Theoretical morphology of developmental asymmetries

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Summary
Morphospaces are theoretical tools to explore the morphological organization of living and fossil organisms. They have been used mostly by the paleontological community in an effort to get the most out of one of the only pieces of evidence that fossil material usually provide: the morphology of hard parts. The expectation with the establishment of theoretical morphospaces is that, by abstracting and modeling the fundamental parts of form, the multiple processes that generate the phenotypes of embryonic and adult structures will be better understood. In this essay, we suggest that ontogenetic trajectories can be used as the generative functions that build morphospaces, and propose approaches to build theoretical models for the establishment of left–right asymmetries during vertebrate heart embryogenesis.

Introduction
Following completion of the human genome and the birth of functional genomics, the analysis of morphology may seem something from the past. Nothing is further from the truth. The study of morphology can be carried out within a proper biological framework, as has been nicely shown by Edelman’s classic Topobiology.(1) The relationship between genes and phenotypes is now seen as a non-linear web of events, involving pleiotropy, self-organization processes, physical forces and environmental cues, all contributing to the final morphology of the organism.(2) A study of these multiple causes is in order, shedding some light on the mechanisms of developmental constraints that set in motion the dynamics of morphological deployment of the embryo.

A developing embryo is a self-organized system that generates spatial structures in a timely manner. In essence, this dynamical process establishes morphological boundaries within and between different organs.(3) These boundaries are established at molecular and cellular levels (e.g., interplay between BMP, chordin and noggin, during patterning of the dorsoventral identity in Xenopus embryos at the molecular level;(4) the formation of the somites at cellular levels.(5) One of the most general, important aspects of embryogenesis is the early establishment of a left–right axis, and the symmetries and asymmetries that appear around the medial plane of an animal. These developmental events can also be viewed as boundary formation processes, in which highly regulated developmental mechanisms have evolved to carry out two main things: a coordinated double-development at left and right sides that generates bilaterality and local ruptures of these mechanisms, forming asymmetries. Although we already know many details about the differential asymmetric gene expression during these processes,(6,7) we are far from understanding the cellular mechanisms that shape the asymmetry of the organs. This leads to a detachment between what is known to happen inside and outside cells and what has been observed in tissues during embryo formation. Most of our current knowledge is restricted to patterns of gene activity. This sort of information is incomplete, inasmuch as it only ties the presence of either mRNA or the protein itself to cellular events, failing to provide a mechanistic connection between both processes. Modeling this relationship can bridge the gap, offering new insights into the sort of restriction that operates during embryonic development.

In this paper, we first introduce an analytical tool, “theoretical morphospace”; that is part of a research program “theoretical morphology” mostly used in paleontology. Second, we elaborate on the notion of ontogenetic trajectory, showing that theoretical morphospaces can be built using ranges of variations for the parameters of the function that describes the ontogenetic trajectory. Finally, we propose the construction of a theoretical morphospace of asymmetries based on the generalization of ontogenetic trajectories to account for the initial morphological symmetry breaking process in vertebrate development, the looping of the heart.

Theoretical morphospaces
When we analyze phenotypic variation within and across taxa, we use different tools that help in partitioning gradation in nature variability. Thus, we recognize the existence of several possibilities embedded in a character (character states), which can vary either in a discrete or continuous manner. In both instances, the problem remains how to recognize that a character is a character and, at the same time, that one given variational grade is “different” from another. Two technical
terms are used to characterize variation across taxa. The term “disparity” characterizes variation in shape and size of a taxon, complementing “diversity” which applies to the number of different taxonomic groups within a given taxon.\(^{(8,9)}\)

Morphospaces provide broad frameworks in which the disparity of organismal form (variation of shape and size) can be suitably studied.\(^{(10)}\) Thus, a morphospace is a space of possible variation for a number of morphological traits. It requires some sort of formalism, which usually (but not always) is unavoidably mathematical. There are two ways to approach the analysis of morphological variation by building morphospaces: empirically and theoretically.\(^{(11)}\) For the sake of clarity, suppose there are two continuous traits \(s, t\) that we are interested in. We can measure these traits and find out that we can divide up their range of variation into two separate clusters. Suppose we have a model of how these characters might originate developmentally, in such a way that we are able to build another morphospace of variation, a theoretical morphospace, which predicts the existence of more character states that form up to five clusters. Now we have a different, theoretical morphological space in which to analyze the real variation that was registered on the empirical morphospace (Fig. 1). Of these five clusters, only clusters 1 and 2 are known to occur in nature, i.e., they have been measured or identified unequivocally by analytical methods. By empirically measuring this variation, clusters 3, 4, and 5 are simply non-existent, and there is no need to hypothesize about them or use them to explain the variation of traits \(s\) and \(t\).

