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THINK AGAIN

Insights & Perspectives

Evo-devo beyond development: Generalizing evo-devo to all levels of the phenotypic evolution

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Abstract

A foundational idea of evo-devo is that morphological variation is not isotropic, that is, it does not occur in all directions. Instead, some directions of morphological variation are more likely than others from DNA-level variation and these largely depend on development. We argue that this evo-devo perspective should apply not only to morphology but to evolution at all phenotypic levels. At other phenotypic levels there is no development, but there are processes that can be seen, in analogy to development, as constructing the phenotype (e.g., protein folding, learning for behavior, etc.). We argue that to explain the direction of evolution two types of arguments need to be combined: generative arguments about which phenotypic variation arises in each generation and selective arguments about which of it passes to the next generation. We explain how a full consideration of the two types of arguments improves the explanatory power of evolutionary theory. Also see the video abstract here: https://youtu.be/Egbvma_uaKc

KEYWORDS
development, evolution, evolutionary theory, protein evolution

INTRODUCTION

Evolutionary theory aims to understand how organisms change over generations. In a nutshell, the theory originally proposed that organisms have changed over generations and that this evolution is explained by natural selection on heritable variation exhibited by organisms.[1] One main effort of early evolutionary biology was to prove that organisms have indeed changed over generations and that this evolution is due to natural selection.[1–3] In the early 20th century the rules of inheritance were discovered.[4–8] These rules, together with population thinking, gave rise to population genetics.[2,9–12] Population genetics studies how gene frequencies change over generations due to processes such as natural selection, drift, migration, mutation, recombination, and so on.[2,9–13]

According to Huxley and others,[3,10–12] population genetics, together with empirical evidence from paleontology, ecology, cytogenetics, zoology, botany, and some other fields, crystallized into what is called the modern synthesis of evolutionary theory. Correspondingly, population genetics is often regarded as the theoretical core of the modern synthesis.[3,10–13] Current evolutionary theory is generally seen as a relatively smooth continuation of the modern synthesis. It shares its main precepts while, at the same time, it includes a more detailed understanding of many of its fundamental processes: inheritance, population dynamics, and so on.[13–15]

This article focuses on the question of the direction of evolution. If one characterizes phenotypes by a set of traits and represents them in the space of their possible values (a trait space or morphospace, see Figure 1), there are three basic evolutionary questions one can ask: an existence question, a rate question and a direction question. The existence question is whether there is evolution. This question has long been answered.[13–15] The rate question is about which factors determine the rate at which organisms evolve (i.e., how fast they move in the trait space). We consider that current evolutionary

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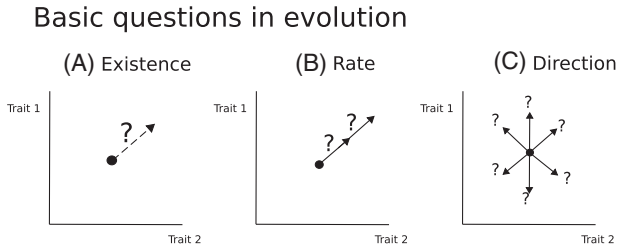

FIGURE 1 The figure shows an idealized phenotypic space characterized by two traits. The black circle represents the population mean. (A) Existence question: whether a population would change the values of its traits from over time. (B) Rate question: how fast change occurs. This is the magnitude of the vector going from the mean trait values in one generation to those in a latter generation. (D) Direction question: How would the traits change in the population over time? This direction is the components of the same vector

theory has a relatively good grasp on this question. For the direction question, however, there is still considerable room for improvement. The question of direction is about how phenotypes change over time. Schematically, one can describe this direction as a vector going from the values of traits in one generation to the values of traits in the next generation in a given population, see Figure 1. The direction question is about the processes that determine how each trait changes over time, in itself and in relationship to others.

The dominant view in the modern synthesis was that natural selection is the main factor determining the direction of phenotypic evolution.[3,12,16] Other processes (e.g., migration, linkage disequilibrium, etc.) were also considered to affect evolution and its rates, but without having a major deterministic influence in its direction.[4,9–14,16] An exception is drift, that can affect the direction of phenotypic evolution in small populations but in an unpredictable, stochastic manner.[17]

Natural selection can only act on existing phenotypic variants. This implies that for natural selection to be the main determinant of the direction of evolution, phenotypic variation has to be possible and equally likely in all directions (i.e., isotropic).[18–21] If that is not the case, then the factors determining which phenotypic variation is likely, and which is not, have also a crucial role in determining the direction of phenotypic evolution. This is, in fact, a major tenet of evo-devo (also called evolutionary developmental biology or developmental evolutionary biology)[18–30]: morphological variation is not isotropic and the process of development is a crucial factor determining which morphological variants are likely to occur in each generation and population (i.e., the directions of morphological variation). Because of its role in determining morphological variation, development is seen in evo-devo as a major factor determining the direction of morphological evolution.[18–30] Natural selection would be the other major factor: In each generation and population, development would "propose" directions of morphological variation and natural selection would choose among these.[18–30] This way, development and natural selection will determine, together, how morphology changes (i.e., the direction of evolution) (see Figure 2).

