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Evolutionary constraints or opportunities?

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ABSTRACT

Natural selection is traditionally viewed as a leading factor of evolution, whereas variation is assumed to be random and non-directional. Any order in variation is attributed to epigenetic or developmental constraints that can hinder the action of natural selection. In contrast I consider the *positive role* of epigenetic mechanisms in evolution because they provide organisms with opportunities for rapid adaptive change. Because the term "constraint" has negative connotations, I use the term "regulated variation" to emphasize the adaptive nature of phenotypic variation, which helps populations and species to survive and evolve in changing environments. The capacity to produce regulated variation is a phenotypic property, which is not described in the genome. Instead, the genome acts as a switchboard, where mostly random mutations switch "on" or "off" preexisting functional capacities of organism components. Thus, there are two channels of heredity: informational (genomic) and structure-functional (phenotypic). Functional capacities of organisms most likely emerged in a chain of modifications and combinations of more simple ancestral functions. The role of DNA has been to keep records of these changes (without describing the result) so that they can be reproduced in the following generations. Evolutionary opportunities include adjustments of individual functions, multitasking, connection between various components of an organism, and interaction between organisms. The adaptive nature of regulated variation can be explained by the differential success of lineages in macro-evolution. Lineages with more advantageous patterns of regulated variation are likely to produce more species and secure more resources (i.e., long-term lineage selection).

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1. Introduction

The theory of evolution is one of the most challenging endeavors in science because it attempts to integrate an enormous amount of information about living organisms, including genetics, molecular biology, physiology, ecology, population dynamics, systematics, and phylogeny. Another challenge is the slow rate of evolutionary change and the lack of detailed information on its intermediate steps. The data supporting hypotheses on short-term evolutionary change include a few observations in natural and laboratory populations, whereas evidence of long-term evolutionary change comes almost exclusively from paleontology and comparative morphology. Existing data on macro-evolution usually do not include information on molecular and developmental mechanisms. These challenges result in the resilience of traditional views on evolution, which are difficult to refute based on data or logic. One of such persistent claims is the notion of randomness and non-directionality of heritable variation. This notion is supported by the randomness of nucleotide substitution in the DNA. Known biases in the probability of nucleotide substitution do not cause any adaptive change in the phenotype and do not make evolution faster or more efficient. Another evidence of randomness comes from the variation of phenotypic qualitative traits such as measures of various body parts. Neo-Darwinism portrays variation as random and "blind" in order to defend the primary role of natural selection in evolution and prove the absence of goal-directed agents in nature (Dawkins, 1986; Dennett, 1995). For example, Dennett wrote about Teilhard de Chardin: "He emphatically denied the fundamental idea: that evolution is a mindless, purposeless, algorithmic process" (p. 320).

However, phenotypic variation is not random but regulated by various internal and external factors, and this regulation generally facilitates organism functions and increases survival and reproduction rates. This implies that developing organisms are *active, self-organizing, and goal-directed agents*. Actual variation always represents only a narrow subset of all logically possible

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forms, which indicates that variation is subject to strong constraints. As Huxley wrote, "A whale does not tend to vary in the direction of producing feathers, not a bird in the direction of developing whalebone" (Huxley, 1893, p. 181). These constraints are present before any selection takes place, and thus, they should not be confused with correlations enforced by purifying selection (Schwenk, 1995).

Darwin was well aware of the constraints on variation. In the "Origin of species", he discussed the phenomenon of "correlation of growth": "I mean by this expression that the whole organization is so tied together during its growth and development, that when slight variations in any one part occur, and are accumulated through natural selection, other parts become modified. which are responsible for coordinated change in many traits if one of the traits is subject to selection." (Darwin, 1987, p. 133). However, he apparently assumed that natural selection can always find a way to overcome these constraints if they hinder the emergence of useful combinations of traits. Darwin accepted only one evolutionary consequence of such correlations: the change of some traits may be caused by their correlation with other traits which undergo change under the pressure of natural selection. In contrast, Gould and Lewontin argued that constraints may be so strong that they "become more interesting and more important in delimiting pathways of change than the selective force" (Gould and Lewontin, 1979, p. 581). Thus, the study of constraints may help in predicting possible directions of evolutionary change. But constraints were considered only in relation to their negative role in evolution–the role of barriers that prevent the development of perfect adaptations.

In this paper I argue that factors regulating phenotypic variation play a *positive role* in evolution by providing opportunities for rapid adaptive changes, which would not exist otherwise. Because the term "constraint" has negative connotations, I use another term "regulated variation" to emphasize the adaptive nature of phenotypic variation, which has evolved to provide the functionality of organisms not only in current conditions, but also in possible alternative conditions. Regulated variation helps populations and species to survive in variable environments, although occasionally it may appear non-adaptive and becoming a constraint. Metaphorically speaking, regulated variation can be compared to handrails on a narrow hanging bridge that provide an opportunity for a person to cross the river. Although this idea is old and was discussed by Cuénot, Goldschmidt, Schmalhausen, Lewontin, Gould (Section 2), now we have not only more evidence of this phenomenon, but also more insights into its molecular and genetic mechanisms (Section 3). This interpretation of evolution does not diminish the importance of natural selection. But in contrast to Neo-Darwinism, it emphasizes the active role of organisms in evolution. In particular, it is based on the notion that organisms build up their evolutionary potential (i.e., adaptability) by developing resources for future heritable variations. The effects of adaptability, phenotypic plasticity, and developmental correlations in evolution fit into the category of "extended evolutionary synthesis" (EES) (Pigliucci and Müller, 2010), which goes beyond the "modern synthesis" (MS) presented in writings of Huxley, Fisher, Dobzhansky, Haldane, Wright, and Mayr. The notion of regulated variation provides a generalized approach to these phenomena and may help to develop a unified theory. Moreover, it prompts to reconsider some basic ideas about heredity and evolution. For example, the "blueprint" metaphor of the genome has to be replaced with a "switchboard" metaphor, and organisms have to be recognized as active agents capable of controlling their phenotypes and increasing their adaptability. In Section 4, I discuss types of evolutionary opportunities that differ in the degree of their novelty and level of organization at which they appear. Finally, in Section 5, I argue that selection of lineages can explain why evolutionary opportunities tend to accumulate in macro-evolution.

2. Overview of theories that accounted for evolutionary opportunities

Studies of evolutionary opportunities have a long history. Lucien Cuénot proposed a hypothesis that large heritable changes are more important in adaptive evolution than small changes, and these changes often appear as adjustments of already existing organs and capacities to new functions (Cuénot, 1914). He called this phenomenon "preadaptation", which seems to capture better the expanded evolutionary potential of current adaptations than the term "exaptation" suggested much later by (Gould and Vrba, 1982). Cuénot criticized Darwin's idea of the primary role of environment in evolution. He argued that the structure of organisms often plays the leading role in evolution by narrowing down the set of new functions that can be acquired with the use of already existing structures. Environment does not *determine* structures of organisms because there are various ways of life and function in each environment. Cuénot considered his theory fully compatible with Darwin's idea of natural selection; he thought that natural selection adjusts existing organs for specific functions of organisms. The theory of nomogenesis developed by Berg (1922) included many examples of preadaptations. However, Berg did not understand the explanatory power of the theory of natural selection, and hence, failed to differentiate between strong and weak aspects of Darwin's heritage.

Richard Goldschmidt argued that macro-mutations in insects (e.g., aristopedia, tetraptera) generate highly-organized novel structures which may appear functional, and hence, may provide opportunities for adaptive evolution (Goldschmidt, 1940). He also noticed that organisms possess a capacity to produce mutant phenotypes under stress conditions without any mutation (he called them "phenocopies"), which implies that the norm of reaction exists independently from mutations (Goldschmidt, 1935). The term "norm of reaction" means morphological responses to environmental factors (Woltereck, 1909).

