

CANALIZATION, ROBUSTNESS AND DIVERSIFICATION

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Summary

In this paper I review concepts affecting canalization from different areas of biology. Canalization has been studied mostly by geneticists and evolutionary ecologists. I discuss the relation of canalization with robustness, a concept recently emerged from molecular biology. Both phenomena produce stability of phenotype, but only canalization insures preservation of variability. Research on mechanisms for genetic robustness, properly discussed, shed light in the proximal causes of canalization. It is a consensus to say that robustness is produced as an outcome of the complexity of gene networks, but in this paper I show how different authors have different ideas of what complexity is. I present a definition of complexity that hopefully clears up the contradictions present in the field. Afterwards I present connections between canalization and founder-event mediated divergence. I claim that this much debated view of divergence is renewed if considered under the light of canalization research.

Keywords

Robustness, genetic architecture, redundancy, epistasis, novel environments, founder event

Introduction

We commonly assume that existing genetic variation is expressed, promoting adaptation. Genetic variation then, becomes the fuel of selection. The existence of canalization contradicts this common believe. A phenotype is canalized when existing genetic variation is not expressed. This paper reviews canalization research and links concepts relevant for canalization from different fields of biology.

With canalization, I connect two current issues of evolutionary biology. They are genetic robustness and divergence via founder events. I will show that this three topics share a common backbone, and more important, they are complementary. They have remained mostly unconnected in the literature because each of them has been developed by different traditions of research. Canalization has been mostly approached by experiments tackling life-history evolution; robustness is a topic relevant for molecular biologists, and the relevance of founder effects has been acknowledged mainly in evolutionary field biology. Nevertheless, I think that the time is ripe to discuss them together, underlining the shared concepts and mechanisms.

This paper has three sections, one for each of the mentioned phenomena. Inside each section, and after having defined the phenomenon, I will discuss its proximate causes, and the evolutionary hypothesis about its origination and maintenance. Using this structure, the relation and, more important, complementarities among issues are easy to observe. We will see that canalization research has a long tradition in evolutionary biology, and quite some effort have been done to define canalization clearly. Evolutionary reasoning mostly agrees that canalization occurs to prevent important traits to change. Proximal causes of canalization had received attention recently. Perhaps as a reaction to the long debate on the concept of canalization, genetic robustness had always enjoyed a very clear definition: resistance to mutational perturbation. The focus of its research has been on its proximate causes, and different evolutionary hypotheses still compete to explain its appearance. Last and certainly not least, long debates have surrounded the concept of founder event. Interestingly enough, the proximate causes of divergence via founder events are strongly related with the mechanisms that produce canalization and/or genetic robustness, i. e. epistatic interactions. Finally, supporters of founder event speciation claims that founder events produce diversification, the exactly opposite claimed effect of canalization and robustness: stability of phenotype. In contrasting known results I show how far and still how close these areas of research are.

This review is not only a rhetorical renaming of common mechanisms. In discussing the connections just outlaid, I present questions that stemming from canalization research illuminate the debate of founder event diversification. I also discuss hypothesis on the architecture of traits diverging via founder events that emerge from the definition of genetic robustness. It is my believe that a shared discussion of interconnected topics provides much needed conceptual unity and new hypotheses to explain long known phenomena.

CANALIZATION

Early definitions of canalization

The concept of canalization stems from the empirical observation that phenotypes are often expressed stably despite genetic, environmental or developmental perturbations. A phenotype is said to be canalized when it is stably expressed despite perturbations (Waddington 1942). Different levels of phenotypic change in response to different perturbations were taken as an evidence for varying degrees of canalization (Scharloo 1991). The decrease of phenotypic variance in a trait under stabilizing selection has been regarded as evidence for selection leading to canalization (e.g. Schmalhausen 1949, Waddington 1957, Rendel 1967).

Since Waddington's original work, several experiments – mostly on *Drosophila* - have lent support to his concept of canalization. These early experiments demonstrated that (a) the properties of the developmental system determine the pattern of response to genetic, environmental or developmental perturbations and that (b) wild-type phenotypes are often less sensitive (more canalized) to perturbations than mutant phenotypes (reviewed in (Scharloo 1991)). Canalization can thus be described as developmental buffering against perturbations.

There is also evidence for canalization as a result of selection. Early on, Rendel reviewed evidence for the idea that artificial stabilizing selection can increase canalization (Rendel 1967). Successful selection for constant number of bristles in *Drosophila*, supports the concept of canalizing selection leading to invariant phenotypes (Pineiro 1992a; Pineiro 1992b; Pineiro 1992c). Some discussion exists on the statistical methods used in this approach. Most experiments with stabilizing selection used probit statistics to infer canalization, i.e. a reduction in phenotypic variance (Rendel 1967). This approach, however, is problematic since it rests on the untested assumption of a normal distribution of the underlying variables (see discussion in (Scharloo 1991; Wagner *et al.* 1997). This problem is typical in the testing of phenomenological concepts. Even though the existence of a pattern (as reduction in phenotypic variance) can be checked statistically, doing so might require the compliance of unrealistic assumptions in the structure of the data. The more mechanistic the concepts that are being tested become, the less important this problem is. Nowadays the literature already counts with mechanistic-based concepts, useful in the definition of canalization (Gibson & Wagner 2000). In the following, I will discuss them.

A distinction, variation and variability

Canalization has been broadly defined as the reduction of phenotypic variation. However, fixation or loss of alleles by selection or drift might also produce reduction in phenotypic variation. Thus, evolution may reduce phenotypic variation without increasing canalization. There is more to canalization than the reduction of phenotypic variation. The original concept of canalization as introduced by Waddington namely developmental buffering, assumes properties of developmental processes that make the phenotype, to a certain extent, buffered against perturbations while keeping, to a certain extent, the potential to respond to these

perturbations. Canalization is then the property of a trait of being resistant to modification while still being modifiable.

The intrinsic tension between no-modification and being modifiable underlines the distinction of variation and variability (Wagner & Altenberg 1996). This distinction has been discussed in other contexts than canalization (Houle 1992). Variation refers to realized differences between entities while variability refers to the potential or propensity to vary. The reduction of variation is a realized pattern observed at phenotypic level. Variability refers to processes that have the potential to engender phenotypic variation. Therefore, canalization occurs if (a) we observe a pattern of reduced phenotypic variation and (b) the variability of the trait (i.e. its potential to vary) remains present.

