Convergent occurrence of the developmental hourglass in plant and animal embryogenesis?

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Background
The remarkable similarity of animal embryos at particular stages of development led to the proposal of a developmental hourglass. In this model, early events in development are less conserved across species but lead to a highly conserved ‘phylotypic period’. Beyond this stage, the model suggests that development once again becomes less conserved, leading to the diversity of forms. Recent comparative studies of gene expression in animal groups have provided strong support for the hourglass model. How and why might such an hourglass pattern be generated? More importantly, how might early acting events in development evolve while still maintaining a later conserved stage?

Scope
The discovery that an hourglass pattern may also exist in the embryogenesis of plants provides comparative data that may help us explain this phenomenon. Whether the developmental hourglass occurs in plants, and what this means for our understanding of embryogenesis in plants and animals is discussed. Models by which conserved early-acting genes might change their functional role in the evolution of gene networks, how networks buffer these changes, and how that might constrain, or confer diversity, of the body plan are also discussed.

Conclusions
Evidence of a morphological and molecular hourglass in plant and animal embryogenesis suggests convergent evolution. This convergence is likely due to developmental constraints imposed upon embryogenesis by the need to produce a viable embryo with an established body plan, controlled by the architecture of the underlying gene regulatory networks. As the body plan is largely laid down during the middle phases of embryo development in plants and animals, then it is perhaps not surprising this stage represents the narrow waist of the hourglass where the gene regulatory networks are the oldest and most robust and integrated, limiting species diversity and constraining morphological space.

Key words: Embryogenesis, developmental hourglass, convergent evolution, developmental networks, comparative transcriptomic analysis, gene expression.

EMBRYOGENESIS
Embryogenesis describes the development of a single fertilized cell into a mature embryo, in which the basic tissue types and body plan for that organism are established. It begins with the fusion of male and female gametes to create the single-celled zygote, with the potential to form a whole organism through cell division and expansion, along with cell type-specific specification, differentiation and maturation. While embryogenesis does not occur in single-celled eukaryotes, it has evolved independently in two of the major multicellular lineages, animals (Animalia) and land plants (Embryophyta) (Meyerowitz, 2002). Producing multicellular offspring through embryogenesis enables the basic body plan to be established, while nutrition and protection provided by the mother increase the chance of survival. In seed plants, embryogenesis occurs as part of seed development and so aids in the dispersal of offspring.

While evolutionary change that affects the embryonic process does occur, ultimately viable offspring must be produced, presumably constraining the total variation that may arise. In the case of the developmental hourglass model, where early events in development are less conserved across species, but lead to a highly conserved ‘phylotypic period’ during mid-embryogenesis before again diverging, how do early-acting events in development evolve whilst providing the scaffold on which all of embryogenesis is built? Here we compare morphological and molecular processes that occur during plant and animal embryogenesis, suggesting that these independently evolved processes both show an hourglass pattern, to draw out general processes that may lead to hourglass models.

THE HOURGLASS MODEL OF EMBRYOGENESIS IN ANIMALIA
Nineteenth century German embryologist Karl Ernst von Baer, from his morphological observations of embryogenesis in a range of animal species, noted that embryos from species in the
same phylum often show considerable variation early in embryogenesis, then converge to a similar form during mid-embryogenesis, before diverging again late in embryogenesis (von Baer, 1828). These observations have led to the development of the hourglass model of embryo development in animals (Fig. 1). This model divides metazoan animal embryogenesis into three stages. The first stage encompasses early embryogenesis and begins with the formation of the zygote. During this stage, multiple rounds of mitotic division generate undifferentiated cells, which are then distributed into layers, through gastrulation. Additionally, the main axes of the adult morphology are established, and the broad domains of the body plan defined at this stage. Morphological observations have consistently indicated that these early events can be very different between metazoan species from the same phylum, as shown in insects (Sander, 1975), nematodes (Goldstein et al., 1998) and vertebrates (Immler et al., 2004). The subsequent middle period of embryogenesis is also known as the ‘phylotypic period’ (Richardson, 1995), defined as the stage at which all body parts are represented in their final positions as undifferentiated cell condensations, or the stage at which organisms within a common phylum show the maximum degree of morphological similarity despite differences in the early stages (Slack et al., 1993). During this stage, patterning along the axes, especially that reflected in Hox gene expression (Harding et al., 1985; Akam, 1987), is established. In the last stage of embryo development, the limbs, organs, eyes and other structures form, resulting in the final structures of the adult or larvae. By the end of development, the differing growth and patterning trajectories of different species lead to increased divergence and diversity in adult body plans.

