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

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Abstract

More than 30 years ago, synergistic effects of the interaction between evolutionary biology and physiology gave rise to the field of 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 inheritance that regulates physiological traits may also arise in coming years, which would represent an overlooked enhancer of natural selection to explain physiological evolution. Synergistic interactions at these intersections and other areas will lead to a novel understanding of organismal biology.

Enhanced Abstract

Background

More than 30 years ago, synergistic effects of the interaction between evolutionary biology and physiology gave rise to the new field of evolutionary physiology. Explanations for how organisms work do not require knowledge of their evolutionary origin nor of ecological circumstances that cause ongoing natural or sexual selection, but it was realized that considering these factors provided a more integrative view of organismal biology. This view caused comparative and ecological 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. This cross-fertilization resulted in the development and wide application of phylogenetic comparative analyses that allowed separation of the effects of common ancestry from recent adaptation. Selection experiments elucidated diverse pathways of physiological evolution that were then traced to genetic variants. The genetic/genomic revolution also fostered studies of the molecular basis of physiological variation and evolutionary adaptation in natural populaitons of a wide range of organisms, including humans.

Advances

We briefly review the significant progress that has occurred as a consequence of reciprocal illumination between evolutionary biology and physiology since the advent of evolutionary physiology three decades ago. Evolutionary biology has led physiology back to its original aim of providing a comprehensive view of organismal function and human pathology, by providing a rigorous framework within which to conduct comparisons among species. The study of wild animals beyond traditional laboratory models has revealed

performance in many physiological processes superior to humans, which has facilitated the understanding of the synthesis of biologically active compounds, including peptide-processing enzymes and estrogens for medical purposes. This has also inspired new strategies for combating various disorders and conditions, such as porphyrias, macular degeneration, aging, and tissue loss. Studies on wild animals have informed our understanding of the endogenous contraints limiting physiological adaptation to the environment. At the same time, physiology has shown potential to explain both the evolutionary origin of particular traits, such as honest communication mechanisms, and the process of genetic adaptation, which directly depends on physiological variation. Physiological explanations of adaptations are exemplified by experiments on birds, where fluctuating environmental factors have been shown to induce epigenetic modifications in genes of cysteine metabolism, in their expression, and in the production of associated pigments that affect the external body appearance. These changes have been proven physiologically adaptive as they offer protection from environmental stressors, and the associated changes in pigmentation are open to sexual selection. These types of studies reveal a nexus between environment, physiology and evolution. In fact, advances in the molecular basis of epigenetic modifications have revealed these as a source of phenotypic plasticity in multiple organisms, underpinning previously unsuspected mechanisms of physiological adaptation to the environment.

Outlook

Notwithstanding its many successes, we argue that evolutionary physiology has yet to reach its full potential. Here we propose new avenues that may lead to unexpected advances in this field. Viewing physiological adaptations in wild, non-model species of animals as potential solutions to human diseases offers enormous possibilities for biomedicine, and may lead to novel perceptions of the human condition. New evidence of

environmentally induced transgenerational epigenetic inheritance that regulates physiological traits may also arise in coming years, which would represent an overlooked enhancer of natural selection to explain physiological evolution. Synergistic interactions at these intersections and other areas will lead to a novel understanding of organismal biology.

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*–*7*)). 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 (*8*–*13*). We believe, however, that evolutionary physiology, as originally outlined (*14*–*16*), 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 (*7*, *17*–*24*).

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 sexual selection in the wild, as dictated by ecological

127 circumstances (e.g., see (*25*–*27*)). Rather, understanding the origin and maintenance of
128 traits and characteristics at all levels of biological organization is the provenance of
129 evolutionary biology. Understanding the evolution of physiological mechanisms equals
130 understanding their causes at both proximate and ultimate levels (*25*), which promotes
131 comprehension of factors that facilitate and constrain evolutionary processes (e.g., see
132 (*27*–*31*)), as well as the causes of and solutions to human pathologies (*32*, *33*). The
133 influence of rigorous evolutionary thinking on physiology has resulted in the rise of
134 evolutionary medicine (*34*, *35*), but it has also led to more sophisticated analyses and
135 approaches in non-medical physiology.