Note that the tension “reduced-variation versus preserved-variability” conditions the evolvability of a trait. The immediate effect of adaptive evolution is the decrease of variation, but total disappearance of variation dooms evolution all the same. Thus the importance of mechanisms that preserves some variation from the erosion of selection, i. e. a mechanism that preserves variability. If such a mechanism exists, we say that canalization exists. That is the relevance of canalization. To research the mechanisms of canalization is to search into a basic dilemma of evolutionary theory. Being canalization a phenomenon in which evolvability is preserved, its research becomes a far-reaching task.

A resulting definition of canalization

Thus, canalization is a *reduction in the expression of existing genetic variation*. This definition makes clear that to identify canalization we have to observe a pattern (i.e. a *reduction in the expression of*), and relate it to the existence of a process (i.e. *existing genetic variation*).

This definition is in agreement with Waddington’s original view of developmental buffering: a trait with a reduced expression of its underlying genetic variation will express relatively stable phenotypes when faced with mutational or environmental perturbations. Canalization thus describes developmental mechanisms that reduce phenotypic sensitivity to genetic and environmental perturbations. The definition also incorporates the more recent view of (Wagner & Altenberg 1996): a *reduction of expression* refers to variation and *existing genetic variation* refers to variability. Previous definitions of canalization biased the following research (Gibson & Wagner 2000). The definition presented above does not lead to any bias in regard to the causes of such a phenomenon. In certain scenarios, canalization may arise from selection, but in principle, it can also arise from neutral evolution or as an emergent property of gene networks. There may be genetic variation for canalization, so canalization may evolve adaptively when advantageous, but this does not always have to be the case. A given developmental mechanism able to canalize a trait doesn't have to evolve adaptively.

Now that a definition of canalization is set, we are ready to discuss the different kinds of canalization present in the literature. Early on, Waddington recognized that there were different causes of perturbation to the phenotypes. Nowadays the different causes of perturbation are thought to originate different kinds of canalization. This issue follows.

Genetic and environmental canalization

Parting from canalization as a buffer against perturbations, different kinds of perturbations to which the phenotype remains stable has been distinguished. Accordingly, genetic canalization refers to cases in which mutation is the source of perturbation and environmental canalization refers to cases where the perturbations arise from exposure to different environments. This difference is already implicitly present in the early work of Waddington. Recent work has made the distinction precise (e.g., (Stearns 1989; Wagner *et al.* 1997)) and found experimental evidence for it (Stearns *et al.* 1995).

Two kinds of environmental perturbations have been described. Macro-environmental perturbations refer to different environments faced by all the members of a population (e.g. different habitats, temperature, salinity, etc.). Micro-environmental perturbations arise from developmental noise and random environmental fluctuations within a macro-environment, leading to differences among individuals of a same genotype (i.e. clone).

Even though the distinction between genetic and environmental canalization is conceptually clear, theoretical analysis suggests that selection for environmental canalization might lead to the evolution of genetic canalization (Gibson & Wagner 2000). Moreover, in some real situations they might be difficult to distinguish. This is the case in the exploration of novel habitats. Since novel environments are considered to be stressful to the organisms, and mutations keep on occurring, it is difficult to unambiguously identify the kind of perturbation that is being buffered, or the perturbation that de-canalize variation. In a stressful novel habitat, micro-environmental disturbance might produce an effect similar to a mutational perturbation.

Despite the problem just mentioned, different authors discuss the exploration of novel habitats as de-canalization agents (Clarke 1998; Holloway *et al.* 1990; Peck *et al.* 1998). More directly, evidence has been accumulated for novel environments being a cause of de-canalization. Exposure to new environments may result in the failure of canalization, leading to the phenotypic expression of developmental noise and genetic perturbations. Different developmental environments may uncover genetic variation for bristle number in *Drosophila* (Garcia Vazquez & Rubio 1988). There is also experimental evidence for an effect of habitat quality and exposure to new environments on the degree of canalization (Joshi & Thompson 1997; Kawecki 1995). (Kawecki 1995) supports the hypothesis that canalization against environmental perturbation is less efficient in poor habitats. However, he did not find an effect of exposure to new environments on canalization. Lastly, in an innovative paper in which canalization is seen as a displacement in a curved phenotype space, (Rice 1998) has shown how the pure change of the optimum to which a phenotype has been canalized is enough to decanalize variation and generates further divergence. A sudden change of optima might occur at the exploration of a novel environment, so the geometric approach of Rice provides reasons to support the exploration of novel environments as a decanalizing cause.

So far, canalization has been presented to a reasonable degree. In the following discussion, proximal causes of canalization, the relation on canalization and plasticity takes the first part. This is so because as early as Waddington's work, it was clear that canalization and plasticity must share the modulation of gene expression as

mechanism. The connection generated debates that I review below. Afterwards, I will discuss more detailed evidence for the existence of canalization. This evidence directly illuminates mechanisms able to produce canalization.

Mechanisms of canalization: plasticity and a phenotypic perspective.

Waddington believed that the capacity of a developmental system to react to environmental stimuli is under genetic control. He recognized that the key to the understanding of trait stability is the modification of phenotypic expression of the genetic information. This idea indicates that phenomena such as canalization and phenotypic plasticity may be closely related at the proximate level of causation. Both phenomena involve the modification of phenotypic expression of genes, probably by other genes. This suggests that phenotypic plasticity (the capacity of a genotype to produce different phenotypes across varying environments) and canalization (the capacity of different genotypes to produce the same phenotype despite perturbation) are mutually exclusive (e.g., (Stearns 1993)).

An example of this view can be found in an extensive review on the developmental modes of arthropods (Higgins & Rankin 1996). Modes of development broadly vary between arthropod taxa. Some of the modes have been interpreted as producing more canalized or more plastic development. The authors found a prevalence of plastic traits, “making it important to document the physiological basis of canalization”. So here canalization or plasticity are **different** outcomes of a developmental process. A more inclusive view is presented by (Nylin 1992). The author found that variation in the developmental time of the butterfly *Polygonia c-album* was due more to plasticity in growth rate than to variation in size or weight, indicating that plasticity in one trait may result in canalization in another correlated trait (Nylin 1992). Thus, the author suggested that canalization and phenotypic plasticity may be “the two sides of the same coin” rather than contrasting phenomena. Pigliucci and co-workers have an even more inclusive point of view. They observed changes in the correlation structure of physiological traits of *Arabidopsis*, following an environmental gradient. Individual traits were highly plastic but the stability of the correlation structure of traits involved in water use across environments suggested that the phenotypes were highly canalized (Pigliucci *et al.* 1995). Here plasticity and canalization occurred in the same set of traits.