Ivan Schmalhausen pioneered in the analysis of the role of phenotypic plasticity and robustness in evolution (Schmalhausen, 1949). He introduced the notion of "stabilizing selection" which means selection for phenotypic plasticity and robustness to cope with heterogeneous environment in space and time. In contrast to the negative "purifying selection" that eliminates deleterious alleles, "stabilizing selection has a positive and constructive role, for it leads to the establishment of new morphogenetic correlations" (p. 93). Phenotypic plasticity facilitates changes of heredity via natural selection that adjust the norm of reaction. This mechanism was later re-discovered and named "genetic assimilation" (Waddington, 1961). Schmalhausen proposed that plasticity of variation is an evidence of species' capacity for evolution, and this capacity can be first reserved and then mobilized in stressful and changing conditions. Accumulation of variability is achieved via genetic dominance, neutralization of harmful mutations, and balance of harmful and advantageous effects of mutations. Mobilization of variability includes the increase of homozygosity due to the fragmentation of populations as well as release of phenotypic variability through elimination of regulatory correlations and direct induction of new phenotypes by stress conditions. Finally, Schmalhausen was among the first to analyze the phenomenon of adaptability at both individual and species levels. The theory of Schmalhausen was far ahead of his time. He complemented the Darwin's theory of selection with deep understanding of the self-regulatory capacity of living organisms and their active participation in the phenotype-building and evolutionary process. Unfortunately, his theory was mostly ignored because it contradicted to the "passive sieve" metaphor of evolution promoted by MS. Very few western biologists were familiar with the theory of Schmalhausen, of whom it is necessary to mention Theodosius

Dobzhansky, who edited the English translation of Schmalhausen's book, and Rupert Riedl (1977).

Unlike morphological or physiological traits, hidden internal capacities of organisms are not seen directly. But their existence can be deduced from repeated and independent appearance of specific features in various species or higher level lineages. Russian botanist Nikolai Vavilov called this phenomenon a "law of homologous series in variation" (Vavilov, 1922). Similar repeated patterns of variations were discovered in bacteria (Zavarsin, 1974), amphibians and mammals (Kovalenko and Popov, 1977). Russian paleobotanist Sergei Meyen analyzed plant leaf variability and revealed a set of shape transformations by which a full morphospace of leaf shapes can be generated (Meyen, 1987). Only a fragment of this diversity has been realized in the process of evolution. Some phenotypes are seen only in defective specimen, but they indicate the directions of possible evolutionary change. Thus, patterns in the morphospace of existing species can be used for reconstructing underlying internal capacities of organisms to generate various phenotypes (Brakefield, 2009). Facts that indicate the existence of hidden evolutionary opportunities can be grouped in the following three categories. (1) Phenotypic variation is a product of dynamic self-regulation that is targeted at preserving the functionality of organisms (i.e., homeostasis). Self-regulation in embryogenesis is supported by the existence of macro-mutations and phenocopies. (2) Organisms have hidden capacities to perform additional and often novel functions after minor change or even without change (preadaptation). (3) Nearly identical adaptations emerged repeatedly in related lineages of organisms (parallelism), which resulted in a combinatorial pattern of variation, whereas other logically possible morphologies never materialized.

A recent attempt to incorporate intrinsic factors into the theory of evolution was undertaken by Stuart Kauffman who suggested that "self-organization" is as important in evolution as natural selection (Kauffman, 1993). However, this theory has several shortcomings. Major examples of self-organization were taken from non-living systems, which erased the qualitative difference between life and non-life. According to Kauffman, self-organization is so abundant in the inorganic nature that it is readily utilized by living organisms for free, as compared to the costly natural selection. This theory, does not acknowledge that living organisms have to internalize self-organization and encode it either in the genome or other memory storage. This process has substantial evolutionary costs in the form of genetic selection or behavioral costs of trials and errors.

The role of embryonic development in reshaping evolutionary opportunities is explored in the novel research area of "Evo-Devo" (Brakefield, 2011; Laubichler, 2009). It attempts to trace the origins and patterns of phenotypic variation and recognizes that certain types of heritable phenotypic change predominate. Topics discussed in Evo-Devo include parallel evolution, developmental correlations, evolvability, robustness in unstable environment, and evolutionary progress. Besides theoretical analysis it includes experimental and comparative studies of real developmental and genetic mechanisms that can affect the directions and rates of evolution. However, to compete with the traditional MS, this area of studies needs a more elaborate theory, which recognizes the active role of organisms in evolution and clarifies notions of variation, heritability, and adaptation.

3. Regulated phenotypic variation is a source of evolutionary opportunities

Evolvability of phylogenetic lineages depends on the capacity of organisms to produce some progeny with modified heritable phenotypes that can survive and reproduce in changed environments better than their parental generation can. Such phenotypes represent evolutionary opportunities. Regulation exists even in the process of DNA replication, which results in differential mutation rates within certain segments of the genome. Those segments that carry genes responsible for adaptation to varying environments or gene copies are usually more mutable than segments that carry unique genes of vital importance (Jablonka and Lamb, 2005). Mutation rates also tend to increase in stress conditions and this additional variability raises the chance for a population to survive and adapt to changed environments.

However, regulated genetic variation is not sufficient for supporting evolvability. Even in the case of high genetic variation, the selection will not lead to novel adaptations if all mutated organisms appear non-viable or barely viable. Thus, in this paper I focus on regulated phenotypic variation generated by various epigenetic and developmental mechanisms and produce highly-functional phenotypes from mutated genotypes. These phenotypes may even provide specific adaptations to novel conditions. Classical models in population genetics assume that each genotype produces a fixed phenotype (Fisher, 1930). Moreover, there is a common misconception that heritable traits are fixed and uniquely determined by genotype. This simplification may help to get elegant mathematical models in population genetics, but it is definitely not true because fixed traits do not exist. Instead the relationship between genotypes and phenotypes is dynamic and *regulated* by various internal and external factors (Laubichler, 2009). Effects of internal factors include compensatory changes in gene regulatory networks and developmental correlations, whereas effects of external factors include phenotypic or behavioral plasticity in varying conditions. Phenotypic traits emerge in a dynamic way at various structural levels from intracellular components to tissues, organs, hormone and immune systems, and behaviors. During organism growth and development, each cell, tissue, and organ interacts with other components of the organism as well as with the environment. Regulated variation may yield both stabilizing and diversifying phenotypic effects. It can support the stability of phenotypes and functions despite of internal and external disturbances (i.e., mutations and environment change) (Laubichler, 2009). But also it can generate diverse phenotypic variants that are not only viable but may show certain benefits in changed environments or internal context of the body. These two roles of regulated variation are interdependent because stabilizing effects help the population to accumulate more genetic variability (e.g., heterozygocity), which later can be converted into increased phenotypic variation in changed conditions (e.g., under stress). If stabilizing effects depend on certain environment conditions, then they become released in changed conditions.

Regulated variation is not always heritable. For example, the capacity of mammals to develop thick hair in cold conditions is heritable, but thick hair itself is not heritable. Because only heritable traits can change in evolution and persist through generation, we need to consider criteria of heritability. But how regulated dynamic traits can be heritable if they are acquired during organism development? According to the common view, acquired traits are not heritable by definition. Obviously, the naïve notion that heritable traits are fixed should be replaced by a more rigorous definition of heritability. In genetics, heritability is assessed by twin-studies which determine if closely related organisms have more similar phenotypes than non-related organisms (Cardon and Neale, 1992). The most valuable are comparisons of monozygotic twins that carry the same genotype, however heritability analysis is possible even if the population has no monozygotic twins. In this case, phenotypic similarity is compared with a measure of relatedness between each pair of individuals. In humans, the relatedness is assessed mostly from pedigrees, but in animals it is evaluated on the bases of genetic similarity (Frentiu et al., 2008).