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

143

144 Three well-established approaches

145

146 Phylogenetically informed comparative studies

147 Of the various tools that evolutionary physiology has adopted from evolutionary
148 biology, none has had a greater impact than the use of phylogenetic comparative methods
149 (*36*). These approaches were in rapid development when comparative and ecological
150 physiologists were first encouraged to take advantage of them (e.g., (*15*, *37*–*42*)).
151 Formalized procedures for phylogenetically based statistical analyses (*36*, *43*, *44*) have
152 caused a mini-revolution in evolutionary biology, and this has been reflected in

153 comparative physiology (*3*, *45*). Phylogenetically informed analyses have improved, for
154 example, the understanding of aging mechanisms in animals (*46*, *47*), the evolution of
155 endothermy (*48*) and of diving (*49*), and the diversity of photosynthesis types in plants
156 (*50*).
157
158 ## Selection experiments and experimental evolution
159 Moving from macroevolutionary to microevolutionary analyses, selection
160 experiments and experimental evolution in both laboratory and field settings have
161 provided unique insights regarding adaption, coadaptation, and the genetic/genomic
162 mechanisms of evolutionary change (*4*, *51*, *52*). For example, Lenski and colleagues had
163 maintained 12 populations of *E. coli* in the laboratory for more than 25 years and 60,000
164 generations (*53*, *54*). Among various results, they discovered a trade-off between growth
165 on glucose and acetate involving two metabolic "ecotypes" that can stably coexist. Each
166 ecotype has a competitive advantage when rare, which it loses when it becomes more
167 common.
168 As a vertebrate example, Garland and colleagues began replicated artificial
169 selection for voluntary exercise behavior in laboratory house mice in 1993, and the
170 experiment has now proceeded for more than 90 generations. Numerous correlated
171 responses have been documented at the levels of both motivation for physical activity and
172 ability to sustain aerobic exercise, including increased endurance and maximal oxygen
173 consumption during forced exercise, changes in muscle size and fiber type composition,
174 skeletal alterations, endocrine changes, and brain changes (*55*–*59*).
175
176 ## Evolutionary genetics and genomics
177 The low cost of sequencing has led to a genetic and genomic revolution that has
178 found its way into all approaches and areas of biology, including selection experiments

and experimental evolution (*60*–*62*), the study of adaptation in natural populations (*63*–*67*), and the study of human morphological and physiological evolution (*68*, *69*). 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 (*15*, *70*)). 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 (*71*).

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 (*72*). 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., (*73*)), 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 (*74*), 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 (*33*, *75*). For example, Mathew Kluger's studies of thermoregulation and behavioral fever in lizards (*76*–*78*) and Fred White's studies of acid-base balance during

hypothermia in reptiles (references in (*15*)) 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 (*33*, *79*, *80*)). Beyond species that produce substances such as venoms that are useful for the preparation of drugs (*81*), many wild non-model species present physiological processes that are similar to those of humans, and sometimes have superior performance. We refer to species whose maintenance and breeding in captivity are not as easily achievable as in laboratory animals. Such species represent a great potential to offer solutions to human pathology.

Examples include many species of frogs that store in the skin an extraordinary diversity of biologically active peptides at high concentrations, many of which 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 (*82*). 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 (*83*). 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 (*84*). 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

231 conditions (*85*). In humans, congenital erythropoietic porphyria is caused by a defect in
232 uroporphyrinogen III synthase that leads to a similar low enzymatic activity and
233 uroporphyrin I overproduction, which allowed researchers to propose the fox squirrel as an
234 animal model for this disease (*86*). The Honduran white bat (*Ectophylla alba*) has recently
235 been reported as the first mammal that has evolved the physiological capacity to esterify
236 and deposit high amounts of carotenoid pigments in the skin, thus constituting a model
237 that may help to improve the assimilation of carotenoids in humans and avoid macular
238 degeneration (*87*). The study of all these species was not primarily motivated by
239 physiological questions. Instead, these studies were started by researchers investigating
240 evolutionary and ecological aspects of these species (e.g., (*88*)), and interest in
241 physiology arose later.

