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Evolutionary physiology at 30+: Has the promise been fulfilled?

Advances in Evolutionary Physiology

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Abstract

Three decades ago, interactions between evolutionary biology and physiology gave rise to evolutionary physiology. This caused comparative physiologists to improve their research methods by incorporating evolutionary thinking. Simultaneously, evolutionary biologists began focusing more on physiological mechanisms that may help to explain constraints on and trade-offs during microevolutionary processes, as well as macroevolutionary patterns in physiological diversity. Here we argue that evolutionary physiology has yet to reach its full potential, and propose new avenues that may lead to unexpected advances. Viewing physiological adaptations in wild animals as potential solutions to human diseases offers enormous possibilities for biomedicine. New evidence of epigenetic modifications as mechanisms of phenotypic plasticity that regulate physiological traits may also arise in coming years, which may also represent an overlooked enhancer of adaptation via natural selection to explain physiological evolution. Synergistic interactions at these intersections and other areas will lead to a novel understanding of organismal biology.

KEYWORDS

adaptation, comparative method, cross-organism integration, epigenetic inheritance, evolutionary medicine, non-model species, physiological performance

INTRODUCTION

Since the first appearances of an identifiable field of evolutionary physiology more than three decades ago,[1,2] both evolutionary biology and physiology have benefited. Evolutionary biology has provided physiology with such tools as phylogenetic analyses,[3] selection experiments,[4] and genetic/genomic analyses (e.g.,[5]). At the same time, physiology and biochemistry have enhanced knowledge of the functional mechanisms that underlie various evolutionary processes and phenomena, including epigenetic inheritance, adaptation, allometric relationships, trade-offs, constraints, and convergence.[6–10] We believe, however, that evolutionary physiology, as originally outlined,[11–13] has yet to reach its full potential. We provide a brief perspective on the field, from the outlook of vertebrate biologists, with the goal of pointing the way towards its enhancement and maturation. We would also direct readers to other papers that provide partial reviews of evolutionary physiology and discussions of future directions.[5,14,15]

Evolutionary physiology sits at the intersection of evolution, ecology, and organismal biology (Figure 1). Most generally, physiology is the study of how organisms work (we include within "physiology" such related areas of biochemistry, neurobiology, endocrinology, functional morphology, and biomechanics). Elucidating the mechanisms that underpin organismal function does not require an explanation for their origin, nor does it require an understanding of why these mechanisms continue to be favored (or become disfavored) by ongoing natural or

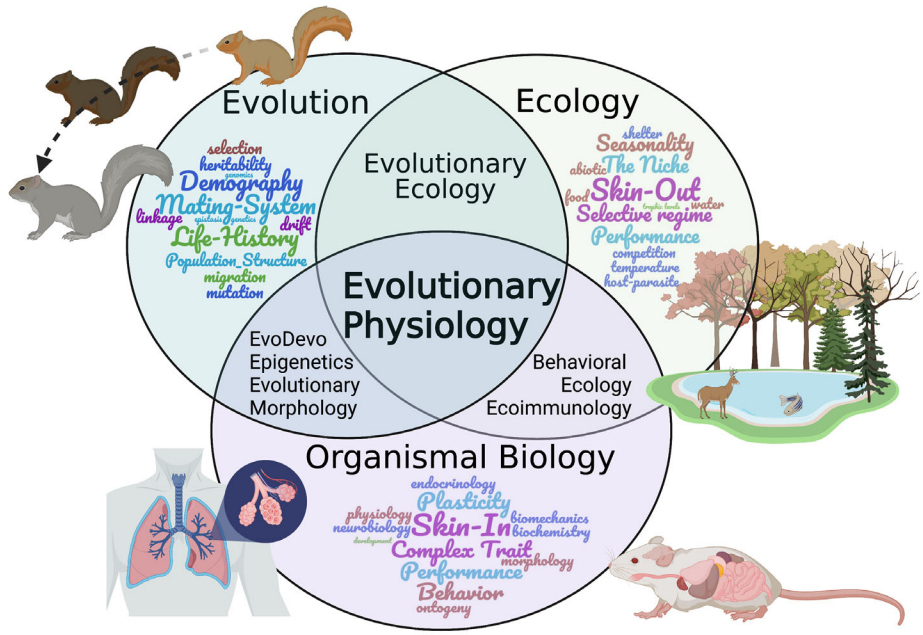


FIGURE 1 Modern evolutionary physiology resides in the intersection of evolution, ecology, and organismal biology

sexual selection in the wild, as dictated by ecological circumstances (e.g., see Ref.[16]). Rather, understanding the origin and maintenance of traits and characteristics at all levels of biological organization is the provenance of evolutionary biology. Understanding the evolution of physiological mechanisms equals understanding their causes at both proximate and ultimate levels[17], which promotes comprehension of factors that facilitate and constrain evolutionary processes (e.g., see [18]), as well as the causes of and solutions to human pathologies.[19,20] The influence of rigorous evolutionary thinking on physiology has resulted in the rise of evolutionary medicine,[21] but it has also led to more sophisticated analyses and approaches in non-medical physiology.

We believe that the aims and scope of evolutionary physiology should now be revisited to explore new possibilities derived from the synergy between evolutionary biology and physiology. We first highlight three now-familiar approaches in modern evolutionary physiology, none of which were common three decades ago. We then provide some examples illustrating how evolutionary thinking has influenced physiology and vice versa, and in so doing we propose new avenues that may lead to unexpected advances in both disciplines.

THREE WELL-ESTABLISHED APPROACHES IN MODERN EVOLUTIONARY PHYSIOLOGY

Phylogenetically informed comparative studies

Of the various tools that evolutionary physiology has adopted from evolutionary biology, none has had a greater impact than the use of phylogenetic comparative methods.[22] These approaches were in rapid development when comparative and ecological physiologists were first encouraged to take advantage of them.[12,23] Formalized procedures for phylogenetically-based statistical analyses[2224] have caused a mini-revolution in evolutionary biology, and this has been reflected in comparative physiology.[3,25] Phylogenetically informed analyses have improved, for example, the understanding of the evolution of endothermy[26] and of diving,[27] and the diversity of photosynthesis types in plants.[28]

Selection experiments and experimental evolution

Moving from macroevolutionary to microevolutionary analyses, selection experiments, and experimental evolution in both laboratory and field settings have provided unique insights regarding adaption, coadaptation, and the genetic/genomic mechanisms of evolutionary change.[4] For example, Lenski and colleagues had maintained 12 populations of *Escherichia coli* in the laboratory for more than 25 years and 60 000 generations.[29] Among various results, they discovered a trade-off between growth on glucose and acetate involving two metabolic "ecotypes" that can stably coexist. Each ecotype has a competitive advantage when rare, which it loses when it becomes more common.

As a vertebrate example, Garland and colleagues began replicated artificial selection for voluntary exercise behavior in laboratory house mice in 1993, and the experiment has now proceeded for more than 90 generations. Numerous correlated responses have been documented at the levels of both motivation for physical activity and ability to

sustain aerobic exercise, including increased endurance and maximal oxygen consumption during forced exercise, changes in muscle size and fiber type composition, skeletal alterations, endocrine changes, and brain changes.[30–32] Together this body of work demonstrates how behavior and morphology/physiology coadapt in response to directional selection, multiple solutions in response to selection, sex-specific responses for some traits, the evolution of adaptive plasticity, changes in genetic correlations over time, possible causes of selection limits, and genomic-level underpinnings of adaptive evolution. It also has implications for human health and well-being in relation to exercise physiology and addiction, the control of body weight, and the control of physical activity.

Evolutionary and functional genomics

The low cost of sequencing has led to a genomic revolution that has found its way into all approaches and areas of biology, including selection experiments and experimental evolution,[33] the study of adaptation in natural populations,[34,35] and the study of human morphological and physiological evolution.[36] As one example, the killifish, *Fundulus heteroclitus*, has been a subject of studies in evolutionary genetics, biochemistry, and physiology since the late 1970s (e.g., see references in [12]). Overall, decades of studies have led to the conclusion that evolutionary adaptation related to the glycolytic enzyme lactate dehydrogenase B has involved small changes in the allele frequencies of many genes, and these changes are manifest at the levels of transcription, biochemistry, metabolism, osmoregulation, and whole-organism physiology.[37]

EVOLUTIONARY BIOLOGY INFLUENCES PHYSIOLOGY

Non-model species widen knowledge in animal physiology

Traditionally, and justifiably, physiology has focused on human beings to find solutions to disease and other pathological conditions.[38] However, given the difficulty, cost, and ethical issues involved with conducting human studies, the use of "animal models" to elucidate aspects of human physiology became widespread. Although other animal models are available for particular physiological processes (e.g., [39]), the house mouse *Mus musculus* is by far the most common animal model in physiology, as it is in most biological sciences.

Early studies in comparative physiology recognized that the neglect of among-species comparisons was retarding the progress of physiology and pathology,[40] but still usually had elucidation of human physiology as the ultimate goal. And comparative physiology has a long history of contributions to basic physiology, including relevance to humans[20]. For example, Mathew Kluger's studies of thermoregulation and behavioral fever in lizards [41] and Fred White's studies of acid-base balance during hypothermia in reptiles (references in [12]) have affected the way physicians view and treat human patients.

Although the majority of animal physiological research has at least an implicit focus on human beings, evolutionary biology addresses all biological diversity. Therefore, by not limiting studies to humans, mice, and other laboratory animals, evolutionary biology necessarily considers physiological systems different from those represented by traditional animal models. This represents an opportunity to widen the general knowledge on animal physiology, and to find unsuspected ways to treat human pathology that could not be approached with traditional animal models (e.g., see [20]). Beyond species that produce substances such as venoms that are useful for the preparation of drugs,[42] many wild non-model species present physiological processes that are similar to those of humans, and sometimes have superior performance through unique adaptations. Such species represent a great potential to offer solutions to human pathology.

