Chapter 13

Systems Evolutionary Biology of Waddington's Canalization and Genetic Assimilation

Alexander V. Spirov, Marat A. Sabirov and
David M. Holloway

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Abstract

In recent years, there has been growing interest in computer modeling of the evolution of gene and cell regulatory networks, in general, and in computational studies of the classic ideas of Baldwin, Schmalhausen, Waddington, and followers, in particular. Two related aspects of Waddington's evolutionary theories are the concepts of canalization and of genetic assimilation. Canalization is associated with the robust development of an individual to diverse perturbations and noise, though, when fluctuations in developmental factors exceed a particular limit, the normal developmental trajectory can be "thrown out" of the robust canal, resulting in an altered phenotype. If selective pressure favors the new phenotype, an initial individual loss of canalization can lead to phenotypic changes in the population (with canalization then becoming established for the new phenotype). Genetic assimilation is the subsequent genetic fixing of the new trait in the population. Recent experimental and theoretical works have established a quantitative basis for these classic concepts of Waddington; this chapter will review these new developments in systems evolutionary biology.

Keywords: canalization, genetic assimilation, gene networks, computer modeling, systems evolutionary biology

1. Canalization and genetic assimilation

Computational studies of the classic concepts of Ivan Schmalhausen [1], Conrad Waddington [2], and their contemporaries (Rendel [3]) have become a major area in evolutionary theory in recent years. A number of these concepts from the 1950s have had a major impact on the evolutionary theory of development, and computation allows for quantitative testing and

characterization of the ideas. Here, we review recent work on canalization, whereby species show low phenotypic variation, despite ample genetic and environmental variation (also termed "robustness"), and on genetic assimilation, in which a phenotypic change induced by an environmental perturbation becomes stabilized in the genotype.

Canalization captures the observation that most developmental phenotypes display a certain degree of stability, despite environmental or genetic perturbations (**Figure 1**). However, for new phenotypes to arise, there must be a limit to this robustness, such that a large enough perturbation will knock the developmental trajectory out of the robust canal, resulting in a new phenotype. If this new phenotype represents higher fitness, it can be reinforced by genetic assimilation (**Figure 2**).

Waddington did a number of perturbation experiments in *Drosophila* (fruit fly) development to characterize such canalization (robustness) and show its underlying genetic basis. In those times Waddington preferred to use simple perturbations of environmental parameters and conditions, such as exposing flies to diethyl ether [5], high sodium chloride concentrations [6], or heat shock (40°C) [7]. Later, other authors have used mutations in key developmental genes as the perturbations [8, 9]. More recent approaches include genetically engineered organisms with loss-of-function [10] or gain-of-function [11, 12] mutations and varying dosages of small interfering RNAs (siRNAs) to quantitatively deplete targeted gene products [13]. Perturbation experiments remain the main approach to study the mechanisms of robustness. Whatever the technique, relative robustness is calculated as the change in variation of one or more specific traits when the experimental perturbation is applied.

In parallel with the new experimental approaches for perturbation and observations from field biology, a large branch of systems biology is now concerned with computer modeling of

