

How many processes are responsible for phenotypic evolution?

Giuseppe Fusco

Department of Biology, University of Padova, Via U. Bassi 58/B, I-35121 Padova, Italy

Correspondence (email: fusco@civ.bio.unipd.it)

SUMMARY In addressing phenotypic evolution, this article reconsiders natural selection, random drift, developmental constraints, and internal selection in the new extended context of evolutionary developmental biology. The change of perspective from the “evolution of phenotypes” toward an “evolution of ontogenies” (evo-devo perspective) affects the reciprocal relationships among these different processes. Random drift and natural selection are sibling processes: two forms of post-production sorting among alternative developmental trajectories, the former random, the latter nonrandom. Developmental

constraint is a compound concept; it contains even some forms of natural (“external” and “internal”) selection. A narrower definition (“reproductive constraints”) is proposed. Internal selection is not a selection caused by an internal agent. It is a form of environment-independent selection depending on the level of the organism’s internal developmental or functional coordination. Selection and constraints are the main deterministic processes in phenotypic evolution but they are not opposing forces. Indeed, they are continuously interacting processes of evolutionary change, but with different roles that should not be confused.

INTRODUCTION

In recent times, from several sources, evolutionary biology students are appealing to a so-called “new synthesis” (e.g., Gilbert et al. 1996; Strohmman 1997; Arthur 2000; Carroll 2000; Hall 2000). In these papers, neo-Darwinism is regarded as incomplete in several respects, and the major omission is thought to be the limited consideration of developmental processes for explaining evolutionary change. “By viewing evolution as a branching tree of adults or genes, theorists have omitted what selection really acts upon: ontogeny. Ontogenies evolve, not genes or adults. Mutated genes are passed on only to the extent that they promote survival of ontogenies; adulthood is only a fraction of ontogeny” (McKinney and Gittleman 1995).

The evolutionary developmental biology approach (evo-devo) aims to integrate development and evolution in a coherent theory (Hall 1992). Some conceptual revisions are useful in order (a) to match the different languages of separate disciplines, (b) to reevaluate biological processes working at different levels of biological organization, and (c) to attempt a new organization of evolutionary and developmental knowledge in a new comprehensive common frame. For instance, with a similar intent, Arthur revised roles and categorization of mutations (Arthur 1997, Chapter 8) and modes of evolutionary change of the developmental processes (“developmental reprogramming,” Arthur 2000).

On the same lines and in the same theoretical context, this article compares some biological processes and attempts to sort them rationally on the basis of their features and their reciprocal relationships. Natural selection, random drift, devel-

opmental constraints, and internal selection are commonly recognized and accepted by a majority of evolutionary biologists as natural processes of primary importance in phenotypic evolution (though internal selection is less widely accepted than the other three). The change of perspective from the “evolution of phenotypes” toward the “evolution of ontogenies” can affect the way we consider, classify, or delimit these different phenomena.

Phenotypic evolution is evolution of individual organisms, but the biological concept of individual is not an easy one, as most definitions provided are troubled by notable exceptions [see Santelices (1999) and references therein]. For the present discussion, I will leave out of consideration chimeric organisms with clonal propagation, genetic instability, and lack of autonomy (as some red algae and fungi) and organisms forming highly specialized colonies. Possibly, to a certain extent, the colony itself could be considered an individual, but in general, the advent of a new level of organization (the colony) involves new complex “developmental” processes that are not simply conceivable as extensions of differentiation and morphogenesis.

Considering the anagenetic dimension of evolutionary change, phenotypic evolution is generally conceived as a two-step process: production of variation and sorting of this variety, mainly through natural selection. Considering together evolution and development does not alter this schema in its main lines, but for one important aspect: variants are not different phenotypes or alternative genotypes but instead different developmental processes. This has substantial consequences.

NATURAL SELECTION AND RANDOM DRIFT

Sorting is a process that changes the frequencies with which different types of elements are represented in a given set. Differential longevity and reproduction unite natural selection and random drift as sorting processes (Vrba and Eldredge 1984), that is they are both forms of sorting running on an offered set of alternative types (e.g., genotypes or phenotypes). Natural selection is a form of nonrandom sorting that is based on some properties or characters of the individuals of a population (longevity and reproduction are correlated with some features of the organisms; it can explain adaptation), while random drift is a stochastic sorting (longevity and reproduction are uncorrelated with any features of the organisms; it can be neutral with respect to adaptation). Herein—after the term sorting, without further specification, will be used for natural selection and random drift together.