Unique physiological adaptations in non-model species

Many species of frogs store in their skin an extraordinary diversity of biologically active peptides at high concentrations, and many of these have mammalian counterparts, thus representing a source for discovering new hormones, neuropeptides, and peptide-processing enzymes that might not be as readily found with conventional animal models.[43] Several species of songbirds and teleost fishes have unusually high levels of aromatase activity that make them interesting models to understand the mechanisms of estrogen synthesis.[44] Wild rodent species have been proposed as a resource for research on immunity and infection, given their high genetic diversity and environmental pressures to which they are exposed as compared with laboratory rodents.[45] In 1971, it was found that fox squirrels (*Sciurus niger)* accumulate large amounts of the pigment uroporphyrin I in internal organs and the skin due a very low activity of the enzyme uroporphyrinogen III synthase in different tissues under healthy conditions.[46] In humans, congenital erythropoietic porphyria is caused by a defect in uroporphyrinogen III synthase that leads to a similar low enzymatic activity and uroporphyrin I overproduction, which allowed researchers to propose the fox squirrel as an animal model for this disease.[46] The Honduran white bat (*Ectophylla alba*) has recently been reported as the first mammal that has evolved the physiological capacity to esterify and deposit high amounts of carotenoid pigments in the skin, thus constituting a model that may help to improve the assimilation of carotenoids in humans and avoid macular degeneration.[47] The study of all these species was not primarily motivated by physiological questions. Instead, these studies were started by researchers investigating evolutionary and ecological aspects of the species (e.g.,[48]), and interest in physiology arose later.

Aging in non-model species

Non-model species have also contributed to our understanding of the process of aging. How animals age is determined by the failures of physiological processes. Understanding why different physiological

processes fail faster or slower in different organisms can bring insight to the evolution of cellular protection and repair processes, as well as the evolution of life histories.[49] For example, a comparison across 18 rodents species with lifespan ranging from two (mice) to 30 years (beavers) determined that the ability to repair double-strand breaks in DNA (via SIRT6) is a tight correlate of long lifespan.[50] The levels of IGF1 hormone has long been recognized as a biomarker of aging. Although humans also express the paralogous hormone IGF2 at high levels as adults,[51] biomedical rodent models do not,[52] resulting in this hormone being understudied in the context of senescence. Recent studies demonstrate that reptiles and birds express IGF2 at high levels in adulthood,[53,54] similar to humans, providing new model systems to study the physiological effects of this hormone in adulthood.

Tissue regeneration in non-model species

Many non-model species exhibit regenerative abilities that are coveted by the biomedical community.[55] Within vertebrates, there is considerable diversity in the degree to which a species can regenerate tissue and which types of tissues can be regenerated,[56] with a clear phylogenetic signal of reduced regenerative abilities moving from fish and amphibians to amniotes and then to mammals. Some species of fish and amphibians have incredible regenerative capacities, including whole limbs, eyes, and internal organs.[56] In newts, for example, after the complete removal of the lens from the eye, the lens can be regenerated *de novo* from the dorsal iris cells that can undergo a dedifferentiation process. [57] Within reptiles the regenerative diversity is more restricted; the best known example being tail regeneration in many lizard species that is associated with autotomy as an anti-predator defense. But brain tissue and optic nerve regeneration has also been demonstrated in lizards.[58] Snakes, which are derived from lizards, have lost the ability to regenerate their tails, but some have rapid organ regeneration. For example, pythons may go months without feeding, during which time their digestive organs regress in size. Within hours to days of refeeding, the intestine regenerates thorough hyperplasia and hypertrophy to accommodate the physiological demands of processing the meal.[59] In contrast, significant regeneration in adult mammals is largely restricted to the liver[60] and antler regeneration in deer,[61] whereas other types of limb loss and tissue damage typically result in scarring. Comparative studies across these non-model species have begun to illuminate common factors in exceptional regenerative abilities, including the maintenance of juvenile physiology or the ability to reactivate an embryonic cellular program, and the need for the regenerating tissues to "hide" from the immune system similar to cancerous tumors.[62]

Examples like those described in the previous paragraphs, with an identified potential to provide solutions to specific human health issues, do not abound in the literature. Furthermore, the utility of these cited systems to widen general physiological knowledge is only beginning to be considered, and only in some cases.[63] A remarkable example is the fox squirrel mentioned above, which was proposed as a model for human congenital erythropoietic porphyria in the 1970s, with a great potential to provide insights into physiological mechanisms that avoid the toxicity of porphyrin accumulation,[46] a proposal that has been overlooked. The use of physiological systems represented in wild, non-model species of animals studied by evolutionary biologists certainly remains an underexplored and promising area for physiologists, especially given that model and non-model species may differ in systematic ways.[64]

Physiological characteristics affect the capacity for physiological adaptation

The concept of adaptation is central to biology, but the term has been used across fields in two distinct ways.[30,65] First, "evolutionary adaptation" refers to multi-generational changes in the allele frequencies of populations in response to natural selection on a particular trait, which could be physiological. Second, "physiological adaptation" has been used in some fields of research to refer to changes that occur within individuals in response to external (or internal) stimuli and that lead to homeostasis and/or improved abilities to perform various tasks and/or improved Darwinian fitness.[66] This second usage encompasses "acclimation" (in the lab) and "acclimatization" (in the wild). Thus, "physiological adaptation" is one aspect of phenotypic plasticity — defined as the ability for an individual's genome to produce different phenotypes in response to varied environmental conditions. This response is also referred to as a reaction norm. It is important to recognize that some plastic responses to external stimuli may not be beneficial, which would cause strong selection on the plastic response to undergo evolutionary adaptation to alter the response (with one possible endpoint being a non-responsive trait). In other words, if sufficient genetic variation is available in the response, then selection is expected to change the shape of the reaction norm over generations.[67] In this way, the capacity for physiological adaptation is, of course, adaptive in an evolutionary sense. In any case, the mechanistic basis of all evolutionary adaptation is necessarily physiological at some level.[68]

Evolutionary studies that include examination of physiological adaptation (i.e., a potentially beneficial response to external stimuli) illustrate the potential to discover the mechanisms by which organisms cope with fluctuating environments, as well as directional climate change (e.g., [69]). In 16 species of birds inhabiting Chernobyl, for example, physiological adaptation occurs in the systemic levels of the master cellular antioxidant (glutathione, GSH) and in the capacity to avoid DNA damage as a response to exposure to ionizing radiation, which generates oxidative stress.[70] The degree of this adaptation, however, depends at least in part on the amount of the pigment pheomelanin that birds produce in their plumage, as pheomelanin synthesis consumes cysteine (a constitutive amino acid of GSH), produces free radicals upon radiation exposure, and may thus cause chronic oxidative stress.[70] Although these studies do not demonstrate the exact mechanism by which physiological adaptation in response to ionizing radiation occurs, they do clearly show that antioxidant-demanding processes, such as pheomelanin synthesis, can be constraining factors in physiological adaptation.

The foregoing avian example illustrates that some characteristics of organisms limit their ability for physiological adaptation. For instance, as in many other organisms, the production of heat-shock proteins is a common response of notothenioid fishes against thermal stress, as this allows restoration of heat-denatured proteins.[71] The activation of this stress response requires modulating the expression of genes that regulate heat-shock protein production in a temperature-dependent manner. However, some species with an evolutionary thermal history that has not favored phenotypic plasticity for temperature-mediated gene expression are limited in their ability to acclimate to increased temperatures.[72]

Similarly, the exposure of birds and mammals to hypoxia activates changes in the expression of some genes that affect O_2 transport and erythropoiesis, but the performance of this physiological adaptation depends on whether the animals are previously acclimatized to living at low or high altitudes.[73] These sorts of characteristics of organisms can be viewed as endogenous constraints and they exemplify how the evolution of certain traits helps explain the capacity of animals to achieve physiological adaptation to the environments where they live, both in terms of phenotypic plasticity and cross-generational genetic changes.[37,65] Detailed investigations of the mechanisms that facilitate or constrain the ability for physiological adaptation are an exciting future direction for evolutionary physiology and may also facilitate finding solutions to diseases related to allostatic load.[74]

Although the examples above come from vertebrate physiology, where our research experience falls, invertebrate studies have also provided great insights into evolutionary physiology (see, e.g., review in [75]). For example, studies of wing-polymorphic crickets have shown that different morphs synthesize different compositions of lipids and allocate them to different tissues, which underlie the existence of alternate life histories.[76] In *Rhagoletis* flies, recent evolutionary shifts between host plants with different seasonality change transcripts that participate in development during diapause, influencing life cycle adaptation.[77] Physiological adaptation associated to photoperiodism varies with different genetic responses to day length in other insects, revealing complex genetic and physiological characteristics that mediate daily rhythms.[78] Other studies provide insight into how natural variation in certain characteristics of insects affects thermal adaptation. This is the case in *Colias* butterflies, whose altitude-related variation in wing solar absorptivities mediates the ability to achieve thermal adaptation under fluctuating environmental conditions, such as wind speed.[79] Studies on willow leaf beetles show that thermal physiological stress effects on mating activity differ between genotypes associated with the glycolytic enzyme phosphoglucose isomerase.[80]

PHYSIOLOGY INFORMS EVOLUTIONARY BIOLOGY

The evolution of honest signals has a physiological basis

Biological communication is mainly driven by signals, traits that evolve because of the benefits obtained by their recipients.[81] When signals can allow the Darwinian fitness (reproductive success) of their recipients to improve, they are considered "honest." This appears to be the case for most biological traits that fulfill a signaling role.[82] Signal honesty is closely related to the concept of individual quality. As stated in the handicap principle, a cornerstone of behavioral ecology, the production of large (expensive) signals is limited to high-quality signalers because low-quality ones cannot afford the costs derived from signal production.[83] However, this explanation has been challenged in recent years because costs for low-quality individuals are frequently not found in empirical studies, and, indeed, natural selection is not expected to favor the evolution of signals when it implies incurring substantive costs.[84] As a consequence, the existence of costs predicted by the handicap principle is not fully accepted by evolutionary biology, which currently lacks an integrated approach to explain the concept of individual quality and the evolution of honesty.

Recent physiological experiments on the classical honest signaling system of the black bib of male house sparrows (*Passer domesticus*) illustrate the possibility that costs are not necessary to explain why low-quality individuals do not develop high-quality signals (i.e., large bibs). Large bibs are associated with low amounts of the pigment pheomelanin in their constitutive feathers, which allows researchers to experimentally create physiological conditions that favor the production of small or large bibs by exposing birds to substances that act as inhibitors or enhancers of pheomelanin synthesis.[85] Despite these induced physiological conditions, the resulting phenotype could be manipulated in high-quality birds (i.e., those with largest bibs initially) only. A physiological mechanism may therefore exist in low-quality individuals that make them less sensitive to environmental factors than high-quality individuals, which prevents low-quality individuals from producing high-quality signals even if they took the "decision" to do so or if environmental conditions favored the production of large signals.[85]

The experiments on the signaling system of male house sparrows exemplify how the details of the machinery controlling the expression of signals can explain their honesty without the costs predicted by the handicap principle. Although specific to visual traits whose production is mediated by the synthesis of melanin pigments, these experiments show that the evolution of honesty can have a physiological basis. Similar studies on the physiological basis of trait production in other honest signaling systems, including those in humans,[86] may provide a more general concept of individual quality and consequently represent a new understanding of this aspect of biological communication.

