



Original research article

Genetic assimilation, robustness and plasticity are key processes in the development and evolution of novel traits

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ABSTRACT

This is a commentary on how C.H. Waddington's experiments in the 1950's, first published in 1953 in a provocatively titled paper "Genetic assimilation of an acquired character," laid the foundation for the field of phenotypic plasticity, and how the ideas he developed eventually led to new ways of understanding phenotypic robustness, plasticity, and how novel traits develop and evolve. The "acquired characters" that Waddington worked with were based on Goldschmidt's ideas of "phenocopies": new phenotypes that develop after an environmental stress that resemble the phenotypes of known mutations. The idea behind genetic assimilation, first outlined by Waddington in 1942, is that existing developmental pathways can be rearranged and redirected through selection to stabilize the phenocopy phenotype, without requiring new mutations. In the short term, Waddington's work led to the discovery of heat shock proteins and the role of Hsp90 in masking defective proteins and allowing the accumulation of cryptic genetic variation. Subsequent studies revealed a host of stabilizing systems that operate at all levels of biological organization that make phenotypes robust to genetic and environmental variation. Many of these resemble homeostatic mechanisms that don't require a stress shock but operate under normal physiological conditions and allow for the accumulation of large amounts of cryptic genetic variation. This cryptic genetic variation can be revealed by mutations or environmental factors that destabilize a homeostatic mechanism. Selection can then act on the phenotypic variants that are produced. This scenario corresponds to the modern phenotype-first hypothesis for the evolution of novel traits that was foreseen by Waddington as early as 1942.

Genetic assimilation is the process by which a novel or aberrant phenotype that was initially induced by an environmental variable can become genetically fixed in a population. The phenomenon was first described by C.H. Waddington some 70 years ago (Waddington, 1953). In the following decades this phenomenon was confirmed by others and research began to focus on discovering the mechanisms by which such a genetic adaptation could occur and the possible role of genetic assimilation in the evolution of phenotypic novelty in general. This subsequent research took several divergent paths. Among these were discoveries of the roles of Hsp90 and a variety of homeostatic mechanisms in regulatory networks play in stabilizing phenotypes. The understanding of these mechanisms led to the realization that genomes in natural populations harbor large amounts of cryptic genetic variation that accumulates over time, and that this genetic variation is the foundation for genetic assimilation and the rapid evolution of novel phenotypes. This commentary is a brief outline of the discovery of genetic assimilations and how the consequences of a simple experiment, even today, continue to expand our view of genetics, development and evolution.

Waddington subjected 21–23 h old pupae of a wild-type strain of *Drosophila melanogaster* to a 40°C heat shock for 4 h and found that some of the resulting adults had a crossveinless phenotype (Waddington,

1953). He bred flies with these phenotypes together and subjected the next generation to a similar heat shock, selecting again for those that expressed the crossveinless phenotype. After about 14 generations of selection some flies showed the crossveinless phenotype even when not exposed to the heat shock. Further selection resulted in a strain that constitutively expressed the crossveinless phenotype when kept at a constant 25°C. He referred to this result as the "genetic assimilation of an acquired character."

This purposely provocative phrase, did not imply any form of Lamarckism but drew attention to an idea he had been developing for over a decade and expressed clearly in a short opinion piece in *Nature* (Waddington, 1942) in which he lays out the general theses that (1) phenotypes are canalized (i.e. relatively insensitive to genetic and environmental perturbation), and (2) that genetic as well as environmental factors or disturbances can be equally effective at setting development on a different trajectory. For the latter he cites Richard Goldschmidt's idea of "phenocopy", referring to an environmentally-induced phenotype which resembles that of a known mutation (Goldschmidt, 1935). Waddington thus referred to his crossveinless phenotypes as phenocopies.

Waddington followed this 1953 study by assimilating the bithorax

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phenotype, using the same stress-response-selection protocol (Waddington, 1956). To demonstrate that genetic assimilation did not just fix aberrant or mutant phenotypes but could also fix ecologically relevant phenotypes, or adaptations, Waddington exposed larvae of *Drosophila* to near-lethal concentrations of sodium chloride and selected for best survivors. This selection was repeated for 21 generations, with increasing concentrations of salt each generation to maintain what he called a roughly constant level of selection (Waddington, 1959). The selected strains had developed much enlarged anal papillae and tolerated salt concentrations up to twice those of sea water. This result suggested that adaptive evolution could occur rapidly without requiring new (and rare) beneficial mutations.

An interest in the effect of environmental factors on phenotypic development long preceded Waddington's work. Particularly in Lepidopteran color pattern studies there is a long tradition of giving temperature shocks to larvae or pupae and finding that the aberrant color patterns produced often resembled those of different geographic races of the species, and occasionally those of closely related species (Merrifield, 1890, 1891; Nijhout, 1984; Serfas and Carroll, 2005; Mahdi et al., 2011).

Several biologists successfully replicated Waddington's assimilation studies (Bateman, 1959; Milkman, 1965; Mohler, 1965; Gibson and Hogness, 1996). In time, both developmental biologists and evolutionary biologists became interested in genetic assimilation. The former because of an interest in the molecular mechanisms underlying the heat-shock and stress responses, the latter because it provided a novel and largely unexplored mechanism for phenotypic evolution.

1. Molecular mechanisms

Molecular studies of the heat-shock and stress response led to the discovery of heat-shock proteins. Heat shock proteins were first identified as a class of proteins whose expression level increased dramatically almost immediately after heat shock was applied (Ritossa, 1962; Tissières et al., 1974). These proteins were subsequently shown to act as chaperones, which attached to other proteins to protect them from denaturation (Ellis and van der Vies, 1991; Santoro, 2000; Julio and Carlos, 2005).

A mechanism by which heat shock proteins could produce the diversity of phenotypes characteristic of the phenocopy response after heat stress was proposed by Mitchell and Lipps (1978). They suggested that heat shock proteins, acting as chaperones, stop development by virtue of their binding to proteins. Then, when the stressor is relieved, proteins gradually become active again and development resumes. But not all proteins become active at the same time, and as development resumes some proteins do not activate in time, and this results in a developmental defect. Some of these phenotypic defects resemble known mutations, presumably in a gene coding for a protein that did not re-activate soon enough to participate in normal development, hence a phenocopy.