Sorting at the level of organisms, traditionally intended as operating on a variety of phenotypic forms, can easily be applied to alternative developmental processes without special conceptual modification. Some ontogenetic processes (individual ontogenetic trajectories) with better reproductive performances proceed in their natural dynamics, producing a larger contribution to the next generation of developmental trajectories than others. We can think of a population as a bundle of ontogenetic trajectories developing in a multivariate morpho-space, each of them representing one individual dynamic-system-organism, where a process of sorting determines which trajectories will give origin, through reproduction, to the new trajectories of the subsequent generation, and in what measure.

Although the present discussion is more concentrated on selection and drift at the level of organisms, it is evident that the shift from a static forms perspective to a dynamical processes perspective is extendible to sorting at any level of biological organization, just because any biological system can be profitably described as a dynamic system. But, beyond the fact that sorting of developmental trajectories constitutes a more complete representation of evolutionary change than sorting of phenotypic forms, what will be stressed here is that natural selection and random drift are post-production processes, that is that sorting, changing the frequency of occurrence of different types in a collection of alternative types, while it can cause the disappearance of certain types, cannot introduce new types. Sorting cannot explain the appearance of phenotypic novelties.

DEVELOPMENTAL CONSTRAINTS

A classical definition of developmental constraint is “a bias on the production of variant phenotypes or limitation to phenotypic variability caused by the structure, character, composition, or dynamics of the developmental system” (Maynard

Smith et al. 1985). Although in past years the notion of developmental constraints has been conspicuously debated, redefined, and supplied with new adjectives to account for a bulk of different phenomena (Antonovics and van Tienderen 1991), in the original definition, developmental constraints were intended to be well differentiated (in principle, not in the history of life) from any kind of sorting. Constraints precede the process of sorting by limiting or in some way conditioning the offer of phenotypic variants among which sorting can then follow. A biased phenotype production is not necessarily a negative concept, because a limited or null production of certain phenotypes means inevitably a production relatively in favor of certain other phenotypes (Arthur 2001).

A biased production is the key concept in distinguishing constraints from sorting, but generally, in part for practical reasons (most studies being based on adult forms), a biased production does not mean exclusively limited (or null) origination of certain developmental trajectories. Cases of precocious failure of development, with low or null viability of the embryo or the larva (especially if these are independent from the environment, where environment has the broad meaning of any factor, biotic or abiotic, external to the organism), or that occur in an environment considered appropriate for other viable conspecifics, may be lumped with developmental constraints. Such cases of “incomplete development” can be considered developmental constraints because they limit young and adult phenotype production.

In an evo-devo perspective, there is no reason for treating the first phase of a life cycle as if it was just a preparatory phase for the production of a living organism that will be sieved by the environment later on. From a formal standpoint, once production has occurred (i.e., when there are new individuals and new developmental processes have started, for instance from fertilized eggs), a downstream process that biases the composition of the “bundle of ontogenetic trajectories” that constitutes a population is functionally a process of sorting, random as the lottery of life, nonrandom as natural selection, or a combination of the two. Obviously, during the whole life of an individual, different ontogenetic stages will experience different kinds of selective pressures and random accidents (for organisms with complex life cycles, these will be dramatically diverse), but in any case it is always a process of sorting because production has already occurred.

To separate the biases on the production of developmental trajectories from the biases on the production of adult phenotypic forms is not an easy task. But, in any case, distinguishing these two components of developmental constraints is important to devise clear models for evolutionary change: an evo-devo perspective needs a narrower concept of constraints.

A narrower definition of constraints

Reproduction consists of the biological processes that produce new organisms from the preexisting ones. It is based on

transmission of initial and boundary conditions to totipotent cellular systems via genes and cytoplasmic organization (Webster and Goodwin 1996).