Without empirical evidence, a theoretical morphospace predicts the existence of the actual character states that form clusters 1 and 2, as well as the “possible” existence of character states that form clusters 3, 4, and 5 under certain conditions. Thus, theoretical morphospaces encompass the possible and the real as a subset of the possible, providing clues about the generative constraints that make their ontological status real, possible, or even impossible. Consequently, by focusing on the generative constraints imposed on the phenotype by developmental dynamics, this kind of analysis might help decide between internal (generative) and external (selective) constraints to explain the absence of morphotypes in the evolution of form. Theoretical morphospaces were first proposed as an exploration tool by David Raup, the founder of Theoretical Morphology, for the analysis of coiling shells, and have ever since appeared in many occasions in the literature for a whole variety of organisms, including invertebrates, vertebrates, and plants.\(^{(10–16)}\)

A non-mathematical morphospace that has been successful in identifying morphological design across taxonomic levels is the so-called “skeleton space”.\(^{(14)}\) This theoretical space is a broad conceptual formalism built with seven variables and three character states, making up a multidimensional space of 1536 possible designs. It has been used to register the disparity and diversity of early faunas and, due to its high level of generality, it can be used to compare the architectural design of widely different metazoan groups.

Theoretical morphospaces owe to Sewall Wright’s concept of adaptive landscape, the graphical metaphor of displaying a phenotype within a broader framework. While Wright’s landscape was based on gene frequency changes, paleontologist George G. Simpson extended this metaphor to directly represent a landscape of changing phenotype.\(^{(17–19)}\) Morphospace theory was also inspired by D’Arcy Thompson’s theory of transformation, by using his mathematical abstractions to show paths of possible changes among related forms\(^{(10,12,20)}\) (Fig. 2). In addition, numerical taxonomy has influenced morphospace theory by providing it with a variety of comparative tools for “empirical” morphospaces.\(^{(21)}\)

**Ontogenetic trajectories**

The spatiotemporal dynamics of embryonic development, along with its response to environmental cues, determines the phenotypic outcome at all stages of the embryo and the future adult organism. There are many ways to visualize this dynamical process. Waddington proposed the concept of...
epigenetic landscape, a rich three-dimensional surface of decision-making processes on top of which the embryo “roles down” until reaching its final morphological organization.\(^{(22)}\) Olson and Miller\(^{(23)}\) proposed the concept of “ontogenetic path,” a curve in three-dimensional space where each point of the curve represents a phenotypic stage. Waddington’s metaphor inspired mathematician René Thom to develop a whole field of mathematics dubbed Catastrophe Theory.\(^{(24)}\)

Olson and Miller’s idea was later used to elaborate the concept of ontogenetic trajectory, which introduces more precise, mathematical tools for the continuous growth of phenotypic traits using, for example, first order differential equations, which characterizes the dynamics of shape change over time.\(^{(25,26)}\)

An immediate option to model ontogenetic trajectories is to use dynamical systems theory, which characterizes mathematically fundamental properties of biological systems, such as stability, robustness, oscillation, or threshold-driven processes.\(^{(27,28)}\) Conversely, many key components of dynamical systems influence the sort of interpretation that can be drawn from studying an ontogenetic trajectory using this mathematical tool. Thus, there is a high-dependence on initial and boundary conditions, which might correspond, in a biological model, to concentration levels of key proteins involved in a signal pathway or the surface boundary between two embryonic tissues, respectively. Furthermore, the analysis of the dynamics itself is theoretically bound to a series of general outcomes. Thus, when evaluating how the concentration or activation level of several proteins change through time, dynamical systems predict three different types of outcomes, states in which the system reaches an equilibrium. If a trait evolves towards a fixed point, it means that there is a unique, unavoidable outcome. Oscillations around two or more predictable outcomes are called limit cycles. Finally, chaotic attractors are those in which the outcome of the trait is unpredictable. The presence of bifurcations is another key predictor of dynamical systems. Bifurcations make the system evolve towards only one outcome out of two possible ones when a given condition is met. In essence, bifurcations show a switch-like behavior, in many occasions irreversible.

