Future directions in the ontogeny of plant defence: understanding the evolutionary causes and consequences

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Abstract

Plant defence often varies by orders of magnitude as plants develop from the seedling to juvenile to mature and senescent stages. Ontogenetic trajectories can involve switches among defence traits, leading to complex shifting phenotypes across plant lifetimes. While considerable research has characterised ontogenetic trajectories for now hundreds of plant species, we still lack a clear understanding of the molecular, ecological and evolutionary factors driving these patterns. In this study, we identify several non-mutually exclusive factors that may have led to the evolution of ontogenetic trajectories in plant defence, including developmental constraints, resource allocation costs, multi-functionality of defence traits, and herbivore selection pressure. Evidence from recent physiological studies is highlighted to shed light on the underlying molecular mechanisms involved in the regulation and activation of these developmental changes. Overall, our goal is to promote new research avenues that would provide evidence for the factors that have promoted the evolution of this complex lifetime phenotype. Future research focusing on the questions and approaches identified here will advance the field and shed light on why defence traits shift so dramatically across plant ontogeny, a widespread but poorly understood ecological pattern.

INTRODUCTION

Plant–herbivore interactions vary over space and time, and extensive research has led to the development of theories aimed at explaining this variation (Stamp 2003). During the past two decades, plant ontogeny has been identified as a key source of variation, and hundreds of species have been reported with significant changes in the levels and even kinds of defence traits as plants develop from the seedling to juvenile to mature and senescent stages. Ontogenetic changes occur in all types of plant defences, including constitutive and induced secondary chemistry, physical traits such as spinescence and leaf toughness, indirect defences involving food rewards and domatia for ants as well as VOC emissions, and tolerance quantified as the maintenance of fitness in damaged compared to undamaged plants. Even escape from herbivores can be variable as plants develop, through intrinsic changes in plant size that make them more conspicuous as they get larger, or through changes in the chemical profiles to avoid being detected by their specialist herbivores (Langenheim & Stubblebine 1983). All these developmental changes, defined as ontogenetic trajectories in plant defence (Box 1), are widespread and have been well described in previous reviews (Boege & Marquis 2005; Barton & Koricheva 2010; Massad 2013; Quintero *et al.* 2013). The key questions now are why plant defence has evolved to vary across ontogeny, what genetic and environmental factors regulate and/or promote these developmental changes, and how natural selection acts on this complex phenotype. Rather than update previous reviews on the patterns of ontogenetic trajectories, the goal of this study was to discuss ideas for why ontogenetic trajectories in plant defence have evolved in most plant species, highlighting insights from recent studies on the physiological and molecular mechanisms underpinning developmental changes in defence, and to identify promising areas for future research. By identifying the ecological and developmental drivers for ontogenetic trajectories in plant defence, we will gain new insights into why these trajectories vary among plant life forms, among types of defence traits, and among biogeographical regions (Boege & Marquis 2005; Barton & Koricheva 2010).

For our purposes here, we focus on whole-plant ontogeny, defined as the development of plants through stages (from seedling to juvenile/sapling, mature and finally senescent stages, Box 1). Development of plant organs has also been referred to as ontogeny, such as 'leaf ontogeny' or 'fruit ontogeny'. Organ ontogeny can also strongly influence defence traits and herbivore damage, however, in contrast with the whole-plant ontogenetic variation, it has received considerable research focus (Coley 1980; Kursar & Coley 1992, 2003; Koricheva & Barton 2012). Moreover, leaf ontogeny and whole-plant ontogeny may differ markedly in their trajectories of plant defence. Whereas a consistent decrease in secondary chemicals have been found during leaf ontogeny (Koricheva & Barton 2012), much more variable and contrasting ontogenetic trajectories have been reported among species for whole-

Box 1

Ontogeny refers to the development of an organism through a series of discrete stages. For plants, ontogenetic stages include seeds, seedlings, juveniles, mature and senescent individuals. Transition between stages can be difficult to identify, particularly when obvious morphological trait changes do not co-occur. For example seedlings are defined in the narrow sense as plants still dependent on seed and maternal reserves (Hanley *et al.* 2004). However, in practice, any small plant is often called a seedling even when those plants are several years old and would more appropriately be called a juvenile plant or sapling. Similarly, transition from the juvenile to mature stages can be most obviously identified when the onset of reproduction coincides. However, phase change from the juvenile to vegetative mature stage precedes reproduction in some plants (Poethig 1990), making it tricky to identify mature-stage plants with confidence. For heteroblastic species undergoing dramatic developmental changes in leaf morphology (Zotz *et al.* 211), pinpointing phase change and identifying ontogenetic stages can be more clear.

