

EVOLUTIONARY PHYSIOLOGY¹

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■ **Abstract** Evolutionary physiology represents an explicit fusion of two complementary approaches: evolution and physiology. Stimulated by four major intellectual and methodological developments (explicit consideration of diverse evolutionary mechanisms, phylogenetic approaches, incorporation of the perspectives and tools of evolutionary genetics and selection studies, and generalization of molecular techniques to exotic organisms), this field achieved prominence during the past decade. It addresses three major questions regarding physiological evolution: (a) What are the historical, ecological, and phylogenetic patterns of physiological evolution? (b) How important are and were each of the known evolutionary processes (natural selection, sexual selection, drift, constraint, genetic coupling/hitchhiking, and others) in engendering or limiting physiological evolution? and (c) How do the genotype, phenotype, physiological performance, and fitness interact in influencing one another's future values? To answer these questions, evolutionary physiology examines extant and historical variation and diversity, standing genetic and phenotypic variability in populations, and past and ongoing natural selection in the wild. Also, it manipulates genotypes, phenotypes, and environments of evolving populations in the laboratory and field. Thus, evolutionary physiology represents the infusion of paradigms, techniques, and approaches of evolutionary biology, genetics, and systematics into physiology. The reciprocal infusion of physiological approaches into evolutionary biology and systematics can likewise have great value and is a future goal.

...each level [of biological integration] offers unique problems and insights,
and each level finds its explanations of mechanism in the levels below,
and its significance in the levels above.

George A. Bartholomew (7, p. 8)

¹Dedicated to George Bartholomew on the occasion of his 80th birthday.

INTRODUCTION

Evolutionary physiology represents an explicit fusion of two complementary approaches: evolution and physiology. This field has been the subject of several recent reviews and symposia (18, 25, 53, 72, and papers following 72). Rather than recapitulating those here, we focus on how perspectives and approaches infused from evolutionary biology, genetics, and systematics are changing the scope and nature of physiological studies and how, in turn, physiological perspectives and approaches may contribute to evolutionary biology.

HOW EVOLUTIONARY BIOLOGY, EVOLUTIONARY AND POPULATION GENETICS, AND SYSTEMATICS HAVE INFORMED PHYSIOLOGY

Physiology has often incorporated both ecological and evolutionary perspectives. Since the field's inception, many physiologists have sought to understand how the environment affects function and how function has undergone evolutionary modification. The field's principal focus, however, has been on the mechanisms of function and description of their variation in cells, species, and environments. Beginning in the 1980s, a complementary focus, "evolutionary physiology," achieved prominence. Evolutionary physiology investigates (*a*) the evolutionary mechanisms underlying or constraining diversification of physiological mechanisms and (*b*) the discrete historical patterns of physiological evolution (104). While physiology has always readily borrowed from other disciplines, evolutionary physiology represents a novel importation of theory, paradigms, techniques, and questions from genetics, population biology, evolutionary biology, and systematics. The variables examined, such as metabolic rate, locomotor speed, thermoregulatory performance, and the physiological mechanisms that underlie them, are those that comparative physiology and physiological ecology have measured for decades. What is new is the analytical context of these studies.

Attributing evolutionary thinking in physiology (and vice versa) exclusively to evolutionary physiology is clearly erroneous. Evolutionary biologists such as Sewall Wright, Theodosius Dobzhansky, and Richard Goldschmidt had major research foci on "physiological genetics" (115, 144, 168). Large numbers of Russian physiologists contributed to a field that they had entitled, in 1914, "evolutionary physiology" (3, 155, 186) and that continues to flourish largely independent of the evolutionary physiology reviewed here. Moreover, many classical studies of comparative and environmental physiology (e.g. 7, 10, 167, 181, 182,) interpreted patterns as the outcome of adaptive evolution (138). Also, physiologists have long exploited the results of evolution in choosing the most appropriate species for investigation of physiological problems (116, 123, 208). Still other investigations, either in advance of or independent of evolutionary physiology, included an

explicit evolutionary analysis of the physiological impact of specific gene alleles (28, 29, 79, 114, 124, 135–137, 211). Nonetheless, physiology and evolutionary biology often remained isolated from one another in the past. For example, standard textbooks on evolutionary biology (67) had little or no discussion of the evolution of physiological traits (more recent editions of this textbook discuss physiological evolution).

But in the late 1970s and early 1980s, several developments (both conceptual and methodological) elicited a substantially increased infusion of evolutionary thinking into the physiological sciences. Undoubtedly the most influential of these was an unwillingness to assume that all patterns of biological traits result from adaptation (174)—a development that achieved its greatest notoriety in a paper by Gould & Lewontin (82). This polemic not only criticized prevailing standards of evidence of adaptation, but also challenged comparative biologists to scrutinize their assumptions about the operation of evolution. One outcome was physiologists' explicit consideration of evolutionary hypotheses alternative to adaptation (e.g. nonadaptive forces such as drift or constraint) (15, 36), which in turn, necessitated explicit examinations of evolution in physiological studies.