242 Non-model species have also contributed to our understanding of the process of
243 aging. How animals age is determined by the failures of physiological processes.
244 Understanding why different physiological processes fail faster or slower in different
245 organisms can bring insight to the evolution of cellular protection and repair processes, as
246 well as the evolution of life histories (*89*, *90*). For example, a comparison across 18
247 rodents species with lifespan ranging from two (mice) to 30 years (beavers) determined
248 that the ability to repair double-strand breaks in DNA (via SIRT6) is a tight correlate of long
249 lifespan (*91*). Although humans express IGF2 at high levels as adults (*92*), biomedical
250 rodent models do not (*93*, *94*), resulting in this hormone being understudied in the context
251 of senescence. Recent studies demonstrate that reptiles and birds express IGF2 at high
252 levels in adulthood (*95*, *96*), similar to humans, providing new model systems to study the
253 physiological effects of this hormone.

254 Many non-model species exhibit regenerative abilities that are coveted by the
255 biomedical community (*97*, *98*). Within vertebrates, there is considerable diversity in the
256 degree to which a species can regenerate tissue and which types of tissues can be

regenerated (*99*), 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 (*97*, *100*–*102*), eyes (*103*), and internal organs (*104*, *105*). In newts, for example, after the complete removal of the lens from the eye, the lens can be *de novo* regenerated from the dorsal iris cells that can undergo a dedifferentiation process (*106*). 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 (*107*). 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 regenerate thorough hyperplasia and hypertrophy to accommodate the physiological demands of processing the meal (*108*–*110*). In contrast, significant regeneration in adult mammals is largely restricted to the liver (*111*) and antler regeneration in deer (*112*), 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 (*113*–*115*).

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 (*116*). A remarkable example is the fox squirrel mentioned above, which was 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 (*85*, *86*), 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 (*117*), although this may often require the development of new tools (e.g., see (*118*, *119*)).

Physiological characteristics affect the capacity for physiological adaptation

The concept of adaptation is central to biology, but the term is used in two distinct ways (*120–122*). First, "evolutionary adaptation" refers to cross-generational changes in the allele frequencies of populations in response to natural selection. Second, "physiological adaptation" refers 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 (the beneficial acclimation hypothesis: (*123–125*)). Some 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 (*126*).

Evolutionary studies that include examination of physiological adaptation illustrate the potential to discover the mechanisms by which organisms cope with fluctuating environments as well as directional climate change (e.g., (*127–129*)). 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 (*130*). 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 (*130*). 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 (*131*). 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 (*132*).

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 (*133*). Also, the capacity of melanins to absorb solar radiation means that the pigmentation pattern of animals partly determines their ability to cope with thermal stress; thus, darker birds may be somewhat limited from occupying environments with high temperatures (*134*). 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 (*71*, *120*, *135*). 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 (*136*).

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 (*137*). 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 (*138*). Signal honesty is closely related to the concept of individual quality. As stated in the handicap principle, a cornerstone of behavioral ecology, ethe production of large (expensive) signals is limited to high-quality signalers because low-quality ones cannot afford the costs derived from signal production (*139*). 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 (*140*, *141*). 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 (*142*, *143*). 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 makes 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 (*142*, *143*).

The experiments on the signaling system of male house sparrows exemplify how the details of the machinery controling 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 (*144*), 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

Evolutionary physiology can play a fundamental role in identifying the mechanisms by which adaptations arise (e.g., see (*8*, *15*, *16*, *31*, *67*, *71*, *120*, *126*, *145–147*)). As evolutionary adaptations directly depend on physiology, physiology has the potential to provide a conjectural background to understand them (e.g., see (*148*, *149*)). Examples of this include simple economical ideas applied to understand the evolution of pigmentation phenotypes (*150*) and theories of sensory cue integration helping to understand the evolution of perception capacity (*151*).