Ontogeny is not synonymous with age or size. While it can be convenient to measure traits or characterise the strength of interactions over time or with respect to increases in size, this is not the same thing as characterising ontogenetic trajectories. This is because phase change between ontogenetic stages involves numerous changes in gene expression, many of which are difficult to detect (Huijser & Schmid 2011; Meldau *et al.* 2012). Nonetheless, size and age may contribute to changes in defence within and across ontogenetic stages because of associated shifts in resource acquisition and metabolic rates, and so accounting for size or age may enhance precision of statistical models describing ontogenetic patterns in defence.

Ontogeny occurs at very different time scales across plant life histories. For annuals living within very short grow-seasons, ontogeny may occur within a few weeks. In contrast, for long-lived woody plants, the juvenile stage may itself last for decades, followed by centuries of the mature stage. Clearly, for experimental studies, ontogeny is much more tractable for annual and short-lived perennials, and previous research on the ontogeny of defence has indeed focused on these species (Barton & Koricheva 2010). However, given the likely greater magnitude of change associated with woody plant ontogeny, it will be important for future research to fill this knowledge gap.

plant ontogeny (Boege & Marquis 2005; Barton & Koricheva 2010), suggesting different selective pressures and underlying regulatory pathways for organ vs. whole-plant ontogeny (Bowers & Stamp 1993; Diezel *et al.* 2011; Maag *et al.* 2015; Brütting *et al.* 2016). The great variation in the ontogenetic trajectories at the whole-plant level provides an excellent opportunity to assess the causes and consequences of the evolution of changing phenotypes across plant development, which has been scarcely explored.

DRIVERS OF ONTOGENETIC CHANGES IN PLANT DEFENCE

The null model to explain whole-plant ontogenetic trajectories of defence is that these arise not because they are adaptive for plants, but rather as a consequence of changes in plant size during development. Such selectively neutral ontogenetic trajectories have been called 'ontogenetic drift' (Evans 1972; Peng *et al.* 2010), and are not expected to enhance fitness for plants exposed to herbivory or other potentially interacting stressors (such as nutrient limitation or drought). Developmental constraints may lead to ontogenetic trajectories that correspond to ontogenetic drift, such as the sudden increase in domatia produced by plants once tissues are large enough to form these structures (Brouat & McKey 2001). In contrast to ontogenetic drift is the idea that ontogenetic trajectories have evolved in response to natural selection pressures. Identifying the agents of selection and unravelling the underlying regulatory mechanisms driving ontogenetic trajectories in plant defence are needed to move this field forward. Several intrinsic and extrinsic factors may select for ontogenetic variation in plant defence, including resource allocation trade-offs, changes in herbivore selection pressure as plants grow, and

the multi-functionality of defence traits in ecophysiological processes. Some of these factors can lead to highly fixed ontogenetic trajectories, whereas other mechanisms are more flexible, may change gradually, and are expected to be variable among species. Ontogenetic patterns in constitutive defence may thus range from canalised to more plastic trajectories, although most studies have not differentiated between these two alternatives. Identifying if ontogenetic trajectories are fixed or plastic is important and can elucidate whether the underlying mechanisms are clearly linked to developmental constraints, and to what degree the dynamic effects of environmental factors and internal resource budgets influence them.