A different role for heat shock proteins in producing phenocopies, that at the same time also enables genetic assimilation of a trait, was revealed by the work of Rutherford and Lindquist (1998). They found that mutations in Hsp90 in *Drosophila* produced a broad diversity of phenotypes. Hsp90 recognizes certain conformational defects or instabilities in proteins produced by mutations and binds to them, blocking their activity. Thus, protein fold variants that could result in a defective or altered function are masked by Hsp90. Mutations in Hsp90, or treatment with geldanamycin, which specifically blocks Hsp90 function, unmask these genetic variants and results in the development of phenotypic defects caused by these mutations. They proposed that Hsp90 effectively acts as a genetic capacitor, storing genetic variability that accumulates over time and that can be released by mutations or stressors that reduce or eliminates Hsp90 function. The accumulated mutations without phenotypic effect are called cryptic genetic variation. Rutherford and Lindquist did selection on several phenotypes produced by defective Hsp90 and were able to observe partial genetic

assimilations that fixed mutant phenotypes under normally-functioning Hsp90 (Rutherford and Lindquist, 1998). The kinds of phenocopies and other phenotypic effects produced by blocking Hsp90 activity depend on the genetic background and precise time in development when the stress-induced blockage occurs (Queitsch et al., 2002; Sangster et al., 2008; Zabinsky et al., 2019).

2. Robustness and cryptic genetic variation

Phenotypes have evolved to be robust to a remarkable diversity of genetic and environmental variation. In Waddington's days this was called *canalization*, which implies that a developmental trajectory becomes increasingly constrained and focused on a target phenotype. Today we call it *phenotypic robustness*, which generally refers only to the final product, not the process or pathway by which the stable phenotype arose. Genetic assimilation produces a canalized phenotype, but this does not imply that it is as robust as a naturally evolved and adapted phenotype, which requires the additional evolution of genes and mechanisms that eliminate deleterious side effects and that further refine and stabilize the phenotype against genetic and environmental perturbations.

Many different mechanisms have evolved that stabilize phenotypes, and they operate at all levels of biological organization from the molecular to the organismal. These include mechanisms that stabilize morphogenetic gradients (Eldar et al., 2002, 2004; Barkai and Shilo, 2009), feedback mechanisms in gene regulatory networks (Kwon and Cho, 2007; Macneil and Walhout, 2011; Arcuschin et al., 2023), allosteric regulatory mechanisms in enzymatic and metabolic systems including signaling pathways (Nijhout et al., 2004, 2018; Fritsche-Guenther et al., 2011; Blüthgen and Legewie, 2013; Radisavljevic, 2013; Li and Elowitz, 2019), and a host of homeostatic mechanisms in development, metabolism and physiology (Schmidt-Nielsen, 1997; Hill et al., 2022). Fig. 1 illustrates the general properties of a system regulated by stabilizing and homeostatic feedback processes. At low input (or some other causal value) there is a proportional response; at an intermediate range of inputs the response stays constant; and at a high input the response becomes proportional again. The intermediate flat region is the *homeostatic plateau* where variation in input strength (or cause) has no effect on the value of the response. This is not a steady-state plateau but is dynamically maintained by other reactions in the system that rise or fall to accommodate and neutralize the effects of varying input. Defects in those regulatory systems can narrow, or tilt, or abolish the plateau.

An example is given in Fig. 2, which plots genotype phenotype

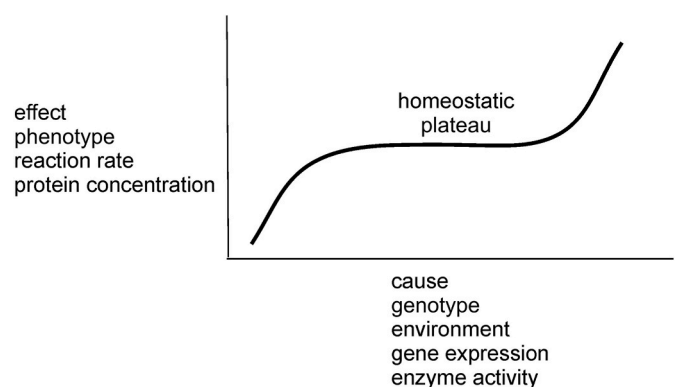


Fig. 1. Homeostatic plateau. The relationship between cause and effect in a generalized homeostatic system. The x-axis represents a causal variable and the y-axis a response variable. In a homeostatic system there is a region, the homeostatic plateau, in which a response variable is independent from the value of a causal variable. The value of the homeostatic plateau is often referred to as a “set point.” The homeostatic plateau is a system property and is dynamically maintained by other variables in the system that compensate for variation in the causal variable.