Developmental constraints in the narrow sense of biases on the production *sensu stricto* could therefore be named reproductive constraints: biases on the production of new ontogenetic trajectories caused by the current mechanisms of transmission of hereditary factors and the laws of epigenetic interaction. In the extreme case, reproductive constraints are manifested by total absence of certain developmental trajectories because it is impossible to originate them from the existing trajectories. Ontogeny can start from a single-cell stage (e.g., a fertilized or a parthenogenetic egg) as well as from a multicellular stage (e.g., a bud or a parental body fragment) (see Fagerström et al. 1998; Grosberg and Strathmann 1998).

For reproductive constraints, as well as for traditional developmental constraints in general, we can distinguish between local and universal constraints (Maynard Smith et al. 1985; Resnik 1995). Local reproductive constraints are taxon-specific in that different ontogenetic trajectories are more or less accessible given the present genetic and epigenetic systems. Local constraints can be active in a lineage for a limited evolutionary time span and are therefore potentially breakable. Universal reproductive constraints are direct consequences of the laws of physics and chemistry, are taxon-independent, and potentially last forever. As noted by Maynard Smith et al. (1985), local and universal are not mutually exclusive categories but rather the terminal points of a continuum of possible situations.

Sources of reproductive constraints

To simplify discussion, we can consider two main (nonindependent and nonexclusive) sources of local reproductive constraints: (a) features of transmission of initial and boundary conditions (genetic and nongenetic heredity) and (b) epigenetic interactions. Their nonindependence lies in the fact that although epigenetics indicates emergent properties of the developmental system not reducible to its components (e.g., its genetic arrangement), nevertheless the latter also have direct influences on development. Therefore initial conditions and epigenetic interactions cannot be completely separated. Initial conditions and epigenesis determine a set of potential ontogenetic trajectories [a “developmental norm of reaction” *sensu* Schlichting and Pigliucci (1998)], the actual realization of the ontogenies depending on the environmental conditions the organisms are exposed to during their life.

Transmission of initial and boundary conditions

Transmission of initial and boundary conditions involves (a) genetic heredity (i.e., genes and their arrangement) and (b) cytoplasmic heredity (also called epigenetic heredity, but this term is not adopted here to avoid possible confusion with epigenetic interaction) that is the spatial configuration and

the physicochemical properties of the physical systems (e.g., the fertilized egg) that is at the base of the new ontogeny. Cytoplasmic heredity can be partially under maternal genetic control.

Mutation, recombination, and syngamy are the main sources of genetic variation and, although their relative importance is taxon-specific, the scope for biases on genotype production is extremely diverse. Type and frequency of possible mutations (not only point mutations, but also frameshift mutations, gene duplication, chromosomal mutations, insertion of transposable elements) and patterns of recombination (independent assortment and crossing over) are affected by the content and organization of the genome (e.g., number of genes, linear arrangement of genes along the chromosome, number of chromosomes). On the other hand, the effects of recombination and syngamy are further affected by the type of reproduction adopted (e.g., amphigony versus uniparental sexual reproduction or asexual reproduction) and the mechanics of gametogenesis. Linkage disequilibrium and meiotic drive are well known examples of transmission genetics biases.

While in asexual reproduction the influence of the specific mechanism adopted reflects directly on the physical properties of the new individual precursor, in sexually reproducing organisms, biases in the setting of the physical structure and properties of the individual’s first cellular precursor can be active in oogenesis. In most species (except for mammals) rate of cell division and placement of blastomeres are completely under control of proteins or mRNA stored in the oocyte by the mother (Gilbert 1997). Well-known examples are the distribution of morphogenetic determinants for antero-posterior polarity in *Drosophila* or for the orientation of cleavage plane in spiral cleavage in the snail *Lymnaea*. Quantity and spatial distribution of yolk in the egg can influence cleavage pattern, and the sea urchin *Clypeaster roseaceus* undergoes facultatively direct or indirect development on the basis of egg size (Raff 1996).

Epigenetic interaction

Conversely, we do not have a general theory for epigenesis and although some ten years ago Alberch (1991) pointed out that “a theory of evolution of complex morphologies has to be based on the global properties of the network of interactions that characterize development,” constraints generated by the dynamics of the epigenetic interactions are still a speculative field of enquiry. Arthur (2000) remarked that this is a neglected field of study and noted that much remains to be discovered.

