**A world of opportunity within constraint: Pere Alberch's early evo-devo**

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## Introduction

The work of Pere Alberch is crucial to study the early stages of evo-devo. In particular, it illustrates very persuasively why developmental systems have so much to say about the course of evolutionary change. In addition to an important empirical work, he elaborated a stimulating framework of theoretical ideas on biological form, morphological variation, and how developmental processes establish possible evolutionary paths previous to the action of natural selection. In this framework, the study of development and evolution are related through the notion of possible morphologies. In his view, the morphology of organisms shows internal coherence and structure, emergent from complex non linear interactions among parts and with the environment. This constitutes a source of determinism absent from other accounts in which novelty is considered to appear only from random mutations. In his words: "In evolution selection may decide the winner of a given game but development non-randomly selects the players" (Alberch, 1980, p. 665).

In the 1970s and 1980s many biologists argued against the prevalent gene-centred view of biology and demanded to study how the organization of multicellular organisms, their *Bauplan*, influences morphological variation and, consequently, evolutionary change. In Alberch's work the link between development and evolution is regained in several ways: (1) by challenging the static hierarchical theories of development in favour of a dynamic, cyclical, and interactive conception that he pursued through experiments and dynamical models; (2) by stressing the relevance of studies of ontogenetic phenomena at the appropriate morphogenetic level, so that morphologies might be regarded as products of complex genetic and epigenetic interactions; and (3) by suggesting that some developmental properties constrain possible paths of evolution in specific directions, because they define the set of variations associated with certain forms (e.g., the tetrapod limb, the pattern of digits, or the form of teeth). Thus, Alberch questioned the theoretical framework that assumes a direct correspondence between genetic structure and morphology with natural selection acting on the effects of random changes.

Although Alberch's work flourished precisely before and during the expansion of developmental genetics, he was more interested in the mathematical techniques of dynamical systems than in the new molecular techniques appearing at the time. His approach was that of a theoretical biologist who considered that experimental and theoretical work together could allow for an a priori and predictive knowledge of living form and its evolution. Thus, Alberch's peculiarity with respect to others investigating development from a dynamical systems approach, like Goodwin (1994) or Kauffman (1993), is the use of experiments in addition to simulations.

In his relatively short life, Alberch wrote many articles on many subjects (he published his first paper when he was only 19 years old), but probably his most important, and most cited, work was done during the decade of the 1980s. Many of the advancements in evo-devo these days are a consequence of ideas and projects in which he, along with many others, was involved at that time.

In this paper we focus on what we consider to be the main early ideas of evo-devo. First we see that Alberch defends the importance of doing research at the morphological level, which he considers as non reducible to lower level molecular factors and that appears as a realm of discrete themes. Then, we focus on the relevance of developmental mechanisms, the role they acquire for studying evolution and how they are conceptualized as developmental constraints to reflect the intrinsic abilities of developmental systems to produce some forms and not others, with consequences for evolutionary opportunities. Finally, in the last section we summarize some conceptual elements of Alberch's views on how to integrate different approaches to explain the morphological organization of living beings.

## 2. The themes of the discrete morphospace

The phenomenological departure point of Alberch's research is the recognition of the ordered character of morphospace, i.e. the space of biological forms: "Nature is not chaos, neither is it a boundless continuum of forms." (1982a, p. 315). Morphological variation is not random and continuous, as the standard view in evolutionary biology maintain, but shows a discrete organization. This “morphological order” has the following properties:

> “ (1) Phenotypes are discrete. That is, points are not distributed uniformly over phenotype space, but tend to cluster around major “themes”, corresponding to taxa or classes of teratologies. (2) While there may be considerable dispersion around a morphological theme, the variability in any trait is definitely limited. (3) When new morphological themes arise, either in ontogeny or phylogeny, the transitions between themes are not random. (4) These properties are largely the result of epigenetic interactions during development.”(Oster and Alberch 1982, p. 444)

The neo-Darwinian approach cannot fully account for the discrete character of morphospace: if evolution depends strongly on the action of natural selection on gradual variations, any living form must be theoretically possible, and the space of biological morphologies, continuous. From this perspective, discrete characters must be the result of functional convergence: unity of type in non-related taxa is seen as the outcome of similar selective pressures.

In Alberch’s view, internal (or developmental) and external (or selective) explanations of evolutionary change are not contradictory, but they deal with different problems: the origin and the fixation of evolutionary change. Standard evolutionary biology has been traditionally concerned with the fate, not with the origin, of variation. The neglect of the origin of variation is linked to the beliefs of the isotropy of molecular variation and the direct correspondence of genotype and phenotype. Alberch challenged both assumptions and vindicated the study of the patterns of variation (the existent and possible order) precisely to understand the mechanisms responsible of their generation, i.e., the variability of developmental systems, as morphological parallelisms suggest that the same mechanisms are in action (Alberch, 1983). Thus, in his view, evolutionary research needs to proceed in two steps: the first is to elucidate an ordered pattern of variation, i.e., to characterize invariance, and the second, to look for the mechanisms behind those patterns (Alberch, 1985b). Without characterizing morphological invariance -he argued- it would be impossible to find the mechanisms responsible of its generation. That is why in Alberch's work morphology recovers the significance as a biological discipline that it lost in the Modern Synthesis: comparative anatomy has a fundamental role in evolutionary theory, since its job is "to determine regularities of structural organization that enable a classification and understanding of the ordered diversity of form" (Shubin and Alberch 1986, p. 377).

