Phenotypic evolution is restrained by complex developmental processes

E. R. Alvarez-Buylla,¹ M. Benítez,¹ and C. Espinosa-Soto¹

¹Instituto de Ecología, Universidad Nacional Autónoma de México, 3er Circuito Exterior, Junto a Jardín Botánico, Ciudad Universitaria, Coyoacán México D.F. 04510 México

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The paper, “Evolution and development of inflorescence architectures” by Przemyslaw Prusinkiewicz, Yvette Erasmus, Brendan Lane, Lawrence D. Harder and Enrico Coen [*Science*, 316, 1452–1456 (2007)], sets to accomplish a longstanding goal: to explain, for the first time, how and to what extent developmental constraints restrict phenotypic evolution. Prusinkiewicz and collaborators provide a relatively simple model that accounts for the variety of patterns of inflorescence architecture found among angiosperms, in which only a few of all possible types are observed. [DOI: 10.2976/1.2749445]

CORRESPONDENCE

E. R. Alvarez-Buylla:
elena.alvarezbuylla@gmail.com

Why are certain phenotypic types, the occurrence of which is theoretically possible, actually not observed? In general, the absence of certain phenotypic types that have equivalent fitness effects than those observed among present living organisms, may be due to constraints of developmental processes. Development involves interactions among parts (genes, cells, tissues, etc.) which affect phenotypic traits in nonindependent manners, thus generating correlated changes among morphogenetic relevant variables. Such developmental constraints could explain the absence of certain phenotypic types.

Until the last decades, developmental processes could not be understood in terms of the interactions of molecular and structural factors at different levels of organization. Such levels go from gene regulatory networks, to collections of cells bearing such networks coupled in spatio-temporal dynamics by intercellular communication processes. At the same time, structural characteristics of the system's components and emerging organs limit the patterns arising during morphogenesis. Mathematical and computational models are required to integrate the concerted action of the many interacting components with correlated and nonlinear behaviors that underlie the complex nature of development. Some recent models of this kind are showing that the whole structure and collective dynamics of gene regulatory networks

are important to obtain robust morphogenetic patterns (Hogeweg, 2000). This implies that models which oversimplify development may predict phenotypic patterns that are superficially similar to those observed in nature, but may not underlie the formation of robust patterns in real organisms.

Prusinkiewicz and co-workers acknowledge that studying the variational properties of developmental processes is important in order to understand phenotypic evolution. Such an endeavor entails postulating explicit molecular genetic models of development that explain why and how the configuration of a morphospace is restricted during the course of evolution. Prusinkiewicz and collaborators propose an imaginative model that yields inflorescence two-dimensional branching patterns that coincide with those observed among angiosperms: panicles, cymes and racemes (Fig. 1). They then provide molecular data for two genes that have been shown to be key in controlling inflorescence branching patterns: *LEAFY* (*LFY*) and *TERMINAL FLOWER1* (*TFL1*) to justify their proposed model for morphogenetic mechanisms. Finally, they map the inflorescence types in a plane with two orthogonal variables and construct a fitness landscape which provides predictions on expected inflorescence types under contrasting environments and enables speculations on evolutionary changes of types.

Figure 1. Alternative simple model that yields the three main types of inflorescence architectures. Types of inflorescences: panicles, racemes and cymes from left to right (A). Network underlying the proposed ‘toy model’ (B). Dynamics of the toy model, which also renders the inflorescence architectural types observed among real plants (C).

The paper of Prusinkiewicz and collaborators is certainly an innovative and important effort in the direction of integrating development and phenotypic evolutionary models. However, we argue that their model might be one of several ad-hoc models yielding inflorescence structures similar to those observed in nature. Thus, it might not be relevant for studying the generation of inflorescence architecture in plants, unless the proposed mechanism could be validated with a broader set of experimental evidence.

The most relevant trait that distinguishes racemose and cymose inflorescences is the relationship between location and age of floral meristems. In racemose inflorescences the youngest flowers are located in the distal part of the inflorescence, just beneath the indeterminate inflorescence main (apical) meristem. In contrast, the main meristem in cymose inflorescences is the first one to become determinate and turn into a flower, while the lateral meristems keep on branching reiterating the same rules: the main meristem terminates in a flower and the derived meristems branch. This results in the youngest flowers occurring farthest from the tip of the main stalk. Hence, any mechanism in which there is a structure that grows by branching will render racemes if, after a bifurcation, the younger tip turns into a floral (determinate) meristem, and the older one does not. In contrast, if the older tips turn into floral (determinate) meristems before the younger ones, cymes will be formed.