Elucidating the physiological underpinnings of evolutionary adaptations

As evolutionary adaptations directly depend on functional aspects of organisms, physiology, and related fields have the potential to provide a conjectural background to understand them (e.g., see [87]). A diversity of approaches in evolutionary physiology can be used to identify the mechanisms by which adaptations arise (e.g., see [6,12,13,18,35,37,65,68]). These approaches include direct gene editing of genes underlying

adaptations, genomic analyses of phenotypes resulting from selection (natural, artificial, or experimental), contrasting repeated evolutionary events of physiological phenotypes using physiological experiments, and the application of physiological knowledge to evolutionary adaptations. We provide examples for these below and in Box 1, including simple economical ideas that have been used to understand the evolution of pigmentation phenotypes[88] and theories of sensory cue integration to understand the evolution of perception capacity.[89]

Research methods in physiology have always strongly relied on experimental manipulations of biological processes[90] and the advent of molecular tools, such as CRISPR, allow manipulations at the level of the genome to prove physiological mechanisms. Although evolutionary adaptations have been linked to specific genes in a growing number of cases (e.g., [7,91–93]), typically these genes fit in to molecular networks — interactions among genes, proteins, and RNAs that are coordinated within the cell — to regulate physiological outcomes. Selection acting on a larger network makes it much harder to detect effects on particular loci because the impact can be shared across loci with relatively small effect, and the probability of pleiotropic effects is high in a network. Moreover, the experimental manipulation of multiple genes concurrently to understand their physiological effects is much more difficult than changing single genes.

Rather than attempting to manipulate genes directly, selection experiments focused at behavioral or other whole-organism levels can be used to understand how evolution can bring about adaptations through shaping of a molecular network. Dogs are a great example, having been under artificial selection for thousands of years, resulting in breeds defined by form, function, and behavior.[94] The evolutionary response to selection that targeted growth, strength, and body size has involved the insulin and insulin-like signaling (IIS) network.[95] This molecular network integrates over 100 genes, and this network has been studied extensively for its pleiotropic effects on both early (growth and reproduction) and late life (rate of aging) traits in various model organisms.[96] Selection has sorted alleles by dog breed for at least seven loci, and most of these genes are in or related to the IIS network.[95] The allelic variation at these seven loci explains over 50% of the variation in body size among breeds. Together, in the context of the function of the IIS network on the cellular and organismal physiology, the alleles in the small-bodied breeds (e.g., Chihuahua) reduce the cellular signaling through IIS network resulting in the correlated phenotypes of small body, small litters, and longer lifespans relative to the larger breeds (e.g., Mastiff).[95,97]

Sensory systems also provide clear illustrations of how physiological knowledge helps us to understand evolutionary adaptations (see also examples in [6]). In the most general sense, the sensory perception of organisms depends on their physiological allocation to the systems involved. This physiological allocation differs among species and even individuals, but this does not mean that perceived objects are only the product of neuronal activity or that the brain produces realistic models without capturing reality itself. The chromatic experience of animals, for example, is not only a type of neural state or process, but also reflects to a large degree the color of the objects being perceived as a physical attribute of these objects. Color perception is thus the combination of an objective and a subjective experience, the latter greatly influencing the ecological/evolutionary implications of perceiving the color of given objects.[98] Color interpretation in some evolutionary studies has been made in a way that gives much weight to the subjective component of color perception (e.g., "Color is not an inherent property of the object; it is a product of the brain of the animal perceiving the object,"[99]), but it must be remembered that color is also a physical attribute of the objects. Considering the objective component of color perception may be useful in interspecific comparisons of animal coloration, and thus provide clues into the adaptiveness of color traits. Indeed, human vision can detect much of the variation in bird coloration in the visible range and also provide a valid proxy for avian perception of such color traits as sexual dichromatism,[100,101] suggesting that considering color exclusively as a neural state may be an incomplete view. That color resides in both the objects being perceived and in the brain of the perceiving animals is known in neuroscience since the 1990s, notably through the work of Francisco J. Varela and others.[98,102] Considering this theoretical background of sensory physiology may therefore help in gaining a deeper insight into the adaptive value of color phenotypes.

In addition statistical analyses are essential to detect patterns in physiological data.[90] It is important, however, that evolutionary inferences from physiological data are not exclusively dependent on statistics, in the sense of using only data that are devoid of clear functional, physiological meaning. Evolutionary biologists should take advantage of research approaches in physiology and related functional fields that allow less dependence on statistics. For example, several studies have reconstructed ancestral proteins and measured or inferred their functional characteristics to gain insight regarding physiological adaptation (e.g., [103,104]).

THE ROLE OF EPIGENETICS IN PHYSIOLOGICAL AND EVOLUTIONARY ADAPTATION

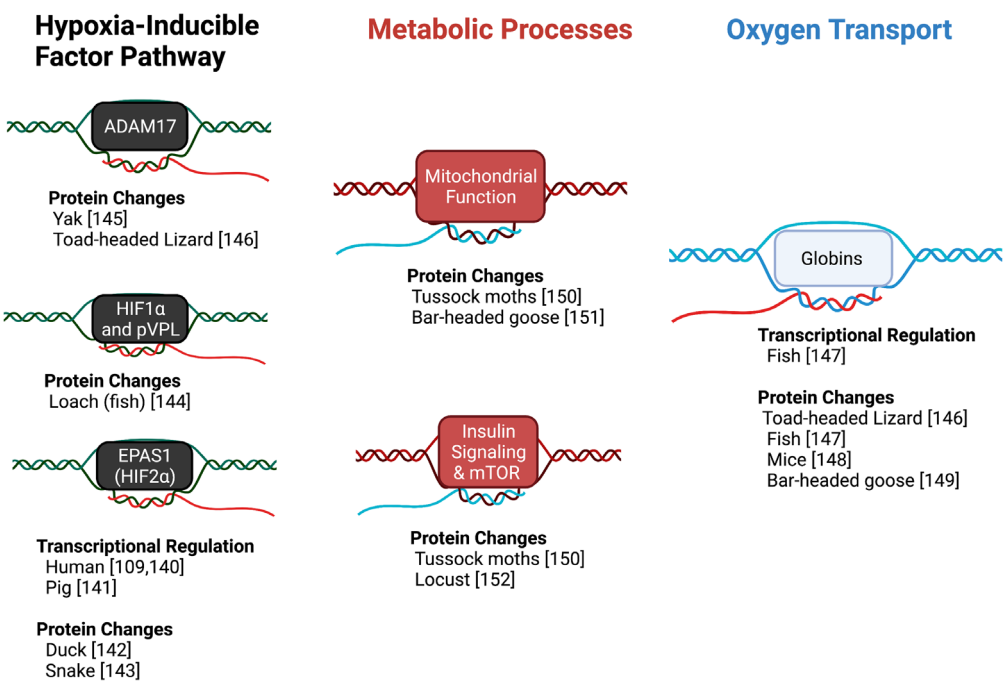
Use of the term "epigenetic" has changed over time, but currently it usually refers to chemical modifications on the DNA, RNA, or associated proteins that regulate the genome (and thereby the expression of genes), without changes in the DNA sequence. In the last 30 years, the molecular basis for how epigenetic modifications can result in phenotypic plasticity has been revealed in different organisms.[105] Such epigenetic plasticity can be induced by environmental factors, and such alterations have been identified as important mechanisms underlying physiological adaptation of organisms to a diversity of environments.[106] Epigenetic plasticity thus acts as a potential enhancer of physiological adaptation. Examples of this are studies of teleost fish where a concerted role for DNA methylation and histone modifications induced by hypoxia, thermal stress, osmotic challenges, and starvation has been shown to regulate the expression of genes involved in, respectively, apoptosis, folate metabolism, osmotic stress transcription factors and autophagy.[107] Such changes may facilitate physiological adaptations to environmental conditions and, in some

Box 1. The use of cross-organism integration to understand physiological evolution

The use of wild species and the integration of findings across organisms is a powerful but underutilized approach to understanding physiological evolution. Here we provide two examples of how we think this has been done well and can provide road maps for others to utilize this approach.

Example 1: Tracking metabolic pathways in different organisms, for example, provides insight into the adaptive value of metabolic products. This is the case of biological pigments, whose whole chemical diversity can be categorized into three common synthesis routes after tracking them across all organisms, suggesting common functional roles.[131] Melanins, pigments that are the result of one of these routes, are probably synthesized by all organisms. From bacteria to humans, broad optical absorption properties make melanins exert a universal protecting role against cellular damage caused by solar UV radiation.[132] Carotenoid pigments resulting from another synthesis route, are synthesized by photosynthetic organisms that benefit from the charge transfer properties of these pigments, which facilitate photosynthesis under exposure to sunlight. Animals that take carotenoids with food, such as insects,[133] benefit from the same properties to quench free radicals and possibly from enhancing properties of the mitochondrial function and thus protect cells from oxidative stress[134] as do birds and other organisms as the cartenoids pass up the food chain.[135] Lastly, porphyrins, pigments corresponding to the third route, fulfill a key role in all vertebrates by acting as intermediates in the synthesis of heme.[130] Marine invertebrates also synthesize porphyrins, and although their physiological role in these groups is unknown, it is likely to be related to the essential role exerted in vertebrates.[136]

Example 2. The mechanistic basis of evolutionary adaptations to high altitude and hypoxia resistance has been informed by genomics studies across species, from mammals and birds to insects.[137–139] The integrated knowledge across these studies reveals the commonalities of approaches that evolution has taken and thereby revealing the higher-order constraints in the physiological system. Compiled across species, the genes underlying the physiological processes of oxygen transport, metabolic processes, and the hypoxia-inducible factor pathway are some of the most prominent targets of selection for living in high altitude environments. In some cases, the same genes are being targeted across species, in other cases different genes in the same process are targets. Genetic variants in and around these genes have been found to be segregating across high and low-altitude populations, and functional studies have demonstrated their importance. Contrasting across these studies reveal the diversity of mechanisms that has been used by evolution to manipulate hypoxia tolerance at many biological levels to achieve similar adaptive benefits to living at high altitude conditions. In some species and populations, the genetic variants differentially regulate transcription of the genes in response to the high-altitude, and thereby altering the amount of the protein product produced in those conditions relative to low-altitude species; in other cases, they alter the amino acid sequence to affect the function of the protein. To illustrate these points, in the figure below we highlight a small subset of the genetic variants that have been identified across different species involved in the evolutionary adaptation to living in high altitude environments (see more extensive reviews[137–139]).



cases, also affect external phenotypic traits on which selection can act (for another example, see Box 2).