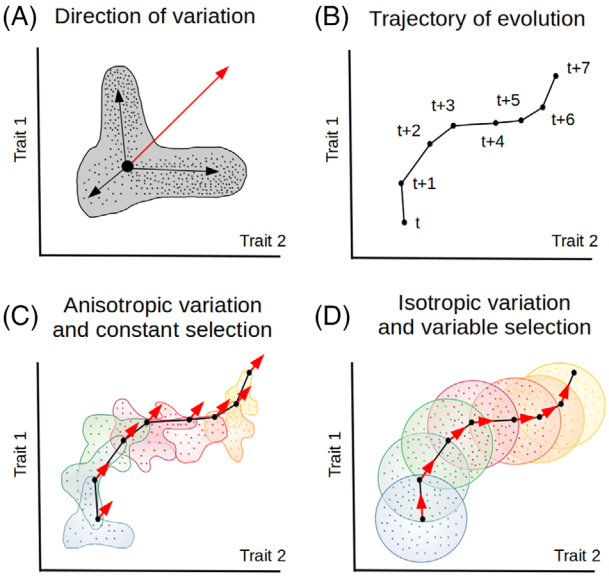

FIGURE 2 Direction of phenotypic variation and phenotypic evolutionary trajectory: (A) Idealization of the possible directions variation in two traits for a given population. The gray area represents the possible variation (variability), the dots represent the encountered variation, the black arrows the directions of variation and the red arrow the direction of natural selection. (B) Idealized trajectory of phenotypic evolution over eight generations from t to t+7 (assuming non-overlapping generations and plotting only the population mean). (C) Evolutionary trajectory explained by phenotypic variation that is not isotropic and natural selection that is constant in direction. The red arrows represent the direction of natural selection and the colored areas show the possible variation in each generation and the dots show the encountered variation in the population. (D) Evolutionary trajectory explained by assuming that the direction of evolution is determined by the direction of natural selection acting on isotropic variation. The red arrows represent the direction of natural selection, the colored circles show the possible variation in each generation (variability) nd the dots show the encountered variation in the population

The aim of this article is to argue that this latter evo-devo perspective on the direction of evolution as being determined by both development and natural selection applies not only to morphological evolution but to evolution in general (e.g., at all phenotypic levels). At phenotypic levels other than morphology (e.g., molecular structure, cell biology, behavior) there is no development as such but there are other processes that can be seen, in analogy to development, as constructing the phenotype (e.g., folding for protein structure, membrane morphogenesis and gene networks for cell biology, learning for behavior, etc.). In here, we call the processes constructing the phenotype generative processes. Since generative processes are responsible for constructing the phenotype, we will argue that phenotypic variation cannot really be random (i.e., isotropic) at any phenotypic level but that, instead, each phenotypic level has some rules of variation (i.e., which phenotypic variants are possible and likely) and these rules are determined by each underlying generative processes. Then, we will argue that evolutionary hypotheses about the direction of evolution should consider,

together, two types of arguments: generative arguments about which phenotypic variants arise from generative processes in each generation and selective arguments about which of these variants pass to the next generation. We discuss how a fully explicit consideration of generative arguments can greatly improve the explanatory and predictive capacity of evolutionary theory.

Our discussion will primarily focus on the morphological level and the protein structure level. Our intention is not to detail the similarities between these two levels but rather to provide two examples of how knowledge on generative processes can be used, together with natural selection, to better understand the direction of phenotypic evolution.

DEVELOPMENT, THE DIRECTION OF MORPHOLOGICAL VARIATION AND EVOLUTION

Development implies a continuous sequence of morphological changes going from the zygote (or similar early structure such as gemmula[31]) to the adult. The morphology of a multicellular organism can be defined as the spatial distribution of its cells, cell types and extracellular matrix (ECM). This implies that for morphology to change, embryonic cells (or ECM) need to change their spatial location or differentiation state (or usually both). Cells move because they regulate force-generating cell behaviors (e.g., cell contraction, cell growth, ECM secretion) or because they are attached to cells doing so (i.e., through cell and ECM adhesion).[31] Specific morphologies also arise because different movements occur in different regions of the embryo. This regionalization is often attained by cells in different regions secreting extracellular diffusible signals. Due to the physical process of diffusion, cells close to one such region receive the signal while the cells far away from it do not, or not at a concentration high enough. This way each signal can create new spatial regions: each region formed by the cells receiving the signal or receiving it within specific concentration interval.[31] Cells may respond to signal concentration by simply differentiating and secreting the same or different signals to further refine the signal spatial distribution (as in reaction-diffusion mechanisms[32]) or create new regions. New spatial regions can also arise through intracellular networks of gene interactions that effectively add or subtract existing regions.[33] Cells also respond to incoming signals by changing their mechanical properties or regulating cell behaviors, thus, leading to further movements. The morphological outcome of these movement also depends on the mechanical interactions of cells and tissues and on their mechanical properties.[31] These movements also affect the cells secreting and receiving signals and, thus, which regions are induced by signaling and with which shapes.[34]