Considering the expression of multiple defences across ontogeny is important to understand lifetime plant defensive strategies, given that synergies or tradeoffs between defensive mechanisms can occur (Agrawal 2011). For example *Plantago lanceolata* plants express simultaneous ontogenetic trajectories of different plant traits that contribute to an overall decrease in leaf quality as food for herbivores: whereas leaves become tougher and have greater concentration of iridioid glycosides as plants age, they also decrease nitrogen and water content (Quintero *et al.* 2014). In other cases, contrasting ontogenetic trajectories have been reported: in *Raphanus sativus*, there is an ontogenetic shift from resistance through glucosinolates early during plant development to tolerance mechanisms in older ontogenetic stages (Boege *et al.* 2007). However, the reverse has been found in *Argemone glauca* which undergoes an ontogenetic shift from high tolerance in young stages to high resistance via prickles and latex in older stages (Barton 2014). Ontogenetic shifts have also been detected between different secondary metabolites: in *Eucalyptus froggattii*, the production of terpenoids increases as plants develop, while the

phenolic compounds decrease (Goodger *et al.* 2013). Hence, the concept of defence syndromes (Agrawal & Fishbein 2006) could be extended to incorporate the simultaneous ontogenetic changes in different defence traits.

Assessing the evolutionary factors driving ontogenetic trajectories in plant defence will provide greater clarity into why ontogenetic trajectories vary among species, biomes and defence traits (Boege & Marquis 2005; Barton & Koricheva 2010). Next, we discuss the evidence for these factors, highlighting fruitful areas for future research that link molecular approaches characterising and manipulating the underlying regulatory pathways with field studies that examine the fitness consequences of ontogenetic trajectories in defence. These new research directions are expected to advance the field from basic descriptions of ontogenetic patterns towards a better understanding of why ontogenetic trajectories in plant defence occur and how they are developmentally regulated.

Developmental constraints

The expression of defence traits may be limited in early ontogenetic stages because key features or pathways are not yet developed in plants. There are several examples for developmental delays in plant traits that attract insects involved in indirect defence. In some cases, developmental constraints can be related to plant size (and thus related to ontogenetic drift), as is the case of seedlings of myrmecophytic species lacking the architectural requirements for producing enough domatia to attract their ant defenders (Brouat & McKey 2000; Trager & Bruna 2006). In other cases, it is not clear what drives the delay in indirect defence, as is found in *Turnera velutina*, which do not secrete nectar from extrafloral nectaries until the exit pore develops in the pre-reproductive stage (Villamil *et al.* 2013). There are also examples of developmental constraints associated with the production of enzymes involved in the biosynthetic pathway of condensed tannins early during plant ontogeny (Murray & Hackett 1991; Fernandez-Lorenzo *et al.* 1999). However, the mechanisms by which enzyme production are regulated have not been fully assessed yet. Developmental constraints may also influence resistance when specialised anatomy is required. For example *Umbellularia californica* seedlings are unable to store terpenes because specialised idioblastic cells are developed only in older stages (Goralka & Langenheim 1996; Goralka *et al.* 1996). In all these cases, however, what remains unclear is if plants are not able to produce domatia or idioblastic cells because they lack the necessary support structures and cell differentiation patterns, or because factors have selected for their absence in early ontogenetic stages. Interesting questions to address in the future are whether the timing of ontogenetic changes varies among species, whether the ontogenetic changes occur when plants surpass size or age thresholds, and what regulatory mechanisms underlie these phase changes.

Some of the developmental constraints described above are consistent with predictions for ontogenetic drift. For example ontogenetic increases in domatia or idioblastic cells that reflect anatomical constraints in smaller plants are not necessarily the result of selection pressure by herbivores or abiotic stressors, but simply the consequence of changes in plant size. Hence, even when it may not be advantageous for plants to delay indirect defences until after the seedling stage, ontogenetic trajectories increasing these defences can become fixed due to ontogenetic drift. In this case, we might predict that plants should express ontogenetic switches from chemical or physical resistance in seedlings to indirect defences after the developmental constraint has been outgrown. Whereas some reports indicate that young myrmecophytic species are less palatable than older stages that support ant defenders (Nomura *et al.* 2001; Llandres *et al.* 2010), others describe increases in both indirect defence and chemical resistance across ontogeny (Del Val & Dirzo 2003).

