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SYNTHESIS PAPER

On the Evolutionary Developmental Biology of Speciation

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Abstract The mainstream approaches to the study of speciation and clade diversification have extensively focused on genetic mechanisms and ecological contexts, while much less attention has been paid to the role of development. In this paper we provide materials to support the thesis that taking development into the picture of evolutionary processes can bring important insights on how species multiply and diversify. Evidence that developmentally entangled evolutionary factors are important in speciation comes from different lines of investigation that can be broadly grouped under three headings: evolvability, phenotypic plasticity, and phenology. Evolvability enters the scene through the complexity of the genotype-phenotype map, the developmental link between transmissible genetic information and selectable phenotypes. Phenotypic plasticity can act as a facilitator for speciation, promoting diversification at different stages of the speciation process, as well as generating novel targets and novel trade-offs for evolutionary processes. The formal inclusion of the developmental time axis in speciation models widens the scope for investigating the onset and/or reinforcement of reproductive barriers through a range of situations along an organism's life cycle. Overall, developmental processes can contribute to speciation and diversification at different stages of the speciation process, at different levels of biological organization and along the organism's whole life cycle.

Keywords Evo-devo · Genotype–phenotype map · Heterochrony · Phenology · Phenotypic plasticity

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Introduction

When evaluating the causes of speciation, authors have focused primarily on geographic scenarios, genetic mechanisms and the possible expansion into new ecological niches through the emergence of novel adaptations (e.g. Schluter 2000; Coyne and Orr 2004; Rundle and Nosil 2005; Grant and Grant 2008), the latter often allowing a clade to give rapidly rise to many descendent species (cf. Schluter 2000).

Speciation processes are very seldom studied from the point of view of the changes in developmental control and timing (cf. Naisbit et al. 2003), although the role of development changes in the origination of key innovations at the roots of often spectacular evolutionary radiations has been long accepted (e.g., Simpson 1944, 1953; Heard and Hauser 1995; Schluter 2000; cf. also Pfennig and McGee 2010).

In this paper we explore some aspects of the hitherto neglected relationships between the evolution of development and speciation, shifting the focus of an evolutionary developmental biology (evo-devo) approach from comparisons between distantly related model organisms to the scale of microevolutionary change. In particular, we review evidence that developmentally entangled evolutionary factors like evolvability, phenotypic plasticity and the control of phenology may have an important role at different stages of speciation processes.

Although the subject of this study is speciation, our meta-analysis is not limited to case studies on the emergence of reproductive barriers between populations. The article can be more aptly defined as a survey of developmental approaches to the cladogenetic dimension of evolutionary change, to complement the more popular evo-devo research focusing instead on the anagenetic dimension of evolutionary change. This obviously encompasses the emergence of reproductive isolation between populations, but also processes that precede it, and can act for instance as facilitators, or follow it, and can act for instance as consolidators of the speciation event. For instance, developmental systems or life history traits inherently disposed to divergent evolution can assume a role at different stages of the speciation process, both before and after the emergence of reproductive barriers. Evolutionary biologists use a variety of approaches to study speciation (The Marie Curie SPECIATION Network 2012), thus, for our exploratory survey, we have opted for a broad scope, based on evidence directly related to the evolution of reproductive barriers to different degrees and with different degrees of observational support.

Our evolutionary developmental biology approach needs some clarifications as well. Some 30 years since the term was introduced in the literature, there is no general agreement, not only on the relevance of evo-devo for evolutionary theory, but also on what evo-devo is all about and on what its main claims are (see Minelli and Fusco 2008). These span from claims about the relative importance of selection vs. constraints viewed as antagonistic factors in evolution (see Fusco 2001), to the relative importance of changes in regulative vs. coding genetic sequence in the evolution of diversity (see Hoekstra and Coyne 2007). In line with the broad view declared in the previous paragraph, we will not pursue these specific claims here. The more general view that we adopt in this review is that including information on developmental processes provides more complete explanations of the observed evolutionary patterns, both for anagenetic change, as most evo-devo literature has always argued, and for cladogenetic change, as we will argue here.

Conceptual Caveats

A major difficulty in the study of speciation is how to isolate morphological traits, if any, directly imputable as causal factors of a speciation event from the often conspicuous set of traits which eventually differentiate newly reproductively separate species, but have been fixed in either species for reasons (drift, adaptive selection) others than speciation per se. Many cases of speciation discussed in this article are not exempt from this limitation, and we can never be sure that we have traced the exact boundary between differences directly involved in the speciation event and simple by-products of incipient or already established reproductive isolation.

A second necessary caveat at the start of our enquiry is that the morphological evidence eventually suggestive of a role of developmental processes in speciation must be searched for with greater awareness than traditional approaches to species diversity would suggest. Indeed, in zoology the morphological approach to taxonomy and phylogeny is mainly, although not exclusively, based on the features of the adults, and in plants obvious character-rich body parts, as are the flowers and fruits borne on mature plants, are mostly given unique attention in the corresponding disciplines. However, reproductive isolation can also depend on differences manifested by two incipient or newly isolated species at earlier developmental stages, or—as we will see below—on differences in gene regulation at any time along the whole ontogeny, rather than on structural traits of the adult only.