Overall, each specific morphological change in development can be seen as arising from a distinct developmental mechanism (i.e., a specific network of extracellular signals, gene interactions and the cell behaviors and mechanical properties these regulate) and a set of associated spatio-temporal interactions.[18–23] Different authors in evo-devo conceptualize developmental dynamics using terms different from the ones we use (e.g., processes, dynamic patterning modules, developmental programs)[18–24,35] but the overall idea is similar: the effect of a mutation (i.e., a change at the DNA-level) on morphology cannot be understood from the gene (or cis-regulatory region) it affects but it needs to be understood from the developmental network of interactions (i.e., developmental mechanism, processes, programs, etc.) in which this gene is embedded to construct morphology.[18–30] In that sense, development (e.g., each developmental mechanism), rather than just random mutation, can be seen as determining the direction in which morphology can vary in a population.[18–30]

Although most evo-devoists consider that development is important to understand evolution because of its role in determining morphological variation,[18–30] different researchers diverge on how, or whether, to use this idea to study evolution and development. From its beginnings, the bulk of evo-devo research has consisted in comparative developmental biology.[20,25,26,29,30] The primary question of this research is not so much about how development affects morphological evolution but about how development itself evolved or, more explicitly, about the genetic and developmental bases of the differences between species.[25,26,29,30] This research focuses on topics such as the changes in developmental genes,[36,37] gene expression[38,39] or gene interactions over phylogenies.[30,40–42] Based on this comparative information, this research also tries to make inferences on some macroevolutionary aspects of morphological evolution, such as the sources of homology,[43–45] convergent evolution[46–48] among others.[29,49,50]

In addition to this comparative development branch of evo-devo, there is also a branch of evo-devo that is more explicitly focused on understanding how development influences morphological evolution.[18–21,26–28] Typically this research tries to understand how development constructs morphology and, from that, understand how morphology can vary. From how morphology can vary and natural selection, this branch tries to understand the direction of morphological evolution.

For most organs or body parts we do not understand how morphology is formed and, then, how it varies. Probably, because of that, most of the research on how development affects the direction of evolution has been eminently conceptual.[18–28,30] One widely-used concept arising from this body of work is that of the genotype-phenotype map or GPM.[51,52] That is the relationship, or correspondence between each genetic change and its phenotypic effect (notice this effect can also depend on the environment.[20,22,53,54]

Some concepts relate to statistical properties of the GPM. An example is genetic robustness, the capacity of organisms to withstand mutations without changing their phenotype, that is, different genotypes are associated with the same phenotype.[52,55] A concept related, but different, from the GPM is that of variational properties.[20] This is not a correspondence between genotypic and phenotypic variants but just the actual ensemble of morphologies that are possible from a given developmental mechanism.[20] By possible we mean the morphologies that would arise in different environments and for different genetic changes as long as these changes do not affect the topology of the developmental mechanism (e.g., which genes regulate which other genes or cell behaviors).

Another related concept is that of evolvability. This concept does not originate in evo-devo[51,52,56] and it is widely used outside it.

Evolvability is understood in different ways by different authors[57,58] but, in many cases, it relates to the capacity to evolve. Since natural selection can only act on existing phenotypic variation, the capacity to evolve depends on the capacity to exhibit phenotypic variation. For some authors[59] then, development would be a major factor determining evolvability.

The study of how development affects morphological variation and evolution does not only rely on concepts, there are also studies on the evolution of specific organs or body parts. This approach is based on mathematical models of organ development. Most experiments in developmental biology rely on relatively gross manipulations of development that lead to morphological changes that are usually much more dramatic than those usually observed in natural populations.[49,60] Nevertheless, the understanding of developmental dynamics arising from experimental developmental biology has allowed to build mathematical models of organ development that can reproduce major aspects of their morphological variation,[61–64] even within populations.[65] There are even models that are applicable to animal development and morphology as a whole.[66–67] In the coming sections we explain how these approaches and similar approaches in protein evolution can be used to better understanding of the direction of phenotypic evolution.