The three examples just mentioned illustrate a gradation of opinion in the relation between canalization and plasticity. Canalization and plasticity are relative concepts. To say that a phenotype is canalized is to say that it present less variation than another. Henceforth it is no surprise that in one study they appear as opposites; while in other studies they are considered as complementary aspects of the same phenomenon. Going back to the original Waddington’s view, it is important to emphasize shared mechanisms (as the modulation of gene expression), more than semantic differences in definitions. Accordingly below I review molecular evidence on mechanisms of canalization.

Mechanisms of canalization, molecular evidence and a closer look at the genes

The search for mechanistic explanations of canalization led to models that are more detailed and experiments in the realm of population biology. But it also led to a more detailed knowledge about the molecular mechanisms that produces canalization.

Consider the work of (Gibson & Hogness 1996) as a first example. Their work suggested that protected polymorphism in the gene *Ubx* affects selection. Later on, the same group showed that canalization might be occurring in the pathway (Gibson & Van Helden 1997). Finally, a result from the same authors (Gibson *et al.* 1999) proved that wild type phenotypes have alleles that can (but do not) show the effect of mutations. This last result demonstrates that the wild type phenotypes showed no variation, although variability still existed. Thus the pathway analysed is genetically canalized.

Similar results have been shown in the development of photoreceptors in *Drosophila*. Mutant screening of wild phenotypes detected higher phenotypic variance than laboratory lines, supporting the old observation that stable wild individuals have a broad and canalized genetic variation below their stable phenotype (Polaczyc *et al.* 1998).

A perhaps more detailed, but still related approach was taken by (Rutherford & Lindquist 1998). The authors showed that if the *Drosophila Hsp83* locus is impaired by mutation, phenotypic variation is elicited. A wide range of defects in the fly morphology is observed. It could be shown that these phenotypes are produced by mutations that are not expressed in the absence of the impaired *Hsp83* allele. Thus, the functional *Hsp83* locus is buffering phenotypes against perturbations; it is a “canalizer” gene.

With the works presented, we can see that precise molecular research can establish whether a pathway is canalized or not. It is possible to observe evidence of canalization, and to design experiments that prove the buffering of mutations. Even better, genes that can be called canalizers have been identified. Having this rather precise information, the research takes two directions. One addresses the evolutionary origins of the canalized phenotypes we observe. The other asks more details on the proximate causes of canalization. In the following section, I will discuss the evolutionary origins of canalization, and in the section on genetic robustness, I will emphasize on the latest characterization of metabolic pathways, proximal causes of canalization.

Canalization as an outcome of selection

Theory suggests that stabilizing selection favors the evolution of micro-environmental canalization (Gavrilets & Hastings 1994). Using the same intuitive argument, the evolution of genetic canalization has been predicted under stabilizing selection (Wagner *et al.* 1997). Their models predict the parameter space in which environmental canalization and genetic canalization can evolve. They investigate two situations. In the first, a single locus affects several others (so called modifier model). In the alternative situation, the model assumes a set of loci interacting freely with each other (so called Universal Mapping Function model). They use standard tools and assumptions from population genetics to define combinations of parameters that favors the evolution of canalization. Simply put, here canalization evolves because it is advantageous to remain closer to an existing optimum. If selection is stabilizing (i.e. a fixed optimum exists), mechanisms that allow the phenotype to remain closer to the attained optimum are selected. In this scenario, canalization will prevent mutations, or other perturbations, from producing phenotypes deviating from the optimum.

In a way, the results of these two pioneering works were disappointing. The parameter space in which canalization could evolve under stabilizing selection turned out to be extremely restricted. The key reason is that selection in itself erodes the needed variation for canalization to evolve. This selective erosion of variation, intrinsic to the evolution of any trait under selection, is particularly troublesome for the evolution of canalization. This is so because the genes that must be selected for canalization to evolve are genes that also decrease variation. So, it is not only selection preventing variation to be expressed, but also the very same genes selected.

The first cautionary point to this perspective has been found in modeling work (Klingenberg & Nijhout 1999). They modeled a phenotypic trait and its underlying developmental control as a diffusion process. This approach was developed to study the evolution of Fluctuating Asymmetry. The result that concerns canalization is that they found that any nonlinear relation among the developmental parameters and the expression of the trait, plus some random developmental noise, is likely to engender variation for developmental stability. In other words, epistasis and developmental noise are enough to produce variation for canalization to evolve. A related result is discussed by (Rice 1998) below. But before going into the discussion of canalization as an intrinsic consequence of epistasis, let us consider related empirical work.

Considering canalization as a way to maintain phenotypes close to an optimum (canalization as a stabilization theory, in the words of (Gibson & Wagner 2000)) leads to considerations about the stability of phenotypic variation of life history traits. As expected, evidence from life history experiments suggests that (a) stabilizing selection for optimal life history traits favors increased canalization and (b) the more important the trait is to fitness, the more canalized it is.

The hypothesis that traits more important to fitness will be more canalized has been tested experimentally (Stearns *et al.* 1995). In their experiments, they used genetically engineered *Drosophila* to assess the fitness effects of genetic perturbations (P-element insertions). They found that there is a positive correlation between the genetic canalization of a trait and its impact on fitness. The authors also found that canalization of life history traits against environmental perturbations increased with the correlation of those traits with fitness. Their result is coherent with the work by (Sherry & Lord 1996). Sherry and Lord found that floral traits (supposed to be important to fitness) of *Clarkia tembloriensis* are canalized.

It is fair to say, then, that the idea that canalization provides stability is connected with the idea that stabilizing selection produces canalization. This association pervades the literature. Other selective and non-selective forces, though, are able to produce canalization. Recent work extends selective scenarios for canalization. (Kawecki 2000) finds that canalization can evolve under fluctuating selection. Imagine an optimum fluctuating between two values with a certain frequency. If the population tracks the changing optimum fast enough, it is doomed to remain maladapted. Once adapted to a value of the optimum, it will change. In this case it pays back to remain canalized to one of the values of the optimum, so at least in some cases the population will enjoy being adapted. This work suggests that fluctuating selection, and not only stabilizing selection, could eventually produce canalization.