Unlike developmental constraints, other mechanisms are likely to promote the evolution of ontogenetic trajectories of defence in response to different selection pressures. In particular, allocation costs, temporal patterns of herbivore selection pressure, multi-functionality of traits, and shifting environmental conditions as plants grow and develop can promote the evolution of the regulatory pathways behind the expression of ontogenetic trajectories of defensive traits.

Allocation costs

Ontogenetic trajectories in defence can be partially explained by an extension of the growth-differentiation balance hypothesis, incorporating the idea that allocation costs vary during plant development (Herms & Mattson 1992). On one hand, resource availability changes as plants grow due to increases in resource acquisition capacities both below- and above-ground. On the other hand, resource allocation priorities also shift as plants develop, particularly during seedling establishment and reproductive maturity. As a consequence of these shifting source-sink dynamics, plants may alter their investment to defence across ontogeny to minimise allocation costs (Boege & Marquis 2005). For example seedlings are typically resource limited due to their small photosynthetic area and shallow roots. Moreover, growth has a high priority during seedling establishment as a consequence of competition for space and light. Therefore, seedlings are predicted to prioritise resource allocation to growth to increase their resource acquisition capacity and outcompete neighbours, at the expense of resource allocation to defences such as secondary metabolites (Boege & Marquis 2005). Thus, while maternally inherited secondary compounds may persist for some time (Chacon *et al.* 2013; Ochoa-Lopez *et al.* 2015), as soon as cotyledon resources are depleted, seedlings are predicted to forgo production of secondary metabolites until enough photosynthetic tissue is produced to reach a balance between below- and above-ground biomass (Bryant *et al.* 1991). Allocation costs are then predicted to peak again later in ontogeny when reproduction has a higher resource priority than defence, particularly in plants that only reproduce once (Boege & Marquis 2005).

Patterns have been found to be quite variable among species and life forms, but some generalities can be drawn, for example in that constitutive secondary metabolites generally increase across ontogeny in both herbs and woody plants (Barton & Koricheva 2010). In addition, growth-defence trade-offs have been detected to be stronger early in ontogeny for some

species (Orians *et al.* 2010), but not for others (Boege *et al.* 2007). In the case of induced chemical defences, herbs show a decline in their responsiveness to herbivore damage as they develop from juvenile to mature individuals (Barton & Koricheva 2010). Hormonal regulation by cytokinins, ethylene and jasmonates has been implicated in this ontogenetic decline in the inducibility of herbs (Diezel *et al.* 2011; Brütting *et al.* 2016). Because induced defences have been proposed as a cost-savings mechanism (Cipollini & Heil 2010), an ontogenetic shift from induced to constitutive chemical defences could reflect a greater importance of allocation costs early in onto-geny, when plants have the priority to establish and grow to outcompete their neighbouring plants. This shift would ensure that seedlings avoid allocation costs of defence until needed (induced by herbivore damage) whereas older plants, having larger resource acquisition tissues, would have sufficient resources to invest in both growth and constitutive defences.

Although growth-defence trade-offs are likely to be ubiqui-tous (Stamp 2003), in many cases, these costs are not detect-able (Bergelson & Purrington 1996; Koricheva 2002), even in young, resource-limited ontogenetic stages (Barton 2007, 2008). Compelling new insights from *Arabidopsis* reveal that growth-defence trade-offs associated with induced responses can be uncoupled when jasmonate suppression of the photore-ceptor phytochrome B is released (Campos *et al.* 2016). Uncoupling growth-defence trade-offs provides evidence that allocation costs are not an inherent consequence of plant growth and resource limitation, as predicted by ontogenetic drift. Instead, allocation costs, when they occur, are most likely driven by natural selection to maximise plant fitness depending on the adaptive value of plant traits and the likeli-hood of attack, as predicted by optimal defence theory (McKey 1979). Further research with genetically modified model systems released from growth-defence trade-offs across plant ontogeny, and exposed to field conditions with variable intensity of herbivore damage and resource availability could provide key insights into the role of allocation costs in the evolution of ontogenetic trajectories of defence.