The study of patterns of variation is thus the first step of Alberch's research and it is also the sole protagonist of many of his papers, where he tries to distinguish between the order of forms internally generated by development and the role of natural selection to favour subsets of them. With this goal, Alberch studied three main morphological phenomena where the patterns of variation cannot be explained by Natural Selection but by the structure of developmental processes.

One case is that of *morphological convergence* where a similar morphology appears linked to different adaptive requirements. In a series of experiments, the functional significance of structural modifications in hand and foot morphology (such as reduction and loss of phalangeal elements, development of interdigital webbing and fusion of tarsal and carpal elements) is explored. The goal is to analyse how to integrate adaptation and ontogeny in a case where a morphological convergence does not imply functional convergence (Alberch, 1981).

A second phenomenon of morphological order is that revealed by patterns of *intraspecific variation*. This is the subject of Alberch's study of phenotypic variation in osteological characters of populations of species of the neotropical salamander genus *Bolitoglossa* (Alberch, 1983). The main finding is a recurrence of the same variants in widely unrelated species, a fact that again, cannot be explained from an externalist approach.

Finally, a constant source of evidence for discrete variation comes from his studies of *teratologies*. Throughout his work, Alberch (like transcendental morphologists Etienne and Isidore Geoffroy Saint Hilaire) demonstrated a special fascination for the development of malformations (Alberch, 1989). Non-functional teratologies were especially appealing for the study of the generative properties of developmental systems. These deviations from “normal” development result in forms which are often lethal or less well adapted than its predecessors. Therefore, following the adaptationist logic, natural selection should prevent their appearance or at least contribute to their disappearance. But in spite of being strongly selected against, teratologies are still being generated in a recurrent way.

All these evidences of the discrete character of morphological order, the regularities of these patterns of variation, indicate the fundamental role of development in specifying the possible forms available for selection: morphological convergence, intraspecific morphological diversification and teratologies show a logic emerging from internal developmental systems.

**Discovering developmental mechanisms**

We have seen that the properties of morphospace demand that morphological evolution be studied from an ontogenetic perspective (Alberch, 1980). This requires to define developmental phenomena at the morphogenetic level: despite the standard position of population genetics according to which all evolutionary novelty comes from random mutations, in a direct one-to-one genotype-to-phenotype relation, the study of ontogeny obliges to pay attention to “epigenetic regulation” and, thus, to a level higher than that of genetics to explain development.

The morphogenetic level is characterized in two ways.  On the one hand, developmental form is not a static entity defined by the spatial position of its component parts, but the result of a dynamical process involving interactions, integration, and regulation among them. On the other hand, the morphogenetic level may be studied in physico-chemical terms. These two points converge in a conceptualization of developmental dynamics as susceptible of mathematical formalization and experimental treatment.

Here we describe Alberch’s conceptualization of the morphogenetic level and the global properties of developmental processes. Then, both work on the mathematical formalization of the dynamics of developmental systems (and the role of mathematical biology) and of their biophysical properties (and the role of experimental embryology) are considered. Both approaches define a mechanistic viewpoint on development and evolution that may provide some degrees of determinism and predictability.

*The morphogenetic level.*-One of the main difficulties for Alberch's theoretical stance was to establish the reality of the morphogenetic level as well as the necessity of working at it despite the success of the genome-centred approach in evolution and development. In his opinion, the latter favours a "hierarchical scheme" of development, which portraits an extreme version of the neo-Darwinist view of genes as directly prescribing developmental processes that, in turn, specify morphology in a straightforward way. This view reduces both morphological evolution and development to purely genetic problems: development to a sequence of gene expression and evolution to a change in gene frequencies.

However, this scheme presents several problems (Alberch, 1991). First, such an "open loop" system would be extremely unstable against the random disturbances that accompany normal development. Second, we do not find a one-to-one correspondence between certain region of DNA and a given morphological trait and (Alberch, 1983). On the contrary, the effect of genes on morphology is mostly indirect: they code for molecules which either regulate the expression of other genes or confer properties on cells (e.g., cell division rates, apoptosis, differentiation timing or cytoskeletal properties), which then construct organs and structures largely in accordance with physico-chemical laws. Thus, the complexity and nonlinearity of the genotype-phenotype mapping increases at higher levels of interaction. Due to the highly context-sensitive character of gene expression (dependent on embryonic stage and local environment), similar genetic changes may yield different morphological effects, and the other way around. In fact, at the morphogenetic level, genetic mutations may not even be expressed, since developmental interactions have properties that emerge from the dynamics of the system and are not encoded in the genome (Oster and Alberch, 1982; Oster et al., 1988; Alberch, 1991). Therefore, phenotypic diversity is not so much the product of new genes as of permutations in context (i.e., the timing and location of expression) of existing genes. The evolutionary consequences of this asymmetry are obvious: there are qualitative differences between modes of evolution of the genetic and epigenetic levels, and therefore there is often no direct correspondence between genetic and morphological divergence (Alberch, 1983).

