



## REVIEW ARTICLE

# Studying phenotypic plasticity: the advantages of a broad approach

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Phenotypic plasticity is traditionally defined as the capacity of a given genotype to render alternative phenotypes under different environmental conditions. Some studies focus on the individual genotype to study ‘true’ phenotypic plasticity, regardless of the level of ecological organization involved in each particular study. We argue that, depending on the research question and the scale, there are advantages of looking beyond the genetic identity of each individual phenotype when addressing phenotypic plasticity. This broad approach may simplify experimental designs, increase their statistical power, and allow a more inclusive estimation of the extent of phenotypic plasticity in natural populations. We also posit that when the focus is on the ecological significance of a given phenotype, the final ontogenetic stage and size of the experimental individuals whose plastic responses are compared should not be necessarily considered as confounding factors. A broad approach to the genotypic basis of phenotypic responses, focusing on the representativeness of the genotypic sample, together with the recognition that any environmentally-induced phenotypic change is legitimate plasticity (and potential target of natural selection), may contribute to the understanding of the ecological significance of phenotypic plasticity. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **105**, 1–7.

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### ADDRESSING PHENOTYPIC PLASTICITY

Phenotypic plasticity, broadly understood as environmentally-induced phenotypic variation (Stearns, 1989), is found in natural populations of a diverse array of organisms and is a major means of adaptation to environmental heterogeneity (Bradshaw & Hardwick, 1989). Paradoxically, being an ecological phenomenon of paramount importance in the wild (Miner *et al.*, 2005; Nussey, Wilson & Brommer, 2007), phenotypic plasticity can hardly be observed in nature. Thus, aside from particular cases where within-individual variation is studied (Cook &

Johnson, 1968), an experimental approach is needed to ascribe observed phenotypic variation in the field to phenotypic plasticity rather than to genetic variation. It is widely known that individuals are capable of modifying their phenotypes in response to changes in the environment, and that these plastic responses may entail fitness benefits (i.e. adaptive phenotypic plasticity; Pigliucci, 2001) and can have significant effects at different levels of ecological organization (Miner *et al.*, 2005). Furthermore, it is clear that phenotypic plasticity itself may evolve independently of the main value of the character (Schlichting & Levin, 1986; Scheiner, 1993). The notion of phenotypic plasticity, however, remains somewhat elusive, with controversy existing regarding the way that it should

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be measured and uncertainty about its role in adaptation in the context of a changing world (Valladares & Gianoli, 2007; Valladares, Sánchez-Gómez & Zavala, 2006; Valladares, Gianoli & Gómez, 2007; Hulme, 2008; Visser, 2008; Matesanz, Gianoli & Valladares, 2010; Nicotra *et al.*, 2010).

Distinguished scholars in the field of phenotypic plasticity have acknowledged that the reaction norm (i.e. the repertoire of phenotypic responses of a given genotype along an environmental gradient) is ultimately a property of individuals (Stearns, 1989; Agrawal, 2001; Schlichting & Smith, 2002; West-Eberhard, 2003). Although we need to replicate ‘individuals’ along an environmental gradient to rigorously assess phenotypic plasticity, the foremost notion is that, by doing so, we are mimicking what occurs when a single individual in nature experiences an environmental change. We argue here that looking beyond the genetic identity of each individual phenotype when addressing phenotypic plasticity in comparative studies not only simplifies experimental designs and increases their statistical power, but also may allow a more inclusive estimation of the extent of phenotypic plasticity in natural populations. In the same vein, we posit that when the focus is on the ecological significance of a given phenotype, the final ontogenetic stage and size of the experimental individuals whose plastic responses are compared should not be considered confounding factors. We will explain why a less strict consideration of all of these factors is a practical necessity in some systems where it is difficult to obtain sets of genetically and developmentally identical individuals, and also a way to better account for genotypic diversity within populations or species for a given sampling effort.

### GENOTYPE $\times$ ENVIRONMENT INTERACTIONS

Phenotypic plasticity is traditionally defined as the capacity of a given genotype (G) to render alternative phenotypes under different environmental conditions (E). Some views emphasize the focus on the individual genotype to study ‘true’ phenotypic plasticity, regardless of the level of ecological organization involved in each particular study or research question. Thus, it has been stated that, for phenotypic plasticity to be adequately addressed, it should be studied with replicated genotypes, i.e., with experiments using the very same genotype (clones) or individuals with known genetic relatedness (genetic families) (Såstad, Pedersen & Digre, 1999; Richards *et al.*, 2006; Rohde & Junntila, 2008; Herrera, 2009; J. M. Gómez, person. comm.). This approach would limit the evaluation of a ubiquitous phenomenon of

paramount importance in nature to only those species either with asexual reproduction, suitable to be cloned or easily manipulated in breeding crosses. When this methodological requirement is relaxed, populations and species can be used as experimental subjects of research on phenotypic plasticity (Valladares *et al.*, 2006). In this regard, Pigliucci (2001: 74) states that ‘there is nothing wrong with considering *mean* plasticities as an attribute of a population or species’.