Temporal variation in herbivore pressure

Herbivore abundance and identity are highly variable over time, and are likely to change the type and intensities of dam-age experienced by plants across ontogeny (Poelman *et al.* 2010; Barton *et al.* 2015). In turn, concomitant developmental changes in plant size and storage can influence how herbivory affects plant fitness (Kitajima & Augspurger 1989; Myers & Kitajima 2007). Together, these temporal shifts in herbivory and their fitness effects should lead to ontogenetic changes in herbivore selection pressure for plant defences. Although no formal studies have assessed changes in selection pressure by herbivores across plant ontogeny, there are several correlative studies supporting a role of herbivores in the evolution of ontogenetic trajectories in plant defence. In boreal systems, for example it has been found that the concentration of plant secondary metabolites decreases as plants develop and out-grow the abundant ground-dwelling mammals that presum-ably have selected for defence exclusively in early ontogenetic stages (Swihart & Bryant 2001). Similarly, myrmecophytic

plants often have greater reward production in extrafloral nec-taries of leaves close to reproductive structures, suggesting a more intense selective pressure of herbivores during the repro-ductive stage (Ochoa-Lopez *et al.* 2015), although this remains to be formally tested. Overall, there is a lack of data on stage-specific herbivore selection pressures, and this represents a fair challenge for our understanding of the evolution of defence ontogenetic trajectories.

Multi-functionality of plant defence traits

Plant defence includes chemical, anatomical and morphologi-cal traits that deter herbivores, but these same traits also func-tion in other interactions and processes in plants. For example plant secondary metabolites are diverse and play many roles both internally with plant biochemistry, storage and regulation, and also externally with decomposition, defence and attraction (Hadacek 2002; Holopainen & Ger-shenzon 2010; Iason *et al.* 2012). Leaf trichomes are impor-tant barriers to herbivores and help moderate water and light stress through light reflection and changes in the leaf bound-ary layer (Jordan *et al.* 2005; Benz & Martin 2006; Liakopou-los *et al.* 2006; Agrawal *et al.* 2009). Spinescence is generally thought to function exclusively as defence, but thorns have been suggested to assist with heat dissipation and photo-pro-tection in desert plants (Nobel 1988), and prickles are induced by high light in prickly poppies (Barton 2014), perhaps to function in reflectance or water balance similar to that of the anatomically similar trichomes. Leaf toughness is well known to function both in defence against herbivores and in abiotic stress tolerance (Turner 1994; Hanley *et al.* 2007; Kitajima *et al.* 2013). In tropical forests, leaf toughness might be the most important trait limiting insect herbivory in tree canopies, leading insects to target developing leaves despite their higher levels of secondary metabolites (Coley 1980; Kursar & Coley 2003; Koricheva & Barton 2012). Nevertheless, leaf toughness is also one of the primary traits defining the leaf economic spectrum, differentiating species along a continuum of leaf carbon budgeting and contributing strongly to seedling sur-vival (Wright *et al.* 2004; Alvarez-Clare & Kitajima 2007).

Ascribing functionality to plant traits as either defensive or ecophysiological can be subjective and overlooks the impor-tance that multi-functionality has for their evolution. More-over, if trait functions shift across ontogeny, it may be difficult to detect clear relationships between trait expression and the strength of an interaction. For example when there is no relationship between plant secondary metabolite concentra-tions and herbivore preference or performance, this is typi-cally interpreted as evidence against their defensive value (Carmona *et al.* 2011; Agrawal & Weber 2015; Smilanich *et al.* 2016). However, most studies have assessed this relation-ship at a single ontogenetic stage, disregarding the possibility that the effectiveness of these as anti-herbivore defences can be relevant at other ontogenetic stages, while at the studied stage can have other functions. To determine the role of trait multi-functionality in the evolution of ontogenetic trajectories in defence, we must incorporate experiments that manipulate both herbivory and abiotic conditions. The use of knockouts in model species with modified trait expression at specific

ontogenetic stages might be particularly useful in such experiments. For example trichomes may function in both mediating light stress and reducing herbivory in juvenile and mature stages, but impose a negative effect on plant fitness by reducing light absorption in seedlings that are shaded by ground vegetation. Identifying the importance of light stress tolerance vs. anti-herbivore defences at each ontogenetic stage could be tested experimentally by subjecting plants with trichome expression enhanced or suppressed at the seedling, juvenile and mature stages subjected to light stress and herbivory.