Pere Alberch’s work on the relationship between developmental trajectories and morphospace occupancy remains a classic reference to date.\(^{(29,30)}\) He used typical dynamical system concepts to argue for gaps in morphospace due to “internal” constraints of development. These constraints were not thought to be derived from genes and proteins exclusively, but from the whole epigenetic relationship between molecules, cells, tissues, and environment. Moreover, in his broad and insightful view, constraints define the realm of possible morphological outcomes, always bound to those equilibrium states accessible to each specific dynamical system describing a developmental trajectory.\(^{(31)}\)

More recently, a general model of ontogenetic growth has been proposed based on metabolic considerations for the production and maintenance of the organism biomass.\(^{(32)}\) The model can be derived from basic cellular properties, such as

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Figure 2. Three space metaphors. \(\text{a,b: Morphospaces have evolved from Wright’s adaptive landscape of gene frequencies and Simpson’s space of phenotypic variation. In both cases valleys (−) and peaks (+) represent adaptive fitness.}^{11,17} \) \(\text{c: In contrast, theoretical morphospaces don’t assess the fitness value of the trait, only its occurrence in nature. They usually depict a full range of impossible and possible forms. In this example, the pelvis of the theropod dinosaur } Deinonychus antiomorphicus \text{ is mathematically distorted, forming an “affine morphospace”.}^{16} \) \(\text{Diagrams of other theropod dinosaur pelvis, colored in black, occupy specific morphological locations in this morphospace. This transformational method highlights the close relationship between theoretical morphospaces and D’Arcy Thompson’s theory of transformation.}^{(16)} \)
necessary energy for cell metabolism, cell division and cell death. While powerful for its level of generality, this model only predicts the mass growth of the life history of an organism. However, its ability to capture the dynamics of growth at different scales of observation (cellular and whole organism) sets the example for future works on form change.

**Ontogenetic trajectories and theoretical morphospaces: ontogenetic spaces**

Ontogenetic trajectories, as long as they are regarded as formalisms that map the dynamics of morphogenetic processes, can be viewed as putative generative functions of a theoretical morphospace. Thus, an ontogenetic trajectory can be generalized into an ontogenetic space; a space where each point represents a particular phenotypic outcome in time for given initial and boundary conditions. The exploration of this space can be done by systematically changing the initial and boundary conditions. The states of morphological outcomes, i.e., the different phenotypes, are mappings of biological parameters unto morphospace. These parameters can vary in nature and scale ranging from molecular (affinities, diffusion coefficients, etc.) to the morphogenetic events that cells engage in, such as proliferation rates, direction and velocity of cell migration, adhesion, growth, shape change, differentiation, necrosis, apoptosis, or amount of morphogen production. The use of a theoretical framework of this sort highlights the complexity of the dynamics of morphogenesis during development leading to the establishment of phenotypes. This complexity can be captured using dynamical systems theory, which is specially suited to characterize the non-linear interactions involving differential gene expression, molecular interactions, cell–cell, and tissue–tissue interactions. Many systems have been characterized with differential equations, from enzymatic reactions to network modules or morphogen dynamics. Asymmetries have also been analyzed in this manner by Graham et al. Here, we identify the generative function of a theoretical morphospace with an ontogenetic trajectory based on the notion of cooperation between cells to form an epithelium. This simple model can explore the different morphologies of the early formation of the heart, the first functional organ that breaks bilateral symmetry in vertebrates.

**Theoretical morphospaces of left–right asymmetries**

Bilateral animals have a complex body plan, involving some asymmetries more often located in internal organs that range from such minute differences in the structure of the eyes to major asymmetries as found in the coiling of the gut or the number of lobes of the human lung. Asymmetries have been traditionally divided into two main types, according to their causal developmental origin: those that appear under the external influence of an environmental or threshold cue (random asymmetries) and those that have an underlying fixed mechanism. Random asymmetries appear in subtle or conspicuous manners. The first ones, random subtle asymmetries, are known in the literature as fluctuating asymmetries. They consist of minute differences between left and right counterpart homologue parts in the same individual (e.g., length of ribs). High levels of fluctuating asymmetry is considered a consequence of developmental instability, sometimes viewed, in populations, as due to the presence of some sort of environmental stress, eventually leading to speciation events. An evolutionary pattern in which random asymmetry precedes the fixation of an asymmetric trait has been suggested elsewhere. Random conspicuous asymmetries (also known as antisymmetry) are typically triggered by an environmental cue or by differential use of the left and right counterpart homologous parts (e.g., the claws of fiddler lobsters). In contrast, fixed asymmetries are due to genetic and/or developmental mechanisms that generate distinct features with respect to the medial axis. Fixed asymmetries appear in different flavors, from handedness as in the heart or gut looping to differential topographic position as it occurs with the bladder (for reviews see Refs. 33–35).