If the epigenetic plasticity is inherited, this may provide an additional, accelerated pathway for evolutionary adaptation.[108] For example, the response to hypoxia involves epigenetic modifications to open the chromatin at regulatory elements to allow transcription of genes, such as EPAS1.[109] A change in the timing of this chromatin opening during the hypoxia response appears be part of the adaptive response to hypoxia in Tibetan relative to Han human populations[109] (also see Box 1).

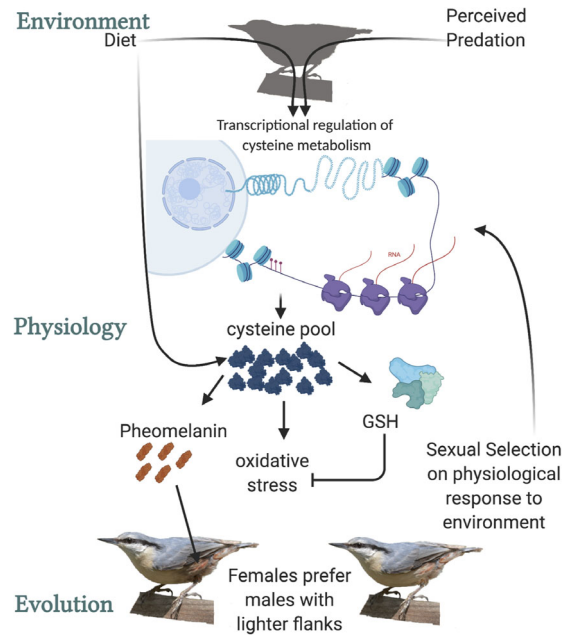
Epigenetic modifications can be transmitted across generations and be an important component of preparing the next generation for the parental environment. In these cases of "epigenetic inheritance," the modifications that occur in response to environmental conditions in the parents are passed on to offspring or even subsequent generations.[110] The importance of epigenetic inheritance in the context of evolution is strongly debated.[111,112] Intergenerational inheritance (where the embryo and its germline are directly exposed to the parental environment while in utero/in ovo) is quite common. In this context, Danchin and Pocheville[113] have made the important claim that "non-genetic inheritance shatters the frontier between physiology and evolution, and leads to the coupling of physiological and evolutionary processes to a point where there exists a continuum between accommodation by phenotypic plasticity and adaptation by natural selection."

Transgenerational epigenetic inheritance, in which the epigenetic marks and consequential phenotypes persist to the generation that has not had direct exposure to the epigenetic defining environment, is prevalent in yeast and plants. But transgenerational epigenetic inheritance occurs substantially less in other organisms,[114] particularly in sexually reproducing species where the germline is separated from the soma, DNA methylation is globally reduced twice in each generation, and histone marks are reprogrammed in the germline and after fertilization. In vertebrates, mechanisms such as histone retention in sperm and ncRNAs are the more likely candidates for transgenerational inheritance.[110] Although evidence of environmentally induced transgenerational epigenetic inheritance in vertebrates is limited, it has been experimentally demonstrated in some species, including rodents,[115] and has been documented in humans,[114] although typically associated with unhealthy or disease phenotypes rather than adaptive responses. For example, in rats, DNA hypermethylation induced by chronic stress exposure has been shown to be transgenerational inheritance to at least three generations.[116] Additionally, injection of herbicides in rats has been demonstrated to cause transgenerational effects (4th generation) via the alteration of histone retention in sperm that associate with diseases.[117]

Epigenetic modifications regulate physiological responses that selection acts on. This link between physiology and evolution agrees with West-Eberhard's[118] idea that genes are "followers" rather than initiators of evolutionary change, when they stabilize phenotypic (physiological) changes that are started by epigenetic processes. Following this idea, epigenetic inheritance has a high potential to affect

Box 2. Environmental epigenetics of pigmentation genes

Expression of the pigment pheomelanin in melanocytes of developing Eurasian nuthatches *Sitta europaea* is regulated, in part, by some genes of cysteine metabolism whose expression can be affected by two environmental factors defined at the top of the diagram: availability of cysteine in the diet and perceived predation risk. At the physiological level, excess dietary cysteine alters the expression of these genes by modifying DNA and RNA methylation levels, promoting pheomelanin synthesis and resulting in flank plumage patches of increased pigmentation intensity. In this way, the epigenetic changes promote usage of the excess cysteine for pheomelanin synthesis. The excess cysteine would otherwise cause cellular oxidative stress, thus the epigenetic changes are physiologically adaptive in an environment that is rich in dietary cysteine.[153] Higher levels of perceived predation risk produce opposite changes in the expression of these genes, limiting pheomelanin synthesis and resulting in flanks of reduced pigmentation intensity. As cysteine is a constituent amino acid of the main cellular antioxidant (i.e., glutathione, GSH), these changes may also be adaptive because they increase the antioxidant capacity and thus allow the animal to avoid oxidative stress/damage expected from predation risk.[154] At the level of evolution, these changes in the pigmentation of nuthatches have consequences for sexual selection, as adult females mate preferentially with males showing flanks of reduced pigmentation intensity.[155] Thus, the physiological responses to perceived predation risk lead to reduced pigmentation intensity, in addition to providing immediate physiological benefits, and are probably favored by sexual selection.



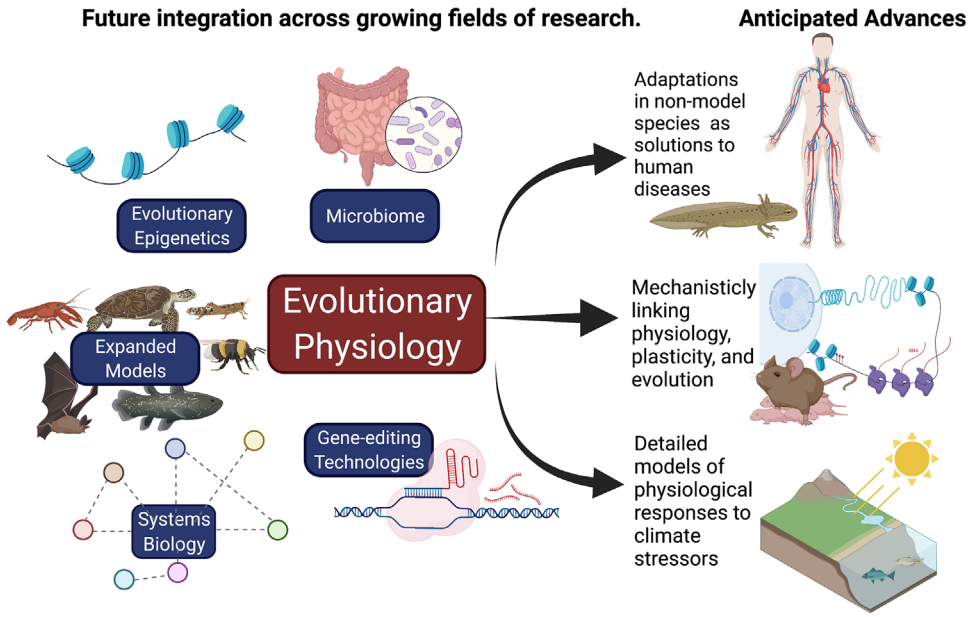


FIGURE 2 The future of evolutionary physiology should embrace new technologies and additional subfields. In addition, the reciprocity between evolutionary biology and physiology needs to proceed under an unbiased interdisciplinary approach that widens the skills of scientists in both fields, from the view that all physiological processes are the result of evolution. Foreseen advances in the field of evolutionary physiology include enhanced mechanistic climate modeling, the use of wild animal adaptations to inform medical treatments, and mechanistic linking of many physiological traits to evolutionary fitness

phenotypic evolution, because epigenetic variation may facilitate the role of natural selection in overcoming stochastic loss of new heritable variants.[119]

CONCLUSIONS AND OUTLOOK

We are optimistic about the future of evolutionary physiology. The future will include embracing other subfields and new research areas (e.g., Figure 2) not often viewed as part of "evolutionary physiology." For example: study of the microbiome and its influences on physiology and the coevolution of organisms and their microbiota (e.g., [120–122]); evolutionary epigenetics to explain the mechanistic bases of phenotypic plasticity and the evolution of reaction norms; integration of -omics technologies and systems biology to facilitate understanding of physiology across hierarchical levels of biology[123]; infusion of physiological analyses into comparative biomechanics/functional morphology/ecomorphology[124]; technological advancements like CRISPR to experimentally test the function of physiologically relevant genes and their impact on organismal fitness. Another opportunity is expanded use of wild species and integration of findings across organisms (e.g., animals, plants, bacteria) to reach a more general understanding of physiological evolution (examples in Boxes). Although this suggestion has been made before (e.g., [125]), little cross-organism integration is evident.

We envision great strides in several areas. First, the linking of physiological traits to evolutionary fitness, across hierarchical levels from genes to ecosystems, in a causal way to inform mechanisms of evolutionary adaptation, constraint, and diversification (e.g., [26,27,124,126–129]). Second, the use of adaptations in wild animals to develop new strategies for combating diseases and injuries.[130] And finally, the development of mechanistic models for the physiological responses (and their evolution) of animals (wild, agricultural, and humans) to extreme external stressors they may encounter with climate change. Although these future achievements are within sight, equally exciting are the unforeseen advances that will undoubtedly unfold over as these opportunities are embraced by the next generation of evolutionary physiologists.

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AUTHOR CONTRIBUTIONS

All authors conceived and wrote the manuscript.

CONFLICT OF INTEREST

None.