A second, contemporaneous intellectual development was the recognition of the nonindependence of species as analytical units for comparative studies. Because of their phylogenetic relatedness, species share common ancestry and common genes to greater or lesser degrees. Consequently, conclusions based on traditional statistical methodologies became suspect. This recognition came first from behavioral ecologists undertaking comparative studies of behavior (e.g. 35). This awareness began to spread to other fields of comparative biology through the primary stimulus of Felsenstein's 1985 paper (62), which not only clearly described the problem but also provided a robust analytical solution (phylogenetically independent contrasts) for the analysis of comparative data. Indeed, immediately studies in evolutionary physiology began to incorporate a phylogenetic perspective. This new perspective not only influenced how investigators compare species, but also motivated comparative biologists to analyze evolutionary patterns from an ancestor-descendent perspective (103, 104). Thus it was a conceptual—and not merely a statistical—advance.

A third factor was the incorporation of the perspectives and tools of evolutionary genetics and selection studies (both field and laboratory (1–2, 125)). The impact here was fundamental. Evolutionary genetics contributed explicit expectations of the patterns of genetic and phenotypic variation that were necessary and/or sufficient conditions for physiological evolution by natural selection. Physiologists could then sample populations to ascertain whether these conditions were met. Also, whereas most previous evolutionary studies (at least those in physiology and morphology) investigated the results of past evolution, evolutionary genetics and selection studies enabled the monitoring of evolution in contemporary populations (i.e. in real time) and prediction of future evolutionary trajectories. In addition, these approaches permitted the design and execution of rigorous evolutionary experiments in which the experimentalist could manipulate putative selective forces,

replicate treatments, and observe outcomes. Thus many assertions and hypotheses concerning physiological evolution, which had previously been only speculative, became falsifiable.

Finally, the techniques of molecular biology and genetic engineering escaped the constraints of standard laboratory model organisms and became broadly applicable to many of the diverse species of interest to evolutionary physiologists (55). While this development is only now yielding information of genomic and proteomic scope, even at the beginning it provided information with either deeper insights or far greater ease than had previously been possible.

The field of evolutionary physiology was greatly influenced by all of these developments and quickly exploited them. The first formative steps in the emergence of the field came from a workshop sponsored by the U.S. National Science Foundation, held in Washington, DC, in 1986, which resulted in an edited volume (53). Pough (163) first used the term “evolutionary physiology” to entitle a review of that volume, Diamond (39, 40) rechristened the field, and Garland & Carter (72) soon codified the term to designate the entire emerging area. In 1994, the U.S. National Science Foundation established a formal Program in Ecological and Evolutionary Physiology. The growth since that time, in both number and breadth of the studies encompassed, has been impressive. We provide only a few examples of relevant studies, and refer readers to successive reviews of this growing field (18, 25, 53, 72).

Major Questions in Evolutionary Physiology

By “physiological evolution,” we mean change (or stasis) through time in traits and characters that are typically the subject of physiological studies. These traits may be at diverse levels of biological organization (molecular through organismal, as well as colonial and symbiotic) and may be biochemical, morphological, and/or behavioral as well as strictly physiological (1, 7–10, 50, 72, 74).

Pattern: What Transformations Has Physiology Undergone as Organisms Have Evolved and Diversified and as Their Environments Have Changed? Organisms inhabit a great range of environments, some seemingly inimical to life, and vary extensively in their physiological processes and capacities, morphology, and behavior. Explaining *how* an organism’s phenotype enables it to exploit its environment was a central heuristic of pre-evolutionary physiology (10) and remains important. The resultant explanations are typically environment- or taxon-specific and post hoc (e.g. seasonal changes in insulation in arctic mammals, increasing cutaneous Na^+ influx and expression of Na^+ channels by amphibians in extremely hypotonic media, and facultative anaerobiosis in animals undergoing temporary hypoxia or anoxia). [Vogel (205) likened such research programs to “shooting at a wall and drawing targets around the bullet holes.”] Evolutionary physiology, by contrast, more often focuses on the discrete transformations occurring during physiological evolution; for example, how and why did endothermic

vertebrates arise from ectothermic ancestors (19, 23, 95, 176, 177)? Alternatively, evolutionary physiology often proceeds from a priori hypotheses or predictions about the distribution of phenotypes in relationship to specific ecological, evolutionary, or genetic regimes and uses taxon-independence (i.e. convergent and/or parallel evolution) and meta-analyses to test the robustness of these predictions. For example, a large body of theory predicts that phenotypic plasticity should be greater in variable than in constant environments (180). This theory would be supported if, in all three of the foregoing examples (arctic mammals, amphibians, and facultative anaerobes), the magnitude of physiological plasticity were correlated with the magnitude of environmental variation. If the theory were supported, then subsidiary predictions would arise concerning the cost of phenotypic plasticity and the magnitude of genetic variability underlying it. Similar issues concern the rate of physiological evolution and how this rate differs in stressful vs benign environments and central vs peripheral populations of a species (99), physiological niche breadth in specialized vs generalized species (78), and the closeness of the match between organismal phenotypes and environment.