In ecological studies of phenotypic plasticity, the main question is seldom whether plasticity occurs in the study system or not; it is hard to find cases of no plasticity at all. Rather, research questions often address whether the study units show differences in plasticity. When the study units are different genotypes within a population it refers to the occurrence of G  $\times$  E interactions. When those research questions are addressed at higher levels of biological organization (e.g. populations within a species, species within a genus), a thorough field sampling followed by random sorting of individuals into experimental treatments should allow to estimate whether the G  $\times$  E interaction analogues are significant, i.e., whether populations (P  $\times$  E interactions) or species (S  $\times$  E interactions) differ in phenotypic plasticity. This entails the reasonable assumption that genetic relatedness is significantly greater within the experimental units than among them. In accordance with such a protocol, Gianoli & González-Teuber (2005), Balaguer *et al.* (2001) and Lind & Johansson (2007) tested for the relationship between phenotypic plasticity and environmental heterogeneity in populations of a perennial herb, an evergreen tree, and a frog, respectively. Similarly, ecological hypotheses on the expression of phenotypic plasticity have been tested comparing the plastic responses of related species of plants (Schlichting & Levin, 1984; Valladares *et al.*, 2000) and animals (Rezende *et al.*, 2001; Deere & Chown, 2006). The within-population approach usually addresses the occurrence of genetic variation for phenotypic plasticity among different clones or genetic families, which is reflected in nonparallelism among reaction norms (Pigliucci, 2005). Examples of these G  $\times$  E interactions are found in the ecophysiological responses to light availability displayed by widespread annual plants (Sultan & Bazzaz, 1993; Godoy *et al.*, 2011), and in the life-history changes induced by food type exhibited by freshwater crustaceans (Ebert, Yampolsky & Van Noordwijk, 1993; Yampolsky & Scheiner, 1994).

A *sensu lato* approach to the genotypic identity in plasticity studies (i.e. related but not identical genotypes being exposed to different environments) has been applied a number of times (Smith & Palmer, 1994; Zhang & Lechowicz, 1994; Schlichting &

Pigliucci, 1995; Valladares *et al.*, 2002; Gianoli, 2003; Saldaña, Gianoli & Lusk, 2005; Iwami, Kishida & Nishimura, 2007; Cavieres & Sabat, 2008; González-Teuber, Segovia & Gianoli, 2008; Visser, Holleman & Caro, 2009). However, it is still a controversial issue. Thus, the *sensu lato* approach has been criticized for relaxing the requirement of genotypic uniformity in common garden measurements of conspecific individuals, of unknown genetic relatedness, from the same population (Herrera, 2009: 121–122). The traditional, *sensu stricto* approach to the study of phenotypic plasticity is strongly recommended: (1) when research is aimed at the genetic basis of phenotypic plasticity, thus evaluating whether reaction norms differ among genotypes (Windig, 1994; Pigliucci, 1997; Scheiner & Yampolsky, 1998) and hence there is potential for an evolutionary response provided that such reaction norms are related to fitness (Scheiner, 1993) or (2) when it is of interest to unravel the precise mechanisms involved in the plastic responses (Ballaré *et al.*, 1995; Brakefield *et al.*, 1996; Fairbairn & Yadlowski, 1997; Kalujnaia *et al.*, 2007). In other words, this approach must be used when dealing with phenotypic plasticity questions at the individual or within-population level (Pigliucci, 2005). When posing ecological or comparative questions in phenotypic plasticity studies framed at higher levels of biological organization, a broad approach might be useful, as is discussed below.

Ecologically meaningful evaluations of phenotypic plasticity must include environmental gradients that are representative of those experienced by the subject organisms in nature (Dudley, 2004; Miner *et al.*, 2005; Hulme, 2008). Otherwise, an incomplete view of the relevant portions of the reaction norm would be obtained. We further suggest that advances in the understanding of the ecological significance of phenotypic plasticity in nature can be achieved if representative samples of the genotypic diversity of natural populations are included in the study. For logistic reasons, this representative sampling is hardly possible if researchers are to work with replicated genotypes only. For example, consider a case where it is of interest to compare the phenotypic plasticity to resource availability in populations of a given species that are distributed in contrasting habitats. Greenhouse (or laboratory) space sets a limit for the number of individuals that can be grown, manipulated and measured. If only replicated genotypes (either clones or genetic families) across the environmental gradient are included as experimental subjects, then only a relatively small number of original genotypes would be sampled in the field or taken as parental material. Alternatively, a bulk sampling of propagules from several, widely-spaced parents, which are later sorted into experimental treatments,

will allow the inclusion of a much more representative sample of genotypes from each population and hence would increase ecological realism of the results. Indeed, for a fixed number of experimental individuals under a given experimental design, the number of genotypes sampled in each population with a *sensu lato* approach will be  $N$  times greater than that obtained following a *sensu stricto* approach, where  $N$  is the number of replicates per genotype in the latter approach. Importantly, to avoid pseudoreplication bias, such a bulk sampling must be as extensive as possible, with only one or a few propagules (seeds, eggs) per parental individual.