In order to capture the properties of developmental systems and their evolutionary consequences, Alberch proposes an alternative view of development which he calls the "*cyclical/feedback scheme*". According to it, developmental processes are divided in three interacting levels (1982a, p. 320): interactions among genes within a highly structured genome, proteins and enzymes generating cell properties involved in morphogenesis, and tissue interactions. Following Waddington, Alberch considered that these regulatory interactions specify the epigenetics according to which phenotypes are well buffered systems with respect to both genetic and environmental perturbations during ontogeny (Alberch, 1980). Therefore, in the cyclical view of development "genes are just one step in the chain of interactions, gene expression is both the cause and the effect of a morphogenetic process" (Alberch, 1991, p. 6).

Thus, Alberch's message is that the morphogenetic level of description deserves special attention in evo-devo because it vastly governs both the dynamics of morphogenesis and the appearance of novelty. For Alberch it is at this level where the range of possible variation for evolution is established, introducing a deterministic factor in the production of variation (which was largely left to random mutations in the Modern Synthesis) that allows for some form of predictability, in so far as the variability of a given trait (its potential for producing variations) can be determined.

*Developmental dynamics: from heterochrony to construction rules.*- Despite the efforts of workers like de Beer, Waddington or Schmalhausen, the role of development was absent from standard evolutionary theory since the decline of the recapitulation theory. However, it received a renewed interest by the scientific community after the publication of Gould's Ontogeny and Phylogeny (1977). Two years later, Alberch and co-workers published together with Gould a celebrated paper where they presented a quantitative method for describing the relationship between heterochronic changes and phyletic trends (Alberch et al., 1979). The aim was to improve Gould's "clock model," which was essentially qualitative and "static." Thus, Alberch and co-workers quantified Gould's model, by characterizing those modifications in the developmental processes that produce relative changes in size and shape. Furthermore, they provided a dynamic expression of heterochrony, defined in terms of shifts in specific processes such as onset, cessation, or rate of growth, rather than of end results (see De Renzi, this book). This approach is followed in later publications, where we find empirical work on heterochrony in *Bolitoglossa occidentalis* (Alberch and Alberch, 1980)

This treatment of heterochrony (and their focus on patterns of relative growth) was very influential, defining the way the concept is used in current evo-devo (Smith, 2001; 2002). However, after 1985 Alberch found that this approach had serious problems, and revised his views in accordance to his more dynamical and mechanistic conception of development (Alberch, 1985a; Alberch and Blanco 1996). Alberch opposes two conceptions of ontogeny and phylogeny: a kinematic one, which only classifies and compares developmental stages, and a dynamic one based on an understanding of the underlying mechanisms of development and the comparison of developmental events (Alberch, 1985a; Oster et al., 1988). The conception of development as a sequence of discontinuous morphological stages conserved throughout evolution and the associated mechanism of heterochrony is rooted in the Haeckelian view. But, taking into account the critique made by von Baer to the law of parallelism of Meckel-Serres, Alberch insists that comparative embryology does not show any linear recapitulation, concluding that all the intermediate stages postulated by heterochronic models are meaningless (Alberch, 1985a, p. 51). Instead, changes between two related morphologies "must be searched for in terms of changes in the developmental rules of interaction or initial conditions, rather than in intermediate ontogenetic stages" (p. 51). He uses several examples, including experiments of previous papers (Alberch and Gale, 1983; 1985) to "illustrate how evolutionary models based on heterochrony can be embedded within a more mechanistic and dynamic framework" (Alberch, 1985a, p. 55). He had to admit then that the work done in 1979 was "no longer valid" (p. 55), because this perspective did not fit well with his later views of development as a dynamical system governed by a set of "construction rules" that are able to generate a global pattern.

This new conceptualization of development has important consequences on the definition of homology, a hot question in current evolutionary biology. The distinction between developmental stages and developmental events leads Alberch to "champion the view that many of the difficulties in establishing homologies could be avoided or resolved by basing comparisons between elements on the developmental processes which created them, rather than on their final geometric form." (Oster et al., 1988, p. 877). The best empirical example of this approach is the study of the morphogenesis of the vertebrate limb. The early stages of the skeletal patterning were explained by a mechanistic model of embryonic branching and segmentation in initial chondrogenesis. In this model, the loss of a digit may result from the failure of a branching bifurcation, and then, it is not sensible to ask "which" digit was lost, since it is the basic sequence what has been altered. Thus, not the morphological elements but the morphogenetic processes are the units of comparison (Oster et al., 1988).