Despite morphological analyses in animals suggesting the existence of the developmental hourglass model, it remained controversial due to the complexity of evaluating evolutionary distances between embryos of diverse species simply by comparison of morphogenesis (Hall, 1997; Richardson et al., 1997; Galis and Metz, 2001; Bininda-Emonds et al., 2003; Irie and Sehara-Fujisawa, 2007; Roux and Robinson-Rechavi, 2008; Comte et al., 2010). Development of comparative transcriptomic analysis has enabled comparison of gene expression at different developmental stages (Domazet-Loso and Tautz, 2010; Kalinka et al., 2010; Irie and Kuratani, 2011; Yanai et al., 2011), thereby bringing a more quantitative methodology to the traditionally qualitative discipline of comparative embryology. By comparing gene expression profiles across species of invertebrate (Drosophila melanogaster, Anopheles gambiae and Caenorhabditis elegans) (Kalinka et al., 2010; Schep and Adryan, 2013), chordate (Ciona intestinalis) and vertebrate (Danio rerio and Xenopus tropicalis) (Schep and Adryan, 2013), it has been possible to address conservation in the different stages of embryo development across a range of measures, including the evolutionary age and sequence divergence of gene expression (Domazet-Loso and Tautz, 2010; Yanai et al., 2011). All of these approaches have provided molecular support for the developmental hourglass model, by highlighting that the highest level of conservation occurs during the ‘middle’ stages of embryogenesis, corresponding roughly to the morphologically conserved phylotypic period.

THE HOURGLASS MODEL OF EMBRYOGENESIS IN ANGIOSPERMAE

Embryogenesis evolved early and independently in the land plant lineage, with the bryophytes producing multicellular embryos. As in animals, the basic body plan in plants is established during embryo development, with the mature embryo containing the tissues and organs required for an adult (West and Harada, 1993; Jurgens et al., 1995; Mordhorst et al., 1997; Chandler et al., 2008; Harada et al., 2010; Peris et al., 2010; Wendrich and Weijers, 2013; ten Hove et al., 2015). This body plan can be considered as a series of elements arranged along two axes: a radial axis and a shoot–root axis. In flowering plants, the radial axis consists of concentric tissue layers containing the three basic tissue types that will form all plant structures. These are, from the outside to the inside, the epidermis, the ground tissue and the central vascular tissue. Along the shoot–root axis is the shoot apical meristem and the cotyledons at the one end, the hypocotyl in the middle and an embryonic root, including a root meristem, at the other end. In seed plants, embryogenesis occurs alongside the processes required for seed development, with the mature embryo neatly packaged in the seed for dispersal.

While the basic body plan is established during plant embryogenesis, the mature embryos of plants are anatomically much less complex than those in animals, and much of the morphological variations observed between plant taxa is established post-embryonically. Due to this reduced complexity, the possible existence of an hourglass pattern of development was largely ignored in plant morphological studies as plant embryogenesis was considered not to generate sufficient morphological diversity (Quint et al., 2012; Drost et al., 2015). However, more recent molecular data imply that flowering plant embryogenesis may also follow an hourglass pattern. Gene expression data from Arabidopsis thaliana embryos at a number of stages of development were used to calculate the age and the sequence divergence of the transcriptomic throughout embryonic development (Quint et al., 2012; Drost et al., 2015). Both of these phylotranscriptomic analyses point to an hourglass model, with the mid-embryonic stages (globular to torpedo) (Fig. 1) being the most conserved in terms of gene expression and thus forming the waist of the hourglass, with more divergent early and late phases. With molecular data suggesting that an hourglass pattern of development may be present in plants, it is relevant to review whether there is evidence for a morphological hourglass in plant embryogenesis, albeit one that may be reduced in morphological complexity compared with animals.

There is a long history of morphological studies in plant embryology reviewed in a number of substantial works such as Wardlaw (1955), Gifford and Foster (1989), Johri et al. (1992) and Raghavan and Sharma (1995). Our aim here is not to review these works, but rather to highlight certain aspects to ask if there are some similarities on the morphological level between the hourglass models observed in animals and events in plant embryogenesis. The study of plant embryogenesis often encompasses a wide range of events in plant reproduction such as gametogenesis, pollination, fertilization and seed development. Here our focus will be on zygotic embryogenesis and events that occur from the zygote to the mature embryo. As phylotranscriptomic support for an hourglass model is currently
Is there a morphological hourglass in flowering plant embryogenesis?