Evolvability and Speciation

From the perspective of evolutionary developmental biology, the main focus of evolutionary research shifts from the problem of the ‘survival of the fittest’ to that of the ‘arrival of the fittest’ (see Wagner 2011). Evolvability, i.e. the ability to produce heritable phenotypic variation (Pigliucci 2008), thus assumes a central role in the explanation of evolutionary change (Hendrikse et al. 2007). Operationally, evolvability has been variously defined (Wagner and Draghi 2010), in particular in relation to evolutionary processes on different time scales. Here, we will adopt a broad view, where evolvability is not a purely quantitative concept (e.g., a scalar, proportional to the amount of variation that can be produced), but rather a complex multifaceted measure that includes nature and composition of variation that can be produced.

Addressing the question of trait evolvability in a study of speciation translates into enquiring about the evolvability of traits accompanying the divergent evolution of two incipient or newly separated species. At the core of the question there are the non-linear relationships between genotype and phenotype, as these emerge through developmental genetics, comparative genomics and comparative morphology and life history studies (e.g., Nijhout 1990; Alberch 1991; Keller 2000; Pigliucci 2001; West-Eberhard 2003; Kupiec 2009; Pigliucci and Müller 2010).

Genotype–Phenotype Map

These relationships between genotype and phenotype (the genotype–phenotype map) are uncontroversially acknowledged as complex and non-linear, if only for the pervasive occurrence of pleiotropy and epistasis in the genetic control of development (Wagner and Zhang 2011). These characteristics of the genotype–phenotype map are reflected in variational properties of the phenotype that are particularly favorable for the onset of speciation, e.g., the possibility for the same phenotypic character to be variably expressed based on a complex multi-genetic control in development or, on the opposite, the possibility that the modulation of a limited number of genetic factors may have sizable divergent and complex effects on phenotypes.

Developmental Genetics Studies

Seemingly simple species-specific traits, demonstrably involved—in association with behavioural differences—in determining reproductive isolation between closely related species are sometimes under complex and eventually different genetic control. This is the case of the sex combs on the fore leg tarsi of the males of many species of *Drosophila* and related genera. Species-specific differences in sex combs have different causes (Kopp 2011). In terms of cellular processes, different sex combs are sometimes the result of a different modulation of a homologous set of precursor cells. For example, in *Drosophila biarmica* the rows of bristles forming the male sex comb are turned by 90° in respect to proximo-distal alignment of their neighbours but such a movement does not occur in the closely related *D. mimetica*. On the other hand, similar sex combs may derive from nonhomologous precursors, as between *D. ficusphila* and the species of the *montium* subgroup. Multiple loci are involved in interspecific differences in male sex combs even in the case of very recently originated sibling species, such as *D. simulans* and *D. sechellia* (divergence time estimated at 413,000 years) or *D. simulans* and *D. mauritiana* (divergence time estimated at 263,000 years) (Kliman et al. 2000). In the case of the first pair of species, at least four quantitative trait loci (QTL) are apparently involved in the differences in the male sex combs, and at least two QTL in the second case (Kopp 2011).

Unfortunately, the number of studies addressing the morphological differences between closely related species in terms of developmental genetics is still very small. Worth mentioning here is the analysis of the mechanisms producing differences in beak shape among Darwin's finches (*Geospiza* spp.). Interestingly, the discovery that these differences can be explained by the differential expression of the products of just two genes (*bmp4* and *calmodulin*) (Abzhanov et al. 2004, 2006) accounts for both the prompt adaptive response of beak proportions to the rapidly changing availability of food items (seeds) of specified size (Grant 1999; Grant and Grant 2002) and for the interspecific differences in beak size and shape, and thus in the trophic niche of closely related species.

Two other studies focused on insects. In one of these, Hawthorne and Via (2001) analysed the genetic architecture of key traits in two highly specialized host races of the pea aphid *Acyrthosiphon pisum* and found several complexes of QTL with effects on differential survival on the two hosts, thus increasing assortative mating and eventually promoting divergence towards speciation. In another study, Naisbit et al. (2003) investigated the genetics of Müllerian mimicry in *Heliconius cydno* and *H. melpomene*, two sister species of butterfly that recently diverged to mimic other species in their own genus. Changes in the developmental regulation of wing-patterning genes are in this case essential in obtaining pre- and postmating isolation.

