

On the origins of morphological variation, canalization, robustness, and evolvability

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Synopsis Canalization is a concept, introduced by Waddington that describes the reduced sensitivity of a phenotype to genetic and environmental perturbations. Some research in canalization assumes that lack of variation in a trait in one genotype with respect to another genotype in a population, is due to the existence of buffering mechanisms against environmental and/or genetic variation. This article criticizes this assumption and out points out other possible problems with the concepts of canalization, robustness, and evolvability. These involve: the neglect of alternative explanations for the lack of variation in a trait, the incompatibility with current understanding of development, the way the multivariate nature of morphological variation is considered. In addition, this article tries to explain that these concepts implicitly assume, although not generally acknowledged, that without buffering any genetic or environmental variation should give rise to a distinct phenotypic outcome. This can be avoided by restricting the use of canalization to cases in which, as in *hsp90*, there is direct evidence of buffering. For the other cases it would be clearer to talk about variational properties or simply type of variation. The concept of evolvability is also biased towards univariate comparisons and is dependent on selective pressures. It is suggested that this can be replaced by “type of phenotypic variation” from a genotype or variational properties. Overall, this article proposes that the concepts of canalization and evolvability involve some assumptions that, in most situations, unnecessarily complicate the study of evolution and development.

One of the aims of evolutionary biology is to understand how the disparity and diversity of living beings have been produced by evolution. To understand in which specific way morphologies in a population change over generations (i.e., the direction of evolutionary change) two questions need to be addressed: (1) which heritable phenotypic variants arise in each generation and (2) which of these variants are filtered out by ecological factors in each generation. During the past century, a great deal of understanding has accumulated in regard to question 2. The study of the kinetics of replacement between genetic variants under different selective regimes, populational structures, and inheritance systems, as well as the study of adaptation in the wild and in the laboratory has been very important for the development of evolutionary theory. It has been claimed that populational thinking and populational genetics have laid the foundations of the neo-Darwinian synthesis (Mayr 1982). Question 1 has received, until recently, much less attention. Evo-devo research is quite promising in that sense. However, this research is not, with few exceptions (Alberch 1982; Newman and Müller 2000), devoted to directly understanding

which phenotypic variation can be produced by development (and how that evolves), but rather to identify the genetic basis of the morphological differences among species (Wilkins 2001).

Most of the past century’s evolutionary research developed without much understanding about which morphological variation arises from changes in development. There is a large amount of literature measuring and describing variation, although, until recently (Bookstein 1982), most of it involved either quantitative univariate (or low dimensionality) morphological traits [preferred in populational genetic studies of morphology Wright (1978)] or qualitative descriptions of variation. Nowadays there are studies describing multivariate morphological variation and its correlation with genetic variation (Klingenberg et al. 2004; Cheverud et al. 2004; Polly 2005). However, as I will try to explain, some concepts in evolutionary biology are based on a much simpler conceptualization of morphology and its variation.

Since the nature of morphological variation is not explained from development, most evolutionary studies are restricted to explaining the direction of

From the symposium “Linking Genes and Morphology in Vertebrates” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2007, at Phoenix, Arizona.

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Integrative and Comparative Biology, volume 47, number 3, pp. 390–400

doi:10.1093/icb/icm075

Advanced Access publication July 26, 2007

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evolution on the basis of which part of the available (given) variation is selectively favored by ecological factors (Alberch 1982; Goodwin 1994). The lack of understanding of development is not the only reason for the relative neglect of question 2. Some of the more influential authors on the neo-Darwinian synthesis (Fisher 1930; Haldane 1932) did favor the view that development does not play a major role in evolution.

A large variety of concepts have arisen over time to deal with phenomena at the morphological level that, arguably (Müller 2006), can not be easily described or explained from the neo-Darwinian synthesis. In the present article, it is argued that some of these concepts are still based on some assumptions about variation and development that are not compatible with current understanding of morphological variation and development. This is done, as previously for the concept of developmental constraints (Salazar-Ciudad 2006a), for the concepts of robustness, also understood as canalization, and evolvability. Some practical problems of these concepts, and the research programs they involve, are explained by comparing them with alternative ones in the understanding of the relationship between genetic and morphological variation. Finally, suggestions are offered for ways these concepts can be redefined or refined to smoothly fit into a research program to understand question 1.