Notice, as suggested by Kawecki (2000), that stabilizing selection is not the *sine qua non* cause for canalization to evolve. The need to remain closer to an optimum is the ultimate cause for canalization to occur.

Is selection necessary for canalization's evolution?

In an innovative paper, it has been introduced a novel method to analyze canalization and its evolution (Rice 1998). He sets a geometric description of the space with coordinates given by the developmental factors that determine a phenotype, versus coordinates given by the phenotype itself. In this "phenotypic space", the degree of canalization will increase in areas in which the geometry is steeper. The steepness of the phenotypic landscape is a measure of the relation of change between the developmental coordinates and the phenotype coordinate. An increase in variation of developmental factors without consequences in the phenotypic coordinates will produce an increased steepness. Or a reduced variation in the expression of the same variation of developmental factors will also produce increased steepness. Canalization, then, will be an increase in the steepness of the landscape. The author shows how epistasis, defined as the nonlinear interaction between developmental factors, produces variation in the landscape steepness. So this is a theoretical suggestion in which epistasis is a sufficient requisite for the evolution of canalization.

As mentioned before, this view is similar to the view of (Klingenberg & Nijhout 1999). The authors here depart from a different theoretical setup. The modeling of developmental factors by Klingenberg and coworkers is through diffusion equations, and no geometrical properties of any landscape are discussed. Nevertheless, their results suggest that epistatic interaction among the developmental factors will produce a reduced developmental instability, or a reduced sensitivity to developmental noise, which is a synonym of micro environmental canalization, in the lexicon of this paper.

In a recent contribution, an interconnected Boolean network is used for modeling gene regulatory networks (Bornholdt & Sneppen 2000). They show that the network is able to accumulate mutations, reducing the mutation's phenotype expression, and eventually able to release the variation gained by mutation in short time intervals. Given a similar genetic network, an organism will canalize the phenotype that the genetic network expresses. Here no selection for canalization is required. Other selective forces could have lead to the apparition of an interconnected genetic network. The phenomena observed -trade mark of canalization under the definition presented here- is a consequence of the intrinsic genetic architecture.

Wrapping canalization together.

In the previous lines, I have discussed a concept of canalization that include previous ones and is unbiased to any field of research in biology. Canalization is a phenomenon in which some of the existing genetic variation underlying a phenotype is not expressed. Accordingly, this phenomenon is able to shield the phenotype from perturbation. Historically two kinds of canalization had been set apart. Genetic canalization refers to mutational perturbations and environmental canalization refers to situations in which individuals are faced with different environments. Recent literature has identified mechanisms that produce canalization, from physiological rearrangements of phenotype production to genes able to modulate the expression of others. Good evidence has been provided for the adaptive evolution of canalization, but there is increasing modeling and data that supports also the occurrence of canalization as an intrinsic property of a genetic system.

ROBUSTNESS

What is genetic robustness?

In the section above, we have seen how a concept originated long time ago has been extensively discussed, and still today, discussion is needed to arrive to a satisfactory definition of the phenomenon. (Gibson & Wagner 2000) already noticed that differences among “developmental biologists” and “evolutionary biologists” already fueled a divergence in concepts and questions regarding the same phenomenon, canalization. Perhaps as a reaction of the long-standing rhetorical discussion, or perhaps because it was originated by biologists from other fields, the early nineties witnessed a new -but related- concept. This is the so-called robustness of gene networks.

From 1995 onwards few seminal papers pointed that the noise that gene networks are exposed to, does not perturb their functioning, so the “design” of gene networks make them robust (Somogyi & Sniegoski 1996, and references therein). The analysis of robust circuitry has a long tradition in electric engineer, so tools were borrowed to analyze gene networks (McAdams & Shapiro 1995). Their analyses underscore that the intrinsic noise of gene expression does not affect the phenotype, due to the robustness of the gene networks (McAdams & Arkin 1997; McAdams & Arkin 1999). From then on, the topic of the robustness of genetic networks became a growing field (Weng *et al.* 1999).

The work of Little and coworkers illustrates with clarity what is robustness. They focus on the infectivity control in the phage Lambda (Little *et al.* 1999). Mutants were expected to have a different gene expression pattern regarding the regulation of the infective stages. Those produced mutants, however, behaved the same as the non-mutant strains. The mutations induced on key parts of the regulatory pathways were not expressed as expected, a result that clearly shows the control system as robust to perturbations. In the lexicon of this paper we can say that the constancy of the behaviour of mutant and non-mutant strains is evidence of genetic canalization.

In all the contributions that we will discuss hereafter, the meaning of “robustness of a genetic network” is unequivocal. Robustness is the capacity that a set of genes has to express the same phenotype, despite perturbations. Needless to say, this definition is extremely related to genetic canalization. An important difference prevents us to think robustness and canalization as total synonyms. To find robustness there is no need to find preservation of variability. Henceforth one of the crucial aspects of canalization might, or might not, happens in robustness. It is clear from the work of Little and coworkers that variation is preserved, but the same is not clear at all from the work of McAdams and colleagues. Below I explore consequences of this difference.

How robustness occurs?

We have said that an active line of research is the characterization of metabolic pathways. One word might broadly describe results. Research had uncovered **complex** pathways of gene control. Each pathway that becomes analyzed in more detail seems to become more complex than previously thought (Peifer 1999; Remy & Michnick 2001; Weng *et al.* 1999). In the following lines I'll present the observation that the complexity of metabolic pathways is the proximal cause of genetic robustness, henceforth can be a cause of canalization, if added the shielding of existing variation.

Theory developed by molecular biologists had model gene networks architectures able to buffer intrinsic noise. Stochastic model of genetic networks had been analysed (McAdams & Arkin 1997; McAdams & Shapiro 1995). Stochasticity is introduced modelling the product from a gene as given by a stochastic formulation of chemical kinetics. The basic approach was later extended to test it against the regulation of the phage Lambda infective state (Arkin *et al.* 1998). The authors modelled a bi-stable regulatory system. The stable states are the infectious and non-infectious phases of the phage Lambda. Accordingly, predictions based on Poisson process (theoretical result from their stochastic chemical kinetics formulation) fit the data obtained from the real system well. Nanomolar concentrations of intra-cell products can provide enough random variation in order for it to become significant in a regulatory pathway. The observed complexity of the pathways is considered as a mechanism to shield against this source of variation. For the authors complexity means extensive genetic redundancy and feedback within regulatory pathways (McAdams & Arkin 1999).