Other well-developed cases of study explore the origin of developmental novelties. Admittedly, the very notion of evolutionary novelty is matter of dispute (e.g., Mayr 1960; Müller 1990; Müller and Wagner 1991, 2003; Minelli and Fusco 2005; Moczek 2008, 2009; Moczek et al. 2011). Nevertheless, it is uncontroversial that features such as the head and prothoracic horns of many scarab beetles, or the prothoracic outgrowths of treehoppers (Membracidae) do not have obvious homologues in the closest (and not so close) relatives of these insects. In the case of scarab beetles, the origin of their cephalic and thoracic appendages is dependent on the ectopic recruitment of many genes (Wasik et al. 2010; Snell-Rood et al. 2011; Wasik and Moczek 2011, in press) including also genes such as *Distalless*, otherwise involved in the positional or structural specification of articulated appendages (Moczek and Rose 2009). The horns of treehoppers have been recently interpreted as prothoracic serial homologues of the wings, which are otherwise confined to the meso- and metathorax (Prud'homme et al. 2011) but this interpretation is probably unwarranted (Yoshizawa 2012; Mikó et al. 2012). In these two insect groups, horn shape is very often species-specific, to the extent that specimens (both males and females in treehoppers; the males at least in scarab beetles) can be routinely identified at the species level on the basis of this character, without the need of further evidence; the only potential source of error being the male polyphenism exhibited by many scarab species in the size or complexity of their horns.

Single mutations in genes involved in developmental processes similarly resulting in major morphological differences are putatively associated to many speciation events. These events have been mainly discussed in the literature about speciation in terms of character evolution and adaptation, but would really deserve close revisitation in terms of developmental genetics and developmental evolution. A few examples are presented in the next paragraph.

Comparative Genomics Studies

Comparative genomics studies are profoundly affecting most areas of biology, including evolutionary biology (Wray 2010). At the intersection between evolutionary genomics and evo-devo, we will discuss here only the contribution of genomics to the long-established debate on the association between events of whole-genome duplication and adaptive radiation.

Complete genome duplications have been documented in several major clades, including yeasts (Kellis et al. 2004), angiosperms (De Bodt et al. 2005) and chordates (Holland et al. 2008). In the latter, two rounds of duplication occurred before the origin of gnathostome vertebrates, while a third round of duplication was specific to the teleost lineage (e.g., Crow et al. 2006).

Several studies have suggested that events of genome duplications may promote speciation through differential retention and loss of duplicated genes (divergent resolution) after genome duplication. The loss of different duplicates in geographically isolated populations could reduce the fecundity of hybrids, providing a powerful isolating mechanism (Semon and Wolfe 2007a). This has been observed in yeast (Scannell et al. 2006), in plants (Adams and Wendel 2005) and in teleosts (Semon and Wolfe 2007b). However, while there is compelling evidence that genome duplication can promote radiation, this does not imply that duplication affects speciation through development.

A special role in driving radiation, through duplication and subsequent subfunctionalization and/or neofunctionalization of the paralogs, has been credited to particular classes of metazoan developmental genes. For instance, Wagner et al. (2003) proposed that Hox cluster duplications may temporarily open a ‘window of increased evolvability’ that under favorable ecological and developmental boundary conditions a lineage has the opportunity to exploit, with potential effects on its radiation. Multiplication of the primitive single copy Hox cluster would have been a key evolutionary change during the early evolution of vertebrates, as vertebrate Hox gene cluster would be structurally less evolvable, in the absence of gene duplications, than the Hox gene cluster of non-vertebrate bilaterians.

A more general result on the relationship between genome duplication and developmental processes comes from the comparative analysis of the complete genomes of several chordates. Holland et al. (2008) have observed that gene duplicates from the two rounds of genome duplication that have been retained in modern gnathostome genomes present a significantly larger fraction of genes involved in functions associated with developmental processes (functions such as signal transduction and transcriptional regulation), if compared to the overall retention rate. They speculate that the two rounds of genome duplication in the early history of vertebrates may have provided a higher level of genomic flexibility, resulting in developmental, morphological and physiological novelties. The finding that, after duplication, genes involved in developmental signalling and gene regulation have significantly higher chances to be retained in multiple copies than an average gene, suggests a correlation between developmental regulation and the evolution of vertebrate novelties that can have played a significant role in driving the radiation of the clade.

Comparative Morphology and Life History Studies

Many plant clades provide examples of the unusual woodiness, and the associated giant size, of species or groups of species recently evolved on oceanic islands within genera represented on continents by small herbaceous species, as illustrated by Carlquist (1974) in his extensive treatment of island floras. Among many others, there are composites of the genera *Bidens* in Southern Polynesia, *Senecio* in New Zealand, and *Centaurea* in the Canary Islands; and species of *Echium* (Boraginaceae) and *Euphorbia* (Euphorbiaceae) in Madeira, the Canary Islands and Cape Verde Islands, all of them having evolved giant size supported by secondary growth as woody plants, in striking contrast to the herbaceous habitus of their continental counterparts. A similar phenomenon occurs in the alpine belt of the highest mountains of Africa. The most extraordinary example, however, is offered by the Hawaiian composites traditionally classified in the genera *Dubautia*, *Wilkesia* and *Argyroxiphion*, quite recently derived from a common ancestor probably similar to some living shrubby *Dubautia* species but today including, among other life forms, the unique silverswords (*Argyroxiphium* spp.), thick cushions of densely packed silvery linear leaves. Despite the enormous differences in habitus, these plants are indeed so closely related that a natural hybrid between a *Dubautia* species and an *Argyroxiphium* species has been reported (Carlquist 1980).