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REFERENCES

1. Feder, M. E., Bennett, A. F., Burggren, W. W., & Huey, R. B. (Cambridge, NY 1987). *New directions in ecological physiology*. Cambridge University Press.
2. Pough, F. H. (1988). Evolutionary physiology: New directions in ecological physiology. *Science*, *240*(4857), 1349–1351. https://doi.org/10.1126/science.240.4857.1349
3. Rezende, E. L., & Diniz-Filho, J. A. F. (2012). Phylogenetic analyses: Comparing species to infer adaptations and physiological mechanisms. *Comprehensive Physiology*, *2*, 639–674.
4. Garland, T. Jr., & Rose, M. R. (Berkeley, CA 2009). *Experimental evolution*. University of California Press. https://www.degruyter.com/document/doi/10.1525/9780520944473/html
5. Storz, J. F., Bridgham, J. T., Kelly, S. A., & Garland, T. Jr. (2015). Genetic approaches in comparative and evolutionary physiology. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *309*(3), R197–R214. https://doi.org/10.1152/ajpregu.00100.2015
6. Autumn, K., Ryan, M. J., & Wake, D. B. (2002). Integrating historical and mechanistic biology enhances the study of adaptation. *The Quarterly Review of Biology*, *77*(4), 383–408. https://doi.org/10.1086/344413
7. Rosenblum, E. B., Römpler, H., Schöneberg, T., & Hoekstra, H. E. (2010). Molecular and functional basis of phenotypic convergence in white lizards at White Sands. *Proceedings of the National Academy of Sciences of the USA.*, *107*(5), 2113–2117. https://doi.org/10.1073/pnas.0911042107
8. Noble, D. (2013). Physiology is rocking the foundations of evolutionary biology. *Experimental Physiology*, *98*(8), 1235–1243. https://doi.org/10.1113/expphysiol.2012.071134
9. Natarajan, C., Hoffmann, F. G., Weber, R. E., Fago, A., Witt, C. C., & Storz, J. F. (2016). Predictable convergence in hemoglobin function has unpredictable molecular underpinnings. *Science*, *354*(6310), 336–339. https://doi.org/10.1126/science.aaf9070
10. Garland, T. Jr., Downs C. J., & Ives, A. R. (2022). Perspective: Trade-offs (and constraints) in organismal biology. *Physiological and Biochemical Zoology*, In press.
11. Diamond, J. M. (Oxford 1993). Evolutionary physiology. In C. A. R. Boyd & D. Noble (Eds.),. *The logic of life: The challenge of integrative physiology* (pp. 89–111). Oxford University Press.
12. Garland, T. Jr., & Carter, P. A. (1994). Evolutionary physiology. *Annual Review of Physiology*, *56*(1), 579–621. https://doi.org/10.1146/annurev.ph.56.030194.003051
13. Feder, M. E., Bennett, A. F., & Huey, R. B. (2000). Evolutionary physiology. *Annual Review of Ecology and Systematics*, *31*(1), 315–341. https://doi.org/10.1146/annurev.ecolsys.31.1.315
14. Zera, A. J. (2011). Microevolution of intermediary metabolism: Evolutionary genetics meets metabolic biochemistry. *Journal of Experimental Biology*, *214*(2), 179–190. https://doi.org/10.1242/jeb.046912
15. Noble, D., Jablonka, E., Joyner, M. J., Müller, G. B., & Omholt, S. W. (2014). Evolution evolves: Physiology returns to centre stage. *Journal of Physiology*, *592*(11), 2237–2244. https://doi.org/10.1113/jphysiol.2014.273151
16. Bateson, P., & Laland, K. N. (2013). Tinbergen's four questions: An appreciation and an update. *Trends in Ecology & Evolution*, *28*(12), 712–718. https://doi.org/10.1016/j.tree.2013.09.013
17. Mayr, E. (1961). Cause and effect in biology: Kinds of causes, predictability, and teleology are viewed by a practicing biologist. *Science*, *134*(3489), 1501–1506. https://doi.org/10.1126/science.134.3489.1501
18. Ng'oma, E., Perinchery, A. M., & King, E. G. (2017). (1857). How to get the most bang for your buck: The evolution and physiology of nutrition-dependent resource allocation strategies. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20170445. https://doi.org/10.1098/rspb.2017.0445
19. Nesse, R. M., & Stearns, S. C. (2008). The great opportunity: Evolutionary applications to medicine and public health. *Evolutionary Applications*, *1*(1), 28–48. https://doi.org/10.1111/j.1752-4571.2007.00006.x
20. Singer, M. A. (2011). Insights into biomedicine from animal adaptations. *Comprehensive Physiology*, *1*, 2063–2081.
21. Longo, V. D., & Finch, C. E. (2003). Evolutionary medicine: From dwarf model systems to healthy centenarians? *Science*, *299*(5611), 1342–1346. https://doi.org/10.1126/science.1077991
22. Huey, R. B., Garland, T. Jr., & Turelli, M. (2019). Revisiting a key innovation in evolutionary biology: Felsenstein's "Phylogenies and the Comparative Method. *American Naturalist*, *193*(6), 755–772. https://doi.org/10.1086/703055
23. Harvey, P. H., & Pagel, M. D. (Oxford 1991). *The comparative method in evolutionary biology*. Oxford University Press.
24. Garamszegi, L. Z. (Berlin 2014). *Modern phylogenetic comparative methods and their application in evolutionary biology: Concepts and practice*. Springer-Verlag.
25. Garland, T. Jr., B A., F., & Rezende, E. L. (2005). Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology*, *208*(16), 3015–3035. https://doi.org/10.1242/jeb.01745
26. Rezende, E. L., Bacigalupe, L. D., Nespolo, R. F., & Bozinovic, F. (2020). Shrinking dinosaurs and the evolution of endothermy in birds. *Science Advances*, *6*(1), eaaw4486. https://doi.org/10.1126/sciadv.aaw4486
27. Verberk, W. C. E. P., Calosi, P., Brischoux, F., Spicer, J. I., Garland, T. Jr., & Bilton, D. T. (2020). Universal metabolic constraints shape the evolutionary ecology of diving in animals. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1927), 20200488. https://doi.org/10.1098/rspb.2020.0488
28. Lundgren, M. R., & Christin, P.-A. (2017). Despite phylogenetic effects, C3–C4 lineages bridge the ecological gap to C4 photosynthesis. *Journal of Experimental Botany*, *68*(2), 241–254. https://doi.org/10.1093/jxb/erw451
29. Lenski, R. E. (2017). Experimental evolution and the dynamics of adaptation and genome evolution in microbial populations. *The ISME Journal*, *11*(10), 2181–2194. https://doi.org/10.1038/ismej.2017.69
30. Garland, T. Jr., & Kelly, S. A. (2006). Phenotypic plasticity and experimental evolution. *Journal of Experimental Biology*, *209*(12), 2344–2361. https://doi.org/10.1242/jeb.02244
31. Garland, T. Jr., Zhao, M., & Saltzman, W. (2016). Hormones and the evolution of complex traits: Insights from artificial selection on behavior. *Integrative and Comparative Biology*, *56*(2), 207–224. https://doi.org/10.1093/icb/icw040
32. Wallace, I. J., & Garland, T. Jr. (2016). Mobility as an emergent property of biological organization: Insights from experimental evolution. *Evolutionary Anthropology*, *25*(3), 98–104. https://doi.org/10.1002/evan.21481
33. Hillis, D. A., Yadgary, L., Weinstock, G. M., de Villena, F. P.-M., Daniel, P., Fowler, A. S., Xu, S., Chan, F., & Garland, T. Jr. (2020). Genetic basis of aerobically supported voluntary exercise: Results from a selection experiment with house mice. *Genetics*, *216*(3), 781–804. https://doi.org/10.1534/genetics.120.303668
34. Bay, R. A., Harrigan, R. J., Underwood, V. L., Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359*(6371), 83–86. https://doi.org/10.1126/science.aan4380
35. Zhu, X., Guan, Y., Signore, A. V., Natarajan, C., DuBay, S. G., Cheng, Y., Han, N., Song, G., Qu, Y., Moriyama, H., Hoffmann, F. G., Fago, A., Lei, F., & Storz, J. F. (2018). Divergent and parallel routes of biochemical adaptation in high-altitude passerine birds from the Qinghai-Tibet Plateau. *Proceedings of the National Academy of Sciences of the USA*, *115*(8), 1865–1870. https://doi.org/10.1073/pnas.1720487115
36. Sella, G., & Barton, N. H. (2019). Thinking about the evolution of complex traits in the era of genome-wide association studies. *Annual*