NATURAL SELECTION OF ONTOGENETIC TRAJECTORIES IN PLANT DEFENCE

If variation in anti-herbivore defences across plant development represents an adaptive solution to deal with the factors described above, we can view ontogenetic trajectories in these traits as a complex phenotype targeted by natural selection under particular environments (Atchley 1987). Visualising an ontogenetic trajectory as the slope defining the relationship between plant defence and developmental age or stage, we can ask if the degree of ontogenetic change in plant defence, (i.e. the slope), has genetic variation and is heritable, and further identify the selective agents favouring genotypes with particular ontogenetic trajectories. When ontogenetic trajectories are not linear (Ochoa-Lopez *et al.* 2015), however, finding a parameter to synthesise the ontogenetic trajectories can represent a greater challenge. Although there are some studies reporting genetic variation in the degree of ontogenetic change in plant defence (Bowers & Stamp 1993; Schappert & Shore 2000; Goodger *et al.* 2004; Barton 2007), we lack empirical evidence of natural selection acting on particular ontogenetic trajectories to confirm if indeed they can be considered as an adaptive complex phenotype. Assessing natural selection on such phenotypes could advance our understanding on the evolution of lifetime plant defensive strategies. This would require experiments including plants from genetic families with contrasting ontogenetic trajectories growing under natural conditions, and the assessment of whether particular trajectories provide greater plant fitness (although confounding factors related to multi-functionality of traits or local adaptations would be difficult to rule out). Alternatively, a molecular genetics approach, involving the manipulation of the ontogenetic patterns of plant defence, combined with field experiments with wild type and modified lines, could be revealing as well. For example because cytokinin levels highly correlate with the expression of induced defences in *Nicotiana attenuata*, developmental patterns of secondary metabolites can be altered through the manipulation of cytokinin biosynthesis genes (Brütting *et al.* 2016). To infer how natural selection acts on this regulatory pathway and the resulting ontogenetic trajectories in plant defence, it would be useful to assess plant fitness of wild and modified lines exposed to herbivores. The limitations of this approach are the reduced number of model systems that can be used in experiments under field conditions, and the likely pleiotropic effects of modifying the expression of a major regulatory hormone. Hence, a challenge is to export the molecular tools used in model systems to a greater variety of species that could be studied under natural conditions.

REGULATORY MECHANISMS UNDERLYING ONTOGENETIC TRAJECTORIES IN PLANT DEFENCE

Although empirical evidence suggests that most plants are likely to express changes in their defences across ontogeny, we still have limited information on the regulatory mechanisms that trigger such changes, particularly when plants switch from one defensive strategy to another throughout ontogeny (Goodger *et al.* 2013; Barton 2014; Ochoa-Lopez *et al.* 2015). If ontogenetic trajectories in plant defence are indeed adaptive, the regulatory mechanisms behind their expression are likely to be important targets of selection. Progress has been made describing the genes involved in phase change and heteroblasty (Zotz *et al.* 2011). For example in *Arabidopsis thaliana*, microRNAs miR157 and miR172 play regulatory roles in the heteroblastic changes observed in leaves from juvenile and reproductive stages, including not only changes in leaf size and shape but also in trichome density (Huijser & Schmid 2011). In addition, modern molecular tools have allowed a greater understanding of the regulatory mechanisms involved in the induction of defences across plant ontogeny. For example we have recently learned that growth-defence tradeoffs can be prevented through the regulation of a jasmonate linked transcriptional network involving epistatic interactions between *jazQ* and *phyB* that attenuate growth upon the activation of induced defences with jasmonic acid (Campos *et al.* 2016). Thus, while whole-plant ontogenetic trajectories in defence traits have not been the focus of most of this molecular work, it offers a promising set of candidate genes to be tested. We also know that genes associated with phytohormones are important regulators of most developmental processes, including changes in plant defence. For example in the case of *Nicotiana attenuanta*, both cytokinins (Brütting *et al.* 2016) and ethylene (Diezel *et al.* 2011) have been found to regulate the expression of induced defences throughout plant development. Ethylene expression decreases with plant age, with a marked decline at the onset of reproduction associated with a significant reduction in the ability of plants to induce defences (Diezel *et al.* 2011). As future avenues of research, it would be important to define how phytohormones and epistatic interactions among genes promote ontogenetic shifts between different defensive strategies (Boege *et al.* 2007; Barton 2014).