The “transient mechanism” proposed by Prusinkiewicz and collaborators is an algorithmical rewrite of the definition of cymes, racemes and panicles. The authors consider two kinds of meristems: young (B) and old meristems (A), and each kind requires different lengths of time to achieve flowering (T_A and T_B). When $T_A < T_B$, the older meristem within an inflorescence transits to flowering before derived lateral meristems, hence cymes are formed. When $T_A > T_B$, the younger lateral meristems turn into flowers before the apical meristem that maintains its indeterminacy, and so racemes

are formed. Panicles are branched racemose inflorescences where the flower stalk divides into two or more parts, and therefore flowers are arranged in groups of two or more. When $T_A = T_B$, either the two resulting meristems of a branching event develop as floral meristems or they do not, giving rise to panicles.

Prusinkiewicz and collaborators attempt to use the transient mechanism to explain some experimental data for two floral meristem identity genes in *Arabidopsis*, *LFY* and *TFL1*. In order to do that, it is necessary to make some assumptions about the interactions between these two genes and the rest of the system’s components. The authors recover activity patterns of *LFY* and *TFL1* that resemble those reported experimentally: *LFY* is expressed in young meristems, while *TFL1* is not, but in older meristems *LFY* is not expressed, while *TFL1* is. The authors conclude from this data that their model provides an explanation for the expression patterns observed in *Arabidopsis*. However, this is not the case, since they had assumed, in order to construct this version of the model, that *TFL1* activity is repressed and *LFY* expression is enhanced in young, but not in older, meristems. The model is fed with the observed expression patterns as assumptions, and therefore, does not explain them. In conclusion, what the authors call the transient mechanism is a description of the pattern and not an explanatory mechanism.

It is important to explore and explicitly model developmental processes because only then can we begin to understand how morphological patterns arise as a consequence of the variational properties of such processes, thus enabling the study of phenotypic evolution. However, a valid and useful hypothetical mechanism would have to generate observed patterns from interactions at a lower scale, otherwise, the model would be descriptive but it would not be explanatory. For instance, consider a chessboard pattern. We could propose that the “mechanism” that leads to it consists of alternately placing white and black squares. Such simple recipe would certainly yield the pattern, but would not provide any information regarding underlying proximal causes of the pattern because it only describes or “draws” it. Moreover, this hypothesis cannot be tested because the only way to achieve this would imply showing that such a pattern does not exist. However, this is impossible because this would be done once such patterns are documented and thus makes such a hypothesis scientifically futile. Alternatively, we could propose that the edges of white squares have a high affinity for the edges of black squares and *vice versa*. In such a system, a chessboard is the only two-dimensional pattern that is steady and stable if there are as many black as white squares. In this alternative mechanism we are explaining the pattern as a consequence of the properties of the system’s components. This hypothetical mechanism could be tested experimentally, and hence it is scientifically useful. For example, we could test it by designing white squares with black edges and mix

them with “wild type” white squares and analyze the patterns achieved.

The lack of explanatory power of the morphogenetic mechanism for inflorescence types proposed in the paper by Prusinkiewicz and co-workers is not the main limitation of the paper. Indeed, the transient mechanism restrains developmental possibilities to a set that excludes many variants not found in nature. However, it seems likely that there are a number of different mechanisms that could recover a similar set of inflorescence two-dimensional architectural types. The necessary rules of all such alternative mechanisms would imply producing, after a branching event, exclusively one of three types of structures: (1) those in which older meristems develop into flowers and younger ones do not, (2) those in which younger meristems develop into flowers and older ones do not, and (3) those in which old and young meristems flower at the same time. These cases correspond to cymes, racemes and panicles, respectively.

Here we provide an example of a set of rules that, just as the transient mechanism, produces the observed types of inflorescences. Consider a hypothetical protein “*T*” that is synthesized in leaves and travels through the phloem towards shoot meristems. Some real molecules, such as the messenger RNA of the *FLOWERING LOCUS T (FT)* gene, show this kind of behavior (Wigge *et al.*, 2005). Let us also assume two proteins, “*A*” and “*L*,” that are synthesized and are enabled to exert their function only in meristematic cells. *A* promotes its own synthesis, and that of *L*. *L* is a small protein that inhibits the synthesis of *A*, and diffuses to nearby meristems, where it can also repress the synthesis of *A*.