In order to capture this new view on developmental evolution, Alberch develops the notion of *construction rules,* which crystallized in publications on the morphogenesis of teratologies and tetrapod limbs. The comparative analysis and the experimental manipulation show how these rules determine how a limb can be modified by evolution:

> "An understanding of these, very often simple, rules can provide valuable insights into the apparent complexity of many developmental processes and, furthermore, allow us to determine the relationships among different phenotypes, since the set of possible phenotypic transformations will be constrained by the generative potentialities of the morphogenetic rules involved in the process." (Alberch, 1982, p. 321)

Development is viewed by Alberch as a dynamical system governed by certain rules or constraints which remain stable during long periods of time, but permit certain range of variation through the alteration of certain (genetic and non-genetic) parameters. These rules arise from the interaction of different "resources" at different levels and direct or channel the possible evolution of the system.

*The formal properties of developmental systems and the role of mathematical biology.*- In order to explain the mechanics of developmental programs, Alberch used the conceptual and mathematical tools developed in the framework of dynamical system theory. Developmental processes are viewed as “complex dynamical systems, where a small set of simple rules of cellular and physico-chemical interaction can interact to generate a complex morphology” (Oster and Alberch, 1982, p. 455).

These rules of construction, probably inspired in the conceptual framework of cellular automata, are formally captured as developmental parameters. Alberch postulates that developmental processes, as any pattern-generating system, have an associated parameter space that captures the global properties of the system, defining its “evolutionary potential” or evolvability:

> “This approach views form and pattern emerging as the combination of a set of specific pattern-generating algorithms with a set of initial boundary conditions. Diversification occurs as the result of changes in the developmental parameters of the algorithm (=”program” composed of a set of local rules of biochemical, cell-cell, or tissue interactions). The basic structure of a pattern formation model is to quantitatively state some local rules of interaction that are able to generate a global pattern.” (Alberch, 1985a, p. 50)

The effects of genetic or environmental alterations in the basic developmental properties are mathematically abstracted as parameter perturbations in a dynamical system (Alberch, 1982, p. 323). Thus, “morphological diversity is generated by perturbations in parameter values (such as rates of diffusion, mitotic rate, cell adhesion, etc.) while the structure of the interactions among the components remains constant” (Alberch, 1989, p. 27). In order to visualize all possible pathways of transformation among phenotypes that some specific rules of construction generate, Alberch imported a tool from dynamical systems theory: *transformational diagrams* (Alberch, 1991). Each species or trait will have a unique transformation diagram dependent on its position in the parameter space, and smooth perturbations of the parameters can result in only certain given phenotypes. These properties of developmental systems are illustrated by teratologies, which are not only generated in an organized and discrete way, but also exhibit general transformational rules. In the case of the salamander limbs, the perturbation of developmental paths through a genetic mutation or experimental manipulation can only produce a limited set of transformations.

Alberch considers that the stabilities and bifurcations of Waddington's epigenetic landscape can be now formalized with the language and mathematical tools developed in dynamical system theory: phenotypic stabilities are treated as *dynamical attractors*, regions in parameter space where small perturbations do not disrupt the basic organization. In the same way, Müller and Wagner state that "ontogenies can be understood as systems of temporary equilibria or steady states between developmental entities" (Müller and Wagner, 1991, p. 249). *Bifurcations* are developmental thresholds (such as critical cell number or inductive or spatial relationships) with the opposite result. The way to explain how mechanistic rules can constrain processes of morphological evolution has to do with the presence of mathematical bifurcations and how it "implies discontinuity and directionality in phyletic transformations" (Oster and Alberch, 1982, p. 455). Thus, developmental thresholds explain the nonlinear character of development and the discontinuity of evolution outlined by the theory of punctuated equilibrium (Eldredge and Gould, 1972): modifications that go beyond such thresholds can cause nonlinear effects.

*The physical properties of developmental systems and the role of experimental embryology.*- Although modelling captures the structural properties of developmental systems, the study of the mechanical and chemical laws to which the morphogenetic processes are subjected is necessary (Oster et al., 1988). In addition to his conceptual work as a theoretical biologist, Alberch proved to be a brilliant experimental embryologist. The papers published in collaboration with Emily Gale since 1983 illustrate this work (Alberch and Gale 1985, 1986; Alberch, 1986; Alberch et al., 1986). Here the influence of perturbations of some developmental parameters in the generation of new forms is studied experimentally. The authors compared the results of treating the limb buds of a frog and a salamander with colchicine, a mitotic inhibitor. This treatment results in various abnormal morphologies such as limbs of smaller size that have lost some skeletal elements. Their results were used to contrast the hypothesis that the digital pattern is affected by reduction in the number of mesenchyme cells. Changes in pattern formation took place when the size and the number of cells of the limb bud were reduced under a critical value, an experimental fact consistent with the mathematical models studied with Oster (Oster et al., 1988). However, the main result of these experiments was that the malformations produced by colchicine were not chaotic, but they exhibited a high degree of order. Most of the patterns of diversity of digital morphology in amphibians can thus be explained as a reflection of developmental properties (Alberch and Gale, 1985).