Review of Genomics and Human Genetics, *20*(1), 461–493. https://doi.org/10.1146/annurev-genom-083115-022316
37. Crawford, D. L., Schulte, P. M., Whitehead, A., & Oleksiak, M. F. (2020). Evolutionary physiology and genomics in the highly adaptable killifish (*Fundulus heteroclitus*). *Comprehensive Physiology*, *10*, 637–671.
38. Sherwood, L. (Cengage Learning, Boston 2016). *Human physiology: From cells to systems*. (9th ed.). Cengage Learning.
39. Lee, C. M., Boileau, A. C., Boileau, T. W. M., Williams, A. W., Swanson, K. S., Heintz, K. A., & Erdman, J. W. Jr. (1999). Review of animal models in carotenoid research. *Journal of Nutrition*, *129*(12), 2271–2277. https://doi.org/10.1093/jn/129.12.2271
40. Loeb, J. (Chicago 1905). *Studies in general physiology*. University of Chicago Press.
41. Kluger, M. J., Kozak, W., Conn, C. A., Leon, L. R., & Soszynski, D. (1996). The adaptive value of fever. *Infectious Disease Clinics of North America*, *10*(1), 1–20. https://doi.org/10.1016/s0891-5520(05)70282-8
42. King, G. F. (2011). Venoms as a platform for human drugs: Translating toxins into therapeutics. *Expert Opinion on Biological Therapy*, *11*(11), 1469–1484. https://doi.org/10.1517/14712598.2011.621940
43. Bevins, C. L., & Zasloff, M. (1990). Peptides from frog skin. *Annual Review of Biochemistry*, *59*(1), 395–414. https://doi.org/10.1146/annurev.bi.59.070190.002143
44. Forlano, P. M., Schlinger, B. A., & Bass, A. H. (2006). Brain aromatase: New lessons from non-mammalian model systems. *Frontiers in Neuroendocrinology*, *27*(3), 247–274. https://doi.org/10.1016/j.yfrne.2006.05.002
45. Turner, A. K., & Paterson, S. (2013). Wild rodents as a model to discover genes and pathways underlying natural variation in infectious disease susceptibility. *Parasite Immunology*, *35*(11), n/a–n/a. https://doi.org/10.1111/pim.12036
46. Levin, E. Y., & Flyger, V. (1971). Uroporphyrinogen III Cosynthetase activity in the fox squirrel (*Sciurus niger*). *Science*, *174*(4004), 59–60. https://doi.org/10.1126/science.174.4004.59
47. Galván, I., Garrido-Fernández, J., Ríos, J., Pérez-Gálvez, A., Rodríguez-Herrera, B., & Negro, J. J. (2016). Tropical bat as mammalian model for skin carotenoid metabolism. *Proceedings of the National Academy of Sciences of the USA*, *113*(39), 10932–10937. https://doi.org/10.1073/pnas.1609724113
48. Rodríguez-Herrera, B., Medellín, R. A., & Timm, R. M. (Santo Domingo 2007). *Neotropical tent-roosting bats*. Instituto de Biodiversidad.
49. Hoekstra, L. A., Schwartz, T. S., Sparkman, A. M., Miller, D. A. W., & Bronikowski, A. M. (2020). The untapped potential of reptile biodiversity for understanding how and why animals age. *Functional Ecology*, *34*(1), 38–54. https://doi.org/10.1111/1365-2435.13450
50. Tian, X., Firsanov, D., Zhang, Z., Cheng, Y., Luo, L., Tombline, G., Tan, R., Simon, M., Henderson, S., Steffan, J., Goldfarb, A., Tam, J., Zheng, K., Cornwell, A., Johnson, A., Yang, J.-N., Mao, Z., Manta, B., Dang, W., … Gorbunova, V. (2019). SIRT6 is responsible for more efficient DNA double-strand break repair in long-lived species. *Cell*, *177*(3), 622–638.e22. https://doi.org/10.1016/j.cell.2019.03.043
51. Fagerberg, L., Hallström, B. M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., Habuka, M., Tahmasebpoor, S., Danielsson, A., Edlund, K., Asplund, A., Sjöstedt, E., Lundberg, E., Szigyarto, C. A.-K., Skogs, M., Takanen, J. O., Berling, H., Tegel, H., Mulder, J., … Uhlén, M. (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular & Cellular Proteomics*, *13*(2), 397–406. https://doi.org/10.1074/mcp.M113.035600
52. Yue, F., Cheng, Y., Breschi, A., Vierstra, J., Wu, W., Ryba, T., Sandstrom, R., Ma, Z., Davis, C., Pope, B. D., Shen, Y., Pervouchine, D. D., Djebali, S., Thurman, R. E., Kaul, R., Rynes, E., Kirilusha, A., Marinov, G. K., Williams, B. A., … Ren, B. (2014). A comparative encyclopedia of DNA elements in the mouse genome. *Nature*, *515*(7527), 355–364. https://doi.org/10.1038/nature13992
53. McGaugh, S. E., Bronikowski, A. M., Kuo, C.-H., Reding, D. M., Addis, E. A., Flagel, L. E., Janzen, F. J., & Schwartz, T. S. (2015). Rapid molecular evolution across amniotes of the IIS/TOR network. *Proceedings of the National Academy of Sciences of the USA*, *112*(22), 7055–7060. https://doi.org/10.1073/pnas.1419659112
54. Beatty, A. E., & Schwartz, T. S. (2020). Gene expression of the IGF hormones and IGF binding proteins across time and tissues in a model reptile. *Physiological Genomics*, *52*(9), 423–434.
55. Alibardi, L. (2018). Review: Limb regeneration in humans: Dream or reality? *Annals of Anatomy – Anatomischer Anzeiger*, *217*, 1–6. https://doi.org/10.1016/j.aanat.2017.12.008
56. Tsonis, P. A. (2000). Regeneration in vertebrates. *Developmental Biology*, *221*(2), 273–284. https://doi.org/10.1006/dbio.2000.9667
57. Tsonis, P. A., Madhavan, M., Tancous, E. E., & Rio-Tsonis, K. D. (2004). A newt's eye view of lens regeneration. *International Journal of Developmental Biology*, *48*(8–9), 975–980. https://doi.org/10.1387/ijdb.041867pt
58. Jacyniak, K., McDonald, R. P., & Vickaryous, M. K. (2017). Tail regeneration and other phenomena of wound healing and tissue restoration in lizards. *Journal of Experimental Biology*, *220*(16), 2858–2869. https://doi.org/10.1242/jeb.126862
59. Secor, S. M., & Diamond, J. (1998). A vertebrate model of extreme physiological regulation. *Nature*, *395*(6703), 659–662. https://doi.org/10.1038/27131
60. Michalopoulos, G. K. (2007). Liver regeneration. *Journal of Cellular Physiology*, *213*(2), 286–300. https://doi.org/10.1002/jcp.21172
61. Wang, D., Berg, D., Ba, H., Sun, H., Wang, Z., & Li, C. (2019). Deer antler stem cells are a novel type of cells that sustain full regeneration of a mammalian organ – Deer antler. *Cell Death & Disease*, *10*(6), 1–13. https://doi.org/10.1038/s41419-019-1686-y
62. Vitulo, N., Valle, L. D., Skobo, T., Valle, G., & Alibardi, L. (2017). Transcriptome analysis of the regenerating tail vs. The scarring limb in lizard reveals pathways leading to successful vs. unsuccessful organ regeneration in amniotes. *Developmental Dynamics*, *246*(2), 116–134. https://doi.org/10.1002/dvdy.24474
63. Shartouny, J. R., & Jacob, J. (2019). Mining the tree of life: Host defense peptides as antiviral therapeutics. *Seminars in Cell & Developmental Biology*, *88*, 147–155. https://doi.org/10.1016/j.semcdb.2018.03.001
64. Moatt, J. P., Nakagawa, S., Lagisz, M., & Walling, C. A. (2016). The effect of dietary restriction on reproduction: A meta-analytic perspective. *BMC Evolutionary Biology*, *16*(1), 199. https://doi.org/10.1186/s12862-016-0768-z
65. Bennett, A. F. (Oxford 1997). Adaptation and the evolution of physiological characters. In W. H. Dantzler (Ed.), *Comprehensive Physiology* (pp. 3–16). Oxford University Press.
66. Wilson, R. S., & Franklin, C. E. (2002). Testing the beneficial acclimation hypothesis. *Trends in Ecology & Evolution*, *17*(2), 66–70. https://doi.org/10.1016/S0169-5347(01)02384-9
67. Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*(7569), 372–375. https://doi.org/10.1038/nature15256
68. Hochachka, P. W., & Somero, G. N. (Oxford 2002). *Biochemical adaptation: Mechanism and process in physiological evolution*. Oxford University Press.
69. Gangloff, E. J., Sorlin, M., Cordero, G. A., Souchet, J., & Aubret, F. (2019). Lizards at the peak: Physiological plasticity does not maintain performance in lizards transplanted to high altitude. *Physiological and Biochemical Zoology*, *92*(2), 189–200. https://doi.org/10.1086/701793
70. Galván, I., Bonisoli-Alquati, A., Jenkinson, S., Ghanem, G., Wakamatsu, K., Mousseau, T. A., & Møller, A. P. (2014). Chronic exposure to low-dose radiation at Chernobyl favours adaptation to oxidative stress in