The formalization of the idea of a biased production of ontogenetic trajectories caused by the dynamics of the developmental system can be traced back to Waddington’s (1957) concepts of epigenetic landscape and canalization, recently reviewed by Schlichting and Pigliucci (1998, p. 232): “Epigenesis is the ensemble of processes that propagate pheno-

typic characteristics throughout development. These processes derive from either indirect effects of gene action (emergent properties) or from non-genetic phenomena (e.g., cell-cell or hormone-target communications)."

The irreducible role of the epigenetic processes derives from complexity and nonlinearity of the network of gene interaction and cell communication involved in any developmental process, from differentiation to morphogenesis and growth. Nijhout (1990) remarked that "genes do not provide instructions for development" and that "the network or pattern of gene activation does not constitute a program, it is both the consequences of, and the contribution to, development." It is exactly the complexity and the nonlinearity of the genotype-phenotype mapping that makes it difficult to explore the dynamics of epigenetic interactions (Minelli 1998) and may frustrate attempts to construct a detailed flowchart of the sequence of events linking genotype to phenotype (Schlichting and Pigliucci 1998).

Even if a complete map of epigenetic interactions had never been drawn, the simple observation that development is amazingly robust in the face of perturbations (Nagy 1998) or that diverse perturbations of development are manifested by a limited subset of morphological defects (e.g., axial patterning in both flies and mice) (Hodin 2000) support the idea that morphogenetic fields embody "organizational principles" that themselves impose important constraints on the set of phenotypic trajectories that can be generated (Webster and Goodwin 1996).

The investigation of the generic properties of networks is now a hot spot in current research. The study of behavior and evolution of biological networks, such as genetic networks, metabolic networks, or neural networks, is advancing in parallel with theoretical studies on their generic properties [see for instance Watts and Strogatz (1998) for signal propagation, or Réka et al. (2000) for error tolerance]. Nevertheless, along with computer simulation studies [e.g., von Dassow et al. (2000) on the segment polarity gene network], some noteworthy experimental studies that try to disentangle this complex subject have been successfully conducted (Brakefield et al. 1996; Nielsen et al. 2000).

Selection constrains selection

What about developmental constraints that I consider not real constraints (i.e., reproductive constraints) but instead a form of selection during development? Natural selection, manifested in a population with the termination of the less fit ontogenetic trajectories, can operate at any stage of development. So, it is not wrong to say that selection at an early stage of development constrains selection at a later stage. "Selection constrains selection" just because a life cycle is a historical process: any time we focus on the selective pressure at a specific developmental stage, any preceding process involved in shaping the current offer of phenotypic variants can be seen

as a constraint for that selective process. For instance, for a holometabolous insect, selection at the first larval stage constrains selection from the second larval stage onward, selection at the second larval stage constrains selection from the third larval stage onward, and so on.

Nevertheless, it is important to distinguish when absence of certain phenotypes, or certain developmental trajectories, is due to a limitation of production in the narrow meaning of reproductive constraints or instead when it is caused by the sequential accumulation of the results of selection during development. One could argue that even reproductive constraints are caused by the sequential accumulation of the results of selection during evolution ["historical constraints" *sensu* Resnik (1995)]. "Pan-selectionists" and "structuralists" could express different opinions here, but anyway, even in case of reproductive constraints evolved by means of selection, selection during development is still a distinct process that works on a different time scale.

INTERNAL SELECTION

The recent advances in developmental genetics that are disclosing the complex network of gene interactions involved in morphogenesis and pattern formation are renewing the interest around the controversial concept of internal selection, as was first conceived by Whyte (1965) and recently revived by Arthur (1997). "Internal selection occurs when individuals with different genotypes at a given locus differ in fitness because they differ in their degree of internal coordination. . . . Fitness differences associated with internal selection are likely to remain approximately constant across a wide range of environments; unlike those caused by an external selective agent which are likely to be highly environment specific" (Arthur 1997, pp. 221–222).

Despite the fact that an organism's internal coordination is indisputably a property of primary importance for a living system, internal selection finds it hard to gain a sound acceptance among other, better established processes, namely (external) natural selection and developmental constraints. I think the reason is that the current understanding of internal selection suffers from two main drawbacks: (a) there is clear overlap between internal selection acting in early development and developmental constraints, when the latter are not intended as reproductive constraints; and (b) if the adjective "internal" refers to the agent of selection, this generates a logical difficulty.