Evolution as the accumulation of small changes

Some influential early researchers in population genetics (Fisher 1930; Haldane 1932) made the assumption that selection is the main force determining the direction of morphological evolution. According to this view, development may play a negligible role in affecting the direction of evolutionary change. Since selection acts on existing morphological variation and development is the process whereby genetic information (and its variation) is used to produce morphology (and its variation), this assumption has inevitable logical implications for development (even if at the time these views arose development was not very well understood). For selection to be the main force determining the direction of evolution, a simple relationship between genotype and phenotype is required. Otherwise, many optimal phenotypes may be unreachable by selection. This is because close genotypes may exhibit very different phenotypes (and fitnesses), and then not every optimal phenotype is attainable through a path of progressively

increasing fitness (Salazar-Ciudad 2006a). In addition, it is required that, from any phenotype, offspring can be generated with small variation in any possible phenotypic direction. This means that small variation is possible for any combination of trait values. If that is not the case, populations may not be able to attain the most optimal phenotypes. They will move, instead, inside the sequence of most adaptive phenotypes arising in each generation (in a population from genetic variation). In other words, if small variation is not possible in all directions, the sequence, or set, of most adaptive phenotypes in each generation is not necessarily connected with, or leading to, the optimal phenotype expected from selection alone. Therefore, the direction of morphological change in each generation and over long time intervals depends on which variation (directions of change) is produced from development and which are of it are selected.

It is currently acknowledged (Goodwin 1994; Newman and Müller 2000; Arthur 2004) that there is structure in the patterns of morphological variation arising from development. Thus, in each generation some specific morphological variation is possible, some is unlikely and some is not possible. Which variation is possible depends on the developmental mechanisms by which morphology is generated. It is rarely possible to predict aspects of this variation from current understanding of development but this does not mean that this variation is random or possible in any direction. Development can also change to produce different kinds of variation but, again, some changes are much more likely than others and lead to different kinds of morphological variation (Salazar-Ciudad et al. 2003). Even if development could change to produce any imaginable phenotype the different likelihood of different changes in it would affect the direction of evolution (to the extent that not any imaginable adaptive phenotype could be effectively attained). Thus, the argument that adaptive variation for any selective pressure would eventually be found is hardly tenable.

Neither development nor selections are sufficient, the direction of morphological evolution depends on the interplay between the morphological variation produced by genetic variation on development and the filtering of that by natural selection. In other words, unless phenotypic variation is assumed to be small, isotropic, and simply related to genetic variation, development is also an important factor in determining the direction of morphological evolution. This kind of view about variation will be called, for historical reasons, neo-Darwinian,

although many researchers that would classify as neo-Darwinians may not explicitly adopt that view.

The origins of canalization and developmental constraints

This view about variation and the relative neglect of development has been extensively criticized (Alberch 1982; Maynard Smith et al. 1985; Goodwin 1994; Newman and Müller 2000). Some of these critics have been very influential in the re-emergence of developmental evolutionary biology. Two concepts have been especially relevant in that sense: developmental constraints and canalization.

Canalization is a concept introduced by Waddington (1942, 1957) that describes the reduced sensitivity of a phenotype to genetic and environmental perturbations. He developed the concept of canalization based on the “very general observation... that the wild type of an organism, that is to say, the form which occurs in Nature under the influence of natural selection, is much less variable in appearance than the majority of the mutant races” (Waddington 1957). In a similar way, Waddington attributed the apparent existence of discrete cell types without intermediates to an effect of canalization. According to him the constancy of wild-type phenotypes in the face of genetic and environmental perturbations is best viewed as buffering of the developmental process. Waddington’s view is that buffering of development evolves as a result of natural selection, i.e., stabilizing selection. Canalization can be towards environmental factors (in which case it is, roughly, the contrary of phenotypic plasticity) or genetic changes (in which case it is the contrary of evolvability; but see subsequently). Canalization would be, according to those definitions, a property of a genotype. Thus, canalization is neither a mechanism, nor developmental, nor selective; although after Waddington canalization is often associated with, or equated with, buffering mechanisms. Recently, many reviews and theoretical studies, as well as some experimental work, have been published about canalization (Gibson and Wagner 2000; Rutherford 2000; Brookfield 2001; Debat and David 2001; de Visser et al. 2003; Flatt 2005; Lenski et al. 2006). Some of these reviews claim that the study of canalization is very important for the study of morphological evolution and development (Gibson and Wagner 2000).