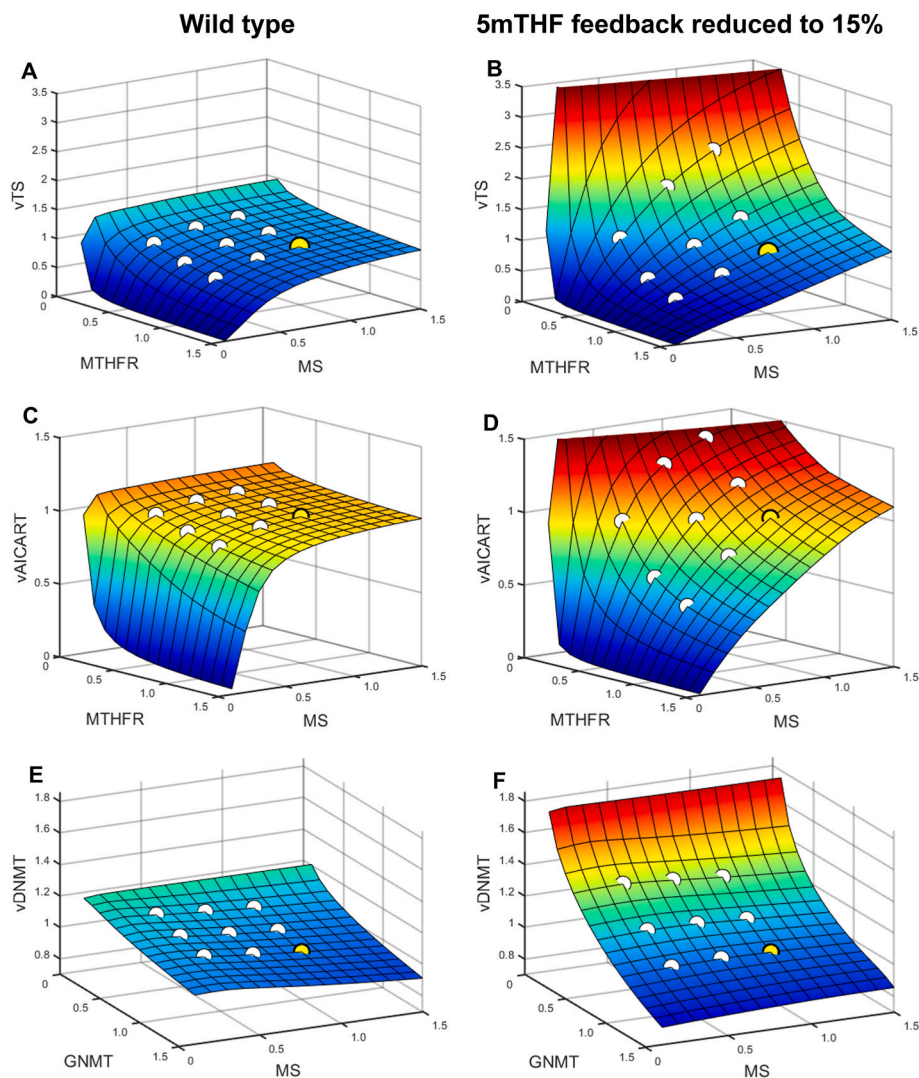


Fig. 2. Homeostasis in the OCM metabolic network. The surfaces are graphs of how a dependent variable, the z-axis, depends on variation in two causal variables (the x- and y-axes). The causal variables are activities of CBS, MS and MTHFR. Change along the activity axes could be due to mutation in the gene for the enzyme or in its cis or trans regulatory system, or it could occur downstream through changes in the allosteric regulation of enzyme activity, or by variation in essential cofactors, or variation in support systems like scaffolds that regulate activity of enzyme clusters. All axes are normalized to wild-type values, so wild-type = 1. The left column illustrates the “wild-type” landscape in which the phenotypes (the velocities of TS, the rate-limiting step in DNA synthesis, or AICART, an early step in purine biosynthesis) have a broad homeostatic plateau and are relatively insensitive to variation in the two enzymes. The right column illustrates the effect of a reduction in a regulatory feedback in the system, mediated by 5mTHF (Nijhout et al., 2006b). The result is that the phenotypes are no longer buffered against variation in the activity of the enzymes. Yellow dots show the position of the wild-type, white dots are at 70 % and 35 % of wild-type. (AICART, Phosphoribosylaminoimidazolecarboxamide formyltransferase; CBS, cystathionine- β -synthase; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; TS, thymidylate synthase, DNMT, DNA methyltransferase; 5mTHF, 5-methyltetrahydrofolate).

relations (Nijhout, 2008) in a fairly complex metabolic network: one carbon metabolism (OCM). This network contains the folate and methionine cycles, the rate limiting reaction for DNA synthesis (thymidylate synthase (TS), DNA methyl transferase (DNMT) that controls DNA methylation, and glutathione (GSH) biosynthesis (the main endogenous antioxidant). Failures in OCM are associated with birth defects (spina bifida, anencephaly), various cancers (colorectal, breast), cardiovascular disease, and psychiatric disorders. Because of its association with many and diverse diseases this network has been exceptionally well-studied (Wagner et al., 1985; Ma et al., 1999; Trinh et al., 2002; Curtin et al., 2004; Stover, 2004; Ulrich et al., 2005; Boyles et al., 2006; Levine et al., 2010; Kennedy et al., 2012; Nazki et al., 2014), and accurate mathematical simulation models have been developed (Nijhout et al., 2004, 2006a, 2009; Reed et al., 2006; Ulrich et al., 2008; Neuhouser et al., 2011). This network is stabilized by a large number of feedback mechanisms that ensure that critical reaction rates and

metabolite concentrations remain within a narrow range in spite of significant genetic and environmental variation (Nijhout et al., 2004, 2006b, 2008, 2014, 2018; Reed et al., 2010, 2015, 2017; Duncan et al., 2013, 2018; Nijhout and Reed, 2014).

Figs. 2 and 3 show phenotypic landscapes: graphs of the dependence of a variable in the OCM system (z axis) on the activity of two enzymes (x and y axes). This activity depends on both genetic and environmental factors that affect expression level of the gene for the enzyme, the presence of cofactors and allosteric regulators, and mutations that affect the efficacy of the enzyme. The location of wild-type activity is shown by the yellow dot. The white dots show activities that are 70 % and 35 % of wild-type. This is a common range of activities of mutations that are phenotypically wild-type but are also statistically associated with rare altered phenotypes in this system (Nijhout et al., 2017, 2018).

The relatively flat regions of the landscape, normal to the z-axis, are the regions where homeostatic mechanisms work to stabilize the

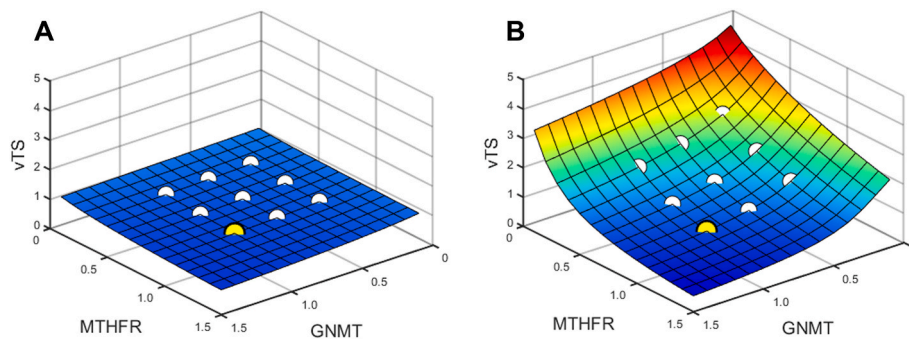


Fig. 3. Phenotypic landscape of the velocity of the TS reaction (the rate-limiting step in DNA synthesis) as a function of activity of MTHFR and GNMT. **A**, wild-type conditions. Yellow sphere indicated wild-type; white spheres are mutations that lower the activity of the enzymes to 70 % and 35 % of wild-type. **B**, vitamin B₁₂ deficiency (which lowers the activity of MS). A B₁₂ deficiency destabilizes the TS reaction and the effects of mutations that increase TS activity are enhanced. (GNMT, glycine-N-methyltransferase; MTHFR, methylenetetrahydrofolate reductase; TS, thymidylate synthase).

phenotype. These regions can be quite large and cover a large range of variation in gene/enzyme activities. These are the regions where mutational variation can accumulate without having an effect on the phenotype. In effect, cryptic genetic variation.

These homeostatic regions are the product of the many regulatory interactions in the system. Fig. 2 shows that reducing one of these (the allosteric effect of 5mTHF on a handful of enzymes) changes the level and slope of the landscape, so that previously phenotypically neutral genetic variants now have an effect on the phenotype.

These stabilizing mechanisms are not static or passive, but, like homeostatic mechanisms in physiology, they are dynamically active and adapt to changes in gene expression and environment. Thus, unlike Hsp90, which masks protein variants, the homeostatic robustness mechanisms keep the defective gene products active but make them irrelevant to the value of the phenotype, whose properties are regulated by a host of feedback and feedforward interactions within the regulatory network.

These diverse evolved mechanisms stabilize phenotypes and make them robust to genetic and environmental variation. Homeostatic mechanisms are dynamic entities, that control numerous processes that ensure that a target phenotype reaches a particular setpoint. These homeostatic properties are probably what gives development and regeneration the appearance of goal-directedness and help explain the valleys in Waddington's epigenetic landscape metaphor (Waddington, 1957).