The hypothesis that such dramatic changes in habitus have accompanied speciation in at least some of these forms is strongly supported by the fact that a corresponding contrast between a stemless plant, with the above-ground vegetative structures reduced to a simple rosette of leaves, and a close relative with elongate stem bearing caulinar leaves in addition to those of a comparatively thinner (if ever recognizable) basal rosette, does actually occur sometimes within one and the same species, as in the European composite *Carlina acaulis* (e.g., Meusel and Kästner 1990).

In animals exhibiting conspicuous directional asymmetry it may be sensible to enquire whether changes in chirality (likely, a developmentally very easy change) may contribute to speciation. Gittenberger (1988) suggested indeed that this may be the case in gastropods such as those of the genus *Partula*, within which both left- and right-handed coiling forms are known, even in sympatry. Gittenberger’s views were initially refuted by Johnson et al. (1990), but the question of possible sympatric speciation following the emergence, within a population of given chirality, of individuals with opposite handedness, has been recently re-opened. As demonstrated by a detailed study in *Euhadra* snails, things are complicated by the maternal inheritance of chirality (Davison et al. 2005) and the question is still far from settled.

Another example of release of extensive variation through mechanistically simple but phenotypically major changes is found in the number of segments in some myriapod clades, where the nearly complete invariance for this trait exhibited by some species-rich clades is contrasted by occasional conspicuous differences in segment number between putative sister species. The most remarkable example is provided by a species pair in the genus *Scolopendropsis*, which belongs to the scolopendromorph centipedes, a clade of ca. 700 species within which segment number was known until recently to be either 21 or 23. A leap in segment number separates however *S. bahiensis*, which includes individuals with 21 pairs of legs and others with 23, from *S. duplicata*, where segments are either 39 or 43 (Chagas et al. 2008). Another example is given by *Mecistocephalus microporus* (Bonato et al. 2001), a geophilomorph centipede with 93-101 pairs of legs, whose closest relatives have the quite lower, and fixed, number of 49 pairs of legs, same as many other species in the large genus *Mecistocephalus* (Bonato et al. 2003). In both instances we can hypothesize that speciation is accompanied by a nearly wholesale duplication of trunk segment number, a phenotypically major result of what is probably a minor change in terms of developmental control.

These conspicuous morphological differences between actual or putative sister species for a character of undoubted adaptive value, along with the recurrence of multiple independent occurrences of this pattern, beyond giving precise indications on the evolvability of the trait(s) (Minelli et al. 2009), suggest the possibility of their involvement in the corresponding speciation events.

Phenotypic Plasticity and Speciation

Many evolutionary biologists have advanced the opinion that phenotypic plasticity, the production from the same genotype of alternative phenotypes in response to different environmental conditions (Fusco and Minelli 2010), can act as a facilitator for evolutionary change in general, and speciation in particular (e.g., West-Eberhard 2003; Pfennig et al. 2010). Here we review the possible role of phenotypic plasticity in speciation in microallopatric or sympatric contexts and discuss how it can promote adaptive radiation by generating novel targets for evolutionary processes.

Speciation Through Resource Polyphenism

Following previous suggestions (e.g., Maynard Smith 1970; Felsenstein 1981; West-Eberhard 1989, 2003, 2005; Smith and Skúlason 1996; Skúlason et al. 1999; Mallet 2008) that phenotypic plasticity often opens the way to a critical, early stage in the speciation process, Pfennig and McGee (2010) have provided extensive evidence in favour of the hypothesis that speciation is sometimes facilitated by polyphenism, a form of phenotypic plasticity in which two or more distinct phenotypes (without intermediates) are elicited by alternative environmental cues (Fusco and Minelli 2010). In particular, Pfennig and McGee (2010) give support to the hypothesis that resource polyphenism, which they define as “the occurrence within a single population of environmentally triggered alternative phenotypes showing differential resource use”, may promote diversification by a) facilitating different stages of the speciation process, from spatial and/or temporal isolation to divergence to reproductive isolation, and b) by reducing the chance of extinction of the clade, because of the occupancy of more diverse habitats, that results in additional opportunities to diversify further. Through a comparative study based on sister-group comparison in amphibians and fishes, they show that the clades in which resource polyphenism has evolved are more species-rich and have broader geographical ranges, occupying more diverse habitats, than closely related clades lacking resource polyphenism.

Sympatric Speciation

The effects of a developmental approach to evolutionary change also have a bearing on a controversial kind of speciation, that is, sympatric speciation (e.g., Butlin et al. 2008; Fitzpatrick et al. 2008; Mallet et al. 2009).