In the case of multi-dimensional phenotypes, heritability is measured by the G-matrix (Lande, 1979). According to the model of Lande, the evolutionary change is proportional to the product of the G-matrix and vector ß of selection direction. In particular, if the G-matrix is singular (or nearly singular) then possible directions of evolution appear limited by internal constraints (Klingenberg, 2005).

If a study covers different environments or geographic populations (as it should be), then these additional factors have to be included into the model to accurately estimate heritability rates. Environmental factors appear to be involved in heritability as conditions that make heredity possible. Mathematically, it is expressed as interaction terms of relatedness and environment as predictors of phenotypic similarity (Brock et al., 2010; Glahn et al., 2013). This interaction is important for environmentally-dependent or age-dependent traits.

This kind of statistical definition of heritability avoids tautology and separates those dynamic traits that are heritable from those that are not heritable. This definition does not require that heredity is passed solely through the DNA and allows one to account for epigenetic transgenerational inheritance (Jablonka and Lamb, 2010). Novel adaptation may become initially preserved by the short-term epigenetic heredity, and later replaced by genetic heredity. Heritable traits may also include behavioral patterns such as innate reflexes, plastic behaviors, and even learned behaviors. Of course, learned behaviors are not described in the DNA, but the genome may encode and control the production of all components that are necessary for the emergence of these behaviors so that they become reliably reproduced in the next generation. It is well established that normal development of immune and neural systems is based on trials and errors at the cellular level (Edelman, 1987). These processes are similar to learning in the sense that they lead to functional solutions that are not prescribed genetically. We can hypothesize that development of other morphological traits may also include elements of learning via trials and errors, where cells try specializing in various directions, and then those cells that appear non-functional in a given organ or tissue become destroyed by apoptosis.

Statistical approach to heredity does not agree with the common notion that the development of an embryo follows a "blueprint" inscribed in the genome. Comparison of a genome with an architectural blueprint appears a misleading metaphor. If a genome was a blueprint of an organism, then any adaptive change would require the emergence of a *description* of that change. To develop dark coloration in a peppered moth, the description should characterize the new color and where it is applied. Such description can be roughly compared to a change of (at least) one sentence in a book. As a metaphoric example let's consider a human book with ca. 10^6 characters in a 30-letter alphabet, which is sequentially copied with random typos. Now we can calculate the probability of one sentence change to another meaningful (and therefore specific) sentence. If we assume that the text remains readable with <10% of typos, then 10^5 character changes can be made in the whole book before the text becomes non-recognizable. The probability of one specific character change to a new correct value equals the product of a typo probability (0.1) and probability of correct change (1/30). If a sentence has 100 characters, then the probability of 90 correct changes out of 100 characters is ca. 10^{-210} (based on the binomial distribution). If the length of a sentence is reduced from 100 to 30 characters (which seems to be a minimum length of a sentence with specific meaning), then the probability of a spontaneous correct change of this sentence becomes 10^{-64}. This probability can be also multiplied by the counts of books in a "population" of 10^8 book copies. But this adjustment does not make a big difference. Events with so low probability simply cannot happen considering that the Earth has only 10^{50} atoms. In other words, biological evolution would not be possible if the genome was a blueprint of organisms.

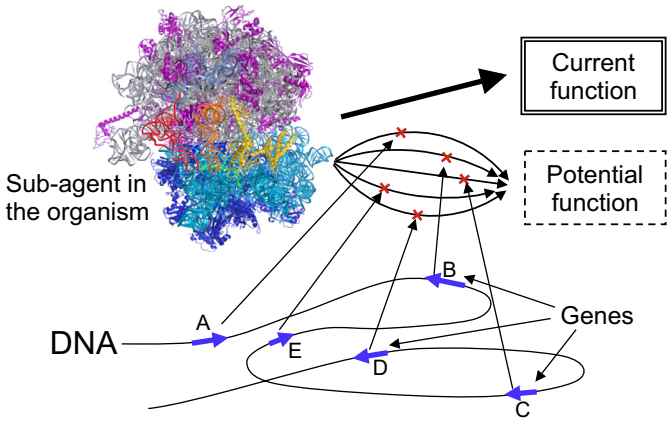

Fig. 1. Phenotype can support alternative potential functions which can be turned "on" or "off" by various genetically controlled pathways.

An alternative view is that the genome contains codes that help in organizing the functions of an organism without providing precise description of these functions. If an organism already has a capacity to develop an adaptation (e.g., it encodes a protein that can perform the function), then this capacity can be activated via one or few single-nucleotide substitutions or single event of genomic rearrangement (e.g., deletion, duplication, inversion, or transposition). The probability of activating the potential function is further increased by the existence of redundant activation pathways (Fig. 1). For example, the amount of protein produced by a gene can be changed through hundreds of pathways such as changes of transcription factor binding to promoters and enhancers of genes, various chromatin modifications, DNA methylation, alternative splicing, alternative polyadenylation of mRNA, mRNA degradation rates that are mediated by non-coding RNA and RNA-binding proteins, alternative protein folding, interference through protein-protein interactions, squelching, protein modifications, and selective protein degradation. As a result the probability of activating a latent potency may become increased by several orders, and such event may happen in a few organisms in a large population. In this model, the genome acts as a *switchboard* rather than as a blueprint. This model explains why comparative genomics tells us so little about positive selection. Because adaptations arise via non-local change in the genome (Fig. 1), they cannot be captured using statistical models.

The switchboard metaphor of the genome is similar to the Waddington's notion of epigenetic landscape (Waddington, 1968), where the developmental trajectory of an embryo or a cell emerge as a track of a ball that rolls along the valleys separated by ridges in a ragged landscape. Genes are assumed to change the curvature of the surface and modify the topology of valleys and ridges. In such indirect way they change the phenotypic outcome of the developing organism. Similar logic is used in the Evo-Devo research projects: "Randomly arising genomic variations converted into non-random form by the rules operating in the existing ontogeny, a process often referred to as 'developmental constraint.'" (Raff and Raff, 2009). In biosemiotics, information is characterized as a "difference that makes a difference" for a certain living agent (Bateson, 1972, p. 459). Because the vast majority of differences is ignored and only a small portion is picked as a sign, any description is fundamentally incomplete (Hoffmeyer and Emmeche, 1991).