A consensus definition of developmental constraints is: “A developmental constraint is a bias in the production of a variant phenotype or a limitation on

phenotypic variation caused by the structure, characteristics, composition, and dynamics of the developmental system” (Maynard Smith et al. 1985). The existence, nature, and consequences of developmental constraints have been an intensively debated issue in evolutionary biology (Alberch 1982; Charlesworth et al. 1982; Arthur 2004 Brakefield and Roskam 2006). The concept of developmental constraint was developed as a criticism of the neo-Darwinian views about morphological evolution (mentioned in the previous section). In that sense, development may be a constraint because the complex genetic and epigenetic interactions that it involves mean that some morphological variation can not be produced (Oster and Alberch 1981; Alberch 1982), and this affects the direction of morphological evolution. As argued previously (Salazar-Ciudad 2006a), however, it is because of these complex networks of genetic and epigenetic interactions in development that any morphological variation is possible at all. In fact, without the developmental interactions that are supposed to be responsible for the existence of developmental constraints morphology would be simple and unvariable. No animal develops in ways that produce the kind of morphological variation required for selection to be the only factor driving the direction of morphological evolution. In fact, some studies (Salazar-Ciudad 2006a) suggest that developmental mechanisms with a simple relationship between genotype and phenotype are unlikely to evolve by random genetic mutation and selection on morphology. Contrarily, developmental mechanisms with a complex relationship between phenotype and genotype are suggested to arise earlier and more easily in evolution (Newman and Müller 2000; Salazar-Ciudad et al. 2001). In that sense, simple relationships between genotype and phenotype, if possible, would be a rather derived feature, especially for complex morphologies (Salazar-Ciudad and Jernvall 2005).

Overall, development can only act as a constraint as long as the mentioned neo-Darwinian assumptions about variation and development can sometimes hold true: otherwise development is more properly described as a factor that, together with natural selection, determines the direction of morphological evolution by producing variation in specific directions. This is not a mere question of semantics because it leads to different research agendas. From this perspective, it is not possible to test for the relative importance of developmental constraint versus selection. Any study in the direction of morphological evolution implies some hypothesis about which morphological variation (or aspects of it) is possible from development

(as seen in the case of the neo-Darwinian view). Thus, the studies that claim to test the existence, or lack (Brakefield and Roskam 2006) of developmental constraints can be understood, instead, as testing for different and not always explicit, views about how variation and development are in a specific system.

The present article explains how the concepts of canalization and evolvability share some problems with the concept of developmental constraints. In fact, the concept of developmental constraints is based on canalization and its underlying epigenetic bases (Alberch 1982). Both canalization and developmental constraints involve the lack of phenotypic variation in respect to some expected variation in some special situation. These situations are, briefly, “no development” (simple relationship between phenotype and genotype) for developmental constraints (Salazar-Ciudad 2006a) and, for some researchers (Waddington 1957), no buffering mechanisms for canalization. In spite of this conceptual overlap, researchers studying developmental constraints and canalization tend to be in different fields and use different concepts and assumptions.

Developmental constraints have traditionally been studied by developmental evolutionary biologists (Alberch 1982). Developmental constraints have their origin at the level of spatiotemporally dynamic interactions between genes, cells, extracellular components, and tissues during development. Canalization is traditionally studied by geneticists. A commonly held view is that variability (as the capacity to vary) arises from sources of variation (genetic mutation and environmental changes) and a set of regulatory processes, including buffering and enhancing mechanisms (Debat and David 2001). Although it is actually acknowledged that, mechanistically, canalization can happen at several levels (de Visser et al. 2003), most of the attention is focused at the level of genes (Rutherford 2000) and, more rarely, networks (Siegal and Bergman 2002), with few considerations of epigenetic interactions in space or of space itself (von Dassow et al. 2000). Many other studies are devoted to estimating canalization through populational studies on phenotypic variation of genetic origin (Waddington 1957; Gibson and Wagner 2000; Dworkin 2005). While researchers in developmental constraints focus on how developmental dynamics affect evolution, researchers in canalization focus on how selection shapes the genotype–phenotype map (even if that is mainly produced by development). Some research in evolutionary developmental biology focuses