The consequences of the experimental manipulation of developmental systems are twofold. (1) The experimentally generated pattern of variation can be compared with the patterns of natural variation, allowing phylogenetic inferences and to trace possible evolutionary pathways. For example, salamanders seem to develop their limbs in a way very different from the rest of tetrapods because the sequence of digit formation seems to be inverted (Alberch and Gale, 1983). Digit reduction is a phenomenon that has taken place several times independently in amphibian evolution. Frogs usually lose their most internal digit (preaxial), whereas salamanders the most external one (postaxial). The interesting result obtained in this comparative study is that there is a parallelism between experimentally generated patterns and the evolutionary trends towards digit reduction observed in wild animals (Alberch and Gale, 1985). At the same time, the bounded variability generated in the laboratory reveals a fundamental property of developmental systems. Although not all induced malformations produce forms observed in nature, these non-natural morphologies are also limited by the system's morphogenetic properties, i.e., they are the product of rule iterations. Shubin and Alberch (1986) maintain that during the evolutionary history of vertebrates these basic morphogenetic rules have not changed, only the interaction parameters. Quantitative variations of these parameters may produce qualitative alterations such as changes in the branching and segmentation sequences, but since the rules of interaction remain the same, we will never obtain qualitatively new forms in the laboratory, but atavisms, deletions, or duplications of already existing features. If we are dealing with an evolutionarily fixed developmental system we are only able to explore its potentialities, mostly reiterating forms that had already been realized during evolution (Alberch, 1991).

## The role of developmental constraints

Alberch explored in depth the notion of “developmental constraint” both theoretically (Alberch, 1982; 1986; 1989; Maynard Smith et. al., 1985) and experimentally (Alberch and Gale, 1983; 1985). The notion of constraint comes to biology from other fields dealing with complex systems, such as physics or engineering, where it is often understood as a limitation of variation of some kind, sometimes with a creative or enabling character. In fact, this sense of a limitation of variety at one level combined with the emergence of possibilities at a higher level was used in a very influential paper by Jacob (1977). There he conflated contraints as elements of hierarchical organization with historical contingencies. However, in Gould and Lewontin (1979) “developmental constraints” are considered as “phyletic restrictions” caused by the nature of the developmental system, in which earlier events are more “refractory to evolutionary change” than later ones (Gould and Lewontin, 1979, p. 160). Their aim was to criticise the view of evolutionary adaptation as perfect optimization through natural selection, and there the notion of constraint refers to how levels integrate in a complex system considering that evolution has to deal with the temporal order of development, and therefore earlier contingent traits that must develop earlier become frozen. Thus constraints appear as a limitation of the power of natural selection considered as an adaptive force:

> “This programme regards natural selection as so powerful and *the constraints upon it* so few that direct production of adaptation through its operation becomes the primary cause of nearly all organic form, function, and behavior.” (Gould and Lewontin 1979, p. 584-5, emphasis added)

Since then, the notion of constraint has been struggling between the two seemingly difficult to conciliate senses of a) limitations of the morphological variation available to natural selection, and b) intrinsic or generic factors for organizing form. Several attempts have been made to clarify this dichotomy (see Amundson, 1994; Schwenk, 1995; Garcia-Azkonobieta, 2005).

Alberch distinguished a range of constraints that act at various levels and have different degrees of generality, but he was interested on constraint at a particular level: the cellular and tissue aspects of pattern formation and morphogenesis (Alberch, 1985b). These are constraints involving morphogenetic construction rules, causing limited variation from their normal development. Amundson (1994) distinguished between constraints on adaptation–preventing certain changes no matter which environmental pressures are at work—and constraints on form, responsible for the available possible morphologies, irrespective of adaptation. For him, the strength of each of these two meanings was more evident if we look at them as being part of different research programs or traditions, namely that of neo-Darwinism (externalism) in the first case, and that of *Naturphilosophie* (internalism) in the second. Alberch's definition of developmental constraint is Amundson's key example to characterize constraints on form:

> "If the raw material upon which selection operates is generated by effectively random and boundless variation, anything will be possible. Therefore it cannot be predicted, except by using optimality arguments and assuming that, if some phenotype would be optimal, the appropriate genotypes will appear. (…) An alternative approach to this organism - environment dichotomy consists in focusing on the internal organization of the organism and attempting to characterize *the constraints operating on the system*" (Alberch 1982, p.314, emphasis added)

For him, constraints reflect the intrinsic abilities of developmental systems to produce some forms and not others. This framework has certain consequences about the generation of the variation required for evolution to proceed, since:

> "the interplay between genetic indeterminacy and resilient developmental rules suggests the likely recurrence of similar morphological variation and the generation of a biased subset of phenotypes upon which natural selection or population stochastic factors can operate" (Alberch and Gale, 1985, pp. 19-20).