The switchboard model indicates the existence of two components or channels of heredity: (1) the genome transfers information in the form of DNA sequence, and (2) the egg transfers the composition of molecules, structures, and functions (Fig. 2). These

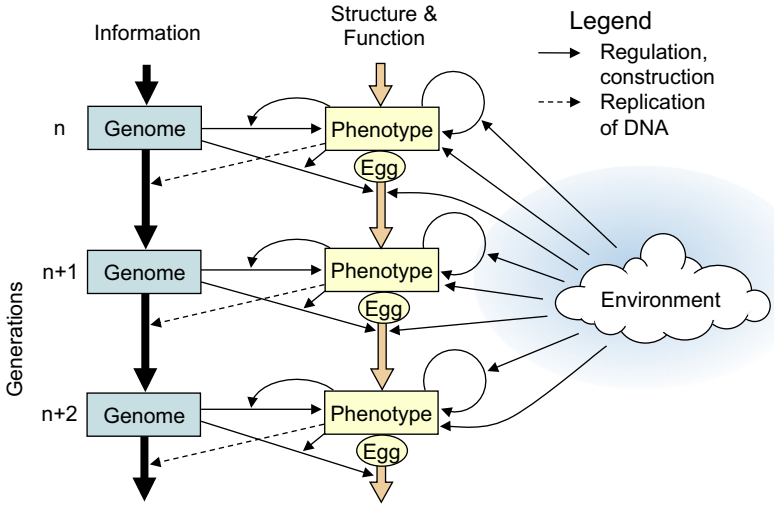

Fig. 2. Two channels of heredity: informational and structure/functional.

components were described as "code-duality", where the genome carries digital information, and the egg carries analog information (Hoffmeyer and Emmeche, 1991). However, the dichotomy of digital/analog does not capture the main difference between them: a functional agency is needed to reproduce functions in the next generation. Agents are produced only by other agents with similar functional complexity (Sharov, 2010), and thus, they are interlinked into potentially infinite construction chains. DNA is not an agent and cannot construct anything; but the egg is an agent, and it uses the genome (DNA) to sequentially derive the structure and function of the embryo, and finally, of the adult organism from basic structures and functions in the egg supplied by the parent. The distinction between the informational and functional channels of heredity also resembles the opposition of replicators versus interactors suggested by Dawkins (1976). However, Dawkins did not consider them as equally important for heredity. Instead he thought that replicators (i.e., genes) fully describe the phenotype of interactors (organisms), and thus, he reduced heredity to only one channel of passive gene copying.

But the "switchboard" model of the genome does not explain how latent capacities emerged in evolution and became heritable. Obviously, these capacities are not inscribed in the genome because "... without readers DNA sequences don't specify anything" (Dennett, 1995, p. 113). All operations with DNA, such as replication, transcription, untangling, and repair are performed by sub-cellular agents that are composed of multiple proteins. These proteins are synthesized by ribosomes, which can be compared to externally programmed robots. The program is represented by mRNA molecule, which is an edited copy of a gene sequence. Each triplet of nucleotides in the mRNA encodes a specific aminoacid in the protein sequence, and hence the sequence of aminoacids matches to the genomic template. The sequence of aminoacids in proteins is the only feature of organisms that is "described" in the genome, in a certain sense. However even this description is incomplete because there is no place in the genome that specifies the structure of 20 aminoacids used for protein synthesis. Thus, a triplet of nucleotides in the mRNA is an abstract symbol, whose meaning is not specified. It's the job of intra-cellular agents to interpret these symbols by initiating catalytic actions that result in the addition of specific aminoacids to the elongating protein chain. Thus, the gene encodes protein in a very abstract way–as a sequence of non-specified tokens. The genome encodes neither the 3-dimensional conformation of the polymer (which depends on physical properties of non-specified monomers) nor its functions. Instead, the capacity of cellular components to perform their functions is an emergent property of the entire cell. Following the metaphor of a cell as a play, the genome is a script, metabolism is a cast, and cellular structure is a stage (Paton, 1997). Heredity, which is the capacity of transferring functions to the next generation of organisms, is a part of this performance. It requires DNA but also needs many other components of a cell that contribute to the transfer of organism functions across generations. Although the construction of these components is encoded in the genome in an abstract way, the cell needs *actual physical subagents* that can interpret genomic symbols and perform cellular functions. Because most heritable variation in phenotypes is linked to some genomic change, irreversible evolution is usually associated with either genetic selection or genetic drift. The role of structure-functional component of heredity can be compared to a short encryption key that unlocks the enormous amount of genetic information. But this encryption key is not constant but slowly changes in evolution. Thus, some functions appear lost in organisms derived from interspecies nuclear transfer (Lagutina et al., 2013).

Functional capacities of living systems are products of a long evolution, and they most likely emerged in a chain of modifications and combinations of more simple ancestral functions. The role of DNA has been to keep records of these changes so that they can be reproduced in the following generations. But the DNA is not editable and cannot serve as a scrapbook where cellular subagents save their records. Instead, DNA changes mostly randomly and subagents keep reinterpreting these changes by modifying and recombining their preexisting functional capacities. If some crucial cellular function fails to emerge in a new organism, then it dies and the corresponding genotype is removed from the population pool (purifying selection). In this way, genetic selection contributes to the change of preexisting internal functional capacities of organisms.

4. Types of evolutionary opportunities

In this section I describe most common types of evolutionary opportunities which result from regulated variation. These types are discussed in the order of increasing complexity, and appear "nested" in the sense that more complex types include primitive types as components (Table 1).

Table 1
Types of evolutionary opportunities.

Type of evolutionary opportunity	Components, functions	Examples
Adjustment	One function	Disappearance
		Reemergence
Multi-tasking	One component, many functions	Redundancy
		Pleiotropy
		Function change
Connection	Many components, many functions	Signal transduction
		Cell communication
		Neural networks
Interaction	Many organisms	Cooperation
		Symbiosis, farming
		Horizontal gene transfer

4.1. Adjustment

Adjustment is the most simple kind of evolutionary opportunity that includes a capacity to activate or repress a certain function. Repressed functions do not vanish entirely but remain present as latent capacities for some time. If they appear useful again in a different environment or body context, they may become reactivated (possibly with minor changes). Although these evolutionary changes provide no novelty, they are important for specialization to various ecological niches or for survival in unfavorable conditions. For example, parasitism in insect eggs requires extreme miniaturization of parasitic wasps, which was accompanied by the degradation of the gut and wing venation. Eyes and pigmentation degraded in cave-living animals.

Because organism functions are interdependent, the adjustment of one function may cause related adjustments in other functions. For example, weakened repressive mechanisms may induce a partial recovery of ancestral functions and structures (i.e., atavisms). Recovery of ancestral traits may offer additional opportunities to find new niches in a changed environment. The demise of molecular and developmental correlations under stress may result in the re-emergence of ancestral functions, recessive genotypes, and thus increase the scope of functional variability (Jablonka and Lamb, 2005). Genetic changes associated with the decline of a function include both neutral evolution and selection. Neutral evolution results from accumulation of mutations in non-functional genomic regions, whereas selection may stem from harmful effects of non-functional organs (e.g., non-functional eyes can still cause inflammation) or from savings in energy and resources released after degradation of these organs.

The loss of phenotypic plasticity following specialization to a narrow ecological niche is an interesting theoretical example of function decline where genetic selection is not required. If previously fluctuating environment becomes stabilized in one particular state, then the benefits of phenotypic plasticity would disappear. As a result, the ability of organisms to adjust their phenotype to the environment may degrade due to neutral evolution, and the phenotype becomes canalized. This model of "adaptive" neutral-driven evolution was proposed independently by Hughes (2012) and Kull (2013). However, this model is "is a theory for specialization rather than for adaptation" (Chevin and Beckerman, 2011, p. 457) because no novel function appears in result. Interestingly, the loss of plasticity due to neutral evolution may generate organisms with a slightly increased fitness because of the release of physiological costs associated with plasticity. However, even if the fitness increases after the loss of plasticity, this model still cannot be viewed as an example of adaptive evolution because it does not show the emergence of novel solutions to life problems, which is a criterion of adaptation, as formulated by Kull (2013).