on explaining specific changes in morphology (Alberch 1982), while research in genetics is more focused on the presence or amount of variation. Indeed, genetic changes alone can not be used to predict or explain in which specific way a morphology has changed unless development is considered. Overall, evolutionary developmental biologists talk about developmental mechanisms and epigenetic and genetic networks while geneticists use more black-box-like concepts such as genetic architecture, genetic background, epistasis, and pleiotropy between genes. Epistasis, for example, is inferred in genetic studies in which the phenotypic effect of a gene is modified by other genes. This does not require any understanding of development itself nor is it indicative of whether these genes directly or indirectly interact during development, or how. It is possible, for example, that one gene is epistatic over another by affecting the same aspect of a phenotype (for example the size of a body part by inhibiting apoptosis in it) but not interacting with it (the other gene could, for example, affect the size of the same body part by promoting proliferation in it). In that sense, genetic studies correlate genetic changes, low level, to morphological changes, high level, without information about developmental dynamics, and intermediate level. Thus, these concepts represent a black-box approach to the explanation of morphological variation without considerations of developmental mechanisms.

Canalization and the production of variation

There are some differences in the literature regarding the definition and meaning of canalization and related concepts (Debat and David 2001). There are also several views about how canalization can be identified and measured (Gibson and Wagner 2000; Dworkin 2005). According to Waddington’s and many other definitions, canalization is a sensitivity, propensity or resistance to change due to genetic or environmental disturbance. Canalization is neither a mechanism (but for some authors some genetic, developmental, or selective mechanisms are associated with it) nor a natural phenomenon. Variation is a natural phenomenon; canalization, as sensitivity, propensity, or resistance, is a quality; a quality of the variation produced in a group of organism. This way to define a concept may produce a substantial amount of confusion and may be partially responsible for the diversity of definitions of canalization and the diversity of similar concepts

in different sub-fields in evolutionary biology (Debat and David 2001).

There is also diversity of views about how canalization can be experimentally identified. For some authors (Gibson and Wagner 2000), canalization can be inferred in an isogenetic lineage if it exhibits less variation compared to another one, over time, or after applying the same (or arguably similar) genetic or environmental perturbations. Some other authors infer canalization when a trait stops responding to selection (reviewed by Debat and David 2001). In both cases the phenomenon identified is phenotypic variation. The question is then: how is it different to state that there is canalization from simply stating that there is more, or less, variation and why should it be useful to make this distinction? In this article, it is argued that, except in a restricted number of situations, it is not.

Canalization is focused on the lack of variation (a trait is canalized if it does not show variation in respect to some genetic or environmental perturbations), but it is not meaningful to define something (like canalization) as the lack of something else (variation), unless it is assumed that this thing (variation) should, by default or in some cases, exist. In the case of canalization it is assumed, as will be discussed, that all genetic and environmental perturbations should lead to morphological variation. Canalization is often identified as the departure from this expectation.

Many of the critiques of canalization in this article are based on the validity and usefulness of this assumption. In addition, some authors (Waddington 1957) argued that this lack of variation in respect to genetic and environmental perturbations is due to the existence of some buffering mechanisms that have been specifically selected for that function. In the following sections, it is also discussed to which extent these buffering mechanisms can be argued to be different from the normal developmental mechanisms by which morphology is produced during development and, if they are not, to which extent the concept of canalization is useful for the study of morphological evolution. The following discussion considers buffering mechanisms as mechanisms that function and have evolved to specifically buffer morphology and are different and independent from the mechanisms by which variation is produced during development.

Canalization as buffering

The existence of buffering mechanisms that specifically function in avoiding the production of

morphological variation implies that, if these buffering mechanisms or properties disappear, more or different variation would be produced. Hence, to identify canalization it is required that: (1) these buffering mechanisms can be experimentally switched off and that (2) there is a clear expectation about how variation would then be.

The aforementioned methods of detecting canalization are based on observed variation and have not been experimentally shown to switch off any buffering mechanism (except for *hsp90*; see subsequently).