The core idea that polyphenism can facilitate speciation was explicitly formulated by West-Eberhard (1986, 1989, 2003). This would occur in four steps. The first step is the fixation of alternative phenotypes in different environments, these phenotypes becoming less and less dependent on the exposure to specific environmental cues but increasingly controlled by a genetic setting divergent from the one going towards fixation in the alternative environment. Genetically controlled fixation of the phenotypes will then act itself as a condition favouring increasing divergence, which represents the second step in the process. A further step is an acceleration of reproductive isolation due to ongoing diverging specialization for different environments, promoting in turn pre- or postzygotic isolation. Eventually—this is the final step in the sequence envisaged by West-Eberhard—the two morphs become compatible in sympatry, because of the expected superiority in competition of a specialist derived from a polyphenic ancestor in comparison with an equivalent phenotype exhibited by a closely related polyphenic population. On the other hand, this competition might in turn drive the originally polyphenic population towards fixation of a contrasting phenotype, thus further increasing divergence in a zone of overlap (West-Eberhard 2003).

A botanical example is offered by the lesser spearwort *Ranunculus flammea* (Cook and Johnson 1968). This plant is heterophyllous, i.e., it produces different kinds of leaves, dependent on the conditions where these develop. Leaves developing in the air, above the water surface, are broad and lanceolate, whereas leaves developing in the water are linear. Both kinds of leaves are usually borne on the same individual plant, but there are also monomorphic populations of *R. flammea* living in wet meadows and in lakes, which only produce lanceolate or linear leaves, respectively. Specimens from monomorphic populations transplanted to habitats where the plant is heterophyllous show some capacity to produce the alternative leaf form, but poorly, and present evidently reduced viability. Unpredictable habitats would thus subject organisms to disruptive selection, consequently acting as generators of evolutionary novelties (Cook and Johnson 1968). This echoes Jablonski's (2005) observation, that higher taxa of post-Paleozoic benthic marine invertebrates tend to appear first in onshore, disturbed habitats, even in the case of groups represented today by deep-water forms only.

According to West-Eberhard (2003), many putative instances of sympatric speciation are possibly better explained as examples of speciation through developmental plasticity. This kind of speciation may also offer the opportunity for replicated, polytopic speciation (Schluter and McPhail 1993).

The occurrence of well-defined 'morphs' within one species, generally corresponding to alternative phenotypes confined to different habitats (e.g., Landry et al. 2007), is well known in many plant and animal species. Particularly intriguing is the occurrence of two or more morphs (up to four, e.g. the deep-, shallow-, river- and bay spawning morphs of the whitefish *Coregonus lavaretus* in Lake Femund, Norway; Østbye et al. 2005) in conditions of strict or approximate sympatry. There is a regular occurrence, both in eastern North America (Pigeon et al. 1997) and in Central Alpine lakes (Douglas et al. 2005) (see also Østbye et al. 2006), of morph pairs of *Coregonus* spp., where the two morphs have demonstrably differentiated in each basin independently of a parallel divergence in other basins. This opens the question whether these products of phenotypic plasticity may eventually represent a step towards speciation, in the case either morph becomes less and less dependent on environmental cues and increasingly fixed genetically.

In these events of rapid morphological divergence of sympatric morphs, a change in the developmental schedules does not simply translate into differences in size and shape of the adult fish. Besides genetically based differences in the time required by the embryo to develop till hatching (Rogers and Bernatchez 2006), differential gene expression is evident throughout the whole embryonic and early post-embryonic development. After hatching, differences in gene expression between sympatric morphs increase in a massive way. In *Coregonus clupeaformis*, genes displaying significant regulatory divergence in 16-week old juveniles were 14 times more numerous than in the embryos (Nolte et al. 2009). Interestingly, while the transcriptome of two sympatric morphs was nearly identical during embryonic development, all hybrids between them exhibited strongly divergent patterns of gene expression, suggesting extensive disruption of regulatory control. Altered expression apparently affected up to over 2,000 genes, including crucial developmental genes (Renaut and Bernatchez 2011).

Based on the evidence available to date, freshwater and brackish-water fishes seem to be particularly prone to these patterns of fixation of initially sympatric, morphologically distinct phenotypes. A phylogenetic analysis of mtDNA variation among seven sympatric pairs of dwarf and normal morphotypes of whitefish from basins in the southern Yukon and in the St. John River drainage, Canada, demonstrated reproductive isolation for all sympatric pairs (Bernatchez et al. 1996; Pigeon et al. 1997; Lu and Bernatchez 1999). Fixation of alternative phenotypes under diverging selective pressure has been shown also among the smelts (*Osmerus*) (e.g., Taylor and Bentzen 1993; Saint-Laurent et al. 2003) and the sticklebacks (*Gasterosteus*) (e.g., Bell 1974; Colosimo et al. 2005), but in all these genera this process may represent only a component of speciation, although likely an important one.