4.2. Multitasking

Multitasking is the ability of living agents and subagents to handle multiple functions. Because of multitasking, every agent has hidden capacities to perform additional tasks that are not currently needed. Multiplicity of upstream functions (e.g., interaction of a membrane receptor with multiple ligants) is known as functional redundancy (Kelso, 1994), whereas the multiplicity of downstream functions is usually called pleiotropy (LaPorte et al., 2008). In some cases, organisms or their subagents are not immediately capable of handling additional functions, but can switch to these new functions after minor change, that can be enforced by genetic selection. For example, the resistance to antibiotics in bacteria develops amazingly fast and some level of resistance is detected even in cells that never encountered antibiotics (Wright, 2010). The ability to develop resistance is based on the presence of multifunctional enzymes capable of degrading toxic molecules; it appears that only minor changes are required to adjust these enzymes for processing a new chemical. If bacteria had no enzymes to destroy toxic molecules then genetic variation would never yield antibiotic resistance. This example shows that variation is not random but depends on the presence of molecular tools that provide an opportunity for performing specific functions. Each molecular tool not only performs a specific function in the cell but also adds a new dimension of additional or alternative functions that may appear in the future evolution. Thus, by expanding the repertoire of molecular tools organisms can build up their evolvability and increase their chances of avoiding extinction and propagating in the long-term.

Another example of multitasking is the use of pigmentation, such as melanin. The change of wing color in peppered moths in England remains the best documented case of genetic selection in natural populations (True, 2003). Originally, moths had light color which helped them to hide on the background of white birch trees. However, after the industrial revolution birches became dark and this change caused a rapid spread of dark colored moths (carbonaria). Obviously, peppered moths had the capacity to produce dark scales even before the industrial revolution. In fact, melanin is multi-functional and it is found not only in the cuticle of insects but also in compound eyes, where it enhances the sensitivity of photoreceptors. At the molecular level, melanin is produced from tyrosine by several enzymes and its production is controlled by such genes as Yellow and Ebony (Wittkopp et al., 2002). However, recent analysis did not confirm the association of any known melanin-related genes to the "carbonaria" morph of peppered moth (van't Hof and Saccheri, 2010). Authors hypothesized that the "carbonaria-gene" may be a high-level developmental factor which regulates the spatial expression of one or more genes related to melanin production. It is also reasonable to hypothesize that the ability to change color is beneficial for many butterfly species because it allows them to rapidly adapt to new conditions, where their hiding places have a different color as compared to the original habitat. Thus, the evolutionary event with the peppered moth in England has been well "rehearsed" in the history of many moth species. This is another example, where evolutionary change does not produce any major novelty; instead organisms simply unfold or tune up already existing hidden capacities. Such kind of "rehearsed" changes are likely to be frequent in the evolution of temperature-dependent processes, photoperiodism, growth rate, and diet.

Multitasking is a common survival strategy for many genes. Genes with only one function may be in danger of extinction if

this function is temporarily not needed. Thus, multi-functional genes are more likely to persist in the long run, even if additional functions are minor and make the difference only in harsh conditions. Multi-functionality of living systems tends to increase as new functions and structures become adjusted to already existing ones. As a result, most ancient components acquire novel functions of coordinating the construction of more recent components. The definite form of an animal develops on the top of the preexisting embryonic form of its ancestors, which is known as Haeckel's biogenetic/recapitulation law (Richardson and Keuck, 2002). Thus, new adaptations develop by reusing already existing structures in a new way, and become dependent on their presence. For example, the presence of eyes in axolotl embryo appears necessary for the development of hypothalamus, which in turn is needed for animal fertility (Van Deusen, 1973). As new functions accumulate, the original function of an organ may disappear (e.g., the notochord). But organisms may retain the opportunity to restore previously lost functions, although the reversibility is never complete. For example skin scales in reptiles and armadillos are similar to the scales of fish but differ in shape and morphogenesis.

Organs may change functions in evolution via multitasking as an intermediate step. For example, organs of the sides of fast-moving animals have a capacity to develop into wings. Insect ancestors used paranatal appendages for maneuvering and gliding, and these appendages eventually transformed into wings. In contrast, reptiles and bird ancestors used legs for maneuvering and gliding, and thus their wings originated from legs. Two important mechanisms can facilitate the change of function: behavioral and developmental plasticity. Behavioral plasticity means that animals can change their behavior habits and start utilizing their organs in unusual way, whereas developmental plasticity can strengthen these organs and adjust them to a new function via various molecular and developmental mechanisms. For example, jumping animals may attempt to use their appendages for gliding, and a different set of muscles will strengthen as a result of these new movements. Behavioral changes were often observed in mammals. For example a goat with non-functional front legs managed to master bi-pedal walking (West-Eberhard, 2005). In theory, behavioral plasticity can accelerate genetic changes toward a new function by reshaping the fitness landscape. This hypothetical mechanism was proposed by James Mark Baldwin (1896) and is known as "Baldwin effect". Learned patterns can become stabilized in populations either by genetic automation or by selection for increased "intelligence" (Dennett, 1995). Although this effect is difficult to prove experimentally, many evolutionary biologists agree that there are no logical flaws in this hypothesis (Dennett, 2003; Depew and Weber, 1995).

In contrast to morphological variations which accumulate slowly over many generations, behavioral changes emerge very fast from individual learning. Animal try to solve the problem by various ways and discover novel solutions within hours or days. There is evidence that mammals and birds can even use primitive logic to solve problems, which further increases the rates of behavioral change as compared to pure trial and error strategy. In experimental conditions, a raven had to combine complex actions to get meat, such as pulling a string with his beak and holding a loose loop of the string with a claw (Heinrich and Bugnyar, 2007). Nevertheless, the bird successfully completed the task without prior teaching. Thus, Baldwin effect may substantially accelerate the adaptive evolution.

4.3. Connection

Connection is the ability of living systems to coordinate changes in previously independent components. The capacity to establish connections increased in biological evolution. In prokaryotes, connections are established mostly via independent evolution of simple proteins. If a protein has two functional parts then it can becomes a proto-index that transfers a signal from one cell component to another (Sharov, 2010). For example, membrane receptors have an outer sensory part that binds to external molecules (e.g., glucose), and the inner part, which activates intra-cellular secondary signals in response to receptor binding. Signal transduction pathways end with activation of transcription factors which activate the transcription of specific genes. In eukaryotes we already see a factory of connection-building via the emergence of large complex genes that combine multiple functional domains, and large multi-protein regulatory complexes. In addition, eukaryotes can interlink distal chromosome regions for transcription regulation via CTCF and cohesin factors (Li et al., 2013). In multicellular organisms, the effects of genes can be easily transferred to another body location (heterotopy), where these genes start interacting with novel tissue-specific genetic networks. Such unusual gene expression patterns result in homeotic transformations, such as development of legs instead of antennae in Drosophila (Goldschmidt, 1940). It was hypothesized that feathers did not originate for flight but appeared on various parts of the body for display, parachuting, or other purposes; however, later birds reused feather-making genes for flight and expressed them on developing wings (Chuong et al., 2003). The most dramatic increase of connection-making is seen in the nervous system, where cells developed specialized connection organelles – synapses, which can be compared to USB ports in computers by the degree of universality.

Connections between regulatory networks result in coordinated variation of cell organelles or multicellular organs. The most common example of such coordination is allometry – a relationship between sizes of various body parts, which quantitatively often follows a power law equation (West and Brown, 2005). The increase of body size without a corresponding increase in the size of legs may produce animals that cannot move. Thus, the embryonic development of animals includes control mechanisms that ensure the proportional change in both body size and legs. Even if the genes that stimulate growth (i.e., growth factors or their receptors) carry mutations that impair normal development, the embryo attempts to compensate these damages by using alternative signaling pathways in order to produce a proportionally shaped organism (Ning et al., 2007). Thus, connections support homeostasis – the capacity of an organism to preserve the functionality of any part of the system despite the change in the environment or in other parts of the system. As a result, developmental correlations provide an opportunity for organisms to survive despite of mutations, and to keep these mutations as a resource for potential future variability. If organisms had no such capacity, then almost every mutation would be lethal, and adaptive evolution would not be possible.