Starting with the pioneering work presented above, many others agree to say that the complexity of gene interactions is the proximate cause of genetic robustness. Alas, the precise meaning of complexity does not enjoy much consensus. In describing a genetic network, 'complexity' has several meanings, hence too many possible interpretations. From an abstract viewpoint, nowadays there are at least three ideas to quantify the complexity of a general system of related components. One is just the number of the parts, another refers to the number of connections between parts, and a third and more elaborated one refers to the information that these parts are able to store. In genetic systems, a good part of research equals complexity with redundancy. The work that relates complexity and robustness in genetic networks has focused in complexity as connectance and complexity as redundancy. Accordingly, I will review the bearings of these two ideas first.

Robustness by complexity-as-redundancy

A pathway with redundant genes is considered as complex. On the origin of this kind of complexity, there is a long tradition of theoretical work that explains the evolution of redundancy using theories developed under neutral or quasi-neutral scenarios. This research has been the subject of extensive reviews [Ohta, 1992 #667] . I discuss recent theoretical work on the adaptive evolution of redundancy.

Classic population genetics conditions in which genetic redundancy could arise and be maintained by selection have been provided (Nowak *et al.* 1997; Wagner 1999). These works put forward that big population sizes and sufficient variation (originating

from mutation) will sustain the evolution of redundancy. They also pose conditions under which the evolution of redundancy of genes yields gene networks, in which different genes cover different functions in a particular pathway (Nowak *et al.* 1997). Under this view, the apparition of redundancy is a plausible origin of the observed complexity of the present genetic networks. In their view complexity as redundancy generates compartmentalisation of functions in a set of interacting genes. In turn, compartmentalisation is a source of genetic robustness

There are more reasons to expect that redundancy is adaptive. Traditional models have underestimated redundancy (Wagner 1998). The reasoning of Wagner is that the evolution of genetic redundancy is a balance among the gene duplication and the subsequent loss of their functions by divergence. Traditional models of population genetics do not include the complexity of genetic networks, so the rates of gene loss are overestimated. The reason is that any mutations leading to loss of functions have been considered neutral or deleterious. However, within a set of interacting genes, a mutant that implies loss of the function might be advantageous in the face of future perturbation, providing backup functions. This is to say that if we take the existence of a genetic network, a loss of function can become just creation of backup, or redundancy. In posterior work, Wagner underlines that the maintenance of redundancy could be due to that mutation in redundant genomes are easily neutral, since there is a backing up -redundant- set of genes (Wagner 1999; Wagner 2000).

Above I have shown that recent work describes plausible ways of the origin of redundancy, one of the elements of the genetic complexity that generates robustness. We can move on to the work that relates complexity-as-connectance with robustness. Recent research shows how epistatic interactions in theoretical and alive models produce more robustness. This is what we will discuss in the next part.

Robustness by complexity-as-connectance

The first widely quoted paper that addresses robustness as an outcome of wide epistatic interactions -or connectance- is the work of (Barkai & Leibler 1997). They pose the connectivity of a biochemical network as the architectural characterisation that insures its robustness. Here robustness is the stable performance of a controlling mechanism despite variation in its components. Simulations and experiments on the genetic network responsible for bacterial chemotaxis shows that it works even under random variations of the components of the biochemical network that controls it. The robustness of the network is due, according to Barkai and Leibler, to the existence of feedback connections in the biochemical system.

A related point has been tackled using an artificial life system (Lenski *et al.* 1999). They showed that different selective scenarios do produce 'complex' or 'simple' organisms, stemming from a common ancestor. In subsequent experiments, mutation stress results in that complex organisms showed higher fitnesses than simple organisms. Statistical tests allowed the authors to conclude that complex organisms have more epistatic interactions in their genome than simpler ones. They suggest a link between complexity and epistasis, without analysis of the explicit pathways that evolved. As a caveat, the authors mentioned that in the selection for simpler organisms, functionally organisms could have arisen with highly redundant genomes. They imply that the appearance of redundant genomes is a problem, because a redundant genome does not have more epistatic effects, henceforth is not more complex (under their definition). This is an example of the need of a consensual definition of complexity.

A mostly undisputed support of the paradigm connectance increases robustness is that increase in connectance increases the presence of feedback loops in genetic networks. Ample evidence exists to support that feedback loops are a source of robustness against the intrinsic noise of genetic networks (Freeman 2000) and references therein. Different organism functions that must be preserved operating in noisy circumstances (chemotaxis (Barkai & Leibler 1997; Yi *et al.* 2000), cell cycle determination (Little *et al.* 1999) or circadian rhythms (Barkai & Leibler 2000; Ueda *et al.* 2001)) has been found to be underlined by feedback loops that guarantee their functioning. Moreover, feedback loops have been recently engineered in vivo systems, showing that feedback loops do induce robustness (Becskei & Serrano 2000).

It is important to mention here that the stability that a feedback loop produces is not stability to a single point attractor, but it is a broader stability. Indeed, feedback loops are also known to produce bistability, and this characteristic has been exploited to analyze, predict and eventually engineer genetic switches in feedback loops (Cherry & Adler 2000; Elowitz & Leibler 2000; Gardner *et al.* 2000; Thattai & van Oudenaarden 2001).

Can we separate connectance and redundancy?

After having presented the evidence of the current opinion trend: “complex is robust”, I will discuss some counterarguments. Part of the presented work stands from the viewpoint that complexity is connectance, and it founds that complexity enhances robustness. From my viewpoint, it is not that clear that a more connected genetic network has to be more robust. Other part of the work presented here stands considering complexity as redundancy. But redundancy and connectance are phenomena not easy to disentangle. It is not clear how redundancy and connectance will interact in a particular network. Imagine the scenario in which we perturb with a mutation a gene affecting many steps of a pathway (high connectance). It might be that the organism is not viable anymore. High connectivity in a gene network enhances the probability that a gene is crucial to many functions. Since the concept of complexity is important and broadly used, it deserves a more precise definition.