Research methods in physiology have always strongly relied on experimental manipulations of biological processes (*152*) 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., (*9*, *64*, *153–156*)), 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. Dog are a great example, having been under artificial selection for thousands of years, resulting in breeds defined by form, function, and behavior (*157*). The evolutionary response to selection that targeted growth, strength, and body size has involved the insulin and insulin-like signaling (IIS) network (*158*, *159*). 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 (*160*, *161*). 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 (*159*). 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

411 resulting in the correlated phenotypes of small body, small litters, and longer lifespans
412 relative to the larger breeds (e.g. Mastiff) (*158*, *159*, *162*, *163*).

413 Sensory systems also provide clear illustrations of how physiological knowledge
414 helps us to understand evolutionary adaptations (see also examples in (*8*)). In the most
415 general sense, the sensory perception of organisms depends on their physiological
416 allocation to the systems involved. This physiological allocation differs among species
417 and even individuals, but this does not mean that perceived objects are only the product of
418 neuronal activity nor that the brain produces realistic models without capturing reality itself.
419 The chromatic experience of animals, for example, is not only a type of neural state or
420 process, but also reflects to a large degree the color of the objects being perceived as a
421 physical attribute of these objects. Color perception is thus the combination of an
422 objective and a subjective experience, the former greatly influencing the
423 ecological/evolutionary implications of perceiving the color of given objects (*164*). Color
424 interpretation in some evolutionary studies has been made in a way that gives much
425 weight to the subjective component of color perception (e.g., 'Color is not an inherent
426 property of the object; it is a product of the brain of the animal perceiving the object',
427 (*165*)), but it must be remembered that color is also a physical attribute of the objects.
428 Considering the objective component of color perception may be useful in interspecific
429 comparisons of animal coloration, and thus provide clues into the adaptiveness of color
430 traits. Indeed, human vision can detect much of the variation in bird coloration in the
431 visible range and also provide a valid proxy for avian perception of such color traits as
432 sexual dichromatism (*166*, *167*), suggesting that considering color exclusively as a neural
433 state may be an incomplete view. That color resides in both the objects being perceived
434 and in the brain of the perceiving animals is known in neuroscience since the 1990s,
435 notably through the work of Francisco J. Varela and others (*164*, *168*). Considering this

436 theoretical background of sensory physiology may therefore help in gaining a deeper
437 insight into the adaptive value of color phenotypes.
438
439 ## The role of epigenetics in physiological and evolutionary adaptation
440 Use of the term "epigenetic" has changed over time, but currently it usually refers to
441 chemical modifications on the DNA, RNA, or associated proteins that regulate the genome
442 (and the expression of genes), without changes in the DNA sequence (*169*, *170*). In the
443 last 30 years, the molecular basis for how epigenetic modifications can result in
444 phenotypic plasticity has been revealed in different organisms (*171–173*). Such
445 epigenetic plasticity can be induced by environmental factors, and such alterations have
446 been identified as important mechanisms underlying physiological adaptation of
447 organisms to a diversity of environments (*174*, *175*). Epigenetic plasticity thus acts as a
448 potential enhancer of physiological adaptation. Examples of this are studies of teleost fish
449 where a concerted role for DNA methylation and histone modifications induced by
450 hypoxia, thermal stress, osmotic challenges, and starvation has been shown to regulate
451 the expression of genes involved in, respectively, apoptosis, folate metabolism, osmotic
452 stress transcription factors and autophagy (*176*). Such changes may facilitate
453 physiological adaptations to environmental conditions and, in some cases, also affect
454 external phenotypic traits on which selection can act (for another example, see **Box 1**).
455 If the epigenetic plasticity is inherited, this may provide an additional, accelerated
456 pathway for evolutionary adaptation (*177*, *178*). For example, the response to hypoxia
457 involves epigenetic modifications to open the chromatin at regulatory elements to allow
458 transcription of genes, such as EPAS1 (*179*). A change in the timing of this chromatin
459 opening during the hypoxia response appears be part of the adaptive response to hypoxia
460 in Tibetan relative to Han human populations (*179*).