Although there is some understanding about the involvement of gene networks and epigenetic networks in the production of morphology during development, in no case are developmental dynamics understood to the extent of precisely predicting which morphological variations can occur through genetic and environmental variation in a specific organ or organism. In other words, there is no realistic expectation about which morphology is possible by genetic mutation (or environmental change) in development (with or without buffering). The only way to do this, currently, is by using mathematical models (Salazar-Ciudad and Jernvall 2002, 2004; Harris et al. 2005; Newman and Müller 2005) that integrate existing experimental evidence on genetic and epigenetic interactions into mechanistic hypotheses to produce virtual organ phenotypes and their change during development.

Since there is no developmentally-grounded expectation about which kind of morphological variation genetic and environmental perturbations should produce, it is difficult to show that specific buffering mechanisms exist or are different from the developmental mechanisms by which morphology is produced. Something similar applies to the commonly held view that variability (as the capacity to vary) arises from sources of variation (genetic mutation and environmental changes) and a set of regulatory processes, including buffering and enhancing mechanisms (Debat and David 2001). Without a default expectation about which variation is possible, or reference about normal variation, there is no way to distinguish or define what is buffering and what is enhancing.

Some researchers claim that, indeed, canalization does not require any special mechanisms (Fraser and Kindred 1962; von Dassow et al. 2000; Nijhout 2002; Siegal and Bergman 2002), but appears as a normal outcome of the developmental processes by which morphology is generated. In other words, during development many genetic and environmental perturbations have no phenotypic effect.

However, as mentioned, development is the process by which morphology is produced and in which genetic variation leads to phenotypic variation. Thus, it may seem contradictory that the same process, development, is responsible for both the generation of phenotypic variation from genetic (and environmental) variation and the lack of, or resistance to, phenotypic variation from genetic and environmental perturbations. Again, the lack of variation is only significant to the extent that there is some expectation that variation should exist in response to genetic and environmental perturbations. Thus, the concept of canalization requires, to be different from the mere description of the amount of variation, the assumption that there is a type of development in which any genetic and environmental perturbation should give rise, in the absence of canalization, to a distinct phenotypic variation (if phenotypically equal variants are produced then it can be argued that these variants are canalized in respect to some mutations or environmental changes). In other words, a one-to-one mapping between genetic (or environmental) changes and phenotypic changes is taken as a default state against which canalized phenotypes are defined (it is not assumed, however, that similar genetic changes lead to similar phenotypic changes).

This assumption, I will argue, is contrary to what is currently known about development and the production of variation. Morphology is constructed, during development, by interactions between genes, the developing phenotype (initially the egg cell itself and later the spatial distribution of cells and extracellular molecules) and the environment. Variation, or perturbations, in each of these factors can lead to phenotypic variation but can also have no effect. Whether this happens or not depends on the specific dynamics of development and is, in general, not well understood.

In that respect it is important to consider that development has had to evolve for phenotypic variation to be possible. It is not because genes interact in complex ways during development that there is not a one-to-one relationship between phenotype and genotype; on the contrary, it is because of these interactions that phenotypic variation is possible at all. It can be expected that there are several ways to organize genetic interactions during development to produce phenotypic variation. Theoretical models of gene networks capable of pattern formation (Salazar-Ciudad et al. 2000) suggest that the simplest gene networks, with few genes and gene interactions, produce complex relationships between genotype and phenotype

in which many genetic and environmental changes have no distinct morphological effect. Networks exhibiting relatively simpler relationships between genotype and phenotype are always complex. Networks in which all genetic or environmental perturbation have a morphological effect were not found. Indeed, these kinds of networks are in the extreme situation in which more distinct phenotypic variants (not considering how different they are) are possible from a given number of genes and gene interactions. Thus, this kind of development, if possible at all, should be regarded as very derived and complex and it is not, thus, a reasonable default.

It is possible that the changes in developmental mechanisms favored in evolution are those that lead to the production of adaptive phenotypes (in a specific generation) and easily arise from mutations in previous development. Developments with a one-to-one relationship are likely to be among the most complex and thus among the most unlikely to arise by random mutation. In addition, selection can be expected to favor developments that have been able to produce adaptive variation in each generation and not necessarily developments that can potentially produce a distinct phenotype per each genetic or environmental perturbation.