A three-step conceptual model by Brown and Wolpert remains as the standard view for the generation of handedness in left–right asymmetry. A first step requires the existence of a molecular biasing mechanism that is conversed into cellular asymmetry (an hypothetical handed “F” protein endows cells with a specific polarity). The second step requires a random asymmetry generator, e.g., a reaction–diffusion mechanism that consistently generates the asymmetry towards one side because it is already biased by the first mechanism. Finally, a third mechanism must interpret this bias at a tissue level, so that different structures end up at left and right sides. Recent research has linked differential cilia rotation in the nodal cells of mice (and possibly other vertebrates as well) and membrane potential disequilibria between left and right counterparts established early during embryogenesis in *Xenopus* as putative processes for the initial biasing mechanism of Brown and Wolpert’s model.

We will restrict our model to the discussion of early heart formation, the first functional organ that breaks the symmetry in the body plan of vertebrates. The formation of the heart roughly follows these different stages: (1) bilateral heart field formation in the anterior lateral plate mesoderm, (2) migration of both heart epithelia towards the midline of the embryo, (3) epithelial fusion and formation of the heart tube, (4) caudal growth of the tube, (5) looping, and (6) valve and chamber formation. The first left–right asymmetry is produced in the looping formation process. In natural populations, from fish to humans, the heart loops to the right in most cases. Several mutants have been identified that either randomize the direction of the looping, make it appear mostly to the left, or show no looping at all.
A theoretical model of left–right asymmetries that generates a morphospace of theoretical forms can be built in the following manner. First, we build a generative function by assuming a threshold-dependent switch-like behavior for the dynamics of each of the left and right epithelial sheets of cardiac precursor cells that originate at the lateral plate mesoderm. This generative function will have the form of a Hill equation, which was originally devised as an algebraic representation of an empirical curve that measures a cooperation effect in multimeric enzymes that are able to bind more than one ligand (e.g., hemoglobin and oxygen). The Hill curve has a switch-like behavior when the Hill coefficient, a measure of cooperation, is sufficiently high. Two equations can characterize, independently, the right and left precardial epithelia:

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T_R = \frac{j^h}{p^h + j^h}; \quad T_L = \frac{j^h}{p^h + j^h} \quad (1,2)
\]

where \(T\) represents the tightness of the precardial right and left tissue, \(j\) is the number or strength of cell-to-cell junctions in the epithelial sheet, the exponent \(h\) is a measure of cooperation, and \(p\) is the point at which half of the possible value for \(T\) is reached. The dynamics of the equation assumes that there is a cooperative effect among cell-to-cell junctions, whereby, above a certain threshold, the tissue forms an epithelium, whereas below it, the tissue remains as loose mesenchyme. The cooperative effect depends on the value of \(h\), as this value increases, the conversion between mesenchyme and epithelium is more abrupt. In biological terms, this implies that, as the density of junctions increases within the tissue, it becomes easier to have a compact tissue that eventually behaves like an epithelium (Fig. 3a, b).

The morphospace of “possible tubes” is restricted to a region of equations 1 and 2 in which both precardial tissues form an epithelium. This is equivalent to the AND logic gate in computation (Fig. 3c). Only when the left and right precardial cells form an epithelium will the heart tube be formed. Subsequently, bending will be determined by the difference between left and right number of junctions. Thus, when the left epithelium is “tighter” than the right one, bending occurs to the right. Conversely, left looping would occur when the right epithelium has more junctional density than the left. A region of no looping heart tubes would occur when left and right epithelia are equally tight. When the difference in junctional density exceeds certain value, the bending cannot be accommodated any longer, those are the non-existent tubes of the morphospace (both extreme tubes in Fig. 3d).