PROTEIN EVOLUTION AS "EVO-DEVO"

The 3D structure of a protein (i.e., its tertiary structure) is a phenotype. The analogy between a protein's structure and morphology is quite straightforward, the latter is the spatial arrangement of cells, cell types and extracellular matrix in 3D space while the former is the spatial arrangement of atoms and chemical bonds in 3D space. The structure of a protein is determined, ultimately, by the DNA sequence coding for it and the surrounding micro-environment within the cell. However, there is not a simple, direct relationship between primary and tertiary structure but a complex physical process of folding of one into the other[68,69] that lasts in the order of milliseconds for most proteins.[70]

In globular proteins, folding results into one or few stable structures.[37] Many other proteins, however, have intrinsically disordered domains that only fold after interaction with other molecules.[71–73] Furthermore, some protein domains, or even entire proteins, may never fold into a stable structure. Instead, their structure fluctuates over time within an ensemble of possible structures that is relatively large but not random.[71–73]

During protein folding, electrostatic, hydrogen bond, hydrophobic, and van der Vaals interactions occur between atoms in a protein and between these atoms and the solvent (e.g., water) or other molecules.[69] The physical interactions occurring at each moment during folding restrict and direct the movement of each part of the polypeptide chain (in respect to the Brownian motion intrinsic to the molecular level) and, thus, facilitate the physical approachment of different protein regions and further interactions.

Usually, the first step in protein folding consists in different segments of the polypeptide chain acquiring different secondary structures. For steric reasons, the angles between the chemical bonds in the polypeptide chain can only take a limited range of values (i.e., the polypeptide chain is relatively rigid). These angles are only compatible with the formation of two major secondary structures: α-helices and β-sheets.[74] Some other structures are possible in intrinsically disorganized proteins but the intrinsically disorganized domains that fold upon interaction, also fold into α-helices and β-sheets.[73]

Both α-helices and β-sheets primarily stabilize through hydrogen bonds that form between N—H and C = O groups within the backbone of the polypeptide chain and without requiring much bending in such chain. Different parts of each α-helix and β-sheet in a protein differ in the degree of hydrophobicity of their amino acid residues. Depending on this hydrophobicity, secondary structures in a protein rearrange in space to maximize the number of hydrogen bonds with the solvent (e.g., water) or screen the hydrophobic parts of the structure from the solvent.[69,75,76] This and other interactions between the amino acid residues lead to the stabilization of a specific protein structure, either permanently or transiently upon binding.

The dynamic network of physical interactions occurring during folding determines not only a protein's structure but also the directions in which its structure will vary with mutation (i.e., variation is not isotropic). This can be understood, for example, by considering that most proteins fold into a combination of α-helices and β-sheets. From this consideration it follows that the structural directions of change possible from mutations changing a single amino acid (i.e., a substitution of an amino acid for another amino acid in the same site in the polypeptide chain) will often consist of slight rearrangements in the relative spatial position of existing secondary elements (e.g., an amino acid substitution leading to the formation of an additional hydrogen bond between two α-helices and a change in their relative positioning) or in relative changes in the sizes of existing secondary elements (e.g., an amino acid substitution leading to a site being incorporated into a nearby α-helix along the sequence). In some rare cases, a single amino acid substitution can lead to global structural changes, for example, changing from an all β-sheets protein to an all α-helix protein.[77]

Determining the structure of a protein experimentally is costly and, thus, there are not many studies experimentally exploring the range of structural changes that are possible in a protein by substituting its amino acids one at a time (i.e., the space of mutant phenotypic changes and, thus, possible directions of variation).[78] There are, however, experimental studies on protein evolution that indirectly exemplify how the possible directions of structural change are determined by the physical interactions occurring between atoms and the structure of the protein at a given moment during folding. Two types of studies are especially revealing in that sense.

The first type of studies are artificial evolution experiments aiming to increase the catalytic activity of specific enzymes for specific reactions (usually different from the one the enzyme naturally catalyzes). In each generation, point mutations are artificially induced at different sites in a gene.[79,80] Each gene variant is then translated into its corresponding protein variant. Fitness is then experimentally measured as the catalytic activity for a specific reaction of interest. In each generation, the set of variant proteins with the highest fitness are cho-

sen as the "parents" for the next generation and the whole process is repeated over generations. This way improvements in the catalytic activity of several orders of magnitude are achieved in few generations (e.g., 18 generations to evolve a phosphotriesterase enzyme to catalyze to an aryl, carboxy-esterase reaction[81]).