Similar arguments can be made from more specific aspects of development functioning. The ultimate effect of many genes is to influence some epigenetic aspect of the embryo and these can, because of their nature, be affected in many ways (Alberch 1982). For example, mutations that make a signaling molecule to diffuse faster can have similar phenotypic effects as mutations that increase the affinity of its receptor (or of the signal itself). In both cases more signal is transduced, the same extra tissue is induced and, likely, the same morphological changes are produced. This multiplicity of ways to arrive at a morphology is an intrinsic property of development, (that is due to the way in which development is constructed in evolution) and does not necessarily arise as a way of restricting phenotypic variation. In fact, this can be considered as a restriction of phenotypic variation only if it is assumed that development should give rise to a one-to-one relationship. But as explained the one-to-one relationship is not based on any understanding of development. Thus, it may be correct to claim that canalization is an intrinsic property of developmental dynamics (Fraser and Kindred 1962; von Dassow et al. 2000; Nijhout 2002; Siegal and Bergman 2002), but this claim does not add anything to the general description of development as the process by which morphological variation is produced from

genetic and environmental variation. In genetics, a one-to-one relationship between morphologic effects and genetic (or environmental) perturbations may seem a reasonable or useful assumption from a black-box approach to development. In this article, it is suggested, instead, that when the genetic and environmental interactions by which development produces the phenotype are taken into account this assumption is no longer tenable. Canalization is not a natural phenomenon it is a description of a phenomenon based on a nondevelopmentally reasonable assumption. Its utility, thus, as guiding the research in the relationship between genotype and phenotype can be criticized. The claim in this article is not that researchers in canalization explicitly consider this default expectation. This article tries to explain, instead, that accepting canalization as something other than the mere description of more or less variation logically implies the feasibility of this expectation. This does not imply that the results found in canalization studies should be discarded but that they can be interpreted without these assumptions (see subsequently).

From this perspective, it is specific phenotypic variation and not its absence (defined on the bases of assumptions that do not consider development) that need to be explained first. Eventually, specific buffering mechanisms restricting variation may be identifiable once variation as such is understood (or, at least, some aspects of it).

Canalization and selection

Stabilizing selection has been suggested to favor canalization. The studies that compare canalization among lineages assume that development or genetic architecture can evolve in ways that stabilize specific phenotypes but, how this happens is not well understood. In the case of morphology, unless there is genetic variation for any imaginable change in reaction norms (or sensitivities to genetic backgrounds and environments), selection cannot be the only force responsible for the shaping of reaction norms or canalization. In which way canalization changes over time may depend on a trade-off between which morphologies are easier to stabilize (from the dynamics and variation in buffering or developmental mechanisms), and which morphologies give an adaptive advantage when stabilized.

Canalization and hsp90

Some studies of *Drosophila* have produced a mutant, hsp83 gene-deletion heterozygote (Rutherford and Lindquist 1998) that has no distinctive typical

phenotype but exhibits, when confronted with different genetic backgrounds and environments, more variation than does the wild-type. The product of hsp83 is hsp90, a ubiquitous heat shock protein that binds to a large spectrum of targets and performs a wide range of functions. The hsp90 and related chaperones are required for the maturational folding and renaturalization of many proteins (Rutherford 2003). Some of the target proteins are components of several signal-transduction pathways and are thus involved in normal signal transduction (Zhao and Houry 2005). The hsp90 is also required for the formation of several multiprotein complexes (Rutherford 2003). In addition, hsp90 is able to interact with chromatin (Sollars et al. 2003) and potentially affects epigenetic inheritance. It has been suggested that hsp90 would be able to bind to many proteins in spite of small variation in their sequence. If this is the case, then the buffering effect of hsp90 may not be morphology-specific but would act generically at the molecular level of protein conformation. If hsp90 interacts with proteins because they are mis-folded and not because they belong to a specific developmental pathways or mechanisms, then hsp90 may be involved in a real buffering mechanism that regulates the amount of morphological variation expressed and not its nature. For question 1, and for the understanding of the direction of morphological variation, hsp90 may not be very important, contrary to what has been suggested previously (Gibson and Wagner 2000; Rutherford 2003).