The idea that sympatric speciation is less improbable when phenotypic evolution is viewed as a result of the evolution of the underlying developmental system is also supported by computational studies. Through a simulation study, Porter and Johnson (2002) showed that when trait evolution is modeled based on regulatory developmental pathways, genetic incompatibilities leading to post-zygotic reproductive isolation arise as frequent by-products of adaptive evolution. This does not occur when phenotypes are modeled using the standard, additive genetic framework. Development processes therefore facilitate interactions among the genes (and their products) fostering speciation, thus contrasting the negative effect of gene flow on population divergence.

Novel Targets for Evolutionary Processes and Novel Trade-Offs

Plasticity can catalyze adaptive radiation by influencing both the likelihood of speciation and the patterns of diversity that are produced through it (Pfennig et al. 2010).

Elaborating on this idea, Moczek (2010) has convincingly argued that phenotypic plasticity has a far-reaching effect on the production of biological diversity, because it multiplies the available targets of evolutionary processes in natural populations. Basically, plasticity opens up new traits—primarily related to the way the phenotypic response is modulated as a consequence of the exposure to an environmental clue—for selection to act on. This fact can be visualized noting that once a character turns from non-plastic (flat reaction norm) to plastic (non-flat reaction norm), domain and shape of the reaction norm itself can become a target of selection (Rice 2004). Numerous studies have shown that plastic responses can evolve independently of other aspects of the phenotype. For instance, in *Onthophagus* scarab beetles there is a remarkable inter-specific disparity in the relationship between body size, that depends on nutrition, and horn size. Examples of divergence in the scaling relationship between body size and horn length in these beetles span from inter-specific, among relatively distant related species (divergence time ~20–40 Myr ago) to between sister species (divergence 10 Kyr ago), to intra-specific, between allopatric populations of *O. taurus* established less than 40 years ago from the same ancestral population. Thus populations can significantly diverge in these parameters within a relatively short time.

Moczek (2010) has also argued that plasticity evolution can elicit correlated changes in traits other that those directly involved in the plastic response, thus becoming a potential source of trade-offs both in development and evolution. In their most simple form, trade-offs during development arise when the morphogenetic processes of two or more structures compete for the same pool of energy or material resources to support their growth (Nijhout 1998). Such trade-offs are particularly important in closed systems, like embryos of non-viviparous animals, or in the pupa of holometabolous insects, where no supplementary energy or material intake is allowed. Resource allocation trade-offs are not exclusive of plastic traits, however few recent works have suggested that plasticity can represent an additional source of trade-offs. For instance, comparative studies among different *Ontophagus* species and between different populations of *O. taurus* have shown that evolutionary changes in the threshold body size that separates horned from hornless male morphs are associated to the expression of other traits, noteworthy the size of the male copulatory organ, which is smaller in species and populations with larger horns (Parzer and Moczek 2008). A trade-off that involves the size of the copulatory organ is of particular significance for these insects, as evolutionary changes in male genitalia are thought to play a major role in reproductive isolation in arthropods (Eberhard 1985; Eberhard et al. 1998). In this case, speciation would represent a by-product of plasticity-induced trade-offs.

The extraordinary diversity and species richness exhibited by the horned scarab beetles of the genus *Onthophagus* (described to date in the order of more than two thousand taxa) testify the potentials of a synergic interaction between powerful generative forces of variations (in this case, through developmental plasticity) with equally powerful diverging selective forces (e.g., sexual selection) in producing adaptive radiation.

Developmental Timing and Speciation

In the previous sections, to document the possible role of development in speciation, we have focused on morphological characters, as the most obvious outcome of developmental processes. In this section our attention will shift instead towards some temporal aspects of a species' biology possibly contributing to reproductive isolation, suggesting that phenology, in addition to morphology, can be profitably studied from the perspective of evolutionary developmental biology.

We discuss here two aspects of developmental timing which are treated separately for ease of description, but are indeed interrelated. The first aspect brings under focus the temporal dimension of the phenotype, or organism phenology; the second considers the possible consequences of heterochrony on the onset of reproductive barriers where juvenile and even embryonic phenotypes are in some way involved.

Phenological Isolation

Seasonal differences between conspecific populations in the time of maturity or actual reproductive activity are an obvious component of many traditional analyses of incipient speciation and eventual reinforcement of isolation between sympatric populations in more advanced stages of divergence. However, the fact that these differences in phenology are one of the products of differences in developmental schedules is generally overlooked. Current evo-devo research focuses in fact on the role of developmental evolution in the production of novel forms, but time is ripe to broaden this perspective to the temporal dimension of the phenotype. In the following paragraphs we provide a few examples of the value of this evo-devo perspective in illustrating the role of phenological differences in facilitating speciation or reinforcing divergence.

Alexander and Bigelow (1960) introduced the term 'allochronic speciation' to single out those cases of sympatric speciation where the isolation between two divergent populations is based on temporal differences in their life cycles. Putative examples of allochronic speciation would be frequent in North American crickets, with a number of species exhibiting geographic variation in traits that would easily lead to temporal isolation of conspecific populations.