It is reasonable to consider that main (apical) meristems receive an amount of *T* that is higher than that received by derived (lateral) meristems, maybe because of differences in the width of vascular bundles in the main and derived shoots. Besides, we consider that *T* promotes *A* activity. Hence, in the apical meristems there will be a high level of *A* and *L* expression, and in nearby lateral meristems there will only be *L* activity, because of the diffusive and regulatory properties of *A* and *L*. This mechanism suffices to distinguish between apical and derived meristems. Now, let us also assume that the activity of *A* affects somehow the decision of turning into a floral meristem. If *A* represses flowering, then the apical meristems will remain indeterminate and lateral meristems, where there is *L* but not *A* activity, will turn into floral meristems; hence racemes will arise. In contrast, if *A* promotes flowering, then the apical meristems will flower and lateral meristems will keep on branching; therefore cymes will result. Different kinds of racemes and cymes could be obtained by tuning parameter values such as branching, decay and diffusion rates. We will refer to this hypothetical process of inflorescence development as our “toy model” (Fig. 1).

We are not claiming that the transient mechanism, by Prusinkiewicz and collaborators, is wrong and our toy mechanism corresponds to a more accurate description of in-

Figure 2. Different developmental models give rise to different morphospaces which may include the same types of “phenotypes” (“blue but no red” is not observed in either case), but these have different distributions in the morphospace and different transition probabilities among them. Blue and red triangles stand for the presence of a certain phenotypic trait. Whereas in the top panel a single genetic change suffices to transit from “red but no blue” phenotype to the “neither red nor blue” phenotype, two changes are required for the same change in the bottom panel. ‘B’ denotes a functional allele, and ‘b’ denotes a null nonfunctional allele of the B locus, whereas ‘A’ denotes a functional allele, and ‘a’ denotes a null nonfunctional allele of the A locus.

florescence development. The point we are trying to make is that there might be several alternative mechanisms, such as the transient and toy ones, that could reproduce similar sets of patterns. Hence, the replication of patterns observed in nature does not guarantee that the mechanism that underlies it in the real world has been discovered or explained. More importantly, two different mechanisms that produce the same structures might generate different adaptive landscapes if the relative positions of the structures inside the landscape depend on the developmental model being postulated (see Fig. 2). Hence, development does not restrain evolution just by limiting the set of available forms, but also by making some structures more probable than others given an ancestral phenotype, therefore biasing the production of variation (Lewontin, 1974; Maynard-Smith *et al.*, 1985). It follows that not any mechanism that generates the set of observed structures will be useful to predict the proximity of phenotypes within the morphospace, and hence the likelihood of going from one to another one during the course of evolution.

There are two nonexclusive and mutually supporting ways to discern among alternative developmental mechanisms. The first one is to ground the model on experimental evidence. This implies incorporating all interactions that have been supported experimentally, and not only taking *a priori* components which behaviors seem to be sufficient to generate similar patterns to those of structures found in nature. Then, such models can be validated by altering them *in silico* and addressing if such alterations recover observed

patterns in mutants. Recently, gene network models grounded on experimental data aiming at uncovering the logic of gene regulation, that underlies observed patterns of gene expression during development in plants and animals, have been proposed (e.g. von Dassow *et al.*, 2000; Albert and Othmer, 2003; Espinosa-Soto *et al.*, 2004; Jonsson *et al.*, 2005; Li *et al.*, 2006; Benítez *et al.*, 2007). However, these gene regulatory network models have not yet been explicitly incorporated in evolutionary models of the studied structures.

The second way to validate if a proposed mechanism is congruent with experimental data is to test if predicted behaviors derived from the models assumptions are indeed found in nature or can be induced in some way. For example, the model by Prusinkiewicz and collaborators creates patterns because each meristem is able to sense its own age and follow some rules accordingly. Hence, it is assumed that the fate of a meristem depends only on its intrinsic dynamics and not on interactions with other meristems or on communication mechanisms between this and the other parts of the plant. If the transient mechanism indeed operates in plants during inflorescence development, one would predict that it would be possible to find somatic mutations that could change the behavior of some meristems resulting in chimeric inflorescences. However, this might not be the case because communication mechanisms, such as diffusion of morphogens (Crick, 1970; Kim and Zambryski, 2005), active transport such as that of the phytohormone auxin; (Vieten *et al.*, 2007), signaling among cell layers (as in meristem patterning by WUS and CLV proteins; Doerner *et al.*, 2000) among many others, might constitute mechanisms important for coordinated responses in organisms. However, our skepticism is not sufficient to discard the transient mechanism, and experiments would be needed to this end.