Evolutionary physiology and other aspects of physiology are clearly not separate endeavors, but may examine identical phenomena with similar techniques from their different starting points. Mechanistic physiology often makes predictions from principles of physics and/or engineering regarding distributions of phenotypes. These might concern, for example, the nature and diversity of respiratory gas and ion exchangers in air vs water (38), the general features of the design of gas exchangers (162), morphologies of organisms living in high vs low flow regimes (205), and the maximum body temperatures of animals (91, 96, 195). Evolutionary and other physiological approaches perhaps most closely coalesce in studies of physiological optimality. Evolutionary optimality models have a long heritage (184), but within the past 20 years, physiologists have undertaken explicit examinations of whether physiological supply and demand are in fact closely matched ("symmorphosis") or whether overdesign and safety margins are commonplace (39, 40, 219). This issue is still contentious (43, 219).

Process: How Important Have the Known Evolutionary Processes (Natural Selection, Sexual Selection, Drift, Constraint, Genetic Coupling/Hitchhiking, and Others) Been in Engendering or Limiting Physiological Evolution? The Modern Evolutionary Synthesis has long recognized the multiplicity of processes that result in or constrain evolution. Nonetheless, the footprint of one of these (adaptation as the outcome of natural selection) upon physiological diversity has been so manifest that many physiological investigators have understandably focused on it to the near or total exclusion of the others. Stepping back from this focus, evolutionary physiology ideally asks, How much of physiological diversity (or its lack) is due to each of the known evolutionary processes (15)? At a more basic level, can we rigorously deduce the evolutionary processes that led to and/or maintain the extant array of physiological phenotypes? One approach to these questions has been to take from evolutionary theory the conditions that

are necessary and/or sufficient for each evolutionary process to occur (effective population size, genotypic and phenotypic variability, heritability, differential survival/reproduction, and so on) and to survey these conditions in natural or experimental populations with reference to physiological traits. The rigor of this approach will only increase with time as the genetic basis of complex physiological traits becomes better understood with the advent of functional genomics and proteomics.

Another approach is to survey the rate of physiological diversification in taxa separated naturally or experimentally in different environments for known lengths of time. Both approaches, however, are tempered with the realization that the outcomes of evolution are heavily contingent on the genetic and demographic conditions prevailing at its outset.

As explained below, laboratory and experimental evolution studies (77, 173, 175) are especially promising in that the experimentalist can manipulate these starting conditions, run multiple replicated evolutionary trials, and determine the probability of specific evolutionary outcomes directly. Moreover, modern techniques of genetic engineering allow this manipulation to occur at the level of the single gene or even nucleotide, with all other factors controlled. Obviously such work is still not feasible for every species of interest to physiologists. Nonetheless, it permits evolutionary physiologists to address still more significant questions.

First, within the range of feasible evolutionary outcomes, does physiological evolution generally follow only one, a few, or perhaps a multitude of these? If the evolution of a biological lineage were to occur again, would it result in the same, similar, or entirely different results (81)? In other words, are evolutionary trajectories predictable, given similar starting conditions?

Second, what is the importance of neutral evolution, specifically the neutral fixation of traits or genes? Such work collectively may reveal the relative importance of history, adaptation, and chance in the formation of physiological diversity (36, 199–202).

Components: How Do the Genotype, Phenotype, Physiological Performance, and Fitness Interact in Influencing One Another's Future Values? Physiologists have long studied the detailed chain of events that ensue between the reception of a physiological stimulus and the manifestation of its corresponding physiological response, the molecular and cellular components of these events, and the impact of each component on each subsequent element of the chain. The elucidations of signal transduction, homeostatic mechanisms, and neurotransmission, for example, are only a few of the success stories of mechanistic physiology. By contrast, we know much less about the detailed events that ensue between the reception of an ecological or evolutionary stimulus (e.g. stress) by a natural population and the manifestation of the corresponding response (selection, response to selection, extinction, and so on) in terms of physiological traits. Evolutionary physiology strives to discover these connections. The general paradigm is that genes encode