Selectionist, intrinsic and convergent explanations of canalization

Several groups of explanations have been given for the existence of canalization of genetic changes. Some studies explore the populational genetic contexts under which natural selection would favor an increase in the canalization of a trait (Wagner et al. 1997; Hermisson and Wagner 2004). These studies show that selection for canalization can, under some circumstances, occur but do not help in understanding whether canalization, or variation on it, can originate. Thus, it would be required to show that at least some incipient mechanisms of buffering can occur as a result of genetic variation. As mentioned, some other studies suggest that canalization may be a phenomenon intrinsic to development (Fraser and Kindred 1962; von Dassow et al. 2000; Nijhout 2002; Siegal and Bergman 2002). Alternatively, congruence hypotheses suggest that genetic canalization may appear as a by-product of

selection of canalization to environmental changes (Meiklejohn and Hartl 2002; de Visser et al. 2003). In this case, it would still remain to be explained how canalization of environmental changes appears, and in which way (if any) it is mechanistically different from the canalization towards genetic changes.

There are several proposed intrinsic properties of development and genetics that can be interpreted as buffering against genetic and/or environmental changes. Gene redundancy and epistasis are commonly suggested to produce buffering. Two or more genes coming from recent genetic duplications or phylogenetically unrelated genes with similar biochemical functions (or developmental roles) can be expressed at the same time and place. Thus, the failure of one of these genes due to the environment or to genetic variation may have no phenotypic effect because the other gene is still functional. Many knock-outs of developmental genes have been found to have no obvious phenotypic effect. Although genetic redundancy has been claimed to be the cause underlying these findings, it is unlikely that gene function can persist against random genetic mutation without some kind of selection. An explanation for this can be found if knock-outs have selectable phenotypic variation in environments other than the laboratory (something that is rarely checked).

The existence of thresholds for the amount of signal that a protein needs to receive to undertake a conformational or chemical change (for example by binding or phosphorylation) has been proposed as an intrinsic explanation for canalization (Rutherford 2000). In fact, binding between molecules naturally gives rise to a sigmoidal relationship between the concentration of free ligand and the concentration of ligand bound to its receptor. Steep sigmoidal relationships effectively behave as discrete switches that would easily buffer any variation in signal concentration to a 0 or 1 response.

More general explanations for canalization to genetic changes involve the dynamic and structural properties by which morphology is produced (Fraser and Kindred 1962; von Dassow et al. 2000; Nijhout 2002; Siegal and Bergman 2002; de Visser et al. 2003). In simple terms, the multiple positive and negative feed-backs existing in developmental processes inevitably lead to the insensitivity of the system to many environmental and genetic changes.

Intrinsic explanations of canalization suggest that there may not be a distinction, or separation, between the developmental mechanisms by which phenotypic variation is produced, and the

mechanisms by which some genetic and environmental variation is buffered.

Evolvability, canalization, and the univariate nature of evolutionary explanations

Evolvability is defined (Wagner and Altenberg 1996) as the ability of the genome to produce adaptive variation [there is a previous more technical definition restricted to the field of populational genetics that will not be discussed here (Houle 1992)]. It is a relative concept that is only definable in comparisons. Since adaptation depends on the environment, the evolvability of a genotype also depends on the environment. Evolvability is often discussed in conjunction with canalization (Wagner and Altenberg 1996; Lenski et al. 2006). Canalization would serve the resistance to genetic and environmental variation, while evolvability would allow genetic variation to produce more phenotypic adaptive variation (de Visser et al. 2003). In other words, these concepts describe the capacity to produce more or less variation and are perceived as two complementary ways to describe the same underlying phenomena. However, they are different from the mere statement that variation is produced, because canalization assumes that every genetic variation would, in principle, produce unique phenotypic variation and evolvability is only concerned with adaptive variation. Hence, they are not strictly complementary concepts. There is a large body of speculation about how the canalization of some aspects of the phenotype or/and development can lead to an enhanced evolvability of some other aspects of the phenotype or/and development (Wagner and Altenberg 1996; Kirschner and Gerhart 1998; Raff and Raff 2000; Lenski et al. 2006).

Some research in evolvability (Wagner and Altenberg 1996; Kirschner and Gerhart 1998) describes many features of the development, cell biology or genetics of animals that may facilitate the production of adaptive variation. Although these authors do not claim that these features have evolved because of their evolvability, it is worth considering that the types of genetic, cellular or developmental features that are more likely to have an important role in evolution are not necessarily the ones that can produce more or better variation (the more "evolvable"). Many of these features may have appeared in evolution because they are easier ways to produce some adaptive variation (they require fewer mutations to arise from previous ones). Hence, the likelihood of a feature appearing by genetic

variation is not related to its evolvability (it would be its “arisability”).