In an attempt to validate their transient mechanism, the authors postulate a developmental model that incorporates the action of only two genes (*LFY* and *TFL*) on a continuous hypothetical variable (*veg*) which levels determine if shoots are vegetative or reproductive and may therefore represent a flower identity factor. *LFY* and *TFL* are components of a larger and complex regulatory network of which many more components have been characterized already. To reduce all the known elements and regulatory interactions relevant for this system to a pair of interacting genes implies averaging, at least, the genetic context in which these genes are embedded. But biological systems are typically nonadditive and, therefore, the loss of information due to averaging is hard to justify, especially in a case where a large amount of experimental information is available. To elaborate on this issue, to us critical, we mention below just some of the elements and interactions that were mistakenly obviated in the developmental account presented by Prusinkiewicz and collaborators.

It has been shown that *LFY* activates different targets

depending on its interactions with other factors (e.g., Percy *et al.*, 2002) whose spatio-temporal expression patterns are far from trivial. Furthermore, *LFY* and *TFL* themselves are regulated in a complex manner by other genes that are not included in the author's account, such as *APETALA1*. Importantly, the genes that regulate *TFL* and *LFY* do not seem to be all acting in the same direction as plant age in their model and, more importantly, some of them are also regulated by *TFL* or *LFY*, so they cannot be collapsed in one input with no feedback. *LFY*, *TFL* and other floral and shoot meristem identity genes have been shown, instead, to hold complex interactions (Baurle and Dean, 2006; and references in Prusinkiewicz *et al.*, 2007).

In the transient mechanism the levels of a hypothetical substance, *veg*, define whether a meristem is reproductive or vegetative. However, we argue that the available experimental evidence suggests very different properties for a meristem identity crucial factor. For example, the MADS-box gene, *APETALA1*, is a floral identity marker, however *veg* may not represent *API* because the effect of *LFY* and *TFL* over *API* is opposite to that proposed for *veg* and *API* does not change gradually as *veg* and *LFY* do. The existence of *veg* could be compatible with the existence of *API* if *veg* mediated the interactions between *LFY* and *API*, but this is not the case, since *LFY* binds directly to the *API* promoter (Wagner *et al.*, 1999). Furthermore, the activity of *API* does not depend only on *LFY* and *TFL*, but also on other independent factors, such as *FT* and *FD* (Wigge *et al.*, 2005). Therefore, there is no reason to reduce the network by excluding *API* among other characterized genes that affect inflorescence architecture. Moreover, including *API* could avoid making up a new variable, such as *veg*, which behavior may not be monitored in real plants. Furthermore, other genes, such as *AGL24* seem to have an important role on the decision of vegetative *vs.* floral meristems in *Arabidopsis* inflorescences and has a feedback regulation with *LFY* (Yu *et al.*, 2004), being only one more of the factors that should be included in a model that addresses inflorescence patterning.

The unjustified reduction of a complex network constituted by several genetic and nongenetic elements, to a pair of interacting genes, leads to conclusions that might not be accurate. The evolution of development and phenotypical traits is a longstanding question in evolutionary biology. However, to address this issue by studying the variational properties of a developmental mechanism, this has to be independently validated. Otherwise, even if the evolutionary assumptions and formalisms are correct, and if the fitness value associated to each genotype is accurate, we can end up studying the evolutionary consequences of a mechanism that has never existed. Therefore, the inferred dynamics and transitions might be misleading. In conclusion, the transient mechanism, by Prusinkiewicz *et al.*, seems too simplistic and *ad hoc* to yield the observed types of inflorescence architectures.