Internal selection and developmental constraints

Organisms are complex biological systems that make themselves through a concert of many parallel physicochemical processes that have to harmonize with each other in order to produce a functional, integrated system. Coordination is fun-

damental at any level of the complex machinery that constitutes an organism: in interaction among cross-regulating genes and gene products, from short- to long-range cell-cell communication, in functional integration among morphological units.

When an insufficient degree of internal coordination affects genetic or epigenetic developmental processes, this affects the normal course of development and in extreme cases can lead to the failure of development itself. In these cases, internal selection is indistinguishable from developmental constraints, as already noted by Arthur and Farrow (1999). Take for instance the consequences of a mutation in *Drosophila* consisting of deletion of the whole Bithorax Complex of homeotic genes. The mutant is normal from the head down to the prospective II thoracic segment that is then iterated until the posterior end of the body: such a case would fit in both categories. It is a case of internal selection because such a modification cannot be integrated in the present gene-regulation network in order to develop a viable larva and adult: the embryo fails to hatch. It is a case of developmental constraint because it constitutes a limitation to adult phenotype production: the regional specification of abdominal segments' identity does not allow the production of phenotypes with abdominal legs. There is overlap between internal selection affecting development and developmental constraints (excluding reproductive constraints) because the former depicts the causes and the latter draws the consequences of the same phenomenon.

On the other hand, when the level of internal coordination is manifested as poor physiological or behavioral performances of the phenotype, at any stage of development and in a wide range of environments, internal selection may be difficult to distinguish from ordinary natural selection. An example could be selection against a mutation that causes low fertility independent from environmental conditions.

Internal selection and natural selection

The core of the definition of internal selection is the nature of the selective agent. A selective agent could be defined as the entity causing the selection, and in the case of internal selection this entity is assumed to be interior to an individual, part of its "internal environment."

The identification of the agent is considered fundamental to qualify selection, and on the basis of the agent's nature several alternative classifications of natural selection have been devised. But sometimes the agent cannot be easily identified: an organism's trait strongly correlated with survival and reproduction could be known (e.g., a "lethal gene"), but there is not an obvious agent, external to the organism, that can be considered responsible for selection. Apparently, there is scope for appealing to an internal agent, but I want to show that (a) even in these cases lacking evident external selective agents, the agents of selection are external to the ele-

ments to be selected (e.g., the organisms), and (b) these cases, while doubtless deserving a special category within natural selection, are not characterized by the special (internal) "position" of the agent.

Which kind of selection is natural selection?

For a population, any biotic or abiotic factor or process that gives rise to differential survival and reproductive performances can be seen as a cause of selection (i.e., a selective agent). But, more precisely, in which way does an agent cause selection? Selection on a set of objects can proceed in several different ways, and this has a fundamental relevance for the scope of an agent.

The simplest form of selection is a mathematical operation within set theory: the result of a selection on a given set is a new set composed of all the elements of that set that match a certain criterion (for instance in a set of natural numbers, all the numbers larger than a given number X). A classical physical transposition of this operation could be the selection of a bucket of sand through a sieve. The width of the sieve's mesh determines which grains of sand will be captured. The sieve is the agent of selection as it is at the same time the device for realizing selection and the entity that supplies the criterion of selection (i.e., a critical grain size). Several natural processes are ascribable to this form of selection as, for instance, sediment sorting during geomorphological processes of deposition.

Within dynamic system theory, another form of selection, quite different from the simple mathematical operation, is realized through the competition for resources among concurrent dynamical processes. Depending on the environment of the competition, certain processes override some others because their dynamics propagate them more effectively. In this case, the agent does not provide directly a criterion of choice but instead (less directly) supplies an arena with a set of rules able to influence the result of selection. An example of this kind of selection is the dynamics of different chemical reactions that are catalyzed by the same substrate in a common chemical system. Reagents can have different affinity for the substrate and the products can have different stability, depending on the physicochemical conditions of the system. Such conditions determine which (if any) dynamical equilibrium state will be reached.