the phenotype, the phenotype determines the performance of organisms in natural environments in response to ecological or evolutionary stimuli, the performance determines the evolutionary fitness of alternative genotypes, and the fitness determines the frequency of genotypes in the next generation, in recursive fashion (1, 7, 61, 72, 74, 102). Determining the details of each step is an ongoing challenge for evolutionary physiologists (61). For genotype to phenotype, for example, what is the genetic basis of complex physiological traits, what is the importance of epigenetic and nongenetic factors in determining physiological phenotypes, and how is the genotypic specification of the phenotype manifested during ontogeny? For phenotype to performance, what exactly is the impact of phenotypic variation on the performance of unrestrained organisms in nature? Mechanistic physiologists are routinely successful in explaining the impact of phenotypic variation on proxies of performance in the laboratory; are these explanations extensible to nature (16)? Similar questions apply to the other links as well. That evolutionary physiologists have one foot in mechanistic physiology and another in evolutionary biology prospectively poises them to make major contributions in this area, where multidisciplinary work is clearly needed.

Analytical Approaches in Evolutionary Physiology

Evolutionary physiology currently exploits two major approaches. The first is to analyze the outcome of “natural experiments”; that is, the extant and paleontological genotypes and phenotypes of evolved organisms, with environment and/or phylogeny (i.e. the genes and traits present at the start of evolution) as variables that have differed in the past. This approach, then, is one of historically based comparison and correlation, and usually best suits (by necessity) studies of physiological evolution among species or higher-level taxa. The second approach is to manipulate genotype, phenotype, and/or environment directly and to observe subsequent evolutionary outcomes.

Phylogenetically Based Comparisons: Examining Extant and Historical Variation and Diversity to Test Hypotheses The statistical analysis of physiological evolution within an explicit phylogenetic framework began only in the mid-1980s but has already become a central paradigm in evolutionary physiology and other comparative fields (76, 94, 104, 133, 141, 206). Phylogenetically based comparative studies analyze physiological and/or ecological variation and covariation against an independently derived phylogeny of the taxa involved. The first of two primary motivations for developing this approach was the realization that the best way to choose species for comparison was with respect to phylogenetic relatedness (104, 206). Many early studies compared very distantly related species (e.g. a hibernating marsupial and a white rat), undoubtedly to increase signal-to-noise ratio, but the results of such studies were inherently ambiguous in the sense that one was comparing apples with oranges (103). The second motivation was a growing awareness that species data are nonindependent, such that conclusions

based on standard statistical models (e.g. regression) were at least suspect and potentially misleading (35, 62). Because of these realizations, comparative biologists and systematists have developed and are developing a variety of new analytical procedures (26, 62, 73, 76, 94, 141) that are leading both to more robust answers to pre-existing research questions and to entirely new questions for physiologists.

In evolutionary physiology, a comparative approach can reveal whether a particular character state (e.g. stenothermy or eurythermy) is ancestral or derived (103, 104), the most likely ancestral condition of a discrete or quantitative trait (75, 104), rates of evolutionary change (103), whether prior evolution of a trait has been necessary and/or sufficient for an ecological or evolutionary outcome, and whether evolution of one trait has evolved in advance of, simultaneously with, or after another (see 26, 62, 76, 84, 94, 133, for a discussion of methods and interpretations). Analyses may concern populations, species, genera, or even higher levels of biological organization or may encompass a combination of organizational levels. Any such analysis requires a minimum of three taxa, in part because of the necessity of incorporating a more distantly related "outgroup" into any comparison (69, 103). Examples of the use of phylogenetic approaches to study the evolution of physiological characters are now very diverse and include thermoregulatory patterns in fish (23, 24), evolution of locomotion in lizards (11, 30), locomotor performance in lizards (132), salinity tolerance in mosquitoes (85), diving physiology of pinnipeds (98, 153), metabolic rate in amphibians (207), dietary modulation in omnivorous birds (140), development in Antarctic birds (48), expression of glycolytic enzymes in fish (161), plasma osmotic concentration in amniotes (75), anaerobic metabolic end products in chordates (178), and nocturnality in geckos (4). These have revealed novel insights. For example, Mottishaw et al (153) showed that "diving bradycardia," long assumed to have evolved in mammals to facilitate diving, likely arose long before the evolution of diving habits. Block et al (24) showed that endothermy has evolved multiple times in fishes (as opposed to evolving only once in a common ancestor of endothermic fishes).

Rigorous phylogenetic approaches to physiological comparisons are not without drawbacks. They require physiological data on multiple species, which can be a formidable barrier for sophisticated mechanistic studies (138), as well as nonphysiological data (i.e. a phylogenetic topology with branch lengths and large numbers of taxa) that may be difficult or even impossible to obtain (e.g. too few taxa may exist for adequate statistical power). In addition, the robustness of any phylogenetic interpretation depends on the hypothesized phylogenetic relationships as well as on the model of evolution underlying the formal statistics (62). Moreover, phylogenetic approaches can establish only correlation and not causation (69, 104, 129); unfortunately, historical patterns can seldom be tested by direct and replