hutchings and de Kroon 1994). Thus, *C. chilensis* plants showed typical responses to shading such as elongation of internodes and petioles, reduction of the number of stems, increase of specific leaf area and changes in leaf shape that enhance light capture (González and Gianoli 2004). Likewise, *L. alba* plants subjected to water shortage showed increased biomass allocation to roots and fewer and thicker leaves (Palacio-López and Rodríguez-López 2008). Consequently, it is reasonable to interpret decreased plasticity in the studied traits as departures from the optimal phenotype in a given environment.

There are no published studies explicitly addressing the relationship between phenotypic plasticity and phenotypic integration. Interestingly, working with different lines of *Sorghum bicolor*, Amzallag (2000) reported that the heritability of a trait decreases with its level of connectance (a slightly different measure of phenotypic integration) and hypothesized that linkages among traits generate a ‘developmental noise’ that somewhat disturbs expression of genetic information. Our results are at the phenotype level and indicate that, for a given character, the linkage with other traits is associated with a reduced expression of functional responses to the environment. This finding may have evolutionary implications if the study species show a good match between phenotypic correlations and their genetic counterparts, as has been reported for a number of plant species (Waitt and Levin 1998).

We are far from understanding the mechanisms underlying the negative relationship between phenotypic plasticity and integration, which in principle should be viewed as complementary aspects of organism functioning: flexibility and coherence. Under some conditions, phenotypic plasticity and phenotypic integration apparently behave as alternative mechanisms of plants to deal with stress. First, it is often found that phenotypic integration increases with environmental stress (Schlichting 1989b, Waitt and Levin 1993, Kawano and Hara 1995, Gianoli 2004). Second, it has been reported that plants show decreased phenotypic plasticity when subjected to strong abiotic stress (Valladares et al. 2005a, 2005b, Sánchez-Gómez et al. 2008) or following leaf damage (Quezada and Gianoli 2006, Gianoli et al. 2007, Valladares et al. 2007), which is a biotic stress. To further interpret these patterns with regard to the relationship between phenotypic plasticity and integration, it is necessary to obtain evidence on the functional value of increased phenotypic integration for plants under stressful conditions. To our knowledge, this information is currently lacking. Moreover, even after getting such evidence, it must be elucidated under which circumstances (ecological scenarios) flexibility prevails over coherence, and vice versa. Otherwise we could not understand the causes of the inverse association between phenotypic plasticity and phenotypic integration that is herein shown for the first time. Further research with genetic families differing in the expression of phenotypic plasticity and/or phenotypic integration and experimentally exposed to environmental stress will shed light into this apparent functional tradeoff.

Phenotypic plasticity is essential for plant adaptation to changing environments and therefore the identification of costs and limits to its expression is a key research area (DeWitt et al. 1998, Pigliucci 2005, van Kleunen and Fischer 2005, 2007, Valladares et al. 2007). Limits to plasticity can cause plants to fail in producing the optimal phenotype for a given environment (Pigliucci 2005). This can occur when environmental cues are unreliable (Tufto 2000), when there is a long time-lag between the detection of the environmental cue and the actual phenotypic response (Stomp et al. 2008), when complex environments affect the plant response to the main environmental cue (Weinig 2000), or when elicitation of plasticity in young seedlings limits the extent of plastic responses to the same environment later in plant life (Weinig and Delph 2001). This study provides evidence for another limit to phenotypic plasticity of plants by showing that the phenotypic trait architecture may constrain their plastic responses to the environment. The next step is to address the ecological and evolutionary implications of this finding. In particular, ongoing research will address whether there is a negative association between phenotypic plasticity and phenotypic integration across genetic families.

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References

Agrawal, A. F. and Stinchcombe, J. R. 2009. How much do genetic covariances alter the rate of adaptation? – Proc. R. Soc. Lond. B 273: 1183–1191.

Amzallag, G. N. 2000. Connectance in *Sorghum* development: beyond the genotype–phenotype duality. – Biosystems 56: 1–11.

Burkart, A. 1979. Flora ilustrada de Entre Ríos (Argentina) Parte V. Dicotiledóneas Metaclamídeas (Gamopétalas) A: Primulales a Plantaginales. – Col. Cient. Inst. Nac. Tecn. Agr. 6: 1–606.

DeWitt, T. J. et al. 1998. Costs and limits of phenotypic plasticity. – Trends Ecol. Evol. 13: 77–81.

Donohue, K. et al. 2003. Adaptive divergence in plasticity in natural populations of *Impatiens capensis* and its consequences for performance in novel habitats. – Evolution 55: 692–702.

Ghalambor, C. et al. 2007. Adaptive versus non adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. – Funct. Ecol. 21: 397–407.

Gianoli, E. 2001. Lack of differential plasticity to shading of internodes and petioles with growth habit in *Convolvulus arvensis* (Convolvulaceae). – Int. J. Plant Sci. 162: 1247–1252.

Gianoli, E. 2003. Phenotypic responses of the twining vine *Ipomoea purpurea* (Convolvulaceae) to physical support availability in sun and shade. – Plant Ecol. 165: 21–26.

Gianoli, E. 2004. Plasticity of traits and correlations in two populations of *Convolvulus arvensis* (Convolvulaceae) differing in environmental heterogeneity. – Int. J. Plant Sci. 165: 825–832.

Gianoli, E. and González-Teuber, M. 2005. Environmental heterogeneity and population differentiation in plasticity to drought in *Convolvulus chilensis* (Convolvulaceae). – Evol. Ecol. 19: 603–613.

Gianoli, E. et al. 2007. Interactive effects of leaf damage, light intensity and support availability on chemical defences and morphology of a twining vine. – J. Chem. Ecol. 33: 95–103.