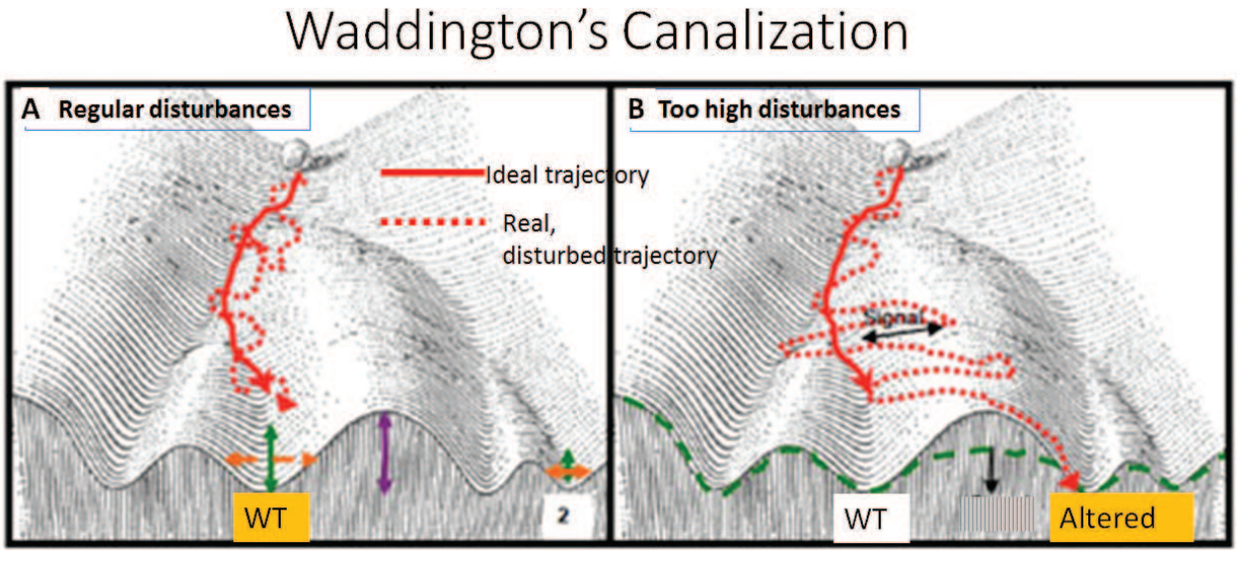

Figure 1. (A–B) Waddington's canalization and epigenetic landscape: Diverse and inevitable environmental disturbances and internal developmental noise systematically disturb developmental trajectory on the epigenetic landscape. However, the developmental process usually returns to the basin of normal development (creod), that is, the development is canalized and the canal walls keep the process in the basin prescribed by the genetic program (after http://www.gen.cam.ac.uk/research-groups/martinez-arias).