This model provides a quantitative basis for assaying the looping phenomenon in the heart tube, controlled by two variables, the density of cell-to-cell junctions responsible for the formation of left and right precardial epithelia. The different morphologies can be predicted efficiently, and even the amount of bending could be calculated in a more complex model incorporating the viscoelastic properties of the heart tissue as well as the resistance opposed by the extracellular matrix. It also provides a basis for experimentation: genes that affect the formation of epithelia should be responsible for the different direction in heart tube looping. Some evidences point...
in that direction. The expression of N-cadherin, a calcium-dependent transmembrane protein that mediates cell-to-cell adherens junctions has been found to correlate with the randomization of the heart looping. Its mRNA is asymmetrically expressed in the left side of the chick node at an early stage, where the left heart field epithelium is forming. Upon treating developing chick embryos with anti-N-cadherin, García-Castro et al. found a randomization of heart looping. In the model presented here, this can be explained by the difference in tightness for each epithelium. Obviously, other proteins must be involved in forming and maintaining the heart field epithelium in the right and left lateral plate mesoderm. For example, in the zebrafish it has been found that the adhesion protein DM-GRASP is involved in left–right asymmetries of the heart and gut. Mercola has recently reviewed the involvement of adhesion molecules such as N-cadherins and claudins in left–right specification; he also notes the relationship between epithelial abnormalities and left–right inversion as demonstrated by the thickening of the epithelium that surrounds the node in inv mutant mice.

An immediate prediction of the model is that, by blocking the action of these other proteins, the epithelia will not be tight enough and, consequently, the heart will not form a tube in the midline. That is equivalent to be in the region of the Hill curve that is not able to reach the threshold needed in density of junctions (Fig. 3a,b). In addition, by varying the density of proteins involved in the formation of each epithelia, it should be possible to direct the left and right looping of the heart.

The model can be seen as a cooperative action among mesoderm cells to form the mesenchyme–epithelium transition. One possibility is that cadherins might act as a “zipper”, in which existent junctions facilitate the formation of new junctions. Evidence for such a behavior in cadherins has been provided, but the actual mechanism of cadherin–cadherin union remains controversial. Another possibility is that each cell as a whole acts as a junction unit; in this case, the cooperative phenomenon occurs as more cells are tightly united, facilitating the union of more cells. Yet another possibility is that the density of cadherins needs to be higher than a certain threshold in order to achieve tight junctions and form an epithelium. Varying this threshold (perhaps as a result of the presence of other adhesion molecules, different concentration of extracellular calcium or specific differences in the catenin–actin intracellular anchorage of the cadherins) would vary the tightness of the epithelium.

Towards a generalized model of left–right asymmetries.

At any rate, a model that generates asymmetries must also account for the evolutionary transitions seen in the fossil record as well as the experiments in population genetics. In addition, fluctuating asymmetry should also be part of the picture. What is lacking is conceptually spelling out the features of such an encompassing framework. In doing so, general predictions immediately appear and experimental scenarios to test such a hypothesis can be readily suggested.

Considerations about fluctuating asymmetry, antisy- mmetry and fixed asymmetry come largely from a population genetic perspective, with a strong linkage to possible evolutionary scenarios. However, in developmental biology, this perspective is not taken into account. Rather, only those molecular and cellular events that take place in model organisms are scrutinized.

The three different asymmetrical patterns seen in natural populations (directional asymmetry, antisymmetry and fluctuation asymmetry) can be integrated using a mathematical metaphor; a dynamical system that presents a bifurcation point and that evolves towards two chaotic equilibrium states (Fig. 4). The important features of this model are as follows. (1) The ontogenetic trajectory of an individual passes through a bifurcation point early during gastrulation (directional asymmetry). (2) The choice of one solution over the other (going to the left rather to the right as in heart positioning in humans) has been evolutionary fixed, but the possibility of reversing this choice remains plausible (antisymmetry). (3) Once a choice has been made, the phenotype fluctuates around a middle point (fluctuating asymmetry). The critical event in the symmetry breaking process occurs at the bifurcation point where morphogenesis chooses one side over the other. The bistability of this process is generally biased towards one trajectory when the asymmetry has been developmentally fixed in a population. In many instances, this fixation does not imply an adaptive advantage. Thus, situs inversus (a mirror image of the normal phenotype) in mammals seems to be as functionally efficient as situs solitus.