One of the mechanisms of homeostasis is modularity, which is the ability of a system to assemble discrete functional units that are self-regulated and protected from undesirable effects from other components of the system as well as from the environment (Schlosser and Wagner, 2004). Modularity provides evolutionary opportunities because it allows functional subsystems to evolve at different rates without affecting the viability of the whole organism (Wagner, 1996). If variation of one component increases the efficiency of some function, this change is not likely to interfere with other functions and structures because developmental correlations tend to neutralize these undesirable effects. Modularity in the coloration of butterfly wings, which is not directly linked with survival, resulted in the amazing combinatorial patterns of variation (Brakefield, 2009).

4.4. Interaction

Interaction is understood here as the ability of organisms to communicate and coordinate their activities. Because interactions

can improve existing functions and establish new functions, they contribute to evolutionary opportunities of populations and species. Many organisms (e.g., polyps, ants, rodents) live in colonies where functions of individuals are regulated by contact or chemical communication. Other examples where additional functionality is gained via interaction with other species include symbiosis and mimicry. Because these interactions often dominate in large phylogenetic lineages (e.g., symbiosis in lichens and mimicry in clearwing moths), they are likely to be supported by specific generic adaptations that provide evolutionary opportunities. It is possible that clear-wing moths exploit body color patterning mechanisms which are similar to those that work in wasps, whom they imitate.

It is possible that the capacity for horizontal gene transfer, which is most abundant in bacteria (Koonin and Galperin, 2003), provides additional evolutionary opportunities that help to acquire novel functions. For example, photosynthetic genes are likely to be transferred horizontally between unrelated lineages of bacteria (Raymond et al., 2002). However, it has not been proven that bacteria developed adaptations specifically to increase the chances of horizontal gene transfer.

5. Evolutionary opportunities are accumulated via lineage selection

Our analysis of regulated variation indicates the inadequacy of evolutionary models that describe single adaptive traits in isolation from the rest of the organism. All traits are highly interdependent, and existing organs and functions reshape the patterns of heritable variation, and in this way, guide the directions of future adaptive changes. Dobzhansky wrote: "A change in the genotype alters the reaction norm, and some of the alterations may enable the new genotype to produce a harmonious response where the ancestral has been a failure." (Dobzhansky, 1937, p. 170). Recently Seaborg described interconnectedness of adaptive events as "sequential evolution": "a change in a trait causing a change in selection on a second trait which leads to a change in the second trait\which in turn causes a change in selection on a third trait changing it and so on." (Seaborg, 1999, p. 1). As organisms develop new structural and regulatory modules, these components open additional opportunities via widening of their functional repertoire. Possession of such modules may benefit populations and species in the long run when they survive natural disasters or spread across a wide range of environments. In addition, animals can explore novel functions behaviorally, and these new behaviors may generate new phenotypes with greater opportunities (i.e., Baldwin effect). In other words, evolutionary opportunities can be constructed, inherited, selected, and even learned.

However, the notion of sequential evolution does not capture the multi-scale nature of adaptations. It is meaningful to talk about opportunities in evolution only if they last *much longer* than the phenotypes they regulate. In other words, we need to distinguish short-term adaptations that are needed for everyday functions, and long-term adaptations (or adaptability) which provide resources and tools for the development of short-term adaptations (Sharov, 2009). This approach indicates that the traditional binary distinction between adaptive and non-adaptive traits is meaningless. Gould and Lewontin argued against the panglossian adaptationalism and demonstrated the abundance of non-adaptive traits (Gould and Lewontin, 1979), but they did not discuss the issue of adaptability.

The effect of adaptability results in providing some benefits to others, specifically, to the long-term descendants (in contrast to altruism, which provides immediate benefits to the members of the group), and therefore it can be viewed as a specific case of *inclusive fitness*. In particular, the Hamilton's rule, which connects costs, benefits, and relatedness (Hamilton, 1964), may eventually appear applicable to adaptability (either in its original or modified form). If the costs of adaptability are high, then corresponding traits have limited chances to get established. However, a mathematical model showed that adaptability-related benefits of sexual reproduction are sufficient to offset the two-fold reduction in the net reproduction rate in sexual populations as compared to parthenogenetic clones (de Vienne et al., 2013). Most adaptability traits probably have low costs (e.g., costs of gene maintenance). Large variability in the size of the genome, known as C-value paradox (Gregory, 2005), indicates that such informational costs are indeed low. However, this does not mean that every trait that supports adaptability emerged due to its contribution to the inclusive fitness. These traits can also emerge as by-products of other evolutionary changes (Pigliucci, 2008). But in this respect, they do not differ from other phenotypic traits, which often emerge to handle one function but become reused later for other functions. Moreover, there are reasons to expect that many adaptability traits have additional everyday functions that may protect the corresponding genes from genetic drift in periods of stable environment (when adaptability is not needed). But the danger of genetic drift should not be overstated as a factor that limits the life of adaptability-related traits because the rate of mutations (ca. 10^{-5} per gene) usually does not exceed the probability of environmental change, and the loss of functionality of mutated genes can be compensated by phenotypic plasticity and mutations in other genes.

Many features of adaptability are locked in the body plan which persists over millions of years, and therefore, is specific to large phylogenetic lineages. Thus, we can expect that less adaptable lineages have a tendency to be gradually replaced in evolution with more adaptable lineages that have expanded opportunities to survive and capture resources. This reasoning, however, does not exclude the possibility that specialized lineages with low adaptability persist in highly specialized niches. Darwin thought that some lineages (e.g., species and genera) have a higher potential for subsequent speciation and divergence, and thus, natural selection occurred not only at the level of individuals but also at the level of species. This idea was further developed by Plate who used the notion "orthoselection" to denote the differential success of lineages in evolution (Plate, 1913). However, association of Plate with Nazi negatively affected the acceptance of his ideas (Levit and Hoßfeld, 2006). Thoday suggested to measure adaptability via long-term fitness measured by the probability that organisms will have offspring 1000 or even 1,000,000 years from now (Thoday, 1958). Van Valen used this idea to assess the probabilities of extinction in lineages of various taxonomic rank based on the paleontological data (Van Valen, 1973). Lineages with advanced developmental and behavioral regulatory mechanisms, which generate phenotypes with higher plasticity and robustness, appear more successful in macro-evolution, and this process of lineage succession resulted in the evolution of evolutionary mechanisms themselves (Depew and Weber, 1995; Pigliucci, 2008). Recently, the selection of lineages was experimentally demonstrated in bacteria (Woods et al., 2011). Thus, the selection of lineages can explain why evolutionary opportunities tend to accumulate in macro-evolution. But its explanatory power should not be overstated. In particular, it does not explain the establishment of any specific kind of evolutionary opportunity, which is a product of individual biological organization at multiple structural and functional levels.

Although biologists tend to agree that progressive evolution is real, very few attempts have been made to quantify the level of progress. At the genetic level, the progress can be seen in the incremental growth of the functional and non-redundant fraction of the genome, which is a biological analog of the Moore's law (Sharov, 2006). At the phenotypic level, progress can be measured by the presence of higher-order innovations: (1) increase of the

dimensionality of the morphospace via providing new axes of variation and (2) overcoming constraints that prevented populating of some non-occupied region of the morphospace, where many unexplored adaptation peaks may be present (Wainwright, 2009). The example of the first kind of novelty is duplication of body segments, or emergence of new behaviors (e.g., flight for birds or diving for whales). The example of the second kind of innovation is the disruption of functional dependency (decoupling) between forelimbs and hindlimbs after the emergence of flight in birds.