Looking at the results of a process of selection, one can always read a "criterion of choice" that, applied to the original set of elements to be selected, can account for the resultant pattern. But if selection proceeds following the latter mode, the criterion of choice cannot be regarded as a "real factor" of the selective process (as for instance, the width of the mesh would be for the sieved sand). It does not give a description of how selection proceeds; it is reduced to a mere (a posteriori) description of the pattern obtained in terms of what it was before.

In general, natural selection is not plain sieving (although its results can be read in this way), it is more a selection of the second type. It is not an operation on a defined set of elements on the basis of a criterion because the criterion is not defined before the selection and often neither is the set (possibly, to some extent, artificial selection can be seen as an operation where the agent supplies a precise criterion of choice). Natural selection cannot “see” fitness: fitness is a measure a posteriori, not a criterion of choice.

Natural selection results from the natural trend for a collection of replicating transient systems (as organisms are) to become relatively richer in those systems that produce more viable quasi-copies of themselves. The process extends ideally ad infinitum toward future generations: a population will enrich in those kinds of individuals that produce more offspring, which in their turn will produce more offspring, and so on. With a parallel in computer sciences (see Hartwell et al. 1999), natural selection can be likened to the collective dynamics of a set of competing (replicating) processes. Computer simulation studies on evolving ecosystems are based on this analogy (Ray 1994).

So, what is the scope for an agent of natural selection? The agent favors certain developmental trajectories (and consequently, certain developmental norms of reaction) over others by shaping the environmental conditions of the competition. It is perhaps necessary to stress that competition does not mean a direct fight (post-Darwinian “struggle for existence”), but just the expression of different survival and reproduction performances.

Can an agent be internal to the elements to be selected?

Conceiving an agent of selection internal to each element of the set to be selected, meaning both inside the organism and potentially specific to the individual, produces a logical difficulty (1) and is unnecessary (2).

1. A circular argument: Undoubtedly, there are factors internal to organisms that can influence the result of selection, but to regard these factors as agents leads to a circular argument because these factors are already part of the explanation for the organism’s features that, affecting survival and reproduction, are winnowed by selection. For instance, considering the level of internal coordination, a selective agent entangles the action of selection itself, because the level of internal coordination is also (I’d say, primarily) a cause of the organisms’ life performances that the agent “internal coordination” would differentiate in terms of survival and reproduction. The same difficulty would result by considering the color pattern of *Biston betularia* as the agent of its selection.

2. Internal selection is always reducible to natural selection: A putative case of internal selection would be the following. For a given locus (say in a haploid organism) the product of allele X coordinates better than that of allele x with genomic context Y . Conversely the product of allele x coordinates better than that of allele X with genomic context y . Selection that favors internal coordination because it allows better survival and reproductive performances will favor combinations XY and xy over combinations Xy and xY . From a gene perspective this is a case of internal selection (what matters is the genomic context Y or y , not the external environment) but from an organism perspective we can more simply record that natural selection favors certain genetic combinations over others.

Selection results from the confrontation of the selective force produced by the agent with the level of adequacy of the biological responses of different organisms. One could say that natural selection has always and simultaneously “internal” and “external” causes: external environmental conditions select organisms’ developmental trajectories among different developmental options (developmental norm of reaction) determined by internal organisms’ features, while the different levels of fitness of the ontogenies depend on an external environment of competition (the agent) that establishes a differential in terms of survival and reproduction. Even when an external agent seems not to be identifiable, because measures of organisms’ performances reveal them to be independent from any environmental condition, it is still in the striving with a physical world (external to the organism) that these performances emerge: a physical world that imposes metabolic (organisms are open systems) and reproductive (organisms are transient systems) performances.

The distinction between internal and external selection is misleading because the accent on the agent forces the mechanism of selection to an unnecessary logical difficulty, confounding the causes of organisms’ life performances with the causes of sorting. On the other hand, the label “internal selection” indicates a class of cases within natural selection that doubtless deserves special attention. But what characterizes this category is not the internal position of the agent, it is the trait that correlates with differential survival and reproduction: the organism’s internal coordination.

In this perspective, the degree of internal coordination is not an agent of natural selection but rather something close to a functional character (sensu Lauder 1990). A functional character (unlike structural characters) is the way in which a complex of structures is used, it is a performance measure, as are for instance the resting metabolic rate or the absorption spectrum in photosynthesis. A functional character, at any level of biological organization, depends on the features and interactions of a complex of structural (e.g., morphological) characters.