As relative concepts, canalization and evolvability easily lead to the comparison of two species, populations, or genotypes as evolving to greater or lesser degrees. It is rarely the case, however, that two genotypes, populations, or species differ only in the amount of (adaptive) phenotypic variation. Essentially, phenotypes are complex and multivariate and two phenotypes rarely diverge in just one measurable trait (Klingenberg et al. 2004; Polly 2005). It may also be rare, however, that selection sees only a specific, single trait of the phenotype. To compare the amount and nature of phenotypic variation as such is not an easy task and multivariate comparison of phenotypes is a rather complex field (Bookstein 1982). When the objective is to understand how the morphological disparity of living beings has arisen during evolution, simple univariate comparisons may be insufficient. This is because the nature of the phenomena to explain, and their underlying causes (development and selection) are intrinsically multivariate. In that sense, evolvability and canalization share with many approaches in the modern synthesis a clear focus on the amount of phenotypic variation but not on types, or nature, of phenotypic variation. This is not to say that studies in canalization and evolvability inevitably imply a univariate approach (de Brito et al. 2005).

It may be better to have a concept to describe variability that is explicitly sensible to the specific nature of variation (and not just to its quantity). In this sense, it may not be very informative to describe variability as arising from and enhancing buffering mechanisms. Instead morphological variability can be described on the bases of different types of developmental mechanisms giving rise to different types of morphological variation from genetic and environmental variation.

In previous work (Salazar-Ciudad et al. 2003; Salazar-Ciudad 2006b) I have proposed the concept of the variational properties of a developmental mechanism. A developmental mechanism is defined as a genetic network that is able to produce a pattern transformation. This is the change from a spatial distribution of cell types, a previous pattern, into another one, the resulting pattern, in the embryo during development. The variational properties of a developmental mechanism is the set of morphological variants (resulting patterns) produced by a mechanisms under all possible environmental changes, genetic mutations (excluding those that alter the topology of the network), and previous patterns. Mutations changing the topology

of a network are described as changing the developmental mechanism itself (that may also have different variational properties). The genetic properties of a developmental mechanism include its genes and their interactions (the network itself) and thus provide an estimation of how likely a mechanism can appear from genetic mutations (from another mechanism or *de novo*). The concept of variational properties could be used as an alternative to the concepts of evolvability and canalization. This concept explicitly considers that variation can be multivariate and of different nature while evolvability (and canalization), although not explicitly univariate, facilitate the comparisons on the basis of being more or less evolvable. The concept of variational properties considers variation without explicit reference its adaptive value. Finally, the variational properties of a developmental mechanism together with its genetic properties help to give simple mechanistic explanations for understanding how development and the morphological variation it produces can evolve. Thus, the recruitment of a developmental mechanism (or its replacement) would depend on its likelihood of arising by mutation and the adaptive value of the morphological variation it produces. Morphological evolution would depend on the morphological variation allowed by the developmental mechanisms used and the selective pressures acting on them.

Variational properties apply to developmental mechanisms and thus provide a concept much narrower than evolvability or canalization. Evolvability applies preferentially to genotypes (Wagner and Altenberg 1996), but it is applied to many other cases (Kirschner and Gerhart 1996; Brookfield 2001). In those cases, it may be possible to simply speak about “variation,” or “the type of variation that can be produced by,” instead of “the evolvability of” or “canalization of.”

Conclusions

Overall, this article proposes that the concepts of canalization and evolvability, in most situations, unnecessarily complicate the study of the relationship between genotype and phenotype and its effect on evolution because they are based on a conceptualization of evolution, variation, and morphology that was not designed to address question 1, and are indirectly based on assumptions about the genotype–phenotype map that are not grounded on experimental evidence from development. Canalization and evolvability are widely used concepts and it is unlikely that any single paper would prevent its use.

However, it is important to highlight that these concepts may not be necessary and may lead to a biased approach to the problem of phenotypic variation and its relationship with genetic variation in evolution and development.

Acknowledgments

I thank Jukka Jernvall, Stuart A. Newman, Sara Zahdy, and Allistair Evans and the organizer of the symposia Linking Genes and Morphology in Vertebrates. This work was supported by Juselius foundation (Helsinki, Finland).

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