To define complexity, consider the two attributes of abstract networks so far discussed, connectance and redundancy. Define the connectance of a net of entities as the average number of interactions of one entity with the others; and define as redundant a set of sleeping genes able to back-up the function of another set of genes. The redundancy of a network is the number of “parallel” sets of connections, able to back up a failed set of genes. Increase in epistasis in a gene network increases its connectance (Weng *et al.* 1999) and -more obvious- increase in redundant genes increases its redundancy. Therefore, the general connectance and redundancy have defined biological meanings. Even better, they allow precise characterization of a genetic network. The architecture of a genetic network (in terms of connectance and redundancy) suggests its response to perturbation. Consider figure 1:

Figure 1

In the figure 1 I depict three idealized pathways, (A), (B) and (C). The circles depict genes and the squares the phenotypes. The arrows depict a controlling relationship. A gene pointed by an arrow will not be active if the gene from which the arrow comes is not active itself. The heavy line is the last step from genotype to phenotype. It is easy to see that pathway (B) is more robust than pathway (A), even though both have the same connectance. Therefore, higher connectance is not a requisite of higher robustness in a gene network. Now compare gene networks (B) against (C) to see that redundancy alone is not enough to cover robustness. Clearly both (C) and (B) have the same level of redundancy, but it is easy to see that (B) is more robust, since a mutation in one of the vertical chain will not affect the parallel chain. But now, if we assume that cross arrows are used only in the absence of vertical arrows (as a backup), then (C) is a more robust net than (B). Anyway, the argument that we need connectance and redundancy to predict robustness is valid under both assumptions.

So far it seemed that redundancy and connectance can thus be separated, and predict robustness for redundancy and fragility (or at least multi-stability) for increase in connectedness. Two sources of confusion arise. In first place, an increase in redundancy has an inherent increase in connectance. This is so because one duplicated gene that acts as a backup if the original gene fails, have to interact with the original gene, at least regarding some exchange of information. In second place, I have reviewed evidence supporting that an increase of connectance is a source of robustness. What is then, the response of a gene network to changes in its architecture regarding redundancy and connectance? So far, no work has addressed both components of complexity together.

A way to test the last question could be the use of artificial genomes (such as the AVIDA platform). This strategy would allow the construction of populations with genomes of controlled redundancy and connectance. Their stability facing perturbations can be assessed in simulations. In principle, I think the essay of robustness increasing connectance or increasing redundancy should be made. Another, but more resource-intensive, method could be the engineering *in vivo* of organisms with controlled complexity in their genetic networks. The pioneering work of (Becskei & Serrano 2000; Elowitz & Leibler 2000) had already created new organisms with genetic networks designed to attain specific roles. So, at least it is theoretically feasible to create individuals with different degrees of connectance and redundancy in their genetic networks. Then, essays for the exploration of novel environments could explore the issues presented above.

The hypothetical different response of different architectures of genetic networks might help to dilemma evolvability versus robustness (Wagner & Altenberg 1996). Evolvability and robustness have been considered as opposed traits (Kirschner & Gerhart 1998). The issue can be clarified by a more precise definition of what is the architecture of a genetic network and what is its response to perturbation. It is clear that an extremely robust, lets say un-changeable genetic architecture (if such a thing could exist) is not evolvable. In this extreme sense, robustness and evolvability are opposites. But we have seen that a feedback loop introduces robustness and multi stability at the same time. One could call a system able to settle in different stable optima, an evolvable system. So, a careful definition of which architecture is considered to be evolvable or robust, can lessen the contrast of evolvable versus robust.

So far, I have defined what is robustness. The characterization of the proximal causes of robustness is a bit more difficult, since the complexity of gene interactions is broadly recognized as the mechanism of robustness, but several definitions of

complexity are available in the literature. I have discussed how different researchers have used these definitions differently. After show how the differences might induce contradictory statements, my contribution to clear this confusion is to propose the abandoning of the vague word complexity, to use the more precise terms connectance or redundancy. In this case, it is possible to refine current hypothesis on the robustness of a gene network. Ongoing dilemmas, as the opposition of evolvable and robust, could be solved with *in silico* systems, as AVIDA, and a careful use of the words. Lets move on the reasons why genetic robustness is present.

Why robustness?

Selection to buffer noise?

The recent accurate descriptions of genetic networks had faced researchers with the complexity of the genome and its mechanisms of control. Moreover, accurate measurements tell us that genes express themselves in a stochastic way, rather than in a deterministic precise pattern. So, noise is built in the mechanisms of gene expression. Then, a general statement arises: complexity is a good solution to noise since complexity yields genome robustness (Hartwell 1997). In the vocabulary of canalization, this is the so-called developmental noise; henceforth robustness is considered to arise as a mechanism to buffer it.

Looking back at the extensive research done into understand the mechanisms trough which complexity buffers noise, make the hypothesis of complexity prevents developmental noise almost undisputable. Nevertheless, I miss in the bibliography research that addresses this particular point directly. We know that the described complexity is able to buffer certain stochasticity, but no model, so far, has shown that in the presence of noise, complex gene networks evolve as a response. The strict test of providing scenarios with different levels of developmental noise and expect different levels of robustness has not been done.

A recent work better tackles the evolution of robust genomes, overcoming other genomes with high replication rates (Wilke *et al.* 2001). Using an artificial life system (Avida platform), the authors selects for organisms with high replication rate. Such organisms, at increasing mutation rates are overcome by organisms having a lower replication rate. This is due to the high genetic robustness that the slow replicating organisms have. This result is important given that traditionally it is considered that selection for replication rate should be able to overcome selection for other traits. Here we saw how robustness is more important.

Have complexity and you will have robustness

Instead of selection to buffer noise, it is possible to say that complexity -regardless its origin- results in noise-buffering mechanisms. This is what has been shown by Lenski and co-workers (Lenski *et al.* 1999). Here different scenarios have been able to select for complexity, and once complex genomes are around, we can see that complexity indeed produce robustness.

We have seen that redundancy is a source of robustness (Nowak *et al.* 1997; Wagner 1998; Wagner 1999; Wagner 2000). And we have discussed work that poses conditions for the evolution or redundancy. Those were examples of the evolution of redundancy, in turn generating robustness.

In recent work, (Lehman *et al.* 2000) analyzed the theoretical consequences of complex genetic interactions. He shows how the complexity of genome landscapes allows a genome to change while the corresponding phenotype remains stable. His argument is comparable to the one used by Nylin in the realm of life history research. It seems that a complex set of interrelated entities might afford some change in their

relations, in order to maintain some of their qualities stable. So here we have another recent work in which genetic complexity leads to robustness of phenotype.