Epigenetics modifications can be transmitted across generations and be important component of preparing the next generation for the parental environment. In these cases of "epigenetic inheritance," the modifications that occur in reponse to environmental conditions in the parents are passed on to offspring or even subsequent generations (*180–183*). The importance of epigenetic inheritance in the context of evolution is strongly debated (*184–186*). Intergenerational inheritance (where the embryo and its germline is directly exposed to the parental environment while *in utero*/*in ovo*) is quite common. In this context, Danchin and Pocheville (*187*) 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 accomodation 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 (*188*), particularly in sexually reproducing species where the germline is seperated 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 (*183*). Although evidence of environmentally induced transgenerational epigenetic inheritance in vertebrates is limited, it has been experimentally demonstrated in some species, including rodents (*189*) and humans (*188*), 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 transgenerationally inherited to at least three generations

(*190*, *191*). 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 (*192*).

Epigenetic modifications regulate physiological responses that selection acts on. This link beween physiology and evolution agrees with West-Eberhard's (*193*) 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 phenotypic evolution, because epigenetic variation may facilitate the role of natural selection in overcoming stochastic loss of new heritable variants (*194*).

Concluding Remarks and Future Directions

We are optimistic about the future of evolutionary physiology. [In passing, we note that current college students may well experience "evolution" as one of the core principles in physiology education (*195*), although perhaps at the bottom of the priority list (*196*).] Among various possibilities, we believe that the way forward needs to embrace other subfields (e.g., Figure 1) that often do not view themselves as part of "evolutionary physiology," including evolutionary endocrinology, evolutionary biochemistry, ecoimmunology, and functional genomics. For example, many studies in comparative biomechanics/functional morphology/ecomorphology attempt to elucidate evolutionary patterns or processes (e.g., (*148*, *149*, *197–203*)), but they typically do not include physiological functions in their analyses (but see (*204*)). 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.

In addition, entirely new areas of research have emerged in the last 30 years, including the microbiome (*205*, *206*). The coming years should provide many opportunities for evolutionary physiology to contribute towards understanding the coevolution of organisms and their microbiota (e.g., (*207*–*209*)).

In addition to the experimental method, statistical analyses are essential to detect patterns in physiological data (*152*). 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., (*21*, *210*, *211*)).

Another way forward is greater integration among studies on different types of organisms (e.g., animals, plants, bacteria) in the hopes of reaching a more general understanding of physiological evolution. Although this suggestion has been made before (e.g., (*212*, *213*)), little cross-organism integration is evident in the current literature. 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 down across all organisms, suggesting common functional roles (*214*).

Finally, we encourage more studies that attempt to tie physiological traits in a causal way to evolutionary adaptation, constraint, and diversification (e.g., (*48*, *49*, *199*, *201*, *204*, *215*–*219*)). In this regard, the view of adaptations in wild animals as potential solutions to human diseases, as pointed out by Singer (*33*) a decade ago, still holds huge potential for biomedical scientists to explore (*220*). An attraction to adaptations in the wild from a variety of fields outside of evolutionary biology may lead to novel perceptions of the

538 human condition, and perhaps new strategies for combating disease and injuries, in the
539 next years.