birds. *Functional Ecology*, *28*(6), 1387–1403. https://doi.org/10.1111/1365-2435.12283
71. Hofmann, G. E., Buckley, B. A., Airaksinen, S., Keen, J. E., & Somero, G. N. (2000). Heat-shock protein expression is absent in the antarctic fish Trematomus bernacchii (family Nototheniidae). *Journal of Experimental Biology*, *203*(15), 2331–2339. https://doi.org/10.1242/jeb.203.15.2331
72. Somero, G. N. (2005). Linking biogeography to physiology: Evolutionary and acclimatory adjustments of thermal limits. *Frontiers in Zoology*, *2*(1), 1. https://doi.org/10.1186/1742-9994-2-1
73. Storz, J. F., Scott, G. R., & Cheviron, Z. A. (2010). Phenotypic plasticity and genetic adaptation to high-altitude hypoxia in vertebrates. *Journal of Experimental Biology*, *213*(24), 4125–4136. https://doi.org/10.1242/jeb.048181
74. Harris, B. N. (2020). Stress hypothesis overload: 131 hypotheses exploring the role of stress in tradeoffs, transitions, and health. *General and Comparative Endocrinology*, *288*, 113355. https://doi.org/10.1016/j.ygcen.2019.113355
75. Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of Marine Science*, *3*, 509–535.
76. Zhao, Z., & Zera, A. J. (2002). Differential lipid biosynthesis underlies a tradeoff between reproduction and flight capability in a wing-polymorphic cricket. *Proceedings of the National Academy of Sciences of the USA*, *99*, 16829–16834.
77. Dowle, E. J., Powell, T. H. Q., Doellman, M. M., Meyers, P. J., Calvert, M. B., Walden, K. K. O., Robertson, H. M., Berlocher, S. H., Feder, J. L., Hahn, D. A., & Ragland, G. J. (2020). Genome-wide variation and transcriptional changes in diverse developmental processes underlie the rapid evolution of seasonal adaptation. *Proceedings of the National Academy of Sciences of the USA*, *117*, 23960–23969.
78. Denlinger, D. L., Hahn, D. A., Merlin, C., Holzapfel, C. M., & Bradshaw, W. E. (2017). Keeping time without a spine: What can the insect clock teach us about seasonal adaptation? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*, 20160257.
79. Kingsolver, J. G., & Watt, W. B. (1983). Thermoregulatory strategies in Colias butterflies: Thermal stress and the limits to adaptation in temporally varying environments. *American Naturalist*, *121*, 32–55.
80. Dick, C. A., Rank, N. E., McCarthy, M., McWeeney, S., Hollis, D., & Dahlhoff, E. P. (2013). Effects of temperature variation on male behavior and mating success in a montane beetle. *Physiological and Biochemical Zoology*, *86*, 432–440.
81. Hasson, O. (1997). Towards a General Theory of Biological Signaling. *Journal of Theoretical Biology*, *185*(2), 139–156. https://doi.org/10.1006/jtbi.1996.0258
82. Searcy, W. A., & Nowicki, S. (Princeton, NJ 2005). *The evolution of animal communication*. Princeton University Press.
83. Zahavi, A. (1975). Mate selection – A selection for a handicap. *Journal of Theoretical Biology*, *53*(1), 205–214. https://doi.org/10.1016/0022-5193(75)90111-3
84. Számadó, S., & Penn, D. J. (2015). Why does costly signalling evolve? Challenges with testing the handicap hypothesis. *Animal Behaviour*, *110*, e9-e12. https://doi.org/10.1016/j.anbehav.2015.06.005
85. Galván, I., & Alonso-Alvarez, C. (2017). Individual quality via sensitivity to cysteine availability in a melanin-based honest signaling system. *Journal of Experimental Biology*, *220*(15), 2825–2833. https://doi.org/10.1242/jeb.160333
86. Havlíček, J., Třebický, V., Valentova, J. V., Kleisner, K., Akoko, R. M., Fialová, J., Jash, R., Kočnar, T., Pereira, K. J., Štěrbová, Z., Varella, M. A. C., Vokurková, J., Vunan, E., & Roberts, S. C. (2017). Men's preferences for women's breast size and shape in four cultures. *Evolution and Human Behavior*, *38*(2), 217–226. https://doi.org/10.1016/j.evolhumbehav.2016.10.002
87. Alfaro, M. E., Bolnick, D. I., & Wainwright, P. C. (2004). Evolutionary dynamics of complex biomechanical systems: An example using the four-bar mechanism. *Evolution*, *58*(3), 495–503. https://doi.org/10.1111/j.0014-3820.2004.tb01673.x
88. Galván, I., & Solano, F. (2009). The evolution of eu- and pheomelanic traits may respond to an economy of pigments related to environmental oxidative stress. *Pigment Cell & Melanoma Research*, *22*(3), 339–342. https://doi.org/10.1111/j.1755-148X.2009.00559.x
89. Fetsch, C. R., DeAngelis, G. C., & Angelaki, D. E. (2013). Bridging the gap between theories of sensory cue integration and the physiology of multisensory neurons. *Nature Reviews Neuroscience*, *14*(6), 429–442. https://doi.org/10.1038/nrn3503
90. Deyl, Z. (Boca Ratón, FL 2019). *Methods in animal physiology*. CRC Press.
91. Linnen, C. R., Poh, Y.-P., Peterson, B. K., Barrett, R. D. H., Larson, J. G., Jensen, J. D., & Hoekstra, H. E. (2013). Adaptive evolution of multiple traits through multiple mutations at a single gene. *Science*, *339*(6125), 1312–1316. https://doi.org/10.1126/science.1233213
92. Vickrey, A. I., Domyan, E. T., Horvath, M. P., & Shapiro, M. D. (2015). Convergent evolution of head crests in two domesticated columbids is associated with different missense mutations in EphB2. *Molecular Biology and Evolution*, *32*(10), 2657–2664. https://doi.org/10.1093/molbev/msv140
93. Pan, S., Lin, Y., Liu, Q., Duan, J., Lin, Z., Wang, Y., Wang, X., Lam, S. M., Zou, Z., Shui, G., Zhang, Y., Zhang, Z., & Zhan, X. (2019). Convergent genomic signatures of flight loss in birds suggest a switch of main fuel. *Nature Communications*, *10*(1), 2756. https://doi.org/10.1038/s41467-019-10682-3
94. Neff, M. W., & Rine, J. (2006). A fetching model organism. *Cell*, *124*(2), 229–231. https://doi.org/10.1016/j.cell.2006.01.008
95. Rimbault, M., Beale, H. C., Schoenebeck, J. J., Hoopes, B. C., Allen, J. J., Kilroy-Glynn, P., Wayne, R. K., Sutter, N. B., & Ostrander, E. A. (2013). Derived variants at six genes explain nearly half of size reduction in dog breeds. *Genome Research*, *23*(12), 1985–1995. https://doi.org/10.1101/gr.157339.113
96. Kenyon, C. (2001). A conserved regulatory system for aging. *Cell*, *105*(2), 165–168. https://doi.org/10.1016/S0092-8674(01)00306-3
97. Doherty, A., Lopes, I., Ford, C. T., Monaco, G., Guest, P., & de Magalhães, J. P. (2020). A scan for genes associated with cancer mortality and longevity in pedigree dog breeds. *Mammalian Genome*, *31*(7), 215–227. https://doi.org/10.1007/s00335-020-09845-1
98. Thompson, E., Palacios, A., & Varela, F. J. (1992). Ways of coloring: Comparative color vision as a case study for cognitive science. *Behavioral and Brain Sciences*, *15*(1), 1–26. https://doi.org/10.1017/S0140525X00067248
99. Bennett, A. T. D., Cuthill, I. C., & Norris, K. J. (1994). Sexual selection and the mismeasure of color. *American Naturalist*, *144*(5), 848–860. https://doi.org/10.1086/285711
100. Seddon, N., Tobias, J. A., Eaton, M., & Ödeen, A. (2010). Human vision can provide a valid proxy for avian perception of sexual dichromatism. *Auk*, *127*(2), 283–292. https://doi.org/10.1525/auk.2009.09070
101. Bergeron, Z. T., & Fuller, R. C. (2017). Using human vision to detect variation in avian coloration: How bad is it? *American Naturalist*, *191*(2), 269–276. https://doi.org/10.1086/695282
102. Varela, F. J., Thompson, E., & Rosch, E. (Cambridge, MA 1991). *The embodied mind: Cognitive science and human experience*. MIT Press.
103. Harms, M. J., & Thornton, J. W. (2013). Evolutionary biochemistry: Revealing the historical and physical causes of protein properties. *Nature Reviews Genetics*, *14*(8), 559–571. https://doi.org/10.1038/nrg3540
104. Tufts, D. M., Natarajan, C., Revsbech, I. G., Projecto-Garcia, J., Hoffmann, F. G., Weber, R. E., Fago, A., Moriyama, H., & Storz, J. F. (2015). Epistasis constrains mutational pathways of hemoglobin adaptation in high-altitude pikas. *Molecular Biology and Evolution*, *32*(2), 287–298. https://doi.org/10.1093/molbev/msu311

105. Ecker, S., Pancaldi, V., Valencia, A., Beck, S., & Paul, D. S. (2018). Epigenetic and transcriptional variability shape phenotypic plasticity. *BioEssays*, *40*, 1700148.
106. Etchegaray, J.-P., & Mostoslavsky, R. (2016). Interplay between metabolism and epigenetics: A nuclear adaptation to environmental changes. *Molecular Cell*, *62*(5), 695–711. https://doi.org/10.1016/j.molcel.2016.05.029
107. Best, C., Ikert, H., Kostyniuk, D. J., Craig, P. M., Navarro-Martin, L., Marandel, L., & Mennigen, J. A. (2018). Epigenetics in teleost fish: From molecular mechanisms to physiological phenotypes. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *224*, 210–244. https://doi.org/10.1016/j.cbpb.2018.01.006
108. Klironomos, F. D., Berg, J., & Collins, S. (2013). How epigenetic mutations can affect genetic evolution: Model and mechanism. *BioEssays*, *35*(6), 571–578. https://doi.org/10.1002/bies.201200169
109. Xin, J., Zhang, H., He, Y., Duren, Z., Bai, C., Chen, L., Luo, X., Yan, D.-S., Zhang, C., Zhu, X., Yuan, Q., Feng, Z., Cui, C., Qi, X., Ouzhuluobu, Wing H. W., Wang, Y., & Su, B. (2020). Chromatin accessibility landscape and regulatory network of high-altitude hypoxia adaptation. *Nature Communications*, *11*(1), 4928. https://doi.org/10.1038/s41467-020-18638-8
110. Perez, M. F., & Lehner, B. (2019). Intergenerational and transgenerational epigenetic inheritance in animals. *Nature Cell Biology*, *21*(2), 143–151. https://doi.org/10.1038/s41556-018-0242-9
111. Laland, K., Uller, T., Feldman, M., Sterelny, K., Müller, G. B., Moczek, A., Jablonka, E., Odling-Smee, J., Wray, G. A., Hoekstra, H. E., Futuyma, D. J., Lenski, R. E., Mackay, T. F. C., Schluter, D., & Strassmann, J. E. (2014). Does evolutionary theory need a rethink? *Nature*, *514*(7521), 161–164. https://doi.org/10.1038/514161a
112. Skvortsova, K., Iovino, N., & Bogdanović, O. (2018). Functions and mechanisms of epigenetic inheritance in animals. *Nature Reviews Molecular Cell Biology*, *19*(12), 774–790. https://doi.org/10.1038/s41580-018-0074-2
113. Danchin, É., & Pocheville, A. (2014). Inheritance is where physiology meets evolution. *The Journal of Physiology*, *592*(11), 2307–2317. https://doi.org/10.1113/jphysiol.2014.272096
114. Cavalli, G., & Heard, E. (2019). Advances in epigenetics link genetics to the environment and disease. *Nature*, *571*(7766), 489–499. https://doi.org/10.1038/s41586-019-1411-0
115. Abbott, C. W., Rohac, D. J., Bottom, R. T., Patadia, S., & Huffman, K. J. (2018). Prenatal ethanol exposure and neocortical development: A transgenerational model of FASD. *Cerebral Cortex*, *28*(8), 2908–2921. https://doi.org/10.1093/cercor/bhx168
116. Gillette, R., Son, M. J., Ton, L., Gore, A. C., & Crews, D. (2018). Passing experiences on to future generations: Endocrine disruptors and transgenerational inheritance of epimutations in brain and sperm. *Epigenetics*, *13*(10–11), 1106–1126. https://doi.org/10.1080/15592294.2018.1543506
117. Thorson, J. L. M., Beck, D., Maamar, M. B., Nilsson, E. E., McBirney, M., & Skinner, M. K. (2020). Epigenome-wide association study for atrazine induced transgenerational DNA methylation and histone retention sperm epigenetic biomarkers for disease. *PLoS ONE*, *15*(12), e0239380. https://doi.org/10.1371/journal.pone.0239380
118. West-Eberhard, M. J. (Oxford 2003). *Developmental plasticity and evolution*. Oxford University Press.
119. Richards, C. L., Bossdorf, O., & Pigliucci, M. (2010). What role does heritable epigenetic variation play in phenotypic evolution? *BioScience*, *60*(3), 232–237. https://doi.org/10.1525/bio.2010.60.3.9
120. Kohl, K. D., Sadowska, E. T., Rudolf, A. M., Dearing, M. D., & Koteja, P. (2016). Experimental evolution on a wild mammal species results in modifications of gut microbial communities. *Frontiers in Microbiology*, *7*, 634. https://doi.org/10.3389/fmicb.2016.00634
121. McNamara, M. P., Singleton, J. M., Cadney, M. D., Ruegger, P. M., Borneman, J., & Garland, T. Jr. (2021). Early-life effects of juvenile Western diet and exercise on adult gut microbiome composition in mice. *Journal of Experimental Biology*, *224*(4), jeb239699. https://doi.org/10.1242/jeb.239699
122. Kohl, K. D. (2017). An introductory "How-to" Guide for incorporating microbiome research into integrative and comparative biology. *Integrative and Comparative Biology*, *57*(4), 674–681. https://doi.org/10.1093/icb/icx013
123. Schwartz, T. S. (2020). The promises and the challenges of integrating multi-omics and systems biology in comparative stress biology. *Integrative and Comparative Biology*, *60*(1), 89–97. https://doi.org/10.1093/icb/icaa026
124. Hein, A. M., Hou, C., & Gillooly, J. F. (2012). Energetic and biomechanical constraints on animal migration distance. *Ecology Letters*, *15*(2), 104–110. https://doi.org/10.1111/j.1461-0248.2011.01714.x
125. Feder, M. E. (2002). Plant and animal physiological ecology, comparative physiology/biochemistry, and evolutionary physiology: Opportunities for synergy: An introduction to the Symposium1. *Integrative and Comparative Biology*, *42*(3), 409–414. https://doi.org/10.1093/icb/42.3.409
126. Herrel, A., Podos, J., Vanhooydonck, B., & Hendry, A. P. (2009). Force–velocity trade-off in Darwin's finch jaw function: A biomechanical basis for ecological speciation? *Functional Ecology*, *23*(1), 119–125. https://doi.org/10.1111/j.1365-2435.2008.01494.x
127. Higham, T. E., Rogers, S. M., Langerhans, R. B., Jamniczky, H. A., Lauder, G. V., Stewart, W. J., Martin, C. H., & Reznick, D. N. (2016). Speciation through the lens of biomechanics: Locomotion, prey capture and reproductive isolation. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1838), 20161294. https://doi.org/10.1098/rspb.2016.1294
128. Miles, M. C., Goller, F., & Fuxjager, M. J. (2018). Physiological constraint on acrobatic courtship behavior underlies rapid sympatric speciation in bearded manakins. *eLife*, *7*, e40630. https://doi.org/10.7554/eLife.40630
129. Lovegrove, B. G. (2019). Obligatory nocturnalism in triassic archaic mammals: Preservation of sperm quality? *Physiological and Biochemical Zoology*, *92*(6), 544–553. https://doi.org/10.1086/705440
130. de Oliveira Neves, A. C., & Galván, I. (2020). Models for human porphyrias: Have animals in the wild been overlooked? *BioEssays*, *42*(12), 2000155. https://doi.org/10.1002/bies.202000155
131. Gudin, C. (Paris 2003). *Une histoire naturelle de la séduction*. Éditions du Seuil.
132. Abram, P. K., Guerra-Grenier, E., Després-Einspenner, M.-L., Ito, S., Wakamatsu, K., Boivin, G., & Brodeur, J. (2015). An insect with selective control of egg coloration. *Current Biology*, *25*, 2007–2011.
133. Matsuno, T., Ohkubo, M., Toriiminami, Y., Tsushima, M., Sakaguchi, S., Minami, T., & Maoka, T. (1999). Carotenoids in food chain between freshwater fish and aquatic insects. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *124*, 341–345.
134. Galano, A., Vargas, R., & Martínez, A. (2010). Carotenoids can act as antioxidants by oxidizing the superoxide radical anion. *Physical Chemistry Chemical Physics*, *12*, 193–200.
135. Powers, M. J., & Hill, G. E. (2022). A review and assessment of the shared-pathway hypothesis for the maintenance of signal honesty in red ketocarotenoid-based coloration. *Integrative and Comparative Biology*, In press. https://doi.org/10.1093/icb/icab056.
136. Moore, M. R. (2009). An historical introduction to porphyrin and chlorophyll synthesis. In M. J. Warren & A. G. Smith (Eds.), *Tetrapyrroles: Birth, life and death* (pp. 1–28). Landes Bioscience and Springer Science+Business Media.
137. Pamenter, M. E., Hall, J. E., Tanabe, Y., & Simonson, T. S. (2020). Cross-species insights into genomic adaptations to hypoxia. *Frontiers in Genetics*, *11*, 743. https://doi.org/10.3389/fgene.2020.00743
138. Storz, J. F. (2021). High-altitude adaptation: Mechanistic Insights from integrated genomics and physiology. *Molecular Biology and Evolution*, *38*(7), 2677–2691. https://doi.org/10.1093/molbev/msab064