The concept of internal selection could be very useful, providing that it does not indicate cases of selection due to an internal agent but instead indicates cases of natural selection for the complex of characters that determines environment-independent, developmental or functional, internal coordination.

Internal selection could profitably indicate those cases of nonrandom sorting that are practically environment-independent, as suggested in Arthur's (1997) concept of a "flat trans-environmental fitness profile," and that, when acting during early development, are the cause of constraints for later developmental stages.

CONCLUSIONS

The concern for keeping selection and constraints well separated is not a new theme in evolutionary biology (see for instance Gould 1989; Williams 1992; Schlichting and Pigliucci 1998; Arthur and Farrow 1999). In this article, special care is taken to maintain this fundamental distinction in the new extended evo-devo context. This is not to say that selection and constraints are antagonists in evolution; on the contrary, I regard them as creative, continuously interacting, processes of evolutionary change, but I think also that they have different roles that should not be confused.

The mutual importance of selection and constraints in evolution is well represented by Wagner and Altenberg's (1996) concept of evolvability: "the genome's ability to produce adaptive variants." These authors developed a model of modular genotype-phenotype mapping, where different morpho-functional units (modules) within an organism have low levels of integration among them, whereas the elements of each module have high levels of integration and are thus expected to evolve in concert, with minor influences on other modules. Integration, intended as pattern, is the morphological association among characters and the correlated change of the state of several characters. When integration depends on a specific genotype-phenotype mapping, the topology of the mapping could be the result of an earlier process of selection among different competing mapping models, but, once established, the mapping works as a constraint, causing limitation to phenotypic variability. Integration as process is the change of the mapping in a direction of stronger association of functionally related characters. These are reproductive constraints that can easily be promoted by natural selection. Integration and modularity, resulting from a combination of selection and constraints, can have a relevant role in evolution guaranteeing evolvability.

A similar view of the cooperative relationship between selection and canalized production (a consequence of ontogeny's "self-organizing" properties) emerges from Kauffman's (1993) model for gene regulation systems ("NK Bool-

ean networks") from which it results that natural selection alone would be unable to escape two "complexity catastrophes" occurring in adaptive searches on a fitness landscape: (a) populations trapped on local (suboptimal) peaks; (b) selection too weak to hold a population in any circumscribed region.

Gould (1989) suggested seeing constraints not only as forbidden ways of evolution but also in a positive sense as channels of possible change, a propensity to vary with some chance to improve. In order to emphasize the positive meaning of biased production, in contrast to the negative sense that the term "developmental constraint" might suggest, von Dassow and Munro (1999) suggested the more comprehensive term "variational tendencies," while Arthur (2001) called the biases in favor of the production of certain phenotypes "developmental drive." Yampolsky and Stoltzfus (2001) illustrated with a token model the potential of "mutation biases," as internal causes of orientation, in driving evolutionary change by introducing novel variants.

Without the aim of suggesting a "new enhanced classification" of the major processes in phenotypic evolution, but with the mere purpose of summarizing the discussion of the present article, below is proposed a schematic classification of the main processes involved in phenotypic evolution, as they are named and organized here respect to their traditional designation (in *italics*):

1. reproductive constraints [*developmental constraints* (exclusively when intended to be seen as biases to the origination of new ontogenetic trajectories)]
2. sorting
 - 2.1. random sorting [*random drift* (at the level of individuals), *contingency* (at the level of populations)]
 - 2.2. nonrandom sorting [*natural selection*]
 - 2.2.1. environment-dependent selection [*natural (external) selection*]
 - 2.2.2. environment-largely-independent selection [*natural (internal) selection, developmental constraints* (when these are the result of environment-independent selection during development)]

Finally, I am expected to answer the question in the title: how many processes are responsible for phenotypic evolution? Obviously this depends on how detailed a classification is adopted, but in my opinion these are mainly two: with Pigliucci and Kaplan (2000) I think that "selection and constraints are the two major deterministic players on the evolutionary stage." Reproductive constraints rank things from "impossible" to "highly probable," while sorting winnows the real world from the world of the possible.

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