Embryo development in flowering plants can be divided into three stages (Raghavan and Sharma, 1995; Harada et al., 2010; Peris et al., 2010; ten Hove et al., 2015). The first stage involves the development of the zygote followed by multiple rounds of mitotic divisions without a large increase in total size, to produce a ball of cells known as the globular embryo. While the cells in the globular embryo are largely undifferentiated, both the apical–basal axis and the radial axis are established by this stage. Morphological surveys have revealed that there is considerable diversity in the pattern of cell divisions, or segmentation, to generate the globular embryo (Wardlaw, 1955; Johri et al., 1992; Raghavan and Sharma, 1995). Based on the sequence and orientation of cell division, six distinct cleavage patterns have been described (Johansen, 1950; Maheshvarl, 1950) (Table 1). Interestingly, these different patterns are distributed across flowering plant taxa and show little relationship to angiosperm taxonomy (Wardlaw, 1955). Similar patterns are found in distantly related families; for example, the Onagrad and Asterad types are found in the orders from the basal eudicots, the core eudicots (both Rosids and Asterad groups) and the monocots (Table 1). Additionally, different patterns are found in related species, such as the order Ranunculales containing genera that display the Onagrad, Solanad and Caryophyllad patterns of early embryo development (Table 1). Some species also display more than one pattern (Caryophyllales), and some species, such as cotton (Gossypium hirsutum) and maize (Zea mays), do not have a precise pattern of mitotic division, with the early division patterns appearing to be random (Pollock and Jensen, 1964; Poethig, 1987). The morphological surveys of early plant embryogenesis thus show considerable diversity in the segmentation pattern as plant embryos progress to the globular stage, and this diversity is not tightly associated with phylogeny. This morphological diversity is reminiscent of the diversity observed in early embryos in animals, providing the wide base to the hourglass (Fig. 1).

As in animals, it is during the middle phase of embryogenesis that patterning occurs in plants, with the basic body plan being laid down (West and Harada, 1993; Mordhorst et al., 1997; Chandler et al., 2008; Harada et al., 2010; Peris et al., 2010; Wendrich and Weijers, 2013). Despite the diversity in cell division patterns in the early stages, the basic architecture established in the middle phase is similar in the major plant taxa only available for the flowering plant Arabidopsis thaliana, the discussion will also be limited to the flowering plants (angiosperms).
The events occurring in the last phase of embryogenesis are quite distinct in plants and animals. In animals, the body plan is built upon as the major organs form, and embryos from related taxa again diversify in morphology. In flowering plants, the third phase in embryogenesis is a maturation phase and does not involve the further elaboration of the body plan (West and Harada, 1993; Harada et al., 2010). The main noticeable change in the embryo is an expansion, with little change in the overall architecture. Other events at this stage relate to the packaging of the embryo into a seed for dispersal such as preparation for desiccation, metabolic quiescence and nutrient storage. Thus, in the third stage of embryogenesis in flowering plants, the morphological hourglass pattern is not followed. Rather than the divergence of morphology and the widening of the hourglass observed in animal embryo morphology, the range of plant diversity remains similar, forming a funnel-like shape rather than an hourglass (Fig. 1). The similarity in embryo

### Table 1. The different patterns of early embryo segregation in plants and some of the Orders in which the patterns have been reported

| Type* | Order reported† | Major clade |
|-------|-----------------|-------------|
| Onagrad (Crucifer) | Myrtales | Core eudicot – Rosids |
| | Lamiales | Core eudicot – Asterids |
| | Brassicales | Core eudicot – Rosids |
| | Malphigiales | Core eudicot – Rosids |
| | Fabales | Core eudicot – Rosids |
| | Ranunculales | Basal eudicot |
| | Sapindales | Core eudicot – Rosids |
| | Asparagales | Monocot |
| | Liliales | Monocot |
| | Poales | Monocot |
| Asterad | Asterales | Core eudicot – Asterids |
| | Geraniales | Core eudicot – Rosids |
| | Lamiales | Core eudicot – Asterids |
| | Oxilidales | Core eudicot – Rosids |
| | Caryophyllales | Basal eudicot |
| | Rosales | Core eudicot – Rosids |
| | Liliales | Monocot |
| | Poales | Monocot |
| Solanad | Solanales | Core eudicot – Asterids |
| | Apiales | Core eudicot – Asterids |
| | Piperales | Basal angiosperm |
| | Malphigiales | Core eudicot – Rosids |
| | Ranunculales | Basal eudicot |
| | Gentianales | Core eudicot – Asterids |
| | Boraginaceae | Core eudicot – Asterids |
| (unplaced order Lamiales?) | Caryophyllales | Basal eudicot |
| | Ericales | Core eudicot – Asterids |
| | Caricaceae | Core eudicot – Asterids |
| | Santalales | Basal eudicot |
| | Caritales | Core eudicot – Asterids |
| | Fabales | Basal angiosperm |
| | Santalales | Basal eudicot |
| | Caritales | Core eudicot – Asterids |
| | Malphigiales | Core eudicot – Rosids |
| | Piperales | Basal angiosperm |

*As described in Johansen (1950).
†Based on information in Wardlaw (1955) and Raghavan and Sharma (1995).