### ARE ONTOGENY AND ALLOMETRY CONFOUNDING FACTORS?

Ontogeny and allometry are considered potentially confounding factors in phenotypic plasticity research (Gedroc, McConnaughay & Coleman, 1996; McConnaughay & Coleman, 1999; Weiner, 2004). First, most organisms change their biomass allocation patterns during ontogeny (Roff, 1992). These changes characterize the life-history strategy of species or populations and are most likely shaped by natural selection (Roff, 1992). Second, biomass allocation patterns often follow allometric trajectories, thus being a function of individual size (Weiner, 2004). Consequently, any environmental factor that influences development and growth rates, and hence the ontogenetic stage and size of individuals at a given time, would also affect the observed allocation patterns (Weiner, 2004). Because plasticity is frequently measured in allocation traits, this rationale has often led to consider these cases as ‘apparent plasticity’ as opposed to ‘true plasticity’ (Weiner, 2004; Geng *et al.*, 2007; Hulme, 2008). This point is illustrated by studies on plant phenotypic plasticity showing that a conventional analysis rendered the expected (from an adaptive plasticity viewpoint) increase in root/shoot biomass ratio in response to low nutrients (Gedroc *et al.*, 1996) and below-ground competition (Cahill, 2003), whereas an allometric analysis (where plant size is accounted for) showed otherwise. Nonetheless, other studies have reported that root/shoot biomass allocation is independent of plant size and support adaptive plasticity prediction (Shipley & Meziane, 2002; Huang *et al.*, 2010). Moreover, the physiological mechanism by which water shortage results in an increase in the root/shoot ratio, another functional response that could be considered as a mere byproduct of a shift in ontogenetic trajectories, has been elucidated (Lambers, Chapin & Pons, 1998). Most studies show mixed results, lending partial support to both optimal partitioning theory and ontogenetic/allometric control

(Gedroc *et al.*, 1996; McConnaughay & Coleman, 1999; Geng *et al.*, 2007).

We assert that, with regard to phenotypic plasticity, the essential fact for a species' ecology is the expression of a given phenotype at a given point in space and time. Whether such plastic responses are a consequence of environmentally-driven changes in size/ontogeny or reflect active plasticity mechanisms does not affect the ultimate, functional implication: the individual fitness. Natural selection acts on traits via individuals, which show differential survival and/or reproduction associated with the expression of certain phenotypic characteristics (Endler, 1986), regardless of the underlying mechanisms. Thus, plastic responses are to be ecologically significant because of the advantages conferred to the organisms displaying them in a changing environment (González-Teuber & Gianoli, 2008) and not because of their dependence or independence on individual size. The latter criterion coincides with the definition of adaptation proposed by Reeve & Sherman (1993), which refers to a phenotypic variant that results in the highest fitness among other variants in a given environment, making no reference to the historical processes, evolutionary mechanisms or genotypic architecture behind the successful phenotype. Summarizing, we think that a consideration of the ecological context of the phenotypic change and its selective implications may lead us to disregard the potentially confounding effects of ontogeny and allometry, particularly when the plasticity research does not aim at proximate causes.

Importantly, by stressing that the ecological significance or adaptive value of phenotypic plasticity can be evaluated without reference to the underlying mechanisms, we do not imply that any phenotypic change observed under different environments is to be considered adaptive (Ghalambor *et al.*, 2007). To properly infer that a given phenotypic response may be an adaptive feature selected in the relevant ecological scenario, and not a byproduct of another process, it is essential to identify a functional link (supported by ecophysiological knowledge) between the observed phenotypic change and the challenging environment (Sultan, 1995; Kingsolver & Huey, 1998). Dudley (2004) discusses how the same phenomenon, plant tissue necrosis, may be interpreted both as a passive symptom of damage, when plants are subjected to nickel deficiency, and as an active adaptive response, when it is associated with a pathogen infection that elicits a hypersensitive response surrounding the pathogen with dead tissue. Similarly, we think that when the consequences of environmental challenges on the organisms are known (e.g. drought may cause plant wilting) and the positive effects of the ensuing responses are identified (e.g. increased root/shoot biomass allocation in response to

reduced soil moisture enhances water uptake), there is no reason to consider this phenomenon as 'apparent plasticity', even when it is associated with delayed/hastened growth or development. The resultant phenotype is the target of natural selection, regardless of size or age, and an evolutionary response may follow, provided that the phenotype with adaptive value shows heritable variation (Endler, 1986). Even if the plant's functional response to environmental change is mediated by a delay in vegetative growth, those individuals showing greater responsiveness would have better fitness than their counterparts and hence would be selected. It was earlier discussed that the phenotype resulting from a plastic response may determine the outcome of competitive interactions among plants, even if the trait state arose from ontogenetic or allometric relationships instead of from functional adjustments (Coleman, McConnaughay & Ackerly, 1994).