In conclusion, we think that the paper has the merit of addressing how developmental processes constrict phenotypic morphospaces and how fitness values can be associated with every point in these morphospaces for a specific case. In doing so it attempts to integrate an architectural model, some molecular genetic data for the trait under analyses and a fitness landscape for evolutionary inferences under contrasting environments. Such an integrative approach could in turn be used to make predictions about which transitions among phenotypes are possible and more probable during evolution. The latter should be approached by evolutionary developmental biology studies in which transitions among types assuming alternative developmental models are mapped onto phylogenetic trees. This is certainly important, innovative and necessary in order to understand phenotypic evolution. However, a developmental approach to this issue should capture the complexity of the processes underlying morphogenetic restrictions, and be validated with data other than the expected results. The challenge remains ahead.

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REFERENCES

- Albert, R, and Othmer, HG (2003). "The topology of the regulatory interactions predicts the expression pattern of the segment polarity genes in *Drosophila melanogaster*." *J. Theor. Biol.* **223**, 1–18.
- Baurle, I, and Dean, C (2006). "The timing of developmental transitions in plants." *Cell* **125**(4), 655–64.
- Benítez, M, Espinosa-Soto, C, Padilla-Longoria, P, Díaz, J, and Álvarez-Buylla, ER (2007). "Equivalent genetic regulatory networks in different contexts recover contrasting spatial cell patterns that resemble those in *Arabidopsis* root and leaf epidermis: a dynamic model." *Int. J. Dev. Biol.* **51**, 139–155.
- Crick, FH (1970). "Diffusion in embryogenesis." *Nature (London)* **225**, 420–422.
- Doerner, P (2000). "Plant stem cells: the only constant thing is change." *Curr. Biol.* **10**, R826–R829.
- Espinosa-Soto, C, Padilla-Longoria, P, Alvarez-Buylla, E (2004). "A gene regulatory network model for cell-fate determination during *Arabidopsis thaliana* flower development that is robust and recovers experimental gene expression profiles." *Plant Cell* **16**, 2923–2939.
- Hogeweg, P (2000). "Evolving mechanisms of morphogenesis: on the interplay between differential adhesion and cell differentiation." *J. Theor. Biol.* **203**, 317–333.
- Jonsson, H, Heisler, M, Reddy, GV, Agrawal, V, Gor, V, Shapiro, BE, Mjolsness, E, Meyerowitz, EM (2005). "Modeling the organization of the WUSCHEL expression domain in the shoot apical meristem." *Bioinformatics* **21**, Suppl. 1, i232–240.
- Kim, I, and Zambryski, PC (2005). "Cell-to-cell communication via plasmodesmata during *Arabidopsis* embryogenesis." *Curr. Opin. Plant Biol.* **8**, 1–7.
- Lewontin, R (1974). *The Genetic Basis of Evolutionary Change*, Columbia University Press, NY.
- Li, S, Assmann, SM, and Albert, R (2006). "Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling." *PLoS Biol.* **4**(10), e312.
- Maynard-Smith, J, Burian, R, Kauffman, S, Alberch, P, Campbell, J, Goodwin, B, Lande, R, Raup, D, and Wolpert, L (1985). "Developmental constraints and evolution: a perspective from the Mountain Lake Conference on development and evolution." *Q. Rev. Biol.* **60**, 265–287.
- Parcy, F, Bomblies, K, and Weigel, D (2002). "Interaction of LEAFY, AGAMOUS and TERMINAL FLOWER1 in maintaining floral meristem identity in *Arabidopsis*." *Development* **129**, 2519–27.
- Prusinkiewicz, P, Erasmus, Y, Lane, B, Harder, LD, and Coen, E (2007). "Evolution and development of inflorescence architectures." *Science* (in press).
- Vieten, A, Sauer, M, Brewer, PB, and Friml, J (2007). "Molecular and cellular aspects of auxin-transport-mediated development." *Trends Plant Sci.* **12**(4), 160–168.
- von Dassow, G, Meir, E, Munro, EM, and Odell, GM (2000). "The segment polarity network is a robust developmental module." *Nature (London)* **406**, 188–193.
- Wagner, D, Sablowski, RW, and Meyerowitz, EM (1999). "Transcriptional activation of APETALA1 by LEAFY." *Science* **285**, 582–584.
- Wigge, PA, Kim, MC, Jaeger, KE, Busch, W, Schmid, M, Lohmann, JU, and Weigel, D (2005). "Integration of spatial and temporal information during floral induction in *Arabidopsis*." *Science* **309**, 1056–1059. Erratum in *Science* **312**, 1600.
- Yu, H, Ito, T, Wellmet, F, and Meyerowitz, EM (2004). "Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development." *Nat. Genet.* **36**, 157–161.