Givnish, T. J. 2002. Ecological constraints on the evolution of plasticity in plants. – Evol. Ecol. 16: 213–242.

González, A. V. and Gianoli, E. 2004. Morphological plasticity in response to shading in three *Convolvulus* species of different ecological breadth. – Acta Oecol. 26: 185–190.

González-Teuber, M. and Gianoli, E. 2007. Tolerance to simulated herbivory in two populations of *Convolvulus chilensis* (Convolvulaceae). – Acta Oecol. 32: 119–123.

González-Teuber, M. and Gianoli, E. 2008. Damage and shade enhance climbing and promote associational resistance in a climbing plant. – J. Ecol. 96: 122–126.

Hutchings, M. J. and de Kroon, H. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. – Adv. Ecol. Res. 25: 159–238.

Kawano, S. and Hara, T. 1995. Optimal balance between propagule output, propagule size, and cost of propagule production in plants with special reference to its evolutionary – ecological implications. – Plant Species Biol. 10: 119–125.

O' Donell, C. A. 1957. Convolvuloideas Chilenas. – Bol. Soc. Arg. Bot. 6: 144–184.

Palacio-López, K. and Rodríguez-López, N. 2008. Plasticidad fenotípica en *Lippia alba* (Verbenaceae) en respuesta a la disponibilidad hídrica en dos ambientes lumínicos. – Acta Biol. Colomb. 13: 187–198.

Pezo, L. and Gonzáles, A. 1998. Caracterización agronómica de pampa orégano *Lippia alba* (Mill). – Folia Amaz. 9: 181–192.

Pigliucci, M. 2002. Touchy and bushy: phenotypic plasticity and integration in response to wind stimulation in *Arabidopsis thaliana*. – Int. J. Plant Sci. 163: 399–408.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. – Ecol. Lett. 6: 265–272.

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

Pigliucci, M. and Marlow, E. 2001. Differentiation for flowering time and phenotypic integration in *Arabidopsis thaliana* in response to season length and vernalization. – Oecologia 127: 501–508.

Pigliucci, M. and Preston, K. 2004. Phenotypic integration. Studying the ecology and evolution of complex phenotypes. – Oxford Univ. Press.

Pigliucci, M. et al. 1995. Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. – J. Evol. Biol. 8: 421–438.

Quezada, I. M. and Gianoli, E. 2006. Simulated herbivory limits phenotypic responses to drought in *Convolvulus demissus* (Convolvulaceae). – Pol. J. Ecol. 54: 499–503.

Richards, C. L. et al. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. – Ecol. Lett. 9: 981–993.

Saldaña, A. et al. 2005. Ecophysiological responses to light availability in three *Blechnum* species (Pteridophyta, Blechnaceae) of different ecological breadth. – Oecologia 145: 252–257.

Sánchez-Gómez, D. et al. 2008. Functional traits and plasticity linked to seedlings' performance under shade and drought in Mediterranean woody species. – Ann. For. Sci. 65: 311.

Schlichting, C. D. 1986. The evolution of phenotypic plasticity in plants. – Annu. Rev. Ecol. Syst. 17: 667–693.

Schlichting, C. D. 1989a. Phenotypic plasticity in *Phlox* II. Plasticity of character correlations. – Oecologia 78: 496–501.

Schlichting, C. D. 1989b. Phenotypic integration and environmental change. – Bioscience 39: 460–464.

Schlichting, C. D. and Pigliucci, M. 1998. Phenotypic evolution. A reaction norm perspective. – Sinauer.

Stomp, M. et al. 2008. The timescale of phenotypic plasticity and its impact on competition in fluctuating environments. – Am. Nat. 172: E169–E185.

Suárez, L. H. et al. 2004. Biología reproductiva de *Convolvulus chilensis* (Convolvulaceae) en una población de Aucó (Centro-Norte de Chile). – Rev. Chil. Hist. Nat. 77: 581–591.

Sultan, S. E. 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. – Ecology 82: 328–343.

Tufto, J. 2000. The evolution of plasticity and nonplastic spatial and temporal adaptations in the presence of imperfect environmental cues. – Am. Nat. 156: 121–130.

Valladares, F. et al. 2005a. Shade tolerance, photoinhibition sensitivity and phenotypic plasticity of *Ilex aquifolium* in continental-Mediterranean sites. – Tree Physiol. 25: 1041–1052.

Valladares, F. et al. 2005b. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. – J. Exp. Bot. 56: 483–494.

Valladares, F. et al. 2007. Ecological limits to plant phenotypic plasticity. – New Phytol. 176: 749–763.

van Kleunen, M. and Fischer, M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. – New Phytol. 166: 49–60.

van Kleunen, M. and Fischer, M. 2007. Progress in the detection of costs of phenotypic plasticity in plants. – New Phytol. 176: 727–730.

Vit, P. et al. 2002. *Lippia alba* N. E. Br. Ficha botánica de interés apícola en Venezuela. – Rev. Fac. Farm. Univ. Venez. 43: 1–14.

Waitt, D. E. and Levin, D. A. 1993. Phenotypic integration and plastic correlations in *Phlox drumondii* (Polemoniaceae). – Am. J. Bot. 80: 1224–1233.

Waitt, D. E. and Levin, D. A. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. – Heredity 80: 310–319.

Weinig, C. 2000. Limits to adaptive plasticity: temperature and photoperiod influence shade-avoidance responses. – Am. J. Bot. 87: 1660–1668.

Weinig, C. and Delph, L. 2001. Phenotypic plasticity early in life constrains developmental responses later. – Evolution 55: 930–936.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. – Oxford Univ. Press.