Robustness of a particular phenotype dissociates that phenotype from variation in the genes that contribute to its function and development. Because natural selection only acts on phenotypes, mutations in those genes will not be visible to natural selection and will therefore accumulate over time as cryptic genetic variation.

When this cryptic genetic variation is revealed by a genetic or environmental factor that destabilizes a robustness mechanism it can lead to the evolution of novel phenotypes as well as the evolution of alternative control mechanisms. It is possible also that cryptic genetic variation enables developmental systems drift: changes in, and divergence of, morphogenetic and regulatory mechanisms of homologous characters over evolutionary time (True and Haag, 2001).

As we will see in the sections that follow, although phenotypic robustness and the associated accumulation of phenotypically neutral cryptic genetic variation have enabled genetic assimilation, the route is via phenotypic plasticity. Phenotypic robustness and plasticity may at first glance seem to be opposite and even contradictory biological phenomena, they easily and commonly coexist for the simple reason that robustness is never perfect and often relies on dynamic regulatory processes that can evolve, as in the case of developmental systems drift, and can also be affected by environmental factors. Moreover, alternative environmentally-induced plastic phenotypes can become stabilized and robust, as in the case of polyphenisms.

3. Phenotypic plasticity and robustness

Today, Waddington's phenocopies occupy but a small corner in what has grown to be the much broader field of *phenotypic plasticity* that developed during the half century following Waddington's seminal work. Broadly defined, phenotypic plasticity is any change in phenotype that is caused by an environmental variable. Despite a general robustness to environmental variation, most traits exhibit plasticity to various degrees. Phenocopies are at one extreme of the plasticity spectrum, and are generally thought of as large, abrupt, and often pathological non-adaptive phenotypic changes due to an environmental stress. Early research on phenotypic plasticity identified so called "norms of reaction" which are gradual and systematic changes in a phenotype that are due to systematic changes in an environmental variable such as temperature or nutrition (Schlichting and Pigliucci, 1998). Reaction norms have been of interest in evolutionary ecology because the phenotypes are not pathological but are viable alterations in phenotypes that have the potential of being adaptive in certain environments (Pigliucci et al., 2006; Schlichting, 2008).

A common form of reaction norm is *allometry*, the changes in the relative sizes of body parts with changes in overall body size. Body size and shape change during development, and those relationships among parts are described as ontogenetic allometries. The final adult body sizes and shapes of most species can vary considerably, producing so-called static allometries (Bertalanffy and Pirozynsky, 1952; Cheverud, 1982; Gayon, 2000; Shingleton et al., 2007; Pelabon et al., 2013). The developmental causes of allometry have been studied in the context of differences in the relative growth of body parts, or of the different components or dimensions of a body part such as appendages, the head, and internal organs (Huxley, 1932, 1950; Miller and German, 1999; Reichling and German, 2000; Shingleton and Frankino, 2018). Some allometries are adaptations, such as the disproportionate increase of rhinoceros beetle horns with body size (Emlen and Nijhout, 2000, 2001; Moczek et al., 2002; Lavine et al., 2015; McCullough et al., 2015), or the disproportionate increase of head size with body size in soldier ants (Wheeler, 1991; Urbani and Passera, 1996; Rajakumar et al., 2018; Nijhout, 2019).

Reaction norms have both genetic and environmental components in the sense that genes affect the degree of sensitivity to the environmental gradient and the type of phenotypic responses that are produced (Schlichting and Pigliucci, 1998; Oostra et al., 2010; Ergon and Ergon, 2017). The evolution of reaction norms would then be due to evolution of the genetic processes that mediate between the environment and the phenotypes. An example can be visualized in Fig. 3. The enzyme methionine synthase (MS) requires vitamin B₁₂ (cobalamin) as an essential cofactor. Reduced B₁₂ levels lower the activity of the enzyme and have the same effect as mutations that lower its expression or enzymatic activity. Vitamin B₁₂ is an environmental factor and must be

obtained from food or the microbiome. Thus the effect of variation along the MS axis in Fig. 2 can also be interpreted as a reaction norm to vitamin B₁₂ levels.

4. Polyphenisms: Combining plasticity and robustness

Reaction norms that have 2 different adaptive phenotypes in 2 different environments may have led to the evolution of polyphenism. Polyphenism are widespread in animals and plants and are manifest as discrete alternative phenotypes that develop when exposed to different environmental stimuli. The most widespread are seasonal polyphenisms, in which animals develop different phenotypes in different season of the year. Mating plumages in male birds and antlers in deer are examples of seasonal polyphenisms in vertebrates. In insects, which typically have life cycles that are shorter than a year, the generations that develop in one season can look dramatically different from those that develop in another. Sometimes the phenotypes are so different that they were initially described as different species (Nijhout, 1991, 2003). Castes in social insects (workers, soldiers, queens) are polyphenisms, as are the winged and wingless forms of many Homoptera such as aphids and planthoppers (Braendle et al., 2006; Hardie, 2010; Xu et al., 2015; Lin et al., 2016; Vellichirammal et al., 2017). Metamorphosis can be thought of as a sequential polyphenism, with highly adapted canalized larval phenotypes that can differ in profound ways from the canalized adult phenotypes (e.g. maggot and fly, caterpillar and butterfly, tadpole and frog) in which an environmental signal such as nutrition or photoperiod triggers an endocrine cascade that leads to metamorphosis: the developmental switch from one adaptive phenotype to another.

Polyphenisms exemplify a coexistence between phenotypic plasticity and robustness. The ability to develop alternative phenotypes in response to different environmental signals is a plastic response, but the alternative phenotypes are highly canalized and robust.

The link between the environmental signal and the initiation of alternative developmental pathways in polyphenisms is mediated by hormones. In general, the brain perceives the relevant environmental signal such as temperature, photoperiod, nutrition, crowding or pheromones, perceived by the relevant sense organs. This information is integrated by the brain over time during a prolonged sensitive period to ensure that the signal is consistent (Smith, 1991; Rountree and Nijhout, 1995; Kooi and Brakefield, 1999). The integrated information then alters the pattern of hormone secretion via the neuroendocrine system of the brain. In insects the terminal hormones are 20-hydroxyecdysone (20E) and juvenile hormone (JH). Both these hormones bind to nuclear receptors and control gene transcription. The physiological control of polyphenic development by hormones occurs during relatively brief and tissue-specific hormone-sensitive-periods, when receptors for the hormones are present (Nijhout, 2003). The exact pathway by which the hormone-stimulated pattern of gene expression causes a switch to an alternative developmental pathway is, at present, not well understood, but significant progress is being made in unraveling the molecular-genetic underpinnings of a polyphenic developmental switch in the nematode, *Pristionchus pacificus* (Wighard and Sommer, 2024), which may serve as a model for other systems as well.