The study of genome-wide transcriptomics has been revealing for our understanding of the regulatory processes of phenotypic plasticity in general (Whitman & Agrawal 2009) and particularly of the inducibility in plant defence (Zheng & Dicke 2008). For example microarrays have allowed the identification of more than 1000 genes that are up-regulated after herbivore damage (Ralph *et al.* 2006). Key questions for future research include: are these genes also involved in the ontogenetic trajectories of constitutive defences? How are these genes up- or down-regulated through major developmental phase changes? Do they influence plant fitness as a function of the ontogenetic stage in which they are expressed? The sequencing of genomes, the use of microarrays and comparative studies of knockouts vs. wild types in the field would provide new insights into these questions.

One approach to understand the regulatory mechanisms behind the expression of ontogenetic trajectories is the

assessment of changes in plant defence and the regulatory mechanisms behind when, as a consequence of severe stress (Grafi *et al.* 2011), hormonal induction (Robbins 1957), and even herbivore damage (Craig *et al.* 1986), plants revert to earlier ontogenetic stages (Craig *et al.* 1986). If reverted plants go back to their previous defensive status, a response called 'induced juvenilization' (Bryant *et al.* 1983; Lindroth *et al.* 2007), this would represent good evidence of processes associated with development driving the expression of plant defence. Evidence from both herbivore preference trials (Craig *et al.* 1986; Price *et al.* 1987; Craig & Ohgushi 2002) and secondary metabolite profiles (Lindroth *et al.* 2007) reveal that shoots developing after herbivory are more similar to juvenile stage plants than the mature stage experiencing herbivore damage. Reverting defensive profiles to juvenile stages can be also achieved by manipulating the onset of reproduction. For example in *Nicotiana attenuata*, herbivore-induced increases in ethylene and jasmonates are suppressed at the onset of reproduction, but the inducibility of these hormones can be restored by removing the inflorescences (Diezel *et al.* 2011). Molecular approaches have also been useful to achieve an over-expression of regulatory enzymes at stages in which plants normally would not produce them. This technique has revealed the regulatory mechanisms associated with some ontogenetic trends. For example the main benzoxazinoid (DIMBOA) that confers resistance to herbivores and pathogens in early stages of maize can be experimentally stimulated in mature stages by overexpressing Bx1 gene, which encodes the signature enzyme of the biosynthetic pathway (Zheng *et al.* 2015). These examples suggest that at least for some species, development-associated phytohormonal regulation is indeed partially responsible for the observed ontogenetic trajectories in plant defence.

SCALING UP FROM ONTOGENETIC TRAJECTORIES OF INDIVIDUALS TO POPULATION DEMOGRAPHY, BIOGEOGRAPHY AND MACROEVOLUTION

Understanding the selective forces shaping ontogenetic trajectories in plant defence will enlighten our knowledge of the consequences of herbivory for plant demography and the regulation of plant populations. Particularly, the use of integral projection models could provide effective tools to identify which stage- or age-related variable (e.g. resistance traits and tolerance) influence population vital rates (i.e. survival, growth and reproduction). These models, in contrast to traditional matrix models, allow the use of a continuous range of plant stages/attributes (Briggs *et al.* 2010) and, the generation of population projections (Merow *et al.* 2014) incorporating genotypes with different ontogenetic trajectories in plant defence. In turn, this information could reveal which ontogenetic trajectories contribute most to population growth. Whereas it has been demonstrated that herbivores can limit plant population growth through their effects on plant survivorship and fecundity throughout different stages of their life cycle (Bastrenta *et al.* 1995; Ehrlen 1995; Stowe *et al.* 2000), and influence the progression or regression of plants between ontogenetic stages (Knight 2004), the link between these effects and particular ontogenetic trajectories in plant defence is still missing.