(Wardlaw, 1955; Peris et al., 2010). In eudicots, the middle phase covers the globular stage to the torpedo stage. It begins with the transition from the globular embryo to the heart stage as cotyledons begin to form at two lateral zones at the apical domain, altering the symmetry from radial to bilateral. During this middle phase, the root and shoot meristems are specified, the hypocotyl becomes apparent and tissue specification takes place, with the precursors of the vascular tissue forming. There is also a considerable increase in size. Notably, the morphology of the embryo during this stage is similar throughout the eudicots regardless of the early segmentation pattern (Wardlaw, 1955; Mordhorst et al., 1997). For example, Mordhorst et al. (1997) noted the high level of similarity in the middle stages between A. thaliana (Brassicaceae), a Rosid that displays the Onagrad pattern in early embryogenesis, and carrot (Daucus carota, Apiaceae), an Asterid that displays a Solanad pattern in early embryogenesis. The morphology of cotton embryos is also similar to that of other dicots in the middle phase, despite the random nature of the early pattern of cell division (Pollock and Jensen, 1964).

The morphology of monocot embryos, however, becomes dramatically different from those of eudicots during the middle phase (Raghavan and Sharma, 1995; Mordhorst et al., 1997; Chandler et al., 2008). The major differences relate to the formation of a single cotyledon, the shoot meristem forming laterally rather than apically, and the resultant shoot–root axis not aligning with the apical–basal axis established early in development. There is also greater variation amongst monocot embryos than the dicots, with the grasses being markedly different, with additional tissues such as the absorptive scutellum and the protective coleoptile and coleorhiza covering the young shoot and root (Raghavan and Sharma, 1995). Thus comparative morphological studies indicate that there is reduced morphological variation within plant taxa in the middle phase of plant embryogenesis compared with the early phase. This reduction in morphological diversity is similar to the narrowing of the hourglass to the constricted waist seen in morphological analyses of animal embryogenesis (Fig. 1). While this similarity to the hourglass model has not been widely commented on previously, the observation that there is a reduction in morphological diversity during plant embryogenesis has been noted (Wardlaw, 1955; Yadegari and Goldberg, 1997; Peris et al., 2010).

In animals, the waist of the hourglass relates to the ‘phylo- typic’ stage where related taxa are more similar than at earlier and later stages based on both morphological and molecular data. The middle period of plant embryogenesis was also defined as the phylotypic stage based on phytotranscriptomic data (Quint et al., 2012; Drost et al., 2015). This then raises the question of whether the middle period of plant embryogenesis can be considered ‘phylotypic’ based on morphology. On a broad basis, the answer appears to be yes, as the differences in morphology at this stage are divided along phylogenetic lines, with the major differences being between the monophyletic eudicots and the monocots. Furthermore additional variations, such as the scutellum, coleoptile and coleorhiza in the grasses, are only found confined to monophyletic groups. Thus, in the middle phase, embryos from related taxa appear similar, with variation between taxa, meaning that this phase could be described as ‘phylotypic’ in flowering plants based on morphology as well as molecular studies.

The events occurring in the last phase of embryogenesis are quite distinct in plants and animals. In animals, the body plan is built upon as the major organs form, and embryos from related taxa again diversify in morphology. In flowering plants, the third phase in embryogenesis is a maturation phase and does not involve the further elaboration of the body plan (West and Harada, 1993; Harada et al., 2010). The main noticeable change in the embryo is an expansion, with little change in the overall architecture. Other events at this stage relate to the packaging of the embryo into a seed for dispersal such as preparation for desiccation, metabolic quiescence and nutrient storage. Thus, in the third stage of embryogenesis in flowering plants, the morphological hourglass pattern is not followed. Rather than the divergence of morphology and the widening of the hourglass observed in animal embryo morphology, the range of plant diversity remains similar, forming a funnel-like shape rather than an hourglass (Fig. 1). The similarity in embryo...
morphology remains high within related taxa, so this stage could also be considered 'phylotypic'.