5. Genetic assimilation and plasticity

As foreseen by Baldwin (Baldwin, 1896, 1902), genetic assimilation cannot only canalize and fix a new phenotype, it can also stabilize phenotypic plasticity and produce a polyphenism. The moth, *Manduca quinquemaculata*, has a green/black larval polyphenism, with black larvae developing in the autumn, and green larvae at other times. *Manduca sexta*, by contrast, only produces green larvae, independent of environmental conditions. There is, however, a recessive mutation that makes *M. sexta* larvae black in the last larval instar (Safrañek and Rid-diford, 1975). The black color is due to a lower level of JH during the molt to the last larval instar, and the wild-type green phenotype can be

rescued by simple treatment with JH (Truman et al., 1973). When the black mutant strain was heat-shocked during a brief sensitive period of the fourth larval instar, the fifth instar developed a range of color phenotypes from normal pure black to nearly wild-type green. Selecting the greenest and blackest larvae separately and repeating the experiment we were able to produce, after 13 generations, one strain that was green at temperatures above 28.5 °C, and black at lower temperatures, plus a strain that was black at all temperatures (Suzuki and Nijhout, 2006, 2008). This was done without using temperature shock or other kinds of stress, but simply by exploring a phenotypic plasticity that was revealed by the enabling black-larva mutation. Selection thus assimilated a canalized black phenotype and a plastic, temperature-dependent polyphenism.

6. Consequences of plasticity and robustness for phenotypic evolution

The most remarkable thing about genetic assimilation is how fast it can occur. A new phenotype can become fixed after only a dozen or so generations of selection. The rapidity of the response to selection, and the fact that the response is gradualistic, makes it unlikely that the fixation of a new phenotype involved new mutations. This in turn implies that the new phenotype arose by reconfiguring existing developmental and regulatory networks, using existing genetic variation.

Plasticity and robustness both degrade the correlation between genetic variation and phenotypic variation. In robust systems mutations can accumulate on a homeostatic plateau (as in Figs. 2 and 3) as cryptic genetic variation. In plastic systems environmental effects override genetic effects on the phenotype and likewise allow for the accumulation of selectively neutral mutations.

Populations of animals and plants must therefore harbor huge amounts of cryptic genetic variation that has been accumulating for eons. Really bad mutations that cannot be masked by homeostasis and other robustness mechanisms will obviously be eliminated by natural selection, but the remainder will persist, masked from selection, until an event, a mutation or an environmental shift, somehow disrupts a phenotypic stabilizing mechanism so that now some of the cryptic variants can have an effect, altering the phenotype.

This insight has significant implications for our understanding of how phenotypic evolution occurs. Although this insight is relatively recent, it was actually foreseen, or at least hypothesized, by Waddington in his 1942 opinion piece cited above (Waddington, 1942).

It is worth quoting the concluding paragraph at length, to illustrate how insightful and prescient it actually was, although it took most of our community almost 60 years to catch up with this idea.

“Summarizing, then, we may say that the occurrence of an adaptive response to an environmental stimulus depends on the selection of a suitable genetically controlled reactivity in the organism. If it is an advantage, as it usually seems to be for developmental mechanisms, that the response should attain an optimum value more or less independently of the intensity of stimulus received by a particular animal, then the reactivity will become canalized, again under the influence of natural selection. Once the developmental path has been canalized, it is to be expected that many different agents, including number of mutations available in the germplasm of the species, will be able to switch development into it; and the same considerations which render the canalization advantageous will favour the super-session of the environmental stimulus by a genetic one. By such a series of steps, then, it is possible that an adaptive response can be fixed without waiting for the occurrence of a mutation which, in the original genetic background, mimics the response well enough to enjoy a selective advantage.”

This idea is echoed by what today we call *phenotype-first* evolution that stands in contrast to the commonly held view of mutation-first evolution of new traits. The standard model is that new traits arise by

a fortuitous, and rare, mutation that produces a trait that improves fitness. That mutation has to be dominant, or the phenotype would disappear in the next generation. This is followed by the accumulation of equally rare mutations that then gradually improve and refine the new phenotype. How often such rare and favorable mutations occur is anyone's guess. Evolutionary biologists who model the evolution of new traits typically start with the assumption that such a mutation already exists and model the mechanisms by which it spreads through a population.

Phenotype-first evolution poses that new phenotypes arise from existing cryptic genetic variation when robustness mechanisms are disrupted. This disruption can be through a mutation in some part of a mechanism that stabilizes phenotypes, or an environmental change that alters the dynamics of a robustness mechanism. In the latter case, if the altered phenotype improves fitness given the environmental change, and if the environmental factor persists for generations, then there will be selection to stabilize and improve that new phenotype in the new environment (West-Eberhard, 2003). Basically, this is genetic assimilation. If we also include the assimilation of plasticity in polyphenism, and the further elaboration, refinement, elimination of deleterious side-effects and stabilization of the trait, then it is called *genetic accommodation* (West-Eberhard, 2003, 2005). Laboratory experiments have shown that the evolution of a new stable phenotype can occur extraordinarily rapidly, requiring only a dozen or so generations of strong selection, and no new mutations.

7. Afterword

Genetic assimilation of an environmentally induced novel trait depends on cryptic genetic variation. Genetic assimilation does not require the induction of a phenocopy by a sub-lethal environmental stressor. Gentler disturbances such as a macro- and micronutrient deficiencies, plant secondary substances (and other toxins in food), temperature gradients and endocrine disruptors, are only a few of the kinds of environmental variables that can alter the effectiveness of a robustness feedback mechanism, release cryptic genetic variation, and result in phenotypic variation. Based on the rapidity with which a new trait can become fixed by artificial selection, it appears that new mutations are not required. New mutations can play a role, however, if they destabilize a homeostatic mechanism.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