Therefore, in this view, epigenetic interactions do not constrain natural selection but how genetic mutations are expressed at the morphological level. This view on constraints (1985b) represents an important turn with regards to the consensus view reached in the paper head by Maynard Smith that same year. Alberch's definition eliminates the statement that constraints are 'limitations on phenotypic variability,' and defines developmental constraints as bias on variability that generate global patterns. This conception suggests that, in order to find out the effect of developmental constraints on phenotypic variation it is necessary first to elucidate the developmental structure of the system. From this perspective, constraints are not only restrictions for certain conceivable novelty in evolution; they provide possible directions for changes and innovation that we actually find in the biological realm. In other words, constraints are not only important to trim adaptationist optimality thinking; they are a fundamental tool for the evolutionist who wants to search both for the source from which certain innovations emerged and possible directions for future evolutionary change. An illustrative example comes from Alberch's study of the evolution of metamorphosis (Alberch, 1987). In this paper, he hypothesizes that the parallelism in the evolution of metamorphic patterns in insects and amphibians suggests a common developmental constraint: the irreversibility of development imposes a general constraint that shapes phylogenetic patterns of metamorphosis, since such distant phyla circumvent it with the similar evolution of redundant systems. Thus, the general constraint of the irreversibility of development does not limit the generation of variation, but makes possible the appearance of certain new developmental "strategies" in the generation of morphological novelty.

Resnik's (1995) distinction between active/passive developmental patterns or constraints can help us to clarify this view. The "rules of construction", as defined above, guide the process of form construction. These rules are then active developmental constraints*:*

> “epigenetic interactions drastically constrain the universe of possible morphological novelties and *impose directionality* in morphological transformations through phylogeny” (1980, p. 654, emphasis added).

The dynamical nature of directionality in transformations among steady states has special relevance, providing a cue to understand the “active” or “positive” meanings of constraint that Alberch had in mind (also defended by Gould, 1989). Dynamical systems tend to fall in their attractors following some specified directions. So, Alberch’s rules or active developmental constraints determine not only the actual form but the set of all possible phenotypic transformations by virtue of its generative potentialities. They are *active developmental constraints* on evolution in Resnik’s sense. Transformational diagrams show the potential evolutionary transformations of phenotypes predicting the most probable ones in absence of external forces. They can be used as a “null hypothesis” of evolutionary transformations, because selection can only drive phenotypes along the internally specified directions. Thus, in Alberch’s view, the dynamical properties of developmental patterns limit the variability producing clusters or attractors in phenotypic space, but at the same time, provide potential directions to evolutionary change.

This positive meaning of developmental constraints is specially illustrated in Alberch’s ideas on evolvability, now a core concept in evo-devo (e.g., Wagner and Altenberg, 1996; Kirschner and Gerhart, 1998). After Dawkins (1989) established the term, he published an article addressing to the different capabilities of developmental systems to evolve (Alberch, 1991). The properties of developmental systems (stability and variability based on developmental thresholds) define their evolvability, which requires that the system has stability against perturbations, but not so much as to be immune to absorb change and variation. These very general properties of developmental systems lead Alberch to propose a new level of selection:

> “…there must have been selection among pattern generating systems favouring the ones that exhibit the adequate balance between stability and

> potentiality to generate sufficient phenotypic variability. It must be emphasized that we are dealing here with a new level of selection, one that it does not act on the phenotype nor on the genotype, but rather on the emergent properties of developmental systems." (Alberch 1991, p. 10)

Alberch presents the generation of form as a process governed by certain generative rules or constraints that both provide stability and promote change in certain directions. But, can this view explain the creative capacity of evolution, for instance, in the origin of new body plans? The answer to this question is, in general, positive, since it seems that to talk about creativity we need a reference to a specific generative system, or to some set of constraints. In this sense, Alberch seems to have thought that the elimination of all constraints and their substitution by random processes would suppress creativity.

However, the conservation of sets of interaction rules within ontogenetic types constrains the range of creativity of developmental systems. The exploration of the space generated by developmental rules can bring more or less creative results, for example, a complex pathway in a transformational diagram, with a lot of transitions, as the introduction of modulation in music. But the mechanism that can produce really creative forms is the transformation of the generative space by changing or eliminating some of the rules. For instance, the suppression of the parallels postulate resulted in the discovery of the non-Euclidean geometries. Alberch presents the Cambrian explosion, one of the mayor creative events in evolution, as an example of this form of creativity:

> "the invention of multicellularity, segmentation or the sequestration of the germ line appear, with hindsight, to have been key developmental events that have speeded up the evolutionary proliferation of lineages." (Alberch, 1991, p. 9).

Alberch thinks that the Cambrian was a period of experimentation in rules of cell-cell interaction, rules that exhibited different form-generating abilities as well as distinct stability properties (Alberch, 1991). After this period, no qualitative new structural body plans seem to have appeared and morphological variation looks as if reduced to variations within themes.