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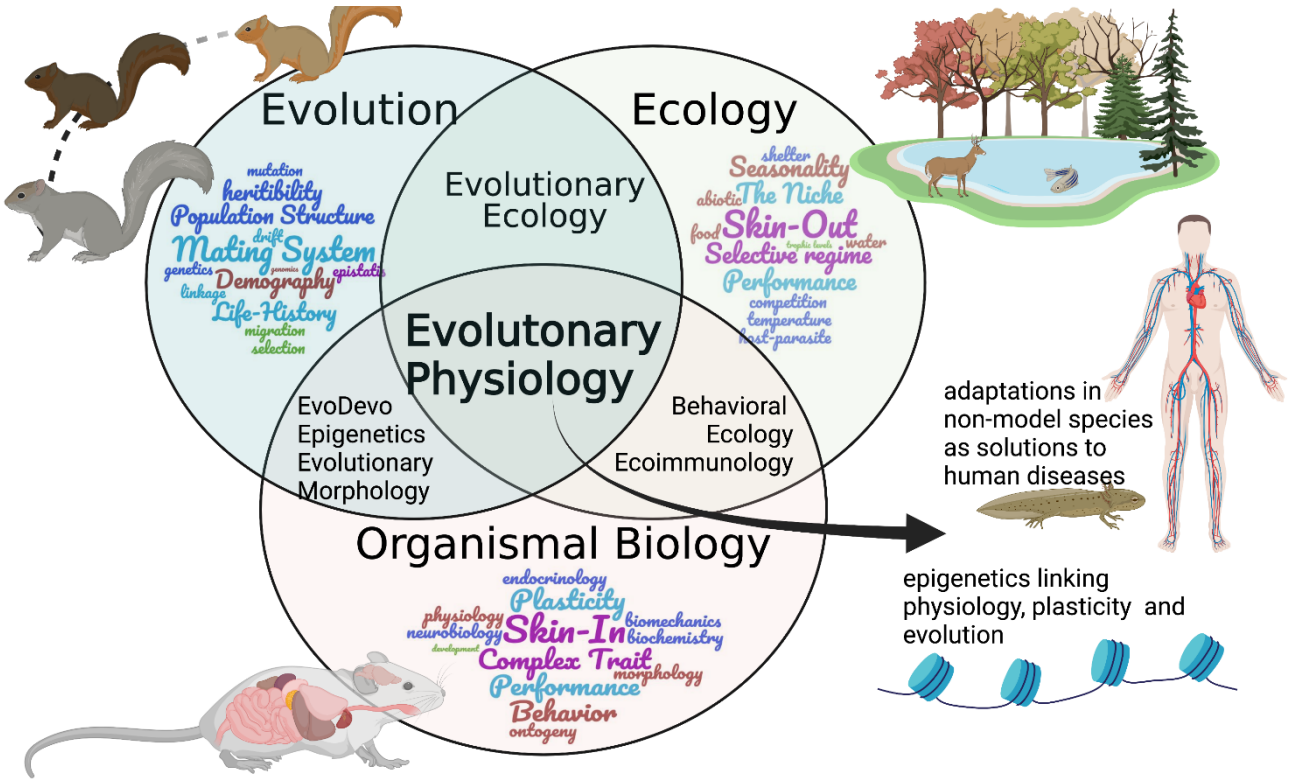
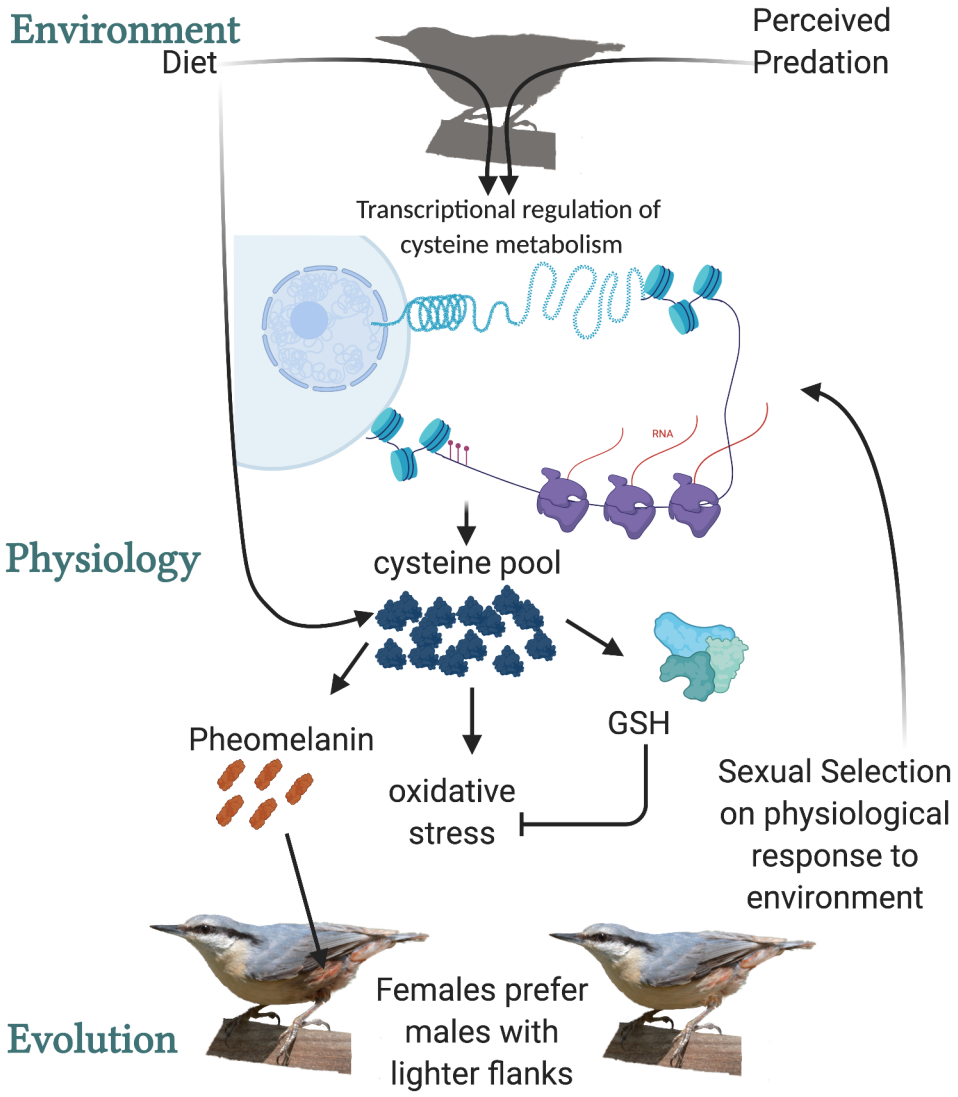