This type of study shows that most of the amino acid substitutions that become fixed in the evolutionary experiments are neutral.[80] Adaptive amino acid substitutions tend to occur early in evolution and, on average, early occurring substitutions are more adaptive than late occurring substitutions. Only a handful of these adaptive substitutions occur in each experiment, even if catalytic activity can experience an up to 1000-fold increase.[80] Most importantly for our discussion, the adaptive value of the substitution of one amino acid for another in a specific site is path-dependent[80,82–84] For example, a specific amino acid substitution at a site may have been neutral when it fixed, but this same amino acid substitution at this same site would have been maladaptive (or adaptive) if it would have occurred later due to a specific trajectory of fixation of other neutral substitutions at other sites in the protein.[82–84] Likewise, an adaptive substitution at a given generation may become neutral (or maladaptive) at a later generation. This occurs because each amino acid substitution alters, locally, the physical space within the protein and, thus conditions, which amino acids substitutions in nearby sites would not alter protein structure, or would alter it in specific ways that the experimenter can see as adaptive or not. Path-dependency is simply another way to say that the physical interactions occurring during folding and maintaining protein structure, determine in which directions would a protein vary when there are further amino acid changes (i.e., no isotropy).

The second type of studies are based on mathematical models that try to predict the structure of biomolecules from their sequence. From these models, some statistical properties of the genotype-phenotype map are obtained and these are used to try to understand some aspects of structural and sequence evolution. In the case of the secondary structure of RNA, these models are relatively precise.[85,86] Protein folding is a complex process and, thus, existing models rely on several simplifying assumptions: considering only two types of amino acids (hydrophobic and polar ones),[87,88] two instead of three spatial dimensions or similar coarse-graining abstractions.[89–93]

The RNA models have been used to suggest that folding can be understood as a process analogue to development and that, thus, theoretical models on folding should help in evo-devo research[86]: specifically some statistical properties of the genotype-phenotype map for RNA should also apply to the protein and morphological level as well. The most important of these are: (i) many genotypes give rise to the same phenotype (also called robustness[55], (ii) some phenotypes are much more common than others and (iii) it is possible to evolve from one phenotype to any other phenotype by accumulating many neutral mutations and one or few non-neutral mutations.[55,86,94–95]

This third property stems from the existence of large neutral networks. These are sets of genotypes that can be connected through accumulating mutations that do not change the phenotype. Some of these networks include genotypes that are one mutation away from most possible phenotypes in the RNA models.[55,86,94–95] This lead some authors to argue that populations can spread over these neutral networks and from these easily access most possible phenotypes.[55] For these authors then, the existence of these networks facilitates phenotypic evolution, or in their terms, robustness leads to evolvability.[55] Similar theoretical studies on general mathematical models of animal development show, however, that the third statistical property does not apply to morphological evolution in general.[67] For the topic of this discussion, the most important property shared by all these systems is that phenotypic variation is anisotropic: from a given phenotype it is easier to mutate to some phenotypes than to others and that this depends on the underlying generative process, that is, folding.

THE ADVANTAGES OF CONSIDERING GENERATIVE ARGUMENTS IN EVOLUTIONARY THEORY

A more realistic depiction of the factors determining the direction of phenotypic evolution

The discussion in the previous sections shows that even at the most basic phenotypic level, that is, RNA and protein structure, phenotypic variation cannot be isotropic. Since these are the levels that are closer to the DNA level, it is clear that phenotypic variation should not be isotropic at any level. Instead, the directions of possible phenotypic variation are determined by how the underlying generative process work to produce the phenotype at each level. In spite of that, most current evolutionary theory does not consider generative processes[18–21,28] and, thus, explains the direction of evolution using, mostly, selective arguments. This requires either assuming that phenotypic variation is isotropic, as explained in previous sections, or taking phenotypic variation as given. In the latter case, one can, for example, measure the phenotypic variation in a population and then explain the direction of evolution based on the selection on this variation, or its heritable part as in done in quantitative genetics.[96] However, this provides an incomplete understanding of the direction of evolution since one is not explaining which phenotypic variants arise in a population and why those and not others. In other words, one only explains which directions selection would choose, but not which directions can be taken. An evo-devo perspective considering generative processes and arguments, on the contrary, can be seen as more general because, if generalized to all phenotypic levels, it considers a larger proportion of the factors determining the direction of phenotypic evolution (i.e., generative processes and natural selection).

Better predicting phenotypic evolution

Evolution is a historic and largely stochastic process that defies accurate predictions, but approximate predictions are possible. In the field of quantitative genetics, for example, these predictions rely on measuring phenotypic variation in a real population, assuming a linear GPM and on information about natural selection.[97]

Generative arguments can also be used to better predict how a phenotype evolves over time. For that purpose the generative argument should be in the form of a mathematical model of the generative process of some phenotype. Such generative model should include the relevant microscopic interactions (e.g., genes and cells for development, amino acids or atoms for folding) and some hypothesis about how they are coordinated in space and time to construct the phenotype of interest (e.g., a protein structure, a morphology). Such model should predict, at least to some extent, how phenotypes arise from the generative process and how they change with mutations or some proxy of it (e.g., changes on how strongly molecules or cells interact in a generative process). For development there are several models of this kind for specific organs: *Drosophila* segmentation,[61] tooth morphogenesis,[65] wing morphogenesis,[62] limb development[63] among others. These models can reproduce the morphologies of specific mutants,[61,62,64] of different species[64,98–100] and subtle morphological variation in real populations.[65]