Another viewpoint is to consider that a neutrally evolving population can evolve to robust architectures, only due to the starting architecture of genetic interactions. Evidence that supports this non-adaptive origin of genetic robustness is discussed by (van Nimwegen *et al.* 1999; Wilke 2001). The work of Wilke shows a characteristic of the evolutionary dynamics of robust genetic networks. They accumulate variation and release it in bursts, moments in which adaptation is almost saltationist. This considered, Wilke's result could be used as well to support the hypothesis of canalization evolves as a subproduct of selected genetic robustness.

Robustness together

In this second part of my review, we have seen that robustness is a phenomenon clearly defined, and that there is consensus on its proximate causes. But a detailed look points to needed precisions in the definition of the causes of robustness. I have posed here complexity has a composite of connectance and redundancy. Then it is posed the question of which element of complexity will produce robustness. This question is still unresolved in the literature. Moreover, I sustain with verbal arguments that the solution of this question (doable at least in silico systems) would solve the apparent contradiction among robustness and evolvability.

The reasons why robustness has appeared are less clear. Less effort has been allocated in evolutionary models of robustness, probably hampered by the well established believe that robustness prevents noise, so this should be its reason to be. I emphasize that specific modeling is required to test this assumption.

Finally, robustness seems to be a trait that has "just evolve". In the past, quite some debate has been about the constraints that design imposes on functionality. Here we are seeing the contrary. Complexity, perhaps non adaptively evolved, produces a desirable quality for the phenotype, that of robustness.

Founder events and divergence

What is founder event diversification?

Here I will briefly define what founder events are, what are the consequences that they can have regarding diversification, and what are the underlying –genetic– mechanisms. Then I draw connections between canalization and founder events. My opinion is that what we have learned on canalization provides a refreshing framework to discuss divergence via founder events.

Divergence via founder events is a controversial issue in evolutionary biology. I will not enter in detailed debates, existing extensive reviews that illustrate conceptual controversies (Barton & Charlesworth 1984; Carson & Templeton 1984); theoretical work that supports or counter-argue founder events as source of diversification (Gavrilets & Hastings 1996; Slatkin 1996; Whitlock 1997) (Charlesworth 1997) and contradicting experimental work on the importance of founder events to speciation (recently (Bryant & L.M. 1996; Galiana *et al.* 1996; Templeton 1999)) (Rundle *et al.* 1998; Rundle *et al.* 1999). Instead of discuss the detail of these and others debates, I choose to argue that de-canalization can occur through founder events. The connection between founder event diversification and canalization brings in new questions and a more inclusive perspective of the interplay among demography and genetic architecture.

A founder event occurs when drastic reductions in population size occur. The name founder occurs because in principle this is the case when few founder individuals from a mother population arrive to a novel environment and start growing a new – daughter– population. The sampling of few individuals can also happen when population size is drastically reduced. Indeed, early formulations of founder events as cause of diversification considers repeated cycles of population growth and crash as producers of genetic divergence (Carson 1968).

The drastic sampling of few individuals can cause that the newly founded population have a rearranged pattern of genetic variation. The demographic perturbation could uncover alleles previously unexpressed (unexpressed because epistatic effects from other alleles, now gone). In turn, this could favor divergence. The newly expressed alleles would allow the population to reach new adaptive peaks, generating differentiation from the mother population.

The claim that founder events produce speciation is the center of a long debate in the literature. I consider founder events a possible source of differentiation. Accordingly, my conclusions do not apply directly to the debate of speciation, but to the broader area of ecological specialization.

So, how does it happen?

The crucial mechanism of founder event diversification is that genetic information not expressed before, goes into the phenotype after a demographic perturbation. For this to happen it is needed a mechanism able to cover genetic information from expression. Epistatic interactions of different kind have been used as such mechanism. Broadly defining epistasis as a phenomenon in which the expression of a genetic substitution depends of its genetic background (Hansen & Wagner 2001), divergence via founder events depends on epistasis, because the sampling of founder individuals is supposed to change the population genetic background. If so, genetic substitutions will be differentially expressed in a daughter population than in a mother population, and divergence might occur.

Notice that the re-arrangement of genetic background implies losing some genetic diversity. Indeed, if the whole diversity of the mother population remains in the daughter population, no genetic background has changed. But divergence also needs the preservation of some genetic diversity. If all genetic diversity is erased by the founder event, there is no potential to respond to novel environments, and the daughter population will die, or at least not diverge from the mother population. This hard-to-strike balance is the requirement for diversification via founder event to happen (Cheverud *et al.* 1999). Interestingly enough, this “hard-to-strike balance” remembers the also hard-to-strike balance required for the evolution of canalization under stabilizing selection. The reasons why these balances are hard to strike are similar. They are the outcome of a process that erode variation (selection in canalization’s evolution and bottleneck in founder event) and a process that needs variation (response to selection and response to the new environment). Anyhow, it has been pointed out that variation lost in founder events might not be enough to rearrange a genetic background. This argument considers that epistatic effects arise from non-additive genetic variation. (Whitlock 1997) showed that the loss of non-additive variation is not directly correlated with the size of a bottleneck.

Divergence by founder events, then, depends in the conversion of epistatic variation into additive variation. Losing some genetic variance might result in the conversion of epistatic to additive variation. Goodnight model this idea (Goodnight 1987; Goodnight 1988; Goodnight 1995). In his model, recombination brings together alleles previously hidden by dominant interactions, which become part of the additive variance expressed at the phenotypic level. The role of dominance in the release of epistatic variation has also been discussed in (Willis & Orr 1993). There is empirical evidence of this phenomenon in populations subjected to experimental bottleneck (Carson *et al.* 1990; Cheverud & Routman 1996; Meffert *et al.* 1999).

So far, I have said that in founder event divergence the interaction of epistasis and demographic event is the mechanism of differentiation. I have mention the idea, naming models and experiments supporting it. A long series of papers have made this point also in the wild. The organism researched is the pitcher-plant mosquito *Wyeomyia Smithii*. Early work showed that the control of dormancy in *W. smithii* is a trait associated with fitness varying along a geographical cline (Bradshaw & Lounibos 1976). Later work showed that the diversification along the geographical cline is due to epistatic effects (Hard *et al.* 1992). The authors showed that in despite of the

intrinsic founder events necessary to the expansion of the species range, genetic variation for the control of photoperiod have not been eroded and it is preserved through epistatic interactions (Hard *et al.* 1993a; Hard *et al.* 1993b). Their proposition is that successive founder events have progressively released genetic variance. In which case demographic perturbation due to founder events is able to express epistatic variation. The points made here are further expanded in two recent works on the same system, (Armbruster *et al.* 1998; Lair *et al.* 1997).