Figure 1. Evolutionary physiology resides in the intersection of evolution, ecology, and organismal biology.



1218

1219 **Box 1. Environmental epigenetics of pigmentation genes.** Synthesis of the pigment
1220 pheomelanin in melanocytes of developing Eurasian nuthatches *Sitta europaea* is
1221 regulated, in part, by some genes of cysteine metabolism whose expression can be
1222 affected by two environmental factors defined at the top of the diagram: availability of
1223 cysteine in the diet and perceived predation risk. At the physiological level, excess dietary
1224 cysteine alters the expression of these genes by modifying DNA and RNA methylation
1225 levels, promoting pheomelanin synthesis and resulting in flank plumage patches of
1226 increased pigmentation intensity. In this way, the epigenetic changes promote usage of

1227 the excess cysteine for pheomelanin synthesis. The excess cysteine would otherwise
1228 cause cellular oxidative stress, thus the epigenetic changes are physiologically adaptive in
1229 an environment that is rich in dietary cysteine (*221*). Higher levels of perceived predation
1230 risk produce opposite changes in the expression of these genes, limiting pheomelanin
1231 synthesis and resulting in flanks of reduced pigmentation intensity. As cysteine is a
1232 constituent amino acid of the main cellular antioxidant (i.e., glutathione, GSH), these
1233 changes may also be adaptive because they increase the antioxidant capacity and thus
1234 allow the animal to avoid oxidative stress/damage expected from predation risk (*222*). At
1235 the level of evolution, these changes in the pigmentation of nuthatches have
1236 consequences for sexual selection, as adult females mate preferentially with males
1237 showing flanks of reduced pigmentation intensity (*223*). Thus, the physiological
1238 responses to perceived predation risk lead to reduced pigmentation intensity, in addition to
1239 providing immediate physiological benefits, and are probably favored by sexual selection.