Some of these models have been combined with population genetics models to explicitly simulate phenotypic evolution. In these models there is a population, mutation on individual genotypes, a phenotype that arises from the genotypes through the modeled generative process and natural selection on the phenotype of individuals (in addition drift noise, sex, phenotypic plasticity or others things depending on the exact model). These "evo-devo" models try to predict the trajectories of evolution of the phenotype under different types of development and selection pressures.[99–105]

In the case of protein folding there are also models that can simulate some aspects of protein folding. Some of these have been used to simulate some aspects of the trajectories of protein evolution, such as the interdependence between amino acid replacements[106,107] or the rates of sequence evolution at specific sites.[108,109]

To our knowledge, mathematical models of generative processes have not been used to predict the evolution of a specific phenotype in a specific population, as for example in actual artificial selection experiments.[110] However, in the accompanying information box we describe how this can already be done from existing models of generative processes.

Understanding the evolution of generative processes

Classical evolutionary theory developed at a time when nearly nothing was known about generative processes and, understandably, it is not well suited to study their evolution Generative processes evolve and this implies that the enhanced understanding of phenotypic evolution that arises from understanding generative processes at a given time may not apply in the long-term. A better understanding of phenotypic evolution over time, thus, requires some understanding of how generative processes themselves evolve. This is an understanding of how the dynamics of generative processes evolve and from that, importantly, about their GPM and variational properties.

To study the evolution of generative processes we propose to use combine generative and selective arguments but for generative processes themselves, that is, *second order generative arguments*. First, one can consider that there would be variants of the generative processes (e.g., different developmental mechanisms or networks) and that these may lead to different phenotypic variants. Then, the direction in which a generative process would evolve from one generation to the next depends on two questions: (1) The directions in which this particular generative process is likely to vary by mutation, (2) Which of these directions of variation consist in generative process that "produce" phenotypic variation that is adaptive. Generative processes would evolve in these directions that are likely by mutation (question 1) and that are also likely to be selected positively based on the phenotypes they lead to (question 2).

To answer question 1 one needs to study which generative processes are possible at a given level and which ones are more likely to arise *de novo*, or from other ones through mutation. Ideally one would study these two questions experimentally. One would choose a phenotype (e.g., an organ or protein), identify the network of interactions underlying its generative process, experimentally vary each interaction, delete interactions and add new interactions (i.e., introduce some changes in the network topology), explore how these manipulations change the generative process dynamics and explore the adaptive value of the resulting phenotypes. To our knowledge, in no organ is development understood enough for this approach to currently feasible. Some of the experiments on protein evolution are compatible with this approach,[79–84] except that they do not yet explain why protein structure changes the way it does in the evolution experiments.

There are theoretical studies using the second-order generative arguments we propose.[101,111] In one of them we built a huge number of gene networks and studied which of them can lead to spatially heterogeneous pattern of gene expression in a mathematical model considering cell signaling in a cell lattice. We then explored how likely these networks can change into each other by mutation. With this approach we suggested how these networks would evolve over time in different environments with different selection pressures on the spatial gene expression patterns[111] and simulated their evolution to confirm these predictions.[101] In other studies we try the same approach but with more complete models that consider also cell behaviors and mechanical interactions.[66,67,112] Other similar theoretical approaches do not explicitly considers question 1, but are similar in considering different possibilities on how development could work (i.e., different variants of development) and which of them would prevail in evolution based on the adaptive value of the morphological they lead to.[30,35,113–116]

Very long-term evolution

Generative arguments can also be used to predict or understand some aspects of phenotypic evolution in the very long-term. This is possible even without mathematical models. The clearest examples are in protein structure evolution. Even if the number of possible protein sequences is huge (20 to the power of a protein's length in amino acids),