For example, *Nemobius carolinus*, *Miogryllus verticalis* and *Teleogryllus commodus* include populations with egg diapause and populations without egg diapause, whereas *Nemobius fasciatus*, *Oecanthus niveus* and *Oe. quadripunctatus* can have either one or two (perhaps also three) generations per year (Alexander 1968). These differences could easily have consequences on the timing of a population's reproductive season, and should thus foster speciation.

Tauber et al. (1986) expressed doubts as to the actual relevance of differences in seasonal cycles in establishing a coexistence of sympatric distinct morphs, but stressed their importance in the evolution of premating isolation between two morphs already differentiated in sympatry, possibly originally due to host shift or habitat diversification rather than differences in phenology. The same authors contributed new clear examples from their own studies on the green lacewings (*Chrysopa* species). For example, two species occurring in sympatry in northwestern United States, *C. carnea* and *C. downesi*, differ in both quantitative and qualitative aspects of their seasonal cycles. *C. carnea* is multivoltine, while *C. downesi* is univoltine and its reproductive season does not overlap with the times when *C. carnea* is sexually active. These differences in the seasonal cycles between these two species have been found to depend on allelic substitutions at two autosomal loci (Tauber et al. 1977).

Morphological differences associated to flowering times are common in plants, but this association becomes especially relevant to our topic only when seasonally distinct, fixed morphs emerge, possibly in syntopy, within one species. Many examples of seasonal dimorphism or polymorphism have been described in the past, beginning with von Wettstein (1895). This phenomenon is widespread for example in *Gentianella* (Gentianaceae) (Prtichard and Tutin 1972; Lennartsson 1997), in *Solidago* (Asteraceae) (e.g., Pors and Werner 1989) and in several genera of the Scrophulariaceae such as *Odontites*, *Euphrasia*, *Melampyrum* and *Rhinanthus* (e.g., de Soó and Webb 1972). In several instances, especially in the last mentioned genus, it has been long believed that haymaking, in high altitude localities where this agricultural practice is operated only once or twice per year, may have substantially helped in forcing a phenological discontinuity between early- and late-flowering plants, thus reducing the potential gene flow between them, with the consequent emergence of largely fixed phenotypes which have been even regarded as distinct species by several authors. More recent literature has to some extent downgraded the relevance of this phenomenon (cf. Briggs and Walters 1997) but modern, adequate studies are not available and the botanist is broadly advised to follow the cautious attitude of *Flora Europaea* authors (e.g., Prtichard and Tutin 1972; de Soó and Webb 1972) who provided keys and descriptions for all the best characterized seasonal phenotypes in these genera without attempting to assess their taxonomic status. One of the most recent studies in this area (Lennartsson 1997, on *Gentianella*) suggests anyway that within one and the same 'species' there can be major regional differences: in some areas a single, long-flowering and morphologically variable form is present, while in other areas there are two phenologically distinct and morphologically distinguishable sympatric forms. The latter condition may eventually represent an incipient stage of speciation. What matters in the context of our analysis is the fact that these alternative phenotypes are produced by a different developmental regulation of flowering time. It would be worthwhile to investigate in these plants the function and the possible polymorphism in genes such as CONSTANS and many others, known to control the transition from vegetative to flowering conditions in plants.

An animal species including phenologically distinct populations with broadly parapatric distribution is the pine bark bug *Aradus cinnamomeus* (Heliövaara et al. 1988). As a rule, this insect takes 2 years to develop into adult and distinct populations are known to mature synchronously (with rare exceptions) either in the odd or in the even years. For example, in western Finland, the bug reproduces mainly in odd years, while in eastern Finland most individuals reproduce in even years. Odd-year bugs are very rare in the even-year area, as are even-year bugs in the odd-year area. The species was known for a while to include two nearly parapatric populations reaching maturity in alternate years, and thus being virtually reproductively isolated, but the whole picture is more complex. In the Åland archipelago, off the eastern coast of Sweden, as well as in the northern Scandinavian provinces close to the Arctic Circle, there are populations of *A. cinnamomeus* with a life cycle spanning over 3 years. Heliövaara and Väisänen (1987) advanced the reasonable hypothesis that these populations may mediate gene transfer between the even- and the odd-year biannual populations. All three temporal cohorts are equally abundant in the areas where development takes 3 years. Here, sympatric but allochronic bugs differ genetically from those of the other two cohorts to about the same extent as do allopatric but synchronic populations (Heliövaara et al. 1988).

The most popular example of speciation involving a major change in developmental timing is offered by the North American cicadas of the genus *Magicicada*, whose short adult season is reached after an embryonic and post-embryonic development lasting either 13 (e.g., *M. tredecim*, *M. tredecassini*, *M. tredecula*) or 17 years (e.g., *M. septemdecim*, *M. cassini*, *M. septemdecula*). The closest relative of a 17-year cicada is not to be found among the other 17-year species, and the same is true for the 13-year cicadas. There are instead pairs of parapatric species with different developmental length, such as the 17-year *M. septemdecim* and the 13-year *M. tredecim* (Marshall and Cooley 2000). Developmental plasticity and speciation in these cicadas have been discussed in great detail by West-Eberhard (2003).