References

- Arcuschin, C.D., Pinkasz, M., Schor, I.E., 2023. Mechanisms of robustness in gene regulatory networks involved in neural development. *Front. Mol. Neurosci.* 16, 1114015.
- Baldwin, J.M., 1896. A new factor in evolution. *Am. Nat.* 30, 536–553.
- Baldwin, J.M., 1902. *Development and Evolution*. Macmillan Co, New York.
- Barkai, N., Shilo, B.-Z., 2009. Robust generation and decoding of morphogen gradients. *Cold Spring Harbor Perspect. Biol.* 1.
- Bateman, K.G., 1959. The genetic assimilation of four venation phenocopies. *J. Genet.* 56, 443–474.
- Bertalanffy, L.V., Pirozynsky, W.J., 1952. Ontogenetic and evolutionary allometry. *Evolution* 6, 387–392.
- Blüthgen, N., Legewie, S., 2013. Robustness of signal transduction pathways. *Cell. Mol. Life Sci.* 70, 2259–2269.
- Boyles, A.L., Billups, A.V., Deak, K.L., Siegel, D.G., Mehlretter, L., Slifer, S.H., Bassuk, A. G., Kessler, J.A., Reed, M.C., Nijhout, H.F., George, T.M., Enterline, D.S., Gilbert, J. R., Speer, M.C., 2006. Neural tube defects and folate pathway genes: family-based association tests of gene–gene and gene–environment interactions. *Environ. Health Perspect.* 114, 1547–1552.
- Braendle, C., Davis, G.K., Brisson, J.A., Stern, D.L., 2006. Wing dimorphism in aphids. *Heredity* 97, 192–199.
- Cheverud, J.M., 1982. Relationships among ontogenetic, static, and evolutionary allometry. *Am. J. Phys. Anthropol.* 59, 139–149.
- Curtin, K., Bigler, J., Slatery, M.L., Caan, B., Potter, J.D., Ulrich, C.M., 2004. Mthfr c677T and a1298C polymorphisms. Diet, estrogen, and risk of colon cancer. *Diet Estrogen Risk Colon Cancer* 13, 285–292.
- Duncan, T.M., Reed, M.C., Nijhout, H.F., 2013. A population model of folate-mediated one-carbon metabolism. *Nutrients* 5, 2457–2474.
- Duncan, W., Best, J., Golubitsky, M., Nijhout, H.F., Reed, M., 2018. Homeostasis despite instability. *Math. Biosci.* 300, 130–137.
- Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B.-Z., Barkai, N., 2002. Robustness of the bmp morphogen gradient in drosophila embryonic patterning. *Nature* 419, 304–308.
- Eldar, A., Shilo, B.-Z., Barkai, N., 2004. Elucidating mechanisms underlying robustness of morphogen gradients. *Curr. Opin. Genet. Dev.* 14, 435–439.
- Ellis, R.J., van der Vies, S.M., 1991. Molecular chaperones. *Annu. Rev. Biochem.* 60, 321–347.
- Emlen, D.J., Nijhout, H.F., 2000. The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.* 5, 661–708.
- Emlen, D.J., Nijhout, H.F., 2001. Hormonal control of male horn length dimorphism in onthophagus taurus (coleoptera: scarabaeidae): a second critical period of sensitivity to juvenile hormone. *J. Insect Physiol.* 47, 1045–1054.
- Ergon, T., Ergon, R., 2017. When three traits make a line: evolution of phenotypic plasticity and genetic assimilation through linear reaction norms in stochastic environments. *J. Evol. Biol.* 30, 486–500.
- Fritsche-Guenther, R., Witzel, F., Sieber, A., Herr, R., Schmidt, N., Braun, S., Brummer, T., Sers, C., Blüthgen, N., 2011. Strong negative feedback from erk to raf confers robustness to mapk signalling. *Mol. Syst. Biol.* 7, 489.
- Gayon, J., 2000. History of the concept of allometry. *Am. Zool.* 40, 748–758.
- Gibson, G., Hogness, D.S., 1996. Effect of polymorphism in the *drosophila* regulatory gene *ultrabithorax* on homeotic stability. *Science* 271, 200–203.
- Goldschmidt, R.B., 1935. Gen und ausseneigenschaft. (untersuchungen an *drosophila*) Z. indukt. Abstamm.- u. VererbLehre, vol. 69, pp. 38–69.
- Hardie, J., 2010. Photoperiodism in insects: aphid polyphenism. In: Nelson, R., Denlinger, D., Somers, D. (Eds.), *Photoperiodism. The Biological Calendar*. Oxford University Press, Oxford, pp. 342–363.
- Hill, R.W., Cavanaugh, D.J., Anderson, M., 2022. *Animal Physiology, fifth ed.* Sinauer, Sunderland.
- Huxley, J.S., 1932. *Problems of Relative Growth*. Methuen, London.
- Huxley, J.S., 1950. Relative growth and form transformation. *Proc. Roy. Soc. Lond. B Biol. Sci.* 137, 465–469.
- Julio, C.B., Carlos, H.I.R., 2005. Protein folding assisted by chaperones. *Protein Pept. Lett.* 12, 257–261.
- Kennedy, D.A., Stern, S.J., Matok, I., Moretti, M.E., Sarkar, M., Adams-Webber, T., Koren, G., 2012. Folate intake, mthfr polymorphisms, and the risk of colorectal cancer: a systematic review and meta-analysis. *J. Cancer Epidemiol.* 2012, 24.
- Kooi, R.E., Brakefield, P.M., 1999. The critical period for wing pattern induction in the polyphenic tropical butterfly *bicyclus anynana* (satyrinae). *J. Insect Physiol.* 45, 201–212.
- Kwon, Y.-K., Cho, K.-H., 2007. Analysis of feedback loops and robustness in network evolution based on boolean models. *BMC Bioinf.* 8, 430.
- Lavine, L.C., Gotoh, H., Brent, C.S., Dworkin, I., Emlen, D.J., 2015. Exaggerated trait growth in insects. *Annu. Rev. Entomol.* 60, 453–472.
- Levine, A.J., Figueiredo, J.C., Lee, W., Conti, D.V., Kennedy, K., Duggan, D.J., Poynter, J. N., Campbell, P.T., Newcomb, P., Martinez, M.E., Hopper, J.L., Le Marchand, L., Baron, J.A., Limburg, P.J., Ulrich, C.M., Haile, R.W., 2010. A candidate gene study of folate-associated one carbon metabolism genes and colorectal cancer risk. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Soc. Preventive Oncol.* 19, 1812–1821.
- Li, P., Elowitz, M.B., 2019. Communication codes in developmental signaling pathways. *Development* 146.
- Lin, X., Xu, Y., Yao, Y., Wang, B., Lavine, M.D., Lavine, L.C., 2016. Jnk signaling mediates wing form polymorphism in brown planthoppers (*nilaparvata lugens*). *Insect Biochem. Mol. Biol.* 73, 55–61.
- Ma, J., Stampfer, M.J., Christensen, B., Giovannucci, E., Hunter, D.J., Chen, J., Willett, W.C., Selhub, J., Hennekens, C.H., Gravel, R., Rozen, R., 1999. A polymorphism of the methionine synthase gene: Association with plasma folate, vitamin b12, homocyst(e)ine, and colorectal cancer risk. *Cancer Epidemiol. Biomark. Prev.* 8, 825–829.
- Macneil, L.T., Walhout, A.J., 2011. Gene regulatory networks and the role of robustness and stochasticity in the control of gene expression. *Genome Res.* 21, 645–657.