Overall it seems that comparative morphology of embryogenesis of flowering plants does show some similarity to the hourglass model described for animal embryogenesis (Fig. 1). There is a high degree of variation in the early stages, and this variation is found within related taxa. The level of variation is then reduced in the middle stages, most notably within related taxa. Thus, these stages reconstitute the hourglass pattern, beginning broad and then narrowing to the waist. However, the later divergence seen in animal embryos does not occur in the flowering plants. While there is some similarity to the hourglass model, it would be speculative to say that plant embryogenesis follows the hourglass model based purely on morphology. However, if we couple the morphological data with the phylogenetic and molecular data (Quint et al., 2012; Drost et al., 2015), there is much stronger evidence that plant embryogenesis follows the hourglass model. This observation is intriguing as embryogenesis evolved independently in plants and animals, suggesting that the hourglass pattern has evolved twice. This raises the question – is this just coincidence or a case of convergent evolution? If it were a case of convergent evolution, this would point to the existence of evolutionary factors that favour the hourglass pattern of embryo development. To try and address if this is convergent evolution, we must consider the interplay between evolutionary and developmental processes, and ask if there are limits or constraints on taxonomic diversity or morphological disparity during embryogenesis. We discuss whether the conserved waist of the hourglass model may be required for spatio-temporal organization and differentiation of complex multicellular life (Quint et al., 2012).

CONVERGENT EVOLUTION OF THE HOU RGlass PATTERN OF EMBRYO DEVELOPMENT

Developmental constraints, defined as ‘...the non-production of variant phenotypes caused by the nature of the developmental system’ (Arthur and Farrow, 1999), imply that the architecture of any given developmental system precludes or biases some developmental outcomes. Although the relationship between the mechanisms that allow variation and those that constrain outcomes is unclear, what is apparent is that these constraints are universal, in that they limit the evolution that can occur, as essentially there are only some changes to the developmental pathways that ‘work’ and a multitude of ‘variations’ that ‘don’t work’ and are non-viable, resulting in constraints on radial evolution. These events in embryogenesis are constrained by the absolute requirement for the generation of a mature embryo, with all the tissues and organs required to mature into a reproductively fit adult. Any alterations in embryo development that impact negatively upon post-embryonic events would be selected against. Convergent evolution of an hourglass pattern of embryo development in animals and plants would imply that the mid-embryonic stages are particularly constrained, and resistant to evolutionary change, compared with the more flexible early and late stages. By considering what general events occur at each stage of embryo development at the morphological and molecular levels in plants and animals, we explore why the hourglass model may fit in these independently evolved ontogenies.

Constraints in early embryo development

We might presume that the earliest stages of embryo development would be the most constrained, as it could be expected that alterations early in development would be more likely to have widespread downstream effects. As development progresses, alterations would then be more widely tolerated, in turn promoting diversity. This funnel-like model predicts that the highest conservation would occur at the earliest stages of development (Fig. 2) (Raff, 1996). Conservation of early embryogenesis is, however, not seen on the morphological or molecular levels in plants and animals, implying that this early stage is less constrained than subsequent mid-embryonic stages. This observation is intriguing as it suggests a measure of plasticity previously not considered permissible, considering the importance of establishing the correct body plan in the mature embryo.

To understand why this stage might be less constrained, we need to consider the events that occur during early embryogenesis. The beginning of embryogenesis in both plants and animals involves the mitotic division of the zygote to produce a multicellular embryo. During this stage, maternal factors and early-expressed zygotic genes establish the major axes of the embryo, while the individual cells are largely undifferentiated and maintain the potential to form multiple cell types. The expression of the genes involved in this stage of embryogenesis is...
Constraints in mid-embryo development

During the middle stages of embryogenesis, the body plan develops further upon the established axes. In most metazoans, the middle stages of embryogenesis are characterized by the expression of the Hox complex of transcription factors, which act to define and regionalize the different areas of the embryo trunk along the anterior/posterior axis of the embryo (Garcia-Fernandez, 2005). This pattern of gene expression is activated by previously acting genes and broad domains (Irish et al., 1989), defines the identity of functional units along the anterior–posterior axis from which the rest of the embryonic structures are built, and forms the basis of different morphologies in each of those units (reviewed in McGinnis and Krumlauf, 1992). It seems that this stage is tightly constrained, perhaps because of the importance of this regionalization in anterior–posterior elongated embryos (Duboule, 1994). A similar situation occurs in plants, with the middle embryonic stages characterized by a number of transcription factors that define regions along the shoot–root axis and cotyledon borders (Chandler et al., 2008; Peris et al., 2010). These transcription factors include the WUSCHEL HOMEBOX LIKE genes: WUS that is critical for shoot meristem activity, WOX5 involved in root specification, SCARECROW (SCR) expressed in the endodermal layer surrounding the vasculature and CUP-SHAPED COTYLEDON (CUC) which is important for the boundary between the cotyledons and the shoot meristem (Chandler et al., 2008; Peris et al., 2010). It is of note that the temporal and spatial expression of these transcription factors is broadly similar in the eudicot A. thaliana and the monocot Z. mays, despite the difference in eudicot and monocot embryos at this stage (Chandler et al., 2008). This conservation in transcription factor patterning in both animals and plants highlights the importance of cells differentiating in the correct spatial and temporal pattern during the middle stages of embryogenesis. Such expression patterns in turn require complex signalling and regulatory pathways and networks. How does the need for the production of tissues/differentiated cells, along with the complex pathways that regulate them, influence the evolution of embryonic development during this middle period?