6. Conclusions

Living organism is not a collection of independent traits encoded by separate genes and optimized for performance of separate functions, as assumed in the MS. Instead, organism is a highly integrated whole where each subsystem is an active generator of forms and functions. Thus, organisms are full of possibilities that far exceed their actual form and function. This hidden potential is needed to produce variations in the phenotype and behavior that help organisms to survive and propagate in varying environments. Hidden potential can be seen in macro-mutations, developmental correlations, environmental plasticity, increased phenotypic variability in stressful conditions, and in the regularity of the morphospace in large phylogenetic lineages. Although developmental regulatory mechanisms restrict the space of generated phenotypes, and therefore can be seen as constraints, these restrictions are generally beneficial for the organisms and thus better considered as evolutionary opportunities. These opportunities can become activated with relatively high probability via minor and non-local mutations in the genome. Thus, the genome is not a "blueprint" but a "switchboard" that releases or blocks preexisting internal capacities of organism subagents. Metaphorically speaking, a business would not survive if a CEO attempts to describe every movement and thought of every worker (which is not possible in the first place). Similarly, organisms would not live and evolve if all details of their structures and functions were described in the genome. Moreover, full description is impossible from the theoretical standpoint. Instead, the genome encodes the phenotype by sequential regulation of various sub-agents, including ribosomes, transcription factors, protein complexes, and other cell components. Developmental mechanisms that regulate phenotypic variation are heritable, and thus, can evolve in the long run toward enhanced adaptability and robustness of living systems. Thus, the adaptive nature of regulated variation can be explained by the differential success of lineages in macro-evolution.

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References

Baldwin, M.J., 1896. A new factor in evolution. Am. Nat. 30, 441–451.
Bateson, G., 1972. Steps to an Ecology of Mind; Collected Essays in Anthropology, Psychiatry, Evolution, and Epistemology. Chandler Pub. Co, San Francisco.
Berg, L.S., 1969. Nomogenesis: or, Evolution determined by Law. MIT Press, Cambridge, MA (orig. 1922).
Brakefield, P., 2009. A focus on both form and function in examining selection versus constraint. In: Laubichler, M.D., Maienschein, J. (Eds.), Form and Function in Developmental Evolution. Cambridge University Press, New York, pp. 112–131.
Brakefield, P.M., 2011. Evo-devo and accounting for Darwin's endless forms. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 366, 2069–2075.
Brock, M.T., Dechaine, J.M., Iniguez-Luy, F.L., Maloof, J.N., Stinchcombe, J.R., Weinig, C., 2010. Floral genetic architecture: an examination of QTL architecture underlying floral (co)variation across environments. Genetics 186, 1451–1465.
Cardon, L.R., Neale, M.C., 1992. Methodology for Genetic Studies of Twins and Families. Kluwer Academic Publishers, Boston.
Chevin, L.M., Beckerman, A.P., 2011. From adaptation to molecular evolution. Heredity (Edinb) 108, 457–459.
Chuong, C.M., Wu, P., Zhang, F.C., Xu, X., Yu, M., Widelitz, R.B., Jiang, T.X., Hou, L., 2003. Adaptation to the sky: defining the feather with integument fossils from mesozoic China and experimental evidence from molecular laboratories. J. Exp. Zool. B: Mol. Dev. Evol. 298, 42–56.
Cuénot, L., 1914. Théorie de préadaptation. Scientia 16, 60–67.
Darwin, C., 1987. The Origin of Species, 4th ed. Penguin Books, Suffolk (orig. 1859, reprint of 1st edition 1859).
Dawkins, R., 1976. The Selfish Gene. Oxford University Press, Oxford.
Dawkins, R., 1986. The Blind Watchmaker. Norton, New York, 1st American ed.
de Vienne, D.M., Giraud, T., Gouyon, P.H., 2013. Lineage selection and the maintenance of sex. PLoS ONE 8, e66906.
Dennett, D.C., 1995. Darwin's Dangerous Idea—Evolution and the Meaning of Life. Penguin Books, Suffolk, London.
Dennett, D.C., 2003. The Baldwin effect, a crane, not a skyhook. In: Weber, B.H., Depew, D.J. (Eds.), Evolution and Learning: The Baldwin Effect Reconsidered. MIT Press, Cambridge, MA, pp. 69–106.
Depew, D.J., Weber, B.H., 1995. Darwinism Evolving: Systems Dynamics and the Genealogy of Natural Selection. MIT Press, Cambridge, MA.
Dobzhansky, T., 1937. Genetics and the Origin of Species. Columbia University Press, New York.
Edelman, G., 1987. Neural Darwinism. The Theory of Neuronal Group Selection. New York, Basic Books.
Fisher, R.A., 1930. The Genetical Theory of Natural Selection. The Clarendon Press, Oxford.
Frentiu, F.D., Clegg, S.M., Chittock, J., Burke, T., Blows, M.W., Owens, I.P., 2008. Pedigree-free animal models: the relatedness matrix reloaded. Proc. Biol. Sci. 275, 639–647.
Glahn, D.C., Kent Jr., J.W., Sprooten, E., Diego, V.P., Winkler, A.M., Curran, J.E., McKay, D.R., Knowles, E.E., Carless, M.A., Goring, H.H., Dyer, T.D., Olvera, R.L., Fox, P.T., Almasy, L., Charlesworth, J., Kochunov, P., Duggirala, R., Blangero, J., 2013. Genetic basis of neurocognitive decline and reduced white-matter integrity in normal human brain aging. Proc. Natl. Acad. Sci. U.S.A. 110, 19006–19011.
Goldschmidt, R., 1935. Gen und Ausseneigenschaft, I. Zeitschrift für induktive Abstammungs- und. Vererbungslehre 69, 38–69.
Goldschmidt, R., 1940. The Material Basis of Evolution. Yale University Press, New Haven, CT.
Gould, S.J., Lewontin, R.C., 1979. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. Proc. R. Soc. Lond. B: Biol. Sci 205, 581–598.
Gould, S.J., Vrba, E.S., 1982. Exaptation—a missing term in the science of form. Paleobiology 8, 4–15.
Gregory, T.R., 2005. The *C*-value enigma in plants and animals: a review of parallels and an appeal for partnership. Ann. Bot. 95, 133–146.
Hamilton, W.D., 1964. The genetical evolution of social behaviour. I. J. Theor. Biol. 7, 1–16.
Heinrich, B., Bugnyar, T., 2007. Just how smart are ravens? Sci. Am. 296, 64–71.
Hoffmeyer, J., Emmeche, C., 1991. Code-duality and the semiotics of nature. In: Anderson, M., Merrell, F. (Eds.), On Semiotic Modeling. Mouton de Gruyter, Berlin, New York, pp. 117–166.
Hughes, A.L., 2012. Evolution of adaptive phenotypic traits without positive Darwinian selection. Heredity (Edinb.) 108, 347–353.
Huxley, T.H., 1893. Darwiniana. Collected Essays. MacMillan and Co, London.
Jablonka, E., Lamb, M.J., 2005. Evolution in Four Dimensions: Genetic, Epigenetic, Behavioral, and Symbolic Variation in the History of Life. MIT Press, Cambridge, MA.
Jablonka, E., Lamb, M.J., 2010. Transgenerational epigenetic inheritance. In: Pigliucci, M., Müller, G.B. (Eds.), Evolution, the Expanded Synthesis. MIT Press, Cambridge, MA, pp. 137–174.
Kauffman, S., 1993. The Origins of Order: Self-organization and Selection in Evolution. Oxford University Press, Oxford.
Kelso, A., 1994. The enigma of cytokine redundancy. Immunol. Cell Biol. 72, 97–101.
Klingenberg, C.P., 2005. Developmental constraints, modules and evolvability. In: Hallgrímsson, B., Hall, B.K. (Eds.), Variation: A Central Concept in Biology. Academic Press, San Diego, pp. 219–247.
Koonin, E.V., Galperin, M.Y., 2003. Sequence – Evolution – Function: Computational Approaches in Comparative Genomics. Kluwer Academic, Boston.
Kovalenko, E.E., Popov, I.Y., 1977. New approaches to the analysis of characteristics of variation. Z. Obshch. Biol. 58, 70–83 (in Russian).
Kull, K., 2013. Adaptive evolution without natural selection. Biol. J. Linn. Soc. 109.
Lagutina, I., Fulka, H., Lazzari, G., Galli, C., 2013. Interspecies somatic cell nuclear transfer: advancements and problems. Cell Reprogram. 15, 374–384.
Lande, R., 1979. Quantitative genetic analysis of multivariate evolution, applied to brain:body size allometry. Evolution 33, 402–416.
LaPorte, S.L., Juo, Z.S., Vaclavikova, J., Colf, L.A., Qi, X., Heller, N.M., Keegan, A.D., Garcia, K.C., 2008. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. Cell 132, 259–272.
Laubichler, M.D., 2009. Form and function in Evo Devo: historical and conceptual reflections. In: Laubichler, M.D., Maienschein, J. (Eds.), Form and Function in Developmental Evolution. Cambridge University Press, New York, pp. 10–46.