- Mahdi, S.H.A., Yamasaki, H., Otaki, J.M., 2011. Heat-shock-induced color-pattern changes of the blue pansy butterfly *Junonia orithya*: physiological and evolutionary implications. *J. Therm. Biol.* 36, 312–321.
- McCullough, E.L., Ledger, K.J., O'Brien, D.M., Emlen, D.J., 2015. Variation in the allometry of exaggerated rhinoceros beetle horns. *Anim. Behav.* 109, 133–140.
- Merrifield, F., 1890. Systematic temperature experiments on some lepidoptera in all their stages. *Trans. Ethnol. Soc. Lond.* 38, 131–159.
- Merrifield, F., 1891. Conspicuous effects on the markings and colouring of lepidoptera caused by exposure of pupae to different temperature conditions. *Trans. Ethnol. Soc. Lond.* 39, 155–168.
- Milkman, R.D., 1965. The genetic basis of natural variation. VI. Selection of a crossveinless strain of *Drosophila* by phenocopying at high temperature. *Genetics* 51, 87–96.
- Miller, J.P., German, R.Z., 1999. Protein malnutrition affects the growth trajectories of the craniofacial skeleton in rats. *J. Nutr.* 129, 2061–2069.
- Mitchell, H.K., Lipps, L.S., 1978. Heat shock and phenocopy induction in *Drosophila*. *Cell* 15, 907–918.
- Moczek, A.P., Hunt, J., Emlen, D.J., Simmons, L.W., 2002. Threshold evolution in exotic populations of a polyphenic beetle. *Evol. Ecol. Res.* 4, 587–601.
- Mohler, J.D., 1965. The influence of some crossveinless-like genes on the crossveinless phenocopy sensitivity in *Drosophila melanogaster*. *Genetics* 51, 329–340.
- Nazki, F.H., Sameer, A.S., Ganaie, B.A., 2014. Folate: metabolism, genes, polymorphisms and the associated diseases. *Gene* 533, 11–20.
- Neuhouser, M.L., Nijhout, H.F., Gregory, J.F., Reed, M.C., James, S.J., Liu, A., Shane, B., Ulrich, C.M., 2011. Mathematical modeling predicts the effect of folate deficiency and excess on cancer-related biomarkers. *Cancer Epidemiol. Biomark. Prev.* 20, 1912–1917.
- Nijhout, H.F., 1984. Colour pattern modification by coldshock in lepidoptera. *Development* 81, 287–305.
- Nijhout, H.F., 1991. *The Development and Evolution of Butterfly Wing Patterns*. Smithsonian Institution Press, Washington, DC.
- Nijhout, H.F., 2003. Development and evolution of adaptive polyphenisms. *Evol. Dev.* 5, 9–18.
- Nijhout, H.F., 2008. Developmental phenotypic landscapes. *Evol. Biol.* 35, 100–103.
- Nijhout, H.F., 2019. Larval development: making ants into soldiers. *Curr. Biol.* 29, R32–R34.
- Nijhout, H.F., Best, J., Reed, M.C., 2014. Escape from homeostasis. *Math. Biosci.* 257.
- Nijhout, H.F., Best, J.A., Reed, M.C., 2018. Systems biology of robustness and homeostatic mechanisms. *Wiley Interdiscip. Rev.: Sys. Biol. Med.*, e1440.
- Nijhout, H.F., Gregory, J.F., Fitzpatrick, C., Cho, E., Lamers, K.Y., Ulrich, C.M., Reed, M.C., 2009. A mathematical model gives insights into the effects of vitamin B6 deficiency on 1-carbon and glutathione metabolism. *J. Nutr.* 139, 784–791.
- Nijhout, H.F., Reed, M., Lam, S.-L., Shane, B., Gregory, J., Ulrich, C., 2006a. In silico experimentation with a model of hepatic mitochondrial folate metabolism. *Theor. Biol. Med. Model.* 3, 40.
- Nijhout, H.F., Reed, M.C., 2014. Homeostasis and dynamic stability of the phenotype link robustness and plasticity. *Integr. Comp. Biol.* 54, 264–275.
- Nijhout, H.F., Reed, M.C., Anderson, D.F., Mattingly, J.C., James, S.J., Ulrich, C.M., 2006b. Long-range allosteric interactions between the folate and methionine cycles stabilize DNA methylation reaction rate. *Epigenetics* 1, 81–87.
- Nijhout, H.F., Reed, M.C., Budu, P., Ulrich, C.M., 2004. A mathematical model of the folate cycle: new insights into folate homeostasis. *J. Biol. Chem.* 279.
- Nijhout, H.F., Reed, M.C., Ulrich, C.M., 2008. A day in the life of cell metabolism. *Biol. Theor.* 2.
- Nijhout, H.F., Sadre-Marandi, F., Best, J., Reed, M.C., 2017. Systems biology of phenotypic robustness and plasticity. *Integr. Comp. Biol.* 57, 171–184.
- Oostra, V., de Jong, M.A., Invergo, B.M., Kesbeke, F., Wende, F., Brakefield, P.M., Zwaan, B.J., 2010. Translating environmental gradients into discontinuous reaction norms via hormone signalling in a polyphenic butterfly. *Proc. Biol. Sci.*
- Pelabon, C., Bolstad, G.H., Egset, C.K., Cheverud, J.M., Pavlicev, M., Rosenqvist, G., 2013. On the relationship between ontogenetic and static allometry. *Am. Nat.* (advance online publication).
- Pigliucci, M., Murren, C.J., Schlichting, C.D., 2006. Phenotypic plasticity and evolution by genetic assimilation. *J. Exp. Biol.* 209, 2362–2367.
- Queitsch, C., Sangster, T.A., Lindquist, S., 2002. Hsp90 as a capacitor of phenotypic variation. *Nature* 417, 618–624.
- Radisavljevic, Z., 2013. Akt as locus of cancer positive feedback loops and extreme robustness. *J. Cell. Physiol.* 228, 522–524.
- Rajakumar, R., Koch, S., Couture, M., Favé, M.-J., Lilloco-Ouachour, A., Chen, T., De Blasis, G., Rajakumar, A., Ouellette, D., Abouheif, E., 2018. Social regulation of a rudimentary organ generates complex worker-caste systems in ants. *Nature* 562, 574–577.
- Reed, M., Best, J., Golubitsky, M., Stewart, I., Nijhout, H., 2017. Analysis of homeostatic mechanisms in biochemical networks. *Bull. Math. Biol.* 79, 2534–2557.
- Reed, M.C., Gamble, M.V., Hall, M.N., Nijhout, H.F., 2015. Mathematical analysis of the regulation of competing methyltransferases. *BMC Syst. Biol.* 9, 69.
- Reed, M.C., Lieb, A., Nijhout, H.F., 2010. The biological significance of substrate inhibition: a mechanism with diverse functions. *Bioessays* 32, 422–429.
- Reed, M.C., Nijhout, H.F., Neuhouser, M.L., Gregory, J.F., Shane, B., James, S.J., Boynton, A., Ulrich, C.M., 2006. A mathematical model gives insights into nutritional and genetic aspects of folate-mediated one-carbon metabolism. *J. Nutr.* 136, 2653–2661.