Accordingly, it is important to keep in mind that although Alberch insisted in predictability and determinism, this is only within the framework of a given developmental system. One of the main criticisms to the notion of developmental constraints is that their force can be broken or suppressed in the course of evolution. Salazar-Ciudad (2005) has proposed to overcome the divide between the standard neo-Darwinian approach and the "Constraint school" by studying the variational properties of different types of development. This idea is congenial to Alberch's thought. If constraints are thought not to be limitations to natural selection but morphogenetic rules that articulate molecular variation, changes of developmental parameters are perfectly expectable. From the point of view of development, switches of developmental systems are not breakings of developmental constraints due to natural selection, since developmental constraints constitute the structure of developmental systems. Changes of developmental constraints are no more than changes in developmental parameters and they must be internally explained. On the other hand, although each developmental type can be said to be constrained in the sense that it generates a given range of possible patterns, the evolutionary switch of developmental types also reflects that what is probable from the point of view of development does not always coincide with what is actualized in evolution. Therefore, although in Alberch's view constraints are not local and historical contingencies as they were in Gould and Lewontin's paper, forms of history and contingency may be taken into account.

Alberch was interested in the epistemology of creative process as well, which he considered to be guided by certain internal rules. For instance, the are some basic rules or phenomenological principles in the creation of mythological creatures, in the same spirit of the rules involved in the construction of tetrapod limbs: a) distortion of size and/or form of existing parts, b) multiplication or suppression of an existing part, c) recombination of parts that eventually have suffered some of the precedent transformations. For him, these universal mythical forms, like natural forms, are not mere historical contingencies, but reflect a deep structure of mind which is dynamical.

As the romantic philosophers and poets that he cites, he suggests that the mental process of creation of a new form is a morphogenetic process that depends on brain dynamics (Alberch, 1998).

**Conclusions: Towards an integrative biology**

Wagner and co-workers (2000) distinguish three phases in the constitution of evo-devo as a discipline. They suggest that the Dahlem conference (Bonner, 1982), where Alberch was one of the central figures, marks the end of the romantic stage. The enthusiastic phase started later with the discovery of homologous genes in *Drosophila* and other animals. We will be nowadays in the process of the constitution of the academic stage, where developmental mechanisms and evolutionary processes should be integrated. It seems to be an overall agreement that the main challenge to current evo-devo is the integration of different theoretical frameworks in establishing the link between development and evolution. The problem is that many authors see developmental evolutionary genetics as the new ”synthesizer” of evolutionary biology (Gilbert, 2003; Amundson, 2005). In this view, *unity of type* is revealed by Hox genes, whereas adaptations to *conditions of existence* are assimilated by genetic networks and filtered by natural selection. However, we think that work of an Alberchian style, involving morphological research to establish patterns of variation and explaining variability at the epigenetic level is fundamental for the integration of the diverse research programs converging in evo-devo (see Müller and Newman, 2005). To, conclude, we would like to summarize some of the philosophical issues stemming from Alberch's work that point towards a true integrative biology:

*Internalism*.- Throughout Alberch's scientific production one perceives a resistance to a given way of thinking about biology (in broad terms, what Gould and Lewontin (1979) called adaptationism), and an appeal to a different view of organisms and their processes for which he used different designations, like "internalism":

> "I focus on the internal rules that control the appearance of morphological variation, on the mechanistic basis of such rules and on the evolutionary consequences of this internally determined order." (Alberch, 1989, p. 28).

We think that Alberch's target of criticism is not the hypothesis of natural selection by itself, but the view that finds that adaptation to an external situation (the environment) is the only source of living organization. Biology, as he conceived it, depends more on mechanisms and regularities stemming from an organic "internal order", but this belief does not lead him to dismiss the role of natural selection:

> "Order in evolution is a combination of deterministic agents at two levels: internally generated order based on the internal dynamics of development, and natural selection, dependent on the properties of organism-environment interaction." (Alberch, 1989, p. 46).

As a matter of fact, both sources of order are not independent "filters". On the contrary, in Alberch's view, the evolutionary process is deeply interactive. When acting upon a particular phenotype, selection indirectly affects the developmental system and this creates a series of contingent history-dependent evolutionary potentialities. Selection operates within a set of internally defined possible transformations and when it determines the probability of survival of each of them, it indirectly affects the morphogenetic properties of the species. It is only because of the stage in which this field of study still is that he prefers to push the internalist approach:

> "At this stage of development of the field, it is more useful to study the organization and evolution of form from the internalist perspective. This approach, based on an understanding of development, naturally incorporates the phenomena of regulation, integration and constraint that are inherent to the concept of organism (i.e. form) and which are absent in the conceptual framework provided by the externalist approach which tends to view the phenotype as a static entity." (Alberch, 1989, p. 48)

One has to envision that within an integrated framework all aspects of evolution must be complementary: the morphological mechanisms for the generation of form, the genetics of molecular details, and the interaction (between forms and with the environment) to integrate functions.