This conceptual model shows the sort of modeling approach that can be implemented to put asymmetries in a general theoretical framework. Since the ontogenetic trajectory can be modeled as a dynamic system, the parameter values can be explored to render a full ontogenetic space. In the case of the looping of the heart tube, the bifurcation point is governed by the threshold response to the Hill equation, a quantitative difference between junctions in the precardial cells of the left and right lateral plate mesoderm.

The attractors as mathematical properties can be translated into biological terms as canalized regions of development in Waddington’s epigenetic landscape. An equilibrium state of the ontogenetic trajectory, in its generalized form, becomes a stable stage of embryonic development in ontogenetic space. The establishment of these kinds of models might shed some light on the mechanisms of each different type of asymmetry by analyzing the properties underlying the generative function. Most importantly, although these have not been done to date, multiscale approaches integrating different levels of analysis, such as gene products interacting, cellular dynamics, and using the actual phenotypic outcome as the
end-mapping layout could also be carried out. Thus, one can envision a system of differential equations to characterize the molecular interactions leading to specific attractors, which would be responsible for the behavior of the cells in a threshold-dependent manner, specifying a morphogenetic process that leads to the symmetry-breaking event at the cellular level.

Conclusion

Little is known about morphogenetic mechanisms that break the bilateral symmetry in metazoans. Our knowledge on gene activity is currently somehow independent of what we know about cellular dynamics. Using paleontological, developmental and mathematical approaches can help in building integrative conceptual frameworks, as we have outlined here. Abstracting an ontogenetic trajectory by a few variables as a dynamic system shows possible models of morphogenesis dynamics. Assigning a generative function to an ontogenetic trajectory converts a theoretical morphospace, a paleontological methodology, into a heuristic developmental tool, which can be used to build computer simulation models. These models (we have shown three cases in the establishment of asymmetries) can be used to hypothesize about the mechanisms that can generate them. In addition, they can be used as a basis to speculate about possible evolutionary scenarios. By accepting the multiplicity of causes in the establishment of phenotypes, developmental biology can use conceptual and mathematical tools to establish theoretical models of development that can be tested experimentally in the laboratory. Morphology, once again, enters the domain of biology as a main actor. Morphospaces and ontogenetic trajectories suggest types of developmental dynamics, and prompt an informed search for the mechanisms that relate the underlying genetic differential expression to the cellular dynamics responsible for form generation. We have argued that the generalization of an ontogenetic trajectory can be seen as an ontogenetic space, a space that the embryo explores as it moves from stage to stage. This “generalization” is all the more important, stimulating a thorough exploration of parameter values that govern the outcome of the trajectory. Multiscale approaches in which molecular dynamics effectively affect the behavior of cells and, in turn, generate morphological features, are the next frontier in modeling embryonic behavior.

The heuristic power of building theoretical morphospaces rests on the capability of generating hypothetical morphologies out of real processes, thus surpassing the usual analytical observation of natural occurrences. At some level, any experimental manipulation involving gain and loss of gene function is a strategy that parallels morphospace building. In both cases, natural occurrences are violated, and new forms appear that have to be explained with normal biological processes. The gain in insight is enormous: looking at the logic of theoretical occurrences can single out the logic of real occurrences.

Figure 4. The ontogenetic space of left–right asymmetries is generated by a generative function G that is species-specific. For each organic structure, the form of the ontogenetic trajectory is such that there are three possible theoretical outcomes (left and right handedness and no-handedness), which are decided at a bifurcation point (no handedness is a special outcome in which the bifurcation point is never reached). In antisymmetry, this decision is random and might be caused by an external trigger. In directional asymmetry, the ontogenetic trajectory is strongly canalized, showing a clear bias towards one side. Fluctuating asymmetry is depicted here as a Gaussian distribution over a mean value of the final end-product of development. This Gaussian distribution has to be generated by a dynamical system with two chaotic attractors at left and right, producing phenotypes that are randomly distributed around the mean symmetry. Variables x and y are putative quantitative characters that can be measured in the resulting morphologies. The parameter space (m,n) represents biological variables that control the state space of left–right, such as number of cells, density of cells, cell adhesive forces, direction and speed of migration, proliferation and death rates. There is a genetic component (gene expression space, lower plane) that underlies the parameter space of cell dynamics (middle plane) bearing a non-linear relationship with it, through the complex interactions among the molecular components of the developing system (depicted as a network of interacting balls). The upper plane represents the morphological outcomes, which are available through experimental manipulation.
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References