To found might be to de-canalize

After a long discussion on canalization, we should be able to say at this point that founder events are environmental perturbations able to de-canalize traits. In re-reading the last paragraph, one could perfectly say that the variability preserved by epistatic interaction has been expressed after the reduction of variation due to successive founder events occurred in the expansion of the geographic range of *Wyeomyia Smithii*. In short: founder events de-canalized variability, occurring divergence consequently.

The original work of Rice (1998) produces connected results. Using geometrical analysis of his phenotype landscapes (described above), he notice that canalization leads to traits that are more and more stable, and at the same moment, they are able to diverge fast under a perturbation. In the terms of dynamics systems, canalized phenotypes are unstable attractors of an epistatic landscape.

A further point of connection among canalization and founder events -now from empirical research- appears in the arguments used by Meffert and coworkers (1999). This study specifically focuses on variance in a trait strongly related to fitness, the courtship repertoire of *Musca*. Since courtship is a fitness important trait, its additive variance is expected to be low and structured by non-additive genetic effects. The study tackles the conversion of non-additive variance in additive variance via bottlenecks. "Translating" this argument to the phraseology of canalization we will expect, according to Stearns and Kawecki (1994), that traits relevant to fitness are highly canalized (low additive variance structured by non-additive variation). Then the environmental perturbation that a population crash provides is able to de-canalize such trait. Precisely the strong version of founder effect speciation claims that traits relevant to fitness are the ones likely to undergo "genetic revolutions" that will produce subsequent isolation.

Where to go, then?

In this last part of my review, I have come to the proposition that founder event diversification and de-canalization are very similar phenomena -if not the same-. For genetic variation be de-canalized it must be buffered from phenotypic expression trough epistatic interactions, and they must be disrupted by genetic or environmental perturbations. Indeed, a population crash, or the sampling of few individuals into a novel environment, can be seen as such perturbation. So, an observer of such a perturbation and the posterior release of variation, could call it de-canalization or founder event divergence, depending of his or her background. If both observers agree with my point, they will appreciate that the difference is just on names. Now, beyond the naming conventions, what can we learn from the connection founder-canalization?

In the second part of this review, we have seen that the complexity of genetic interactions is expected to produce genetic robustness, so that the genetic machinery of an organism produces stable phenotypes in despite of perturbations. In principle, the literature predicts that gene networks that are more complex are more robust. We have discussed the relation canalization-robustness. So then, we could extend the previous argument and say that complex genetic networks are more prone to produce canalized phenotypes than their simple counterparts. So far, this prediction has not been checked in the literature explicitly. It is worth to make a cautionary point. There is more in canalization than robustness. Not all sources of robustness can accumulate, or preserve, variation. So, the study of robust genetic networks might illuminate some of the mechanisms for canalization, but not all. Feedback loops, for example, are able to stabilize noise intrinsic to the stochastic process of gene expression. But feedback loops alone are not able to hide variation from selection. Conversely, genetic redundancy might not be able to provide stable phenotypes when stochasticity is high, but back up mechanisms -as redundancy- accumulates silent variation and can provide robustness from mutation. Some cross talk among robustness and canalization is possible, but must be done carefully.

Conceiving complexity as a compound term, one could then refine the prediction that complexity engenders robustness that engenders canalization. One could say that evolvable traits are underlined by more connected genetic networks than fixed traits, which are expected to be produced by more redundant gene networks. The reason is that the complexity induced by an increment in connectance is likely to induce robustness to developmental noise and multistability. So, at the same time that a trait will become more and more resistant to the stochasticity of the development, it will also become able to explore other stable configurations, perhaps invading new adaptive peaks, *sensu* Wright. Indeed, this is the conclusion that Rice present saying that canalization due to epistasis produces canalized individuals that are also prone to divergence. On the other side, one could predict that redundant gene networks underline long-age fixed traits, since they are able to buffer mutational variation.

Above we have discussed mechanisms that are required for divergence via founder events. We have seen how epistatic interactions are thought to be a requisite, so that they are disrupted by a founder event, eliciting the new rearranged genomes' divergence. Considering the prediction on the evolvability of highly connected gene networks, one can see that both arguments go in the same direction. So traits determined by more connected genomes must be better explorers of novel environments. On the other side, one could say that highly redundant genomes, being more stable to mutations, would not be able to colonize successfully other optima in novel environment. Nevertheless, a current hypothesis for divergence claims that redundant genes can accumulate variation, neutral until a novel environment is explored (for a recent model supporting this argument for canalized phenotypes see (Eshel & Matessi 1998)). So, an interesting question arises. Which architecture is prone to explore novel environments? A largely epistatic gene network, or a largely redundant one? This is one future and interesting line of research amenable to empirical and theoretical work. It would be interesting to consider how different complex genomes (more or less redundant, more or less epistatic) react to the demographic perturbations that founder flush theories conceive as disrupters of coadapted genomes.

Acknowledgments

N. Vouilloz and T. Flat participate in intensive discussions at earlier versions of this paper. F. Mery, A. Kelly and C. Melser commented extensively the manuscript. To all of you I am indebted.

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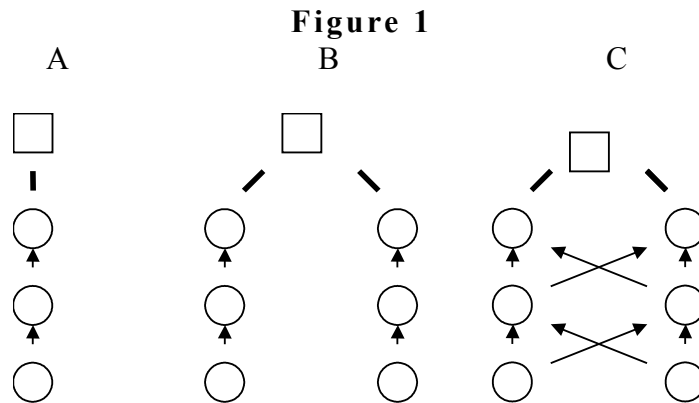
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(A), (B) and (C) are three idealized genetic networks. Circles symbolize genes and a square symbolize a phenotype. Arrows denote controlling relations from the gene from which they depart to the gene that they point to.