Like other aspects of plant defence, it is possible that ontogenetic defence trajectories are contingent on environmental factors. Thus, the study of ontogenetic trajectories within a biogeographical context would be useful. A clear biogeographical pattern has been previously detected in the ontogenetic trajectories of boreal vs. non-boreal trees (Barton & Koricheva 2010). Population and biogeographic variation in the ontogeny of plant defence could result from spatial variation in resource availability and climate that contributes to the intensity of species interactions (Moles *et al.* 2011; Rasmann *et al.* 2014; Moreira *et al.* 2015). Alternatively, geographical variation in plant defence could also be a byproduct of different life-histories strategies that predominate in different regions of the world.

Finally, a macroevolutionary approach will be useful to detect general trends in ontogenetic trajectories and propose hypotheses of major evolutionary transitions in this complex phenotype (Adams & Nistri 2010). Evaluating phylogenetic signals can be informative to assess the influence of life history and particular environmental conditions on the evolution of defensive strategies of plants. Some studies have reported interspecific trends in the ontogenetic trajectories of defensive traits within a phylogenetic context. For example whereas an increase in alkaloid concentration during plant development has been found to be consistent for 12 species of the genus *Datura*, ontogenetic changes in trichome density are considerably more variable (Karinho-Betancourt *et al.* 2015). As a consequence, correlations among different alkaloids and trichomes vary across plant ontogeny and among species. To interpret such correlations, a challenge is to identify the costs and benefits, as well as the selective agents promoting the simultaneous expression of different defensive traits at particular ontogenetic stages and under specific environmental contexts. A phylogeographic approach using comparative methods would also provide hypotheses for the adaptive value of different ontogenetic trajectories. For example for the *Datura* clade, leaf pubescence has been associated with arid habitats, and glabrous leaves with more temperate or sub-tropical regions (Karinho-Betancourt *et al.* 2015), but ontogenetic changes in this trait as a function of the environment have not been investigated in this context.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Ontogeny has emerged as a widespread and common source of variation in plant defence. Despite hundreds of descriptions for ontogenetic trajectories in plant defence traits, we still do not know what factors drive the evolution of these patterns. Although some ontogenetic trajectories in plant defence traits may reflect developmental constraints and thus be consistent with the null model of ontogenetic drift, it is more likely that selection by herbivores, allocation costs, and trait multi-functionality have led to the evolution of these developmental patterns, through the underlying regulatory pathways fine-tuning these complex phenotypes. The challenge now is to design research to investigate the relative importance of these factors during the evolution of ontogenetic trajectories of defence, considering that each one can vary among plant life history strategies, with respect to biogeography, and among defence traits.

In the past decades, we have learned that variation in plant defence is not exclusively genetic, but also due to phenotypic plasticity (Whitman & Agrawal 2009). In particular, an incredible amount of knowledge has accumulated on the induction of plant defence against herbivore damage and has led to a more realistic understanding of the causes and consequences of variation in plant defence. We believe that the inclusion of ontogeny into studies on plant defence could provide equally fulfilling results in the future, given that developmental reaction norms have been considered to be the one of the main targets of selection of phenotypes (Schlichting & Pigliucci 1998), and ontogeny itself as a flexible process subject to environmental input (Whitman & Agrawal 2009). However, most studies in the evolutionary ecology of plant defence have concentrated their efforts on particular plant ontogenetic stages, offering important, but partial information or snapshots of the selective dynamics influencing the evolution of plant defence. Hence, we highlight the importance of incorporating ontogenetic trajectories to further research into the studies of molecular ecology, plant physiology, ecology and phenotypic evolution, to move forward and achieve a better understanding of lifetime defensive strategies evolution, and their consequences for interacting species, communities and ecosystems.

In particular, we encourage the development of molecular tools for non-model systems to perform field experiments aimed at testing the selection pressure on ontogenetic trajectories of plant traits (and thus on the regulatory mechanisms driving these developmental reaction norms), that also incorporates the role of multi-functionality of such traits during plant ontogeny. Broader studies integrating phylogenetic and biogeographic patterns are also recommended to reveal macroevolutionary trends and to clarify the role of ontogeny in the global diversity of plant defences.

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