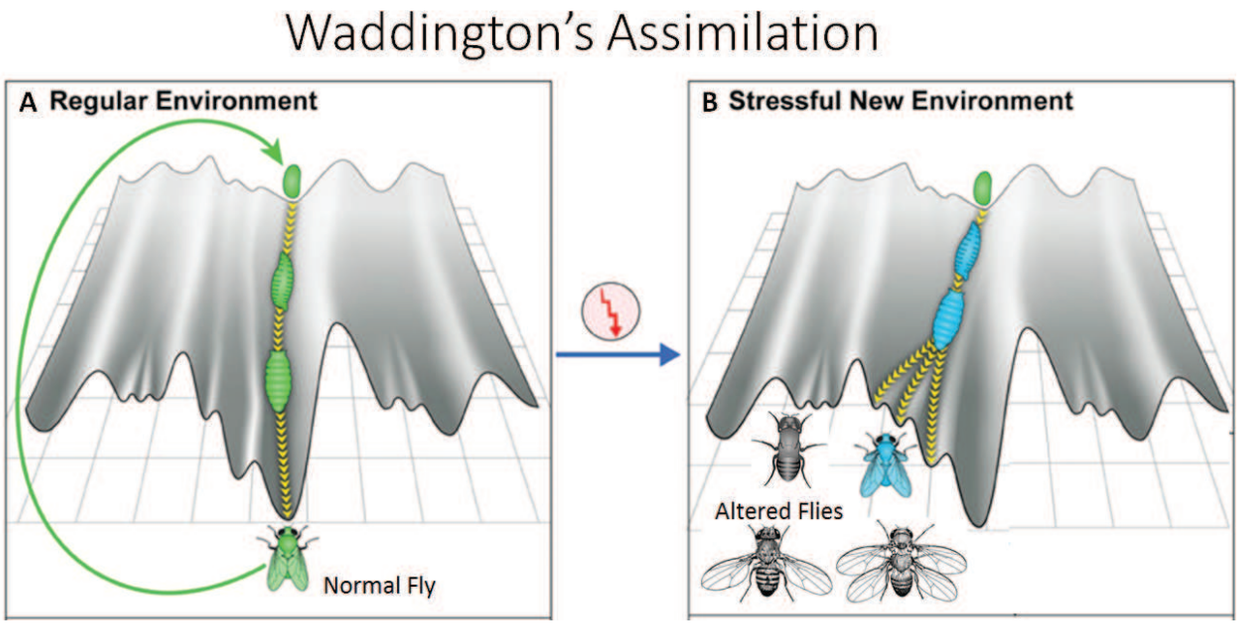

Figure 2. (A–B) Waddington's genetic assimilation: The environmental stress causes a series of the Drosophila's divergent phenotypes. The untypical, high environmental disturbances deform, change the epigenetic landscape. By doing so, it causes the appearance of new phenotypes in the population under stress. If some of the phenotypes are beneficial, it can be stabilized in the genotype by further selection (after [4]).

evolution, in order to test and verify hypotheses for evolutionary mechanisms (e.g., especially in studying evolution over numerous generations and with strictly controlled evolutionary rules). This review focuses on such work which has tested and extended the classic concepts of canalization and genetic assimilation.

2. Simulation of gene network evolution

Andreas Wagner laid out an approach to computational modeling of gene network evolution in two pioneering publications [14, 15]. In his models, an evolving population comprised individuals, each characterized by a genotype and a phenotype. Individuals have a developmental phase of their lifetimes, in which an initial phenotype develops to reach a new stable phenotype. Development is specified by the genotype, which is modeled as a gene regulatory network (GRN). Once an individual has reached its stable adult phenotype, it can reproduce to create the next generation. Reproduction occurs sexually, implying a random mixing of parental genomes in the offspring's genome. Mutations can also occur, modifying gene-gene interactions which may then disrupt the viability of the individual. Stabilization of the adult phenotype is one of the core hypotheses of the Wagner model and its subsequent variants and further developments. Some models have been proposed which constrain selection to be for a particular predetermined phenotype, rather than any stable phenotype. Or fitness functions have been studied in which fitness decreases as the Hamming distance increases between the individual's phenotype and the predetermined one [14–20]. The ways to extend and develop further the approach were reviewed recently [21].

3. How canalization evolves

Computational modeling has shown a number of different aspects of how canalization operates in evolution [21].

3.1. Phenotypic robustness to diverse perturbations

Computation allows for the separate consideration of a number of types of genetic perturbations (such as point mutations, small deletions/insertions, crossover, or gene duplications) and non-genetic perturbations (such as fluctuations in a gene circuit's cellular or nuclear microenvironment [22–24] or changes in an organism's macroenvironment).

Three major reasons for phenotypic robustness to a particular type of natural perturbation have been proposed [25, 26]. First, robustness to a perturbation might have evolved as an adaptation to reduce phenotypic variation in response to a specific perturbation. Second, robustness to the specific perturbation could be a congruent byproduct of evolved robustness to a different perturbation. Lastly, we can hypothesize that robustness is an intrinsic property of biological systems selected for their primary functions. Computational simulations of GRNs suggest both that intrinsic robustness could be widespread and that natural selection can increase robustness under diverse and reasonable sets of parameter values and assumptions [15, 16, 27–31]. Whichever of these options applies, robustness to mutation results in the accumulation of phenotypically cryptic genetic variation (CGV), that is, it allows for changes in genotype which do not affect phenotype (until a strong enough perturbation is made). Partial robustness can lead to preadaptation, and thereby might contribute to evolvability [25] (i.e., accumulated genetic variation may allow for rapid evolution under new selective pressures).

A number of studies have shown that genetic canalization would evolve under stabilizing selection [16, 32–35].

3.2. More realistic models of GRN evolution

Discrete (Boolean, "on/off") GRN models are simplified representations of gene interactions but allow for rapid analysis of some aspects of network evolution. Earlier evolutionary studies were generally with discrete models; more recent developments include continuous treatments of gene states, which are more biologically realistic but more computationally intensive to solve.