- Reichling, T.D., German, R.Z., 2000. Bones, muscles and visceral organs of protein-malnourished rats (*Rattus norvegicus*) grow more slowly but for longer durations to reach normal final size. *J. Nutr.* 130, 2326–2332.
- Ritossa, F., 1962. A new puffing pattern induced by temperature shock and dnp in *Drosophila*. *Experientia* 18, 571–573.
- Rountree, D.B., Nijhout, H.F., 1995. Hormonal control of a seasonal polyphenism in *Precis coenia* (Lepidoptera: nymphalidae). *J. Insect Physiol.* 41, 987–992.
- Rutherford, S.L., Lindquist, S., 1998. Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336.
- Safranek, L., Riddiford, L.M., 1975. The biology of the black larval mutant of the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* 21, 1931–1938.
- Sangster, T.A., Salathia, N., Undurraga, S., Milo, R., Schellenberg, K., Lindquist, S., Queitsch, C., 2008. Hsp90 affects the expression of genetic variation and developmental stability in quantitative traits. *Proc. Natl. Acad. Sci. USA.* 105, 2963–2968.
- Santoro, M.G., 2000. Heat shock factors and the control of the stress response. *Biochem. Pharmacol.* 59, 55–63.
- Schlichting, C.D., 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. *Ann. N. Y. Acad. Sci.* 1133, 187–203.
- Schlichting, C.D., Pigliucci, M., 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Inc., Sunderland, MA.
- Schmidt-Nielsen, K., 1997. *Animal Physiology, fifth ed.* Cambridge University Press, Cambridge.
- Serfas, M.S., Carroll, S.B., 2005. Pharmacologic approaches to butterfly wing patterning: sulfated polysaccharides mimic or antagonize cold shock and alter the interpretation of gradients of positional information. *Dev. Biol.* 287, 416–424.
- Shingleton, A.W., Frankino, W.A., 2018. The (ongoing) problem of relative growth. *Curr. Opin. Insect Sci.* 25, 9–19.
- Shingleton, A.W., Frankino, W.A., Flatt, T., Nijhout, H.F., Emlen, D.J., 2007. Size and shape: the developmental regulation of static allometry in insects. *Bioessays* 29, 536–548.
- Smith, K., 1991. The effects of temperature and daylength on the rosa polyphenism in the buckeye butterfly, *Precis coenia* (Lepidoptera: nymphalidae). *J. Res. Lepid.* 30, 237–244.
- Stover, P.J., 2004. Physiology of folate and vitamin B12 in health and disease. *Nutr. Rev.* 62, S3–S12.
- Suzuki, Y., Nijhout, H.F., 2006. Evolution of a polyphenism by genetic accommodation. *Science* 311, 650–652.
- Suzuki, Y., Nijhout, H.F., 2008. Genetic basis of adaptive evolution of a polyphenism by genetic accommodation. *J. Evol. Biol.* 21, 57–66.
- Tissières, A., Mitchell, H.K., Tracy, U.M., 1974. Protein synthesis in salivary glands of *Drosophila melanogaster*: relation to chromosome puffs. *J. Mol. Biol.* 84, 389–398.
- Trinh, B., Ong, C.-N., Coetzee, G., Yu, M., Laird, P., 2002. Thymidylate synthase: a novel genetic determinant of plasma homocysteine and folate levels. *Hum. Genet.* 111, 299–302.
- True, J.R., Haag, E.S., 2001. Developmental system drift and flexibility in evolutionary trajectories. *Evol. Dev.* 3, 109–119.
- Truman, J.W., Riddiford, L.M., Safranek, L., 1973. Hormonal control of cuticle coloration in the tobacco hornworm, *Manduca sexta*: basis of an ultrasensitive bioassay for juvenile hormone. *J. Insect Physiol.* 19, 195–203.
- Ulrich, C.M., Curtin, K., Potter, J.D., Bigler, J., Caan, B., Slattery, M.L., 2005. Polymorphisms in the reduced folate carrier, thymidylate synthase, or methionine synthase and risk of colon cancer. *Cancer Epidemiol. Biomark. Prev.* 14.
- Ulrich, C.M., Neuhouser, M., Liu, A.Y., Boynton, A., Gregory, J.F., Shane, B., James, S.J., Reed, M.C., Nijhout, H.F., 2008. Mathematical modeling of folate metabolism: predicted effects of genetic polymorphisms on mechanisms and biomarkers relevant to carcinogenesis. *Cancer Epidemiol. Biomark. Prev.* 17, 1822–1831.
- Urbani, C.B., Passera, L., 1996. Origin of ant soldiers. *Nature* 383, 223.
- Vellichrammal, N.N., Gupta, P., Hall, T.A., Brisson, J.A., 2017. Ecdysone signaling underlies the pea aphid transgenerational wing polyphenism. *Proc. Natl. Acad. Sci.* 114, 1419–1423.
- Waddington, C.H., 1942. Canalization of development and the inheritance of acquired characters. *Nature* 150, 563–565.
- Waddington, C.H., 1953. Genetic assimilation of an acquired character. *Evolution* 7, 118–126.
- Waddington, C.H., 1956. Genetic assimilation of the bithorax phenotype. *Evolution* 10, 1–13.
- Waddington, C.H., 1957. *The Strategy of the Genes*. Routledge, London.
- Waddington, C.H., 1959. Canalization of development and genetic assimilation of acquired characters. *Nature* 183, 1654–1655.
- Wagner, C., Briggs, W.T., Cook, R.J., 1985. Inhibition of glycine n-methyltransferase activity by folate derivatives: implications for regulation of methyl group metabolism. *Biochem. Biophys. Res. Commun.* 127, 746–752.
- West-Eberhard, M.J., 2003. *Developmental Plasticity and Evolution*. Oxford University Press, New York.
- West-Eberhard, M.J., 2005. Phenotypic accommodation: adaptive innovation due to developmental plasticity. *J. Exp. Zool. B Mol. Dev. Evol.* 304B, 610–618.
- Wheeler, D.E., 1991. The developmental basis of worker caste polymorphism in ants. *Am. Nat.* 138, 1218–1238.
- Wighard, S., Sommer, R.J., 2024. The role of epigenetic switches in polyphenism control: implications from a nematode model for the developmental regulation of alternative phenotypes. *Biology* 13, 922.
- Xu, H.-J., Xue, J., Lu, B., Zhang, X.-C., Zhuo, J.-C., He, S.-F., Ma, X.-F., Jiang, Y.-Q., Fan, H.-W., Xu, J.-Y., Ye, Y.-X., Pan, P.-L., Li, Q., Bao, Y.-Y., Nijhout, H.F., Zhang, C.-X., 2015. Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* 519, 464–467.
- Zabinsky, R.A., Mason, G.A., Queitsch, C., Jarosz, D.F., 2019. It's not magic – hsp90 and its effects on genetic and epigenetic variation. *Semin. Cell Dev. Biol.* 88, 21–35.