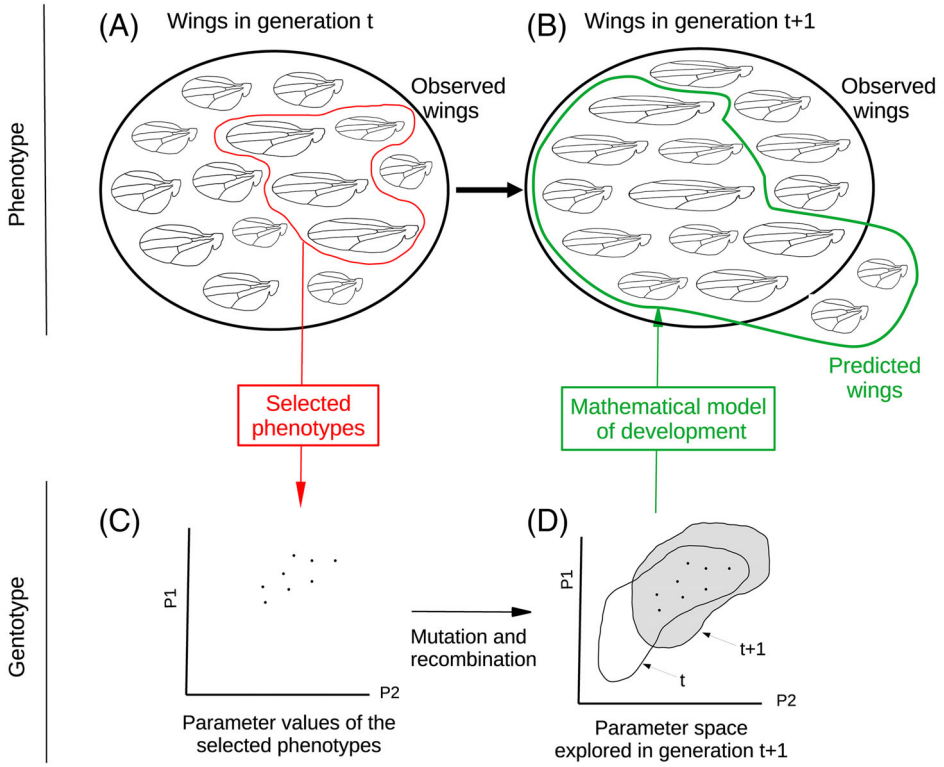

FIGURE 3 Schema showing how development can be used to predict evolution based on a hypothetical model of wing development (similar to that in[62]): (A) Phenotypes observed in the first generation (t) of the experimental population under artificial selection. The phenotypes selected to be the progenitors of the next generation are surrounded by the red line. (B) Phenotypes observed in the second generation (t+1) of the artificial selection experiment. The green line groups the phenotypes that are actually predicted from the model. The phenotypes encapsulated by the green line but outside the black circle are phenotypes that the model predicts but are not observed in the experimental population. (C) Plot representing the genotypes (i.e., actual developmental parameters) that in the model can reproduce the phenotypes selected in generation t. (D) In gray we represent the set of offspring genotypes (i.e., combinations of parameter values assigned to individuals) in t+1 arising from mutation and recombination of the genotypes of the individuals selected from the previous generation (t). Each offspring genotype (i.e., each point in the gray region) is run through the mathematical model of the generative process to obtain the predicted phenotypes (the ones surrounded by the green line in b)

it has long been known that all protein structures can be classified into roughly 10 000 fold classes, at least for globular proteins.[117–119] A fold class is defined as a particular relative arrangement of secondary structures and linking loops in space.

The finiteness of protein fold classes is not the result of natural selection but rather a mere consequence of the physics and geometry of the protein folding process. Because of their many intra-structure hydrogen bonds, secondary structures do not easily deform and can then be seen as relatively rigid bodies tied by short and more flexible protein segments (i.e., loops). It is energetically unfavorable that these loops cross each other or over-cross the polar surface of a secondary structures.[75] In addition, all globular proteins have a hydrophobic center and, thus, it is energetically favorable that this center is not in contact with a polar solvent (i.e., water). This screening occurs by the polar parts of loops and secondary structures coming to surround this center. Due to the size and shape of these structures and loops, there are only a small number of geometrically possible arrangements by which this screening can happen.[75,76] These arrangements are the folds. The finiteness of protein folds and their physical bases constitute a generative argument to predict long-term protein evolution: it occurs within the space of these possible folds. Natural selection would affect protein structure evolution but only by choosing among these possible folds.

Although less obvious, the range of morphologies that are possible for the whole of animals can also be predicted, to some extent, from development. According to Newman,[114,116] animal morphology can be understood as being composed of a small set of morphological motifs (e.g., invaginations, sheets, rods, etc.) and the formation of these motifs during development would be a consequence of some of the bio-physical properties of cells and cell collectives (adhesivity, contractility, etc.). Many of these cell properties were already present in the unicellular ancestors of animals.[35] These generic properties of cells and tissues could themselves evolve but they would do it very slowly and, thus, can be considered as setting the stage for what is possible in animal morphology evolution over hundreds of millions of years.[25,114,116] In other words, the prediction is that animal morphology would be made of combining these morphological motifs. The same has been suggested for plant morphology, although in this case the morphological motives are different because the physical properties of plant cells are different.[120]

Information box 1: How to predict evolution

Through the procedure we describe below, a mathematical model of a generative process (e.g., a mathematical model of wing development[100] or protein folding) can be used to actually predict how the phenotype should change in these experiments (e.g., actual artificial selection experiments on wing morphology as in[110] or as in the enzyme evolution experiments discussed in previous sections).