Draghi and Whitlock [36] developed a GRN model with continuous gene expression levels, affected by environmental cues, forming the phenotype. They showed by computational experiments that the evolution of phenotypic plasticity can produce populations with larger mutational variance and larger standing genetic variance. They found also that plastic populations do not respond much more quickly to selection pressure than do populations that are more static. Furthermore, if the optimal phenotypes of two traits vary together, then larger mutational and genetic correlations were observed. According to their findings, the

quantitative genetic descriptions of traits created by explicit developmental network models are evolutionarily labile, with genetic correlations that change rapidly with shifts in the selection regime [36].

Iwasaki, Tsuda, and Kawata developed an individual-based approach to the GRN modeling [37, 38]. The GRN of each individual had both phenotypic and regulatory genes, each gene was composed of a cis-regulatory region and a coding region, and a cis-regulatory region was composed of cis-sites for specific transcription factors. They showed by the approach that simple GRNs tend to evolve under conditions where genetic canalization is expected, while more complex GRNs tend to evolve in conditions favoring decanalization. Iwasaki and co-authors study showed that complex GRNs display a high mutational robustness (i.e., mutations against core genes have only a small phenotypic effect) and evolvability (i.e., a larger mutational target size and mutation are likely to change the phenotype). In contrast, simple GRNs have mutational robustness only because of their small mutational target size. Iwasaki and co-authors found that the level of CGVs in a population was mainly determined by the order (weighted size) of GRNs and concluded that the outgrowth of GRNs and adaptation to new environments are mutually facilitating, resulting in sustainable evolvability [37].

3.2.1. Evolution of genotype-phenotype mapping

Crombach and Hogeweg [34] did a computational study with an individual-oriented model with population on a lattice subjected to an environment that changes over time. They showed that long-term evolution of complex GRNs in a changing environment can increase the generation of beneficial mutations. The population evolves toward genotype-phenotype mappings that allow for an orchestrated network-wide change in the gene expression pattern, requiring only a few specific gene *indels* (small insertions and deletions), and the genes involved are hubs of the networks or directly influencing the hubs. In addition, the GRNs maintain their mutational robustness throughout the evolutionary trajectory: evolution in a changing environment leads to a network that is sensitive to a small class of beneficial mutations, while the majority of mutations remain neutral—an example of the evolution of evolvability. These evolutionary dynamics showed a number of similarities with experimental studies in yeast (*S. cerevisiae*) [39, 40] and *E. coli*. [41].

4. Genetic assimilation

The understanding of genetic assimilation, similarly, has been extended through computational investigations in recent years.

4.1. Waddington's canalization and genetic assimilation

A heat shock perturbation done by Waddington in 1953 is used as a classic example of genetic assimilation: cross-veinless flies resulted from an initial heat shock and, selected over multiple generations, eventually produced the phenotype without the perturbation [7]. Alternatively,

the cross-veinless phenotype could be due to contributions from multiple genes, which could have a lower heat shock threshold. This Falconer & Mackay threshold model [42] predicts that if selection for the assimilating cross-veinless phenotype was relaxed, genetic assimilation would not occur. Masel tested this prediction with a Wagner-type GRN model [43]. Her results indicated that genetic assimilation can occur in the absence of selection for the trait, supporting Waddington's mechanism.

4.2. Phenotypic plasticity and CGV

Closer consideration of genetic assimilation shows that it must involve some degree of phenotypic plasticity—the capacity for a genotype to produce multiple phenotypes in response to non-genetic perturbations. This plasticity may also be considered from the stand-point of release of CGV (which Masel and Trotter [44] defined as standing genetic variation that does not ordinarily contribute to the phenotype), which increases during neutral drift but may only become visible with a large enough perturbation. CGV accumulation can cause a diversification of genetic backgrounds on which new mutations may arise. It is biologically reasonable to expect that the effect of any new mutation should be background dependent: a given mutation would have a given effect at a particular background and a different or no effects at other backgrounds. The diversity of genetic backgrounds would give the population access to more novel phenotypes than if it were isogenic [45–47], as reviewed in [48]. As Siegal and Leu [48] summarized, this conceptual argument for evolvability correlating positively with mutational robustness has been formalized in mathematical models of so-called neutral networks in genotype space (more recently termed "genotype networks") and has some empirical support [49–51].