It is possible that variation in embryogenesis comes from the evolution of developmental pathways that allow the outputs of the pathways to remain stable while developing new parallel processes that can permit stepwise change to embryogenesis to occur. Although limited in scope and complexity, multiple studies have shown that in developmental evolution, different species use conserved genes in alternative ways (Carroll, 2008; Shubin et al., 2009; Wilson and Dearden, 2009; Wilson et al., 2010), and this altered usage gives rise to morphological variation (Sucena et al., 2003; Prud’homme et al., 2006; Chan et al., 2010). This is especially true for transcription factors that are involved in spatial and temporal regulation of (often) large numbers of other genes (Liang and Biggin, 1998; Li et al., 2008). Both the expression and activity of transcription factors during embryogenesis are tightly regulated. The construction of multicellular organisms relies on complex networks of genes that are regulated by transcription factors that, in turn, regulate other genes. Therefore, networks must themselves change to produce morphological change during evolution (Davidson and Levine, 2008). The complexity of these networks, however,
may begin to constrain morphological variation as a new role for a conserved transcription factor may have pleiotropic effects on the integrated biology of the organism. We know little about how such gene networks act, and less about how they evolve in embryogenesis (Golsteyn et al., 2007; Maduro, 2009), yet changes to such networks may constrain (Cameron et al., 2005), or confer diversity in, an organism. Genetic networks are robust, due to multiple feedback, feed-forward and cross-regulatory mechanisms (von Dassow et al., 2000; Eldar et al., 2000; Davidson, 2010; Hilgers et al., 2010; Holme, 2011; Gavin-Smyth et al., 2013; Zheng et al., 2013). This makes it probable that changes in expression, mediated by transcription factors, of multiple genes are needed to ‘rewire’ these networks, and thereby change their outcome at the morphological level.

Investigation of expression of orthologous transcription factors at different time points during embryonic development across vertebrate (D. rerio and X. tropicalis), chordate (C. intestinalis) and invertebrate (D. melanogaster, A. gambiae and C. elegans) (Schep and Adryan, 2013) phyla showed very similar transcription factor expression patterns between species. Transcription factor expression increases during the initial stages of development, with C2H2 zinc finger transcription factors over-represented and Homeobox transcription factors under-represented in the early stages, but increasing later in development in all species investigated (Schep and Adryan, 2013).

Mid-embryogenesis is thus characterized by precise co-ordination between growth and patterning, and as such is highly sensitive to perturbations in the sequence of temporal and spatial activation of genes (Duboule, 1994). Taking a more global view of conservation, Raff argued that the complexity of interactions between genes, cells and developmental processes reaches a maximum during mid-embryogenesis when the body plan of the organism is being established (Raff, 1996). Common to both models is the idea that changes during mid-embryogenesis are deleterious in nature due to the properties of the developmental system that are unique to this period. However, to what extent variation at mid-embryogenesis is limited purely by selective constraints, or by the interplay between selective and developmental constraints, is not specified in either model. An example of this is the gene-regulatory network controlling root stele development, in which morphological phenotypes were found to be associated with mutations in only 16% of the transcription factors tested, whereas molecular or expression phenotypes were identified for 65% (Brady et al., 2011). Accordingly, the transcriptional network can be affected in a transcription factor mutant despite the absence of a mutant phenotype (van der Graaff et al., 2002; Brady et al., 2011). Therefore, mutations in compensatory genes or changes in the expression of several genes may be needed to allow the creation of a new steady state of the network, resulting in a robust change in morphology.

How do we reconcile changes in the network with the conservation we observe in the developmental hourglass model? The most likely explanation is that the hourglass model represents an evolutionarily constrained central node in the ‘hierarchical’ system structure of the developmental network essential for maintaining core gene networks that regulate essential developmental outcomes, such as an antero-posterior identity of the embryo. Mutations that affect general ‘upstream’ regulators (usually highly connected nodes in a network) of developmental or cellular gene expression are more likely to have pleiotropic effects; therefore, these mutations tend to reduce fitness (Stern and Orgogozo, 2009). In contrast, genes that execute cellular responses downstream often act with other genes in a concerted fashion in basic cellular functions. The expression of these genes needs to be modulated in a co-ordinated manner, usually by transcription factors (Stern and Orgogozo, 2008). Comparative epigenomics in Oryzias latipes and D. rerio identified conserved cis-regulatory nodes active during the phylotypic period in these species. A large proportion of these cis-regulatory nodes provided regulatory input to genes encoding transcription factors, suggesting that these regulatory regions represent constrained nodes from essential gene regulatory networks operating at the phylotypic period (Tena et al., 2014). Therefore, the time at which a gene is expressed relative to the central node will determine how far a gene can evolve, relative to the genes that control the central node (Stern and Orgogozo, 2009).