Levit, G.S., Hoßfeld, U., 2006. The forgotten old-Darwinian synthesis: the evolutionary theory of Ludwig H. Plate (1862–1937). NTM Int. J. History Ethics Nat. Sci. Technol. Med. 14, 9–25.
Li, Y., Huang, W., Niu, L., Umbach, D.M., Covo, S., Li, L., 2013. Characterization of constitutive CTCF/cohesin loci: a possible role in establishing topological domains in mammalian genomes. BMC Genom. 14, 553.
Meyen, S.V., 1987. Fundamentals of Palaeobotany. Chapman and Hall, London/New York.
Ning, Y., Hoang, B., Schuller, A.G., Cominski, T.P., Hsu, M.S., Wood, T.L., Pintar, J.E., 2007. Delayed mammary gland involution in mice with mutation of the insulin-like growth factor binding protein 5 gene. Endocrinology 148, 2138–2147.
Paton, R., 1997. The organisations of hereditary information. BioSystems 40, 245–255.
Pigliucci, M., 2008. Is evolvability evolvable? Nat. Rev. Genet. 9, 75–82.
Pigliucci, M., Müller, G.B., 2010. Elements of an extended evolutionary synthesis. In: Pigliucci, M., Müller, G.B. (Eds.), Evolution – The Extended Synthesis. MIT Press, Cambridge, MA/London.
Plate, L.H., 1913. Selektionprinzip und Problem der Artbildung: ein Handbuch der Darwinismus, 4th ed. Engelmann, Leipzig.
Raff, R.A., Raff, E.C., 2009. Evolution in the light of embryos: seeking the origins of novelties in ontogeny. In: Laubichler, M.D., Maienschein, J. (Eds.), Form and Function in Developmental Evolution. Cambridge University Press, New York, pp. 83–111.
Raymond, J., Zhaxybayeva, O., Gogarten, J.P., Gerdes, S.Y., Blankenship, R.E., 2002. Whole-genome analysis of photosynthetic prokaryotes. Science 298, 1616–1620.
Richardson, M.K., Keuck, G., 2002. Haeckel's ABC of evolution and development. Biol. Rev. Camb. Philos. Soc. 77, 495–528.
Riedl, R., 1977. A systems-analytical approach to macro-evolutionary phenomena. Quart. Rev. Biol. 52, 351–370.
Schlosser, G., Wagner, G.P., 2004. Modularity in Development and Evolution. University of Chicago Press, Chicago.
Schmalhausen, I.I., 1949. Factors of Evolution: The Theory of Stabilizing Selection. Blakiston, Philadelphia, PA.
Schwenk, K., 1995. A utilitarian approach to evolutionary constraint. Zoology 98, 251–262.
Seaborg, D.M., 1999. Evolutionary feedback: a new mechanism for stasis and punctuated evolutionary change based on integration of the organism. J. Theor. Biol. 198, 1–26.
Sharov, A.A., 2010. Functional information: towards synthesis of biosemiotics and cybernetics. Entropy 12, 1050–1070.
Sharov, A.A., 2006. Genome increase as a clock for the origin and evolution of life. Biol. Direct 1, 17.
Sharov, A.A., 2009. Role of utility and inference in the evolution of functional information. Biosemiotics 2, 101–115.
Thoday, J.M., 1958. Natural selection and biological progress. In: Barnet, S.A. (Ed.), A Century of Darwin. Harward University Press, Cambridge, MA, pp. 313–333.
True, J.R., 2003. Insect melanism: the molecules matter. Trends Ecol. Evolut. 18, 640–647.
van't Hof, A.E., Saccheri, I.J., 2010. Industrial melanism in the peppered moth is not associated with genetic variation in canonical melanisation gene candidates. PLoS ONE 5, e10889.
Van Deusen, E., 1973. Experimental studies on a mutant gene (e) preventing the differentiation of eye and normal hypothalamus primordia in the axolotl. Dev. Biol. 34, 135–158.
Van Valen, L., 1973. A new evolutionary law. Evol. Theory 1, 1–30.
Vavilov, N.I., 1922. The law of homologous series in variation. J. Genet. 12, 47–89 (in Russian).
Waddington, C.H., 1961. Genetic assimilation. Adv. Genet. 10, 257–293.
Waddington, C.H., 1968. Towards a theoretical biology. Nature 218, 525–527.
Wagner, G.P., 1996. Homologues, natural kinds and the evolution of modularity. Am. Zool. 36, 36–43.
Wainwright, P.C., 2009. Innovation and diversity in functional morphology. In: Laubichler, M.D., Maienschein, J. (Eds.), Form and Function in Developmental Evolution. Cambridge University Press, New York, pp. 132–152.
West-Eberhard, M.J., 2005. Phenotypic accommodation: adaptive innovation due to developmental plasticity. J. Exp. Zool. B: Mol. Dev. Evol. 304, 610–618.
West, G.B., Brown, J.H., 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J. Exp. Biol. 208, 1575–1592.
Wittkopp, P.J., True, J.R., Carroll, S.B., 2002. Reciprocal functions of the Drosophila yellow and ebony proteins in the development and evolution of pigment patterns. Development 129, 1849–1858.
Woltereck, R., 1909. Weitere experimentelle Untersuchungen über Artveränderung, speziell über das Wesen quantitativer Artunterschiede bei Daphnien. Verhandlungen der deutschen zoologischen Gesellschaft 19, 110–173.
Woods, R.J., Barrick, J.E., Cooper, T.F., Shrestha, U., Kauth, M.R., Lenski, R.E., 2011. Second-order selection for evolvability in a large *Escherichia coli* population. Science 331, 1433–1436.
Wright, G.D., 2010. Antibiotic resistance in the environment: a link to the clinic? Curr. Opin. Microbiol. 13, 589–594.
Zavarsin, G.A., 1974. Phenotypical Systenatics of Bacteria. Nauka, Moscow (in Russian).