Iwasaki with co-authors [38] focused on GRN as an important mechanism for producing CGV and examined how interactions between GRNs and the environment influence the number of CGVs by using individual-based simulations. The authors conclude that interactions with variable environments may promote the accumulation of CGVs by facilitating the evolution of larger GRNs. In turn, the expansion of GRNs could facilitate evolutionary adaptation to novel environments and niche construction [38].

4.3. Phenotypic plasticity and genetic assimilation

Lande defines genetic assimilation in an altered environment as the reduction in plasticity and its replacement by genetic evolution, while maintaining the phenotype initially produced by plasticity in the altered environment [52]. According to Lande, reduction in plasticity during genetic assimilation is often attributed to fitness costs of plasticity.

Lande quantifies the relation between phenotypic plasticity dynamics and genetic assimilation, wherein plasticity must increase to allow evolution to a perturbed environment but then be reduced to maintain the new optimum. During the first generation in the novel environment, the average fitness substantially drops and the average phenotype jumps toward the new optimum by expression of partially adaptive plasticity. Then, transient evolution of increased plasticity accelerates phenotypic adaptation and allows the average phenotype to

come toward the new optimum. Then, the novel phenotype undergoes a slow process of genetic assimilation, with reduction in plasticity [52].

Temporary perturbations that reduce robustness could turn unitary phenotypes into plastic ones. It then gives natural selection a substrate on which to select a particular novel phenotype (i.e., genetic assimilation) [48, 53–55]. High levels of phenotypic variation could increase the chance of population survival in new hostile environments, which in turn would give time for the population to accumulate adaptive mutations [48, 56, 57]. As Siegal, Leu concluded, the connection between robustness and plasticity could be especially important to evolution [48].

Janna Fierst asked herself to what degree can a history of phenotypic plasticity affect the rate of adaptation to a new environment, that is, is plasticity merely a condition for genetic assimilation, or do environmental fluctuations cause phenotypic plasticity, generating genotypic evolvability? She showed that a history of phenotypic plasticity may determine the evolution of genetic architecture and shorten the waiting time for the generation of phenotypic variance from new mutations and recombination. Hence, rather than acting as a short-term alternative, phenotypic plasticity may facilitate future adaptation and genetic evolution [58].

4.3.1. Phenotypic plasticity and evolvability

Non-genetic perturbations, such as environmental change or developmental noise, can induce novel phenotypes. If an induced phenotype appears recurrently and confers a fitness advantage, selection may promote its genetic stabilization. Non-genetic perturbations can thus initiate evolutionary innovation. CGV may play an important role in this process [20]. Populations under stabilizing selection on a phenotype that is robust to mutations can accumulate such variation. After non-genetic perturbations, this variation can produce new phenotypes. Espinosa-Soto with co-authors find that phenotypic robustness promotes phenotypic variability in response to non-genetic perturbations but not in response to mutation. It suggests that non-genetic perturbations may initiate innovation more frequently in mutationally robust gene expression traits [20].

Phenotypic plasticity can facilitate the origin of genotypes that produces a new phenotype in response to non-genetic perturbations. Espinosa-Soto with co-authors find that phenotypic plasticity frequently facilitates the evolution of novel beneficial gene activity patterns in gene regulatory circuits [59]. The fundamental reason is that genotypes that produce occasionally a beneficial phenotype (and thus have a selective advantage) give more easily rise to genotypes where that same phenotype is more strongly genetically determined [59].

The characterization of plasticity, robustness, and evolvability can be studied in terms of phenotypic fluctuations. By numerically evolving GRNs, the proportionality between the phenotypic variances of epigenetic and genetic origins is confirmed by Kaneko [60]. The relationship suggests a link between robustness to noise and to mutation. The proportionality between the variances is demonstrated to also hold over expressions of different genes (phenotypic traits) when the system acquires robustness through the evolution. It was found by Kaneko that both the population's adaptability to a new environment and the population's robustness becomes compatible when a certain degree of phenotypic fluctuations is produced

by the developmental variability and noise [60]. The Kaneko's conclusion is that the highest adaptability is achieved at near-the-threshold noise level at which the gene expression dynamics are near the critical point to lose the robust evolutionary process.