*Form and function.*- Related to the need of bringing forth the internalist perspective is the vindication of a "theory of form" based on the global properties of the network of interactions that characterize development:

> "Evolutionary biology needs a theory of form […] The concepts of regulation and feedback that characterize developmental systems are conspicuously absent from existing theoretical schemes." (Alberch, 1989, p. 39)

Alberch's contribution is in our view relevant to promote the inclusion of studies centred in the interactive dynamics of processes of form generation within the theoretical framework of evo-devo. In fact, his last works were geared towards a general theory of form, not just in biology, but also in technology, and arts. From the study of pattern generation in complex systems he started to approach the problem of forms without functions, i.e., forms not attending to external functional demands (Alberch, 1998). Monsters were definitely for him an example of forms without function, and so were machines without functions, “monstrous machines” as he called them.

However, Alberch does not restrict the meaning of function to the contribution of a character to adaptation, ultimately shaped by natural selection. Recovering the Cuverian tradition (see also Schwenk, 200; Reiss, 2005), he defends an internal approach to function that accounts for the organismal integration of forms, defining functional constraints as those “imposed by functional interactions among different parts of the organism” (Alberch, 1981, p. 84). Alberch foresaw an approach to functions that started with both the generative and interactive properties of patterns, and not with the adaptive advantages assigned to separate parts. From this view, producing integrated and organized phenotypes becomes a three-step process: (1) generation of forms (morphogenesis); (2) integration of those forms through functional interactions; and (3) natural selection and other contingent historical processes. In this process, new morphologies are first produced, and then these novelties have to be integrated in the rest of the organism interacting with the environment (Alberch, 1982b).

*Mechanistic typology*.- Alberch’s distinction between the kinematics (description) and the dynamics (explanation) of evolutionary change reveals an organized synthesis of phylogenetic research: the comparative evolutionary approach to assess phylogenetic patterns and the mechanistic analysis of developmental processes. Thus, morphological disciplines (comparative anatomy, taxonomy, systematics and palaeontology) are necessary to characterize the patterns of variation, while experimental embryology and mathematical biology, in permanent dialogue with evolutionary morphology, help to discover the mechanisms responsible of such variation.

The exclusion of morphology in contemporary evo-devo can be related to its relationships with typology (Love, 2003). The substitution of typological thinking by populational thinking was seen by Mayr as the great conquest of Darwin, and any kind of typology was censured as narrowly linked to essentialism and idealism, the big obstacles to evolutionism. However, if we take into account Alberch’s work, the problem does not lie in the very typology, which accounts for the fundamental morphological dimension of evolution, but in the traditional statical and merely descriptive approach to form:

> "…this typological and static approach is not opposed to evolution or even to natural selection. (…) it does not draw from evolutionary mechanisms based on environmentally defined selection and random mutation. The quest for a general set of principles of form is legitimate if we exchange the metaphysical concept of the Bauplan for a mechanistic one based on principles of morphogenesis and internal integration." (Shubin and Alberch, 1986, p. 377)

Thus, Alberch's work demonstrates a form of typological thinking formulated in a purely mechanistic context, disconnected from metaphysical references to immutable essences (Amundson, 2001; Love, 2003). As we have seen in his analysis of tetrapod limbs, this type is not an ideal entity, but the result of a historically conserved developmental process, non reducible to an exclusively genetic explanation, determining the range of possible variation upon which selection acts.

*Contingency and determinism.-* The study of variability forces a radical epistemological change in the domain of evolutionary theory because it introduces a deterministic factor in the production of variation as well as some form of predictability. Morphological coherence will be a source of determinism absent from those accounts in which the appearance of novelty is considered to come only from random mutations:

> "An alternative approach to this organism-environment dichotomy consists in focusing on the internal organization of the organism and attempting to characterize the constraints operating on the system. These constraints define the potential pathways of transformation and impose an additional

> deterministic component on evolutionary processes.” (Alberch, 1982, p. 314)

However, Alberch does not defend an absolute but a “relative” determinism (Alberch, 1981). We can say that throughout his work, the epigenetic level emerges as a realm of determinism between two sources (physical and historical) of uncertainty. From the point of view of development, Alberch prefers the term “developmental bias” to that of “developmental program”, because the latter has “perhaps a more deterministic connotation than we intend here”. Although embryogenesis may appear deterministic on a macroscopic scale, the cellular level proceeds in a more stochastic fashion: “It is only the aggregate behaviour that exhibits overall coordination.” (Oster and Alberch, 1982, p. 444). The other source of unpredictability is the historical contingency coming from the level of the interaction of developmental constraints and natural selection: the most probable forms in terms of their dynamic properties (stability or accessibility in the transformational diagram) can or cannot be preferred by selection. Maybe an improbable form can be the fittest in a determined environment. Randomness, determinism, and contingency coexist in biological processes. That is why “we live in a world of opportunity within constraint” (Alberch, 1986, p. 8).

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