Analysis of D. rerio and D. melanogaster identified that evolutionarily younger transcription factors seem to be more important in later stages of development, whereas evolutionarily older genes are prevalent in the earlier stages of development. Indeed, transcriptional expression of conserved transcription factors, by itself, marked the phylotypic stage of metazoans (de Mendoza et al., 2013). By changing the expression of input-output genes of the node, morphologies can be modulated in a specific context. In line with this, some types of transcription factors were repeatedly (although not exclusively) recruited to modify organ morphologies in a certain manner in plants. One example is the heterotopic expression of KNOX transcription factors resulting in dissected leaf development (Bharathan et al., 2002; Hay and Tsiantis, 2006; Hay and Tsiantis, 2010). Another example is the recurrent recruitment of CYC-type TCP transcription factors in generating monosymmetric flowers (zygomorphic) across distant eudicot lineages (Busch and Zachgo, 2009). This recurrent recruitment might be linked to an ancestral dorsal expression domain in floral meristems that were selectively expanded and/or switched to later stages of organ development in monosymmetric taxa (Preston and Hileman, 2009; Busch et al., 2012).

Constraints in late embryo development

Late embryogenesis differs markedly between plants and animals. Animal embryos at this stage build on their regionalized axes to produce limbs and organs, elaborating on body plans to produce diverse morphologies. Differences in late embryogenesis produce taxa-specific patterns and morphological variation in adult forms. Such morphological variation is underpinned by selection for the structures of the final form, showing that evolution of this late stage of development can shape and re-shape form to fit the final animal’s environment. At these stages, evolutionary changes in gene expression are presumably less pleiotropic, providing morphological detail rather than fundamental structures on which morphology is built.

In flowering plants, the body plan does not change dramatically during the later stages of embryogenesis, as the basic body plan is elaborated upon post-embryonically. Rather, the embryo
expands, and processes such as nutrient accumulation and preparation for dehydration occur as part of seed development (Harada et al., 2010). While flowering plant embryos do not show the morphological variations in the later embryo stages that are seen in animals, a molecular hourglass pattern is observed in A. thaliana embryogenesis (Quint et al., 2012; Drost et al., 2015). The transcriptome at later stages contains both genes with a greater sequence divergence from close relatives and younger genes that are more recently evolved. Sequence divergence at later stages in flowering plants probably relates to differences in seed biology, such as dormancy potential, and is unlikely to be constrained by the need to produce a functional adult from an established body plan (Fig. 1). As long as the embryo can still successfully germinate, variation may be selected to aid dispersal and adaptation to germinate in response to different climatic conditions. The transcriptome at this stage also contains evolutionarily younger genes, which is not surprising as seeds are a younger invention, arising approx. 350 million years ago (Mya) (Linkies et al., 2010), compared with plant embryogenesis, that probably evolved as plants became adapted to land between 480 and 420 Mya (Bowman et al., 2007). Indeed embryos in the non-seed plants such as the bryophytes do not undergo this late maturation phase (Harada et al., 2010).

Developmental robustness and hourglasses

Developmental networks have evolved dramatic robustness to changes in kinetic parameters associated with most system components (von Dassow et al., 2000; Eldar et al., 2002; Meir et al., 2002). Such robustness to uncertainty and noise is considered as a hallmark of living systems (Kitano, 2004) and has been observed in a number of developmental processes (reviewed in MacNeil and Walhout, 2011). The robustness in embryogenesis is thought to rely on the architecture of the gene regulatory networks underlying developmental systems (Lin et al., 2009). It seems likely that robustness is an evolved characteristic, and there is, in many cases, a selective advantage to evolving robust embryonic systems that produce the same outputs in widely varying environmental situations. Given the propensity to evolve robustness and thus constraint, is the waist of the developmental hourglass just a reflection of its age? Does the ‘phylotypic stage’ represent the oldest, most robust, most integrated part of a developmental system? If this were the case, then the convergent evolution of developmental hourglasses in animals and in plants might be seen as inevitable, rather than coincidence. Indeed research has identified developmental hourglasses in other analogous developmental systems (e.g. Coprinopsis cinerea) (Cheng et al., 2015).