5. Canalization and assimilation in population biology

Current advances in evolutionary systems biology were caused not only by working out of new computational approaches but also by new biological observations performed to verify the computational conclusions.

5.1. CGV in natural populations

Biological systems are highly robust to perturbation by mutations, recombination, and the environmental stress. Robustness to mutation and recombination permits genetic variation to accumulate as hidden genetic diversity (or CGV). CGV might be revealed in response to stress, and "the amount of heritable phenotypic variation available can be correlated to the degree of stress and hence to the novelty of the environment…" [44].

The CGV role in genetic assimilation was extensively studied by computational evolutionary tests (as overviewed in Section 4.2). They are considered to contribute to evolutionary responses to environmental changes by generating phenotypic diversity [61–63]. Furthermore, the CGV's ability to accumulate and release multiple mutations in populations supports some researchers' considerations that CGVs also promote the acquisition of new traits [38, 64, 65].

Some experiments support these considerations. For example, as it was shown by Suzuki and Nijhout [55], a mutation in the larval hormone-regulatory pathway in *Manduca sexta* moth enables heat stress to reveal a hidden larval coloration. The *black* mutant strain of the moth, which was originally green, demonstrated variations in thermosensitivity: heat shocks during the sensitive period generated larvae with colors that ranged from normal black to nearly normal green [55]. Suzuki and Nijhout also successfully established two lines by artificial selection: one selected for increased greenness upon heat treatment (sensitive line) and the other for decreased color change upon heat treatment (insensitive line). Hence, CGVs really could contribute to phenotypic evolution.

5.2. Phenotypic capacitors

Phenotypic capacitor "is a biological switch capable of revealing previously cryptic heritable variation" [25]. This is an analogy with an electric capacitor, which is capable to store and release an electric charge. Many of the capacitors are proteins whose function contributes to robustness and, therefore, whose damage or modification reveals phenotypic variation [66].

In a complex GRN, there are many gene products which could appear as "phenotypic" capacitors, such that their removal increased phenotypic variability. An extensively studied example is the molecular chaperone Hsp90, but GRN dynamics indicated there should be

many more. Experiments in yeast indicated more than 300 gene products whose removal increased variation [10].

In Drosophila, other molecular chaperones—Hsp22, Hsp67, and Hsp70—were also observed to affect either within-individual variation (measured by asymmetry of bilateral traits) or among-individual variation in morphology [67]. In eukaryotes, Hsp90 impairment has been found to reveal CGV in organisms ranging from yeast to flies to vertebrates to plants [68, 69].

Masel and Siegal [25] considers three approaches based on the use of phenotypic capacitors to study robustness. The first approach is a genome-wide screening for genetic perturbations affecting the variance of a given trait. The trait can be morphological [10, 70, 71], or physiological [72] or can be measured as RNA and protein concentrations [73–75]. Good examples of the approach include the studies of cellular morphology in *S. cerevisiae* mutant strains [10, 76] and the genome-wide analysis of more complex and quantitative traits in both *S. cerevisiae* [70, 73] and *A. thaliana* [71, 74].

The second approach is based on usage of a well-characterized model developmental system under the impact of perturbations. Good example of the approach is the consideration of the developmental lineage of the cells comprising the vulva of nematode species of the genus *Caenorhabditis* [77, 78]. Perturbation of *C. elegans* vulva development by mutation or environmental variation revealed changes in the underlying signaling pathways [77, 78]. Robustness of the vulval developmental system to environmental perturbations results through an integration of multiple buffering capacities at the molecular and cellular level [77, 78].

The third complementary approach is to focus on a single well-characterized perturbation and the variety of developmental systems that it affects. Examples include perturbation of translation termination by the yeast prion [*PSI*+] [79–81] and the heat shock protein, Hsp90, which affects a stunning variety of developmental processes [70, 82].

Namely the computational evolutionary experiments with the GRN models revealed possible existence and evolutionary significance of the phenotypic capacitors and brought intent attention to its experimental study.