139. Storz, J. F., & Cheviron, Z. A. (2021). Physiological genomics of adaptation to high-altitude hypoxia. *Annual Review of Animal Biosciences*, *9*(1), 149–171. https://doi.org/10.1146/annurev-animal-072820-102736
140. Beall, C. M., Cavalleri, G. L., Deng, L., Elston, R. C., Gao, Y., Knight, J., Li, C., Li, J. C., Liang, Y., McCormack, M., Montgomery, H. E., Pan, H., Robbins, P. A., Shianna, K. V., Tam, S. C., Tsering, N., Veeramah, K. R., Wang, W., Wangdui, P., … Zheng, Y. T. (2010). Natural selection on EPAS1 (HIF2α) associated with low hemoglobin concentration in Tibetan highlanders. *Proceedings of the National Academy of Sciences*, *107*(25), 11459. https://doi.org/10.1073/pnas.1002443107
141. Ma, Y.-F., Han, X.-M., Huang, C.-P., Zhong, L., Adeola, A. C., Irwin, D. M., Xie, H.-B., & Zhang, Y.-P. (2019). Population genomics analysis revealed origin and high-altitude adaptation of Tibetan pigs. *Scientific Reports*, *9*(1), 11463. https://doi.org/10.1038/s41598-019-47711-6
142. Graham, A. M., & McCracken, K. G. (2019). Convergent evolution on the hypoxia-inducible factor (HIF) pathway genes EGLN1 and EPAS1 in high-altitude ducks. *Heredity*, *122*(6), 819–832. https://doi.org/10.1038/s41437-018-0173-z
143. Li, J.-T., Gao, Y.-D., Xie, L., Deng, C., Shi, P., Guan, M.-L., Huang, S., Ren, J.-L., Wu, D.-D., Ding, L., Huang, Z.-Y., Nie, H., Humphreys, D. P., Hillis, D. M., Wang, W.-Z., & Zhang, Y.-P. (2018). Comparative genomic investigation of high-elevation adaptation in ectothermic snakes. *Proceedings of the National Academy of Sciences*, *115*(33), 8406–8411. https://doi.org/10.1073/pnas.1805348115
144. Chen, J., Shen, Y., Wang, J., Ouyang, G., Kang, J., Lv, W., Yang, L., & He, S. (2020). Analysis of multiplicity of hypoxia-inducible factors in the evolution of Triplophysa fish (*Osteichthyes: Nemacheilinae*) reveals hypoxic environments adaptation to Tibetan plateau. *Frontiers in Genetics*, *11*, 433. https://doi.org/10.3389/fgene.2020.00433
145. Qiu, Q., Zhang, G., Ma, T., Qian, W., Wang, J., Ye, Z., Cao, C., Hu, Q., Kim, J., Larkin, D. M., Auvil, L., Capitanu, B., Ma, J., Lewin, H. A., Qian, X., Lang, Y., Zhou, R., Wang, L., Wang, K., … Liu, J. (2012). The yak genome and adaptation to life at high altitude. *Nature Genetics*, *44*(8), 946–949. https://doi.org/10.1038/ng.2343
146. Yang, W., Qi, Y., & Fu, J. (2014). Exploring the genetic basis of adaptation to high elevations in reptiles: A comparative transcriptome analysis of two toad-headed Agamas. (Genus Phrynocephalus). *PLoS ONE*, *9*(11), e112218. https://doi.org/10.1371/journal.pone.0112218
147. Chao, Y., Xia, M., Wu, R., Chen, Q., Zheng, Z., & Qi, D. (2019). Molecular characterization and expression changes of cytoglobin genes in response to hypoxia in a Tibetan schizothoracine fish, *Schizopygopsis pylzovi*. *Fish Physiology and Biochemistry*, *45*(3), 863–872. https://doi.org/10.1007/s10695-018-0582-1
148. Storz, J. F., Sabatino, S. J., Hoffmann, F. G., Gering, E. J., Moriyama, H., Ferrand, N., Monteiro, B., & Nachman, M. W. (2007). The molecular basis of high-altitude adaptation in deer mice. *PLoS Genetics*, *3*(3), e45. https://doi.org/10.1371/journal.pgen.0030045
149. Natarajan, C., Jendroszek, A., Kumar, A., Weber, R. E., Tame, J. R. H., Fago, A., & Storz, J. F. (2018). Molecular basis of hemoglobin adaptation in the high-flying bar-headed goose. *PLoS Genetics*, *14*(4), e1007331. https://doi.org/10.1371/journal.pgen.1007331
150. Zhang, Q.-L., Zhang, L., Yang, X.-Z., Wang, X.-T., Li, X.-P., Wang, J., Chen, J.-Y., & Yuan, M.-L. (2017). Comparative transcriptomic analysis of Tibetan Gynaephora to explore the genetic basis of insect adaptation to divergent altitude environments. *Scientific Reports*, *7*(1), 16972. https://doi.org/10.1038/s41598-017-17051-4
151. Scott, G. R., Schulte, P. M., Egginton, S., Scott, A. L. M., Richards, J. G., & Milsom, W. K. (2011). Molecular evolution of cytochrome c oxidase underlies high-altitude adaptation in the bar-headed goose. *Molecular Biology and Evolution*, *28*(1), 351–363. https://doi.org/10.1093/molbev/msq205
152. Ding, D., Liu, G., Hou, L., Gui, W., Chen, B., & Kang, L. (2018). Genetic variation in PTPN1 contributes to metabolic adaptation to high-altitude hypoxia in Tibetan migratory locusts. *Nature Communications*, *9*(1), 4991. https://doi.org/10.1038/s41467-018-07529-8
153. Rodríguez-Martínez, S., Márquez, R., Inácio, Â., & Galván, I. (2019). Changes in melanocyte RNA and DNA methylation favour pheomelanin synthesis and may avoid systemic oxidative stress after dietary cysteine supplementation in birds. *Molecular Ecology*, *28*(5), 1030–1042. https://doi.org/10.1111/mec.15024
154. Galván, I. (2018). Pre dation risk determines pigmentation phenotype in nuthatches by melanin-related gene expression effects. *Journal of Evolutionary Biology*, *31*(12), 1760–1771. https://doi.org/10.1111/jeb.13379
155. Galván, I., & Rodríguez-Martínez, S. (2018). Females mate with males with diminished pheomelanin-based coloration in the Eurasian nuthatch *Sitta europaea*. *Journal of Avian Biology*, *49*(9), e01854. https://doi.org/10.1111/jav.01854

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