Here, we are not disregarding the role of allometry or ontogeny in determining the observed phenotypic variation. We agree with earlier ideas regarding the 'developmental reaction norm' (i.e. the set of ontogenies that can be produced by a genotype when it is exposed to different environments) as the ultimate object of natural selection (Pigliucci *et al.*, 1996). In the same vein, we are not suggesting that allometry and ontogeny should be dismissed as potential explanatory factors when interpreting phenotypic responses to the environment; it is a fact that size and age often explain observed patterns of phenotypic plasticity, at least partially (Schlichting & Pigliucci, 1998). Rather, we assert that, even in these cases, the phenotypic outcome can be interpreted in adaptive terms. Below, we illustrate this point with an example that was provided by a reviewer of this manuscript. The tadpoles of some frog species increase the depth of their tailfin (i.e. the maximum body length perpendicular to the tadpole's tail) in response to the presence of insect predators (Dayton *et al.*, 2005). There is some evidence that deeper tails confer an advantage in escape speed (Van Buskirk & McCollum, 2000). Let us consider a case in which the predator-induced phenotype shows a 2-cm absolute increase in tail depth compared to control, predator-free tadpoles. However, after correcting for body size, which is greater in predator-exposed individuals (Van Buskirk & McCollum, 2000), the relative increase is of 0.5 cm. Consequently, these size-corrected tails are statistically similar to those of control tadpoles. Our point is that, if experiments demonstrate that: (1) tadpoles with deeper tails, in absolute terms, are faster and (2) faster tadpoles survive better in the presence of predators, then the plastic response of increased tail depth is to be considered legitimate and adaptive. Therefore, the actual fitness benefit overrides the fact

that size-corrected tails did not show a significant change, which is of importance for understanding the internal mechanism of biomass and energy allocation of tadpoles, although it does not deny the relevance of the plastic response. Of course, caution should be exercised before concluding that it is the change in tail depth, and not an unmeasured correlated trait or even body size, what explains the enhanced tadpole performance in the presence of predators. The precision of experimental approaches and, as discussed above, the connection of observed patterns with functional arguments (e.g. size itself can hardly be connected with escape speed) would shed light into this question.

### CONCLUDING REMARKS

Phenotypic plasticity is inherently an ecological phenomenon and refers to changes in the phenotype induced by the environment. Whether this must be accompanied by a one-to-one identification of a phenotype with its underlying genotype or not should depend upon the scale of the question. When the focus is on the evolution of phenotypic plasticity within a population, or on the mechanisms underlying the plastic response, the use of the traditional approach to the genotypic basis of the phenotype is recommended for gaining precision and predictive power. Knowledge of the particular processes and signals involved in the expression of plastic responses and of the genetic architecture of characters that promote or constrain trait plasticity can be very valuable for understanding the evolution of plasticity or the lack thereof (Pigliucci & Byrd, 1998; DeWitt & Scheiner, 2004). When the study poses ecological questions, such as those related to patterns of population differentiation in plasticity along an environmental gradient (Gianoli & González-Teuber, 2005; Bell & Galloway, 2008), or those inquiring for the role of plasticity in explaining contrasting niches in closely-related species (González & Gianoli, 2004; Saldaña *et al.*, 2005), a broad approach to plasticity, as we have shown here, can be very valuable. By placing emphasis on the phenotype and focusing on the representativeness of the genotypic sample, this approach may contribute to the understanding of the ecological significance of phenotypic plasticity. It also allows the inclusion of a broader range of study systems, thus providing a comprehensive view of the relevance of phenotypic plasticity in nature. This approach has been followed in many previous studies and has been briefly discussed in the context of the realism versus precision dichotomy, as compared to the *sensu stricto* approach (Richards *et al.*, (2006). However, a clear assertion of the associated advantages was pending.

Despite the remarkable expansion of phenotypic plasticity research along the evolutionary and molecular fronts (Pigliucci, 2005; Ellers & Stuefer, 2010), we think that the field will still benefit from more basic, ecological evidence eventually leading to the elucidation of general patterns of phenotypic plasticity in nature, which currently appear to remain elusive (Palacio-López & Gianoli, 2011). We hope that the approach advocated herein will help to promote ecological research on phenotypic plasticity, particularly by young scholars.

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