5.3. Phenotypic plasticity and genetic assimilation in biology

Baldwin [56], Simpson [83], and Waddington [84, 85] proposed that phenotypic plasticity may benefit populations in new environments. In accordance with Waddington's pioneer considerations, artificial selection can turn an alternative phenotype into a native one [5, 7]. More recently, other researchers have confirmed his observation for diverse traits and different species [55, 70, 86].

Many empirical studies of wild populations support the hypothesis that an ancestral alternative phenotype could have facilitated the evolution of novel, adaptive traits [16, 28, 29, 72, 87–93]. For example, severe environments enhance phenotypic differences among fruit fly strains [94], and a temperature rise caused by a lack of shade increases the frequency of abnormal morphologies in fruit flies [95].

The phenotypes where plasticity may have facilitated adaptation are very diverse. They include gill surface area in cichlid fishes [96], pigmentation patterns in the crustacean *Daphnia melanica* [97], and head size in the snake *Notechis scutatus* [98]. Despite an abundance of candidate examples, plasticity's importance for adaptive evolution is not universally accepted, and we still do not know whether existing observations are rare oddities or hint at general principles of evolution [99–105].

5.3.1. Natural populations in changing environments

During millions of years of existence, species repeatedly encounter extreme changes in average environment, and the capacity to accelerate phenotypic adaptation by transient evolution of plasticity may be crucial for long-term persistence. Sudden environmental change often occurs at the start of natural biological invasions and colonizations (reviewed in [106]).

The success of natural invasions, and artificial introductions for biocontrol, may depend on the evolution of increased plasticity during adaptation to novel environments outside the native range of a species [107–109]. Genetic variance in plasticity within and/or among populations has commonly been observed [110, 111], and species invading novel or extreme environments often display increased plasticity compared to populations from the native range [96, 112–117]. Populations of invasive species outside their native range usually maintain substantial genetic variance [118–120].

Experiments on newly established small populations show that intense artificial selection can rapidly create large phenotypic changes, often altering the mean phenotype by several standard deviations within a few dozen generations [121–123]. For extremely large populations undergoing sudden environmental change *in situ*, sustained intense directional selection can cause adaptation by a rare allele of major effect [124, 125].

Many empirical studies suggest that invasive species tend to have an evolutionary history of environmental disturbance [53]. Ecological disturbances constitute fluctuating selection pressures over evolutionary time, and evolutionary genetic theory predicts that patterns of fluctuating selection can cause genetic architectures to take different paths (e.g. [126]).

When environmental changes happen infrequently, populations maximize fitness by producing a single phenotype [58]. When the environment changes more frequently, organisms that can evolve more rapidly are favored by selection. As Janna Fierst concluded, "when environmental fluctuations are rapid, fitness is maximized by genetic architectures that produce a broad, generalist phenotype or short-term phenotypic plasticity" [58].

Increasing amounts of evidence suggest that traits induced by non-genetic factors are important for innovation in nature [98, 127–129]. For example, taxa with genetically determined dextral or sinistral morphologies are frequently derived from taxa in which the direction of the asymmetry is not genetically fixed but where it is a plastic response [128, 130]. Transitions like these imply genetic assimilation of a direction of asymmetry. This was observed for multiple traits, such as the side on which the eye occurs in flat fishes (*Pleuronectiformes*) and the side of the larger first claw in decapods (*Thalassinidea*) [128].

More generally, good candidates for genetic assimilation are the traits where fixed differences among closely related species are mirrored by plastic variation within populations. Amphibian traits, such as gut morphology [129], limb length, and snout length [130], are illustrative examples.

We can conclude that the observations on the environmental dependence of phenotypic and genetic variances evidences accelerated phenotypic adaptation after an extraordinary environmental change [52].

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Author details

Alexander V. Spirov[1]*, Marat A. Sabirov[1] and David M. Holloway[2,3]

*Address all correspondence to: spirov@iephb.nw.ru

1 Lab Modeling of Evolution, I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

2 Mathematics Department, British Columbia Institute of Technology, Burnaby, Canada

3 Biology Department, University of Victoria, Victoria, Canada

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