First, one needs to search for values of the parameters of the model that allow to reproduce the phenotypes observed in each individual in the starting population in an artificial selection experiment (see Figure 3). All mathematical models include a set of parameters that numerically specify some aspects of its microscopic interactions (e.g., how strongly a gene regulates a gene product or cell behavior or the primary sequence of a protein). These values are supposed to be genetically encoded, although in some cases indirectly, and, thus, these parameters can be taken as a proxy for the genotype. We call the in silico genotype of an individual the specific parameter values that allow the model to reproduce the phenotype of such individual.

Second, as in the case of quantitative genetics, one needs some information about which phenotypes within the starting population are selected for the next generation. In artificial selection experiments this information is readily available since it is the experimenter who decides which individuals are selected. One should then take the in silico genotypes of the individuals selected from the starting population to generate, by mutation and recombination of the parameter values, the genotypes of the next generation. The phenotypes of the offspring in the next generation is then simulated from the in silico genotypes using the generative process model (e.g., of development or protein folding). These phenotypes are, in fact, our prediction of evolution in each generation (see Figure 3). The individuals selected from the next generation would then be used to generate the predicted genotypes and then phenotypes for the other next generation and so on. The whole process is slightly similar to the approach of quantitative genetics, except that the underlying genotype-phenotype map is not assumed to be linear but it is simply taken from the mathematical model of the generative process.

Our predictions are actual sets of phenotypes. From these one can obtain other predictions such as trait means, trait covariances or other statistical measures of phenotypic variation (see Figure 3). This is simply not possible in quantitative genetics where one uses trait covariances to predict trait means and, thus, cannot predict trait covariances themselves or anything else. In addition, the generative models may also be able to predict changes in the nature of traits, the arising of fundamentally new traits (i.e., novelty *sensu*[115]).

Information box 2: The direction of phenotypic evolution in the modern synthesis and in current evolutionary theory

The modern synthesis is not a monolithic homogeneous theory and it includes different ideas from different authors. Two ideas on which most of its proponents agreed are the randomness of variation and the primacy of natural selection in determining the direction of evolution.[2,3,12,14–16] In this literature, random variation is understood in two ways: as variation being independent of its adaptive value and as variation being equally likely in all directions (i.e., isotropic). As described in the main text, the assumption that phenotypic variation is isotropic is logically required for the primacy of natural selection.

The assumption that variation is isotropic did not arise from any experimental evidence on how organisms are constructed from genetic information. In fact, at the time of the synthesis, the molecular bases of inheritance and development were poorly understood. What was known at the time of the synthesis was that chromosomes are bearers of inheritance and that changes (i.e., mutations[6]) in specific regions of these (i.e., alleles in specific loci) are statistically associated with specific phenotypic variants.[6,7,8,16] It was also known that these chromosomal regions obey Mendel's laws and that some phenotypic variants also obey these laws in some cases, at least roughly.[6,9,16] From this knowledge, the field evolved to understand evolution as the arising of mutations in loci and their spread, or decline, in populations over time due to the adaptive value of the phenotypic variants statistically associated with these loci (i.e., changes in gene frequencies over generations).[16] This is the understanding of genetics and phenotypic variation on which the modern synthesis was built. Later came the molecular biology revolution in which DNA was established as the basis of inheritance: chromosomes are made of DNA, DNA is replicated, DNA codes for proteins and RNAs, and so on. This allowed to equate loci with protein-coding and RNA-coding regions of DNA (i.e., genes) and alleles with variants in their sequence.[16]

Discovering the DNA bases of phenotypic variation and Mendel's laws was fundamental for the advancement of evolutionary theory but neither of these two discoveries explain, on their own, which directions of phenotypic variation are possible in a given generation. This, as we discuss on the main text, depends on the processes that actually built the phenotype (i.e., the generative processes). In essence, classical evolutionary theory had to either assume that phenotypic variation is isotropic or restrict itself to explain the direction only partially, that is, as selection among existing directions of variation but without explaining the latter.

CONCLUSIONS

In this article we have tried to explain that an explicit consideration of generative arguments in evolutionary explanations can largely improve the explanatory and predictive capacity of evolutionary theory. This we see as a key contribution of evo-devo to evolutionary theory. We consider that, in the same way that the discovery of the rules of inheritance gave rise to a major improvement in evolutionary theory (i.e., neo-Darwinism and the modern synthesis), the understanding of generative processes and its consideration in evolution may also be leading to a major improvement in evolutionary theory (e.g., some devo-darwinism or generative evolutionary theory).

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

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