A transcriptomic hourglass (TAI and TDI) has been observed over the developmental life cycle of the mushroom C. cinerea (Cheng et al., 2015). Although C. cinerea lacks a process of embryogenesis (Kues, 2000), it still exhibits a transcriptomic hourglass waist albeit shifted towards late development (Cheng et al., 2015). This period of the hourglass is characterized by an upregulation of information storage and processing genes, including transcription factors and RNA processing factors, a signature of other developmental networks. The waist of the hourglass is followed by an upregulation of metabolism genes that encode enzymes related to carbohydrate metabolism, which may function to generate osmolytes, synthesize fungal cell wall components and hence support the rapid elongation of the mushroom stems required for spore distribution (Cheng et al., 2015). This observation suggests that the transcriptomic hourglass in C. cinerea represents a developmental network essential for maintaining developmental outcomes even though it is not coupled with a morphological hourglass pattern (Cheng et al., 2015).

Alongside this, there is some tentative evidence that hourglass patterns may form spontaneously due to the nature of developmental networks. Evolutionary modelling of regulatory gene interactions in simulated hierarchical gene networks can produce hourglass patterns (Akhshabi et al., 2014). This pattern is reflected both in the divergence of gene expression and, interestingly, in ages of genes, with the oldest genes being expressed in the ‘waist of the hourglass’ (Akhshabi et al., 2014). These hourglass networks are produced in situations where developmental regulators have increasingly specific functions (Akhshabi et al., 2014), a situation consistent with the biology of embryos. That simulation of random networks produces hourglass patterns indicates a propensity of all such networks to form this pattern.

Possible roles for the developmental hourglass model in developmental robustness and species diversity

It seems likely that developmental hourglasses are a common pattern in embryogenesis-like processes, and may even occur spontaneously because of the architecture of transcription factor-driven gene regulation (Akhshabi et al., 2014; Friedlander et al., 2015). Even if these patterns arise spontaneously, there are implications for developmental robustness and species diversity from the constraints that the hourglass places on development. Hourglass structures imply that the waist of the hourglass is the most sensitive region to perturbation in the network. These observations are supported by studies in C. elegans where genes expressed during the phylotypic period are known, from loss-of-function studies, to have significant effects on nematode morphology or function (Levin et al., 2012). These data imply that the genes expressed at the waist of the hourglass are highly connected and are conserved due to selection against mutations creating deleterious pleiotropic effects (Artieri, 2012). This suggests that there is selection against mutations/variation in genes expressed during the phylotypic period of the developmental hourglass, thus limiting evolvability in this period. Is it possible that the phylotypic stages of animal and plant embryos represent frozen accidents, developmental networks that have been fixed in structure as newer developmental networks have grown to act before them, in response to new developmental signals, and after them to produce detailed morphology shaped by selection? As these stages become ever more integrated, robust and pleiotropic, they become ever more invariant.

Perhaps the frozen phylotypic stages we see today are a reflection, probably highly embryonized, of ancestral networks that allowed the first metazoans and plants to regulate their morphology effectively. This frozen regulation, however, has consequences. The phylotypic stage must constrain the morphological space which an embryo is able to occupy; evolution beyond the phylotypic stage can cause dramatic shifts in
morphology, but these changes are still based on the conserved body plan of each phyla, and are conserved by the ancient gene regulatory network lurking in the waist of the hourglass. Whether produced by selection, or as an unforeseen consequence of the evolution of gene regulatory networks, it seems that developmental hourglasses constrain variation in both plants and animals, linking their morphological range and, in turn, species diversity.

CONCLUSIONS

The phylotranscriptomic hourglass patterns associated with embryogenesis in animals and plants are convergent as embryogenesis evolved independently in animals and plants (Meyerowitz, 2002), but may be a consequence of similar processes in developmental regulation. The hourglass pattern constrains the evolution of embryogenesis, producing a developmental stage that every embryo must pass through with reduced evolutionary, transcriptomic and morphological variation. This stage appears to evolve in both plants and animals, and there is evidence for it in fungi and modelling suggests it may occur spontaneously. Despite this, the developmental hourglass must (and does) limit variation, constraining variation that can exist or survive. Variation readily evolves before and after the phylogenetic stage, providing the glorious diversity of plants and animals alive today, but constraint does seem to exist. It is an intriguing thought that the way transcription factors act to regulate gene networks may spontaneously lead to the waist of developmental hourglasses, and that these in turn constrain the morphology that can be produced, or that will be viable. Is the very nature of developmental gene regulation responsible for the production of phyla?

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