Optimization of life cycle length on a currently accepted phylogeny of *Magicicada* species suggests a 13-year cycle as the primitive condition in this clade, with three independent transitions to a 17-year cycle. The plausibility of this hypothesis is strengthened by occasional records of 13-year cicadas emerging 4 years later than expected (Marshall et al. 2011). Some degree of developmental plasticity is also present in the 17-year cicadas, which sometimes emerge from 6 years early to 5 years late. The environmental trigger of these deviations from the usual schedule is uncertain, although high population densities during nymphal life (Martin and Simon 1990b), peculiar climatic conditions and local fires have been suggested (Marshall et al. 2011). Fixation of otherwise environmentally inducible alternative lengths of development (Martin and Simon 1990a, b; Heliövaara et al. 1994; Williams and Simon 1995) is thus likely at the base of speciation in these cicadas (Martin and Simon 1988).

Very recently, a case of prezygotic allochronic isolation has been described also in yeasts. Sympatric woodland populations of *Saccharomyces cerevisiae* and *S. paradoxus* exhibit a significant differences in mating and germination timing that are responsible for the observed mate discrimination (Murphy and Zeyl 2012).

Divergence at Immature Stages

Reproductive isolation can emerge from phenotypic variation manifested by incipient species at any stage of development, rather than being restricted to structural traits of the adult.

Extensively studied is a pair of sea urchin species, whose very close affinity is not simply revealed by the very similar adult morphology, but also by the possibility to obtain hybrid embryos (Nielsen et al. 2000). Differences between *Heliocidaris tuberculata* and *H. erythrogramma* are, however, dramatic at the egg and larval stage. The eggs of the former are small and poor in yolk, and develop through conventional blastula (coeloblastula) and gastrula stages into a typical, plankton-feeding pluteus larva; the eggs of the other species are instead very large and rich in yolk, so much as to affect whole course of embryonic development, with a yolk-filled sterroblastula eventually transforming into a yolk-feeding massive larva that bears no obvious resemblance to a pluteus. These morphological differences are paralleled by differences in gene expression, mainly of heterochronic nature (Parks et al. 1988; Raff 1992).

Interestingly, a recent study (Tills et al. 2011) suggests that the evolvability of heterochrony can be investigated at population level. Detailed comparisons of the developmental schedules of many individuals within a population of the freshwater snail *Radix balthica* have produced indeed some evidence that populations can be polymorphic for the temporal sequence of developmental events during embryonic life. These results, which deserve further effort and especially extension to a range of species spanning across different taxa, habitats and kinds of development, may provide a link between intraspecific variation and interspecific differences in developmental change.

A peculiar and to some extent extreme example of heterochrony is exhibited by paedomorphic forms when compared to their close relatives with non-abridged development. Developmental changes translating into paedomorphosis have been sometimes advocated as the cause of speciation. A botanical example has been discussed by Box et al. (2008) in orchids; zoological examples are apparently offered by several genera of salamander or newts, which exhibit facultative metamorphosis/paedomorphosis associated with assortative mating, a behaviour likely to favour divergence and eventually speciation (Semlitch and Wilbur 1989; Scott 1993). However, cross-breeding experiments indicate that the normal and paedomorphic morphs interbreed successfully (Denoël et al. 2001, 2005), thus sexual compatibility sustains significant gene flow between them (Krenz and Verrell 2002). As a consequence, this developmental polymorphism would unlikely lead to future speciation events, unless sexual isolation occurs. But this is exactly the case when paedomorphs breed significantly earlier than metamorphs, as recorded in some salamander taxa, thus leading to assortative mating (Denoël et al. 2005).

Concluding Remarks

Far from pretending to offer a complete, systematic treatment of the role of developmental processes in speciation, the collection of case studies we have presented and discussed in this paper provides, in our view, preliminary but convincing support to the thesis that there is a great value in taking development into the picture of speciation processes. This will require, however, a substantial widening of focus in respect to the current trends. Despite the growing mass of studies on the developmental genetic basis of traits responsible for the onset and/or the consolidation of reproductive isolation, the field is still largely dominated by studies of hybrid sterility and inviability (Naisbit et al. 2003).

Future experimental investigations and the accompanying theoretical work will be necessary to address at least some basic questions that nowadays remain open. Are there developmental characters with a special role in the formation of reproductive barriers? How do developmental systems and evolutionary forces interact in originating and/or fixing phenotypic divergence that qualify as a speciation process?

Last but not least, we believe that temporal phenotypes, briefly considered in this review because of the relevance of phenological isolation in speciation events, deserve to be taken on board by evolutionary developmental biology, as a legitimate companion to the morphological phenotypes on whose developmental origin and evolution evo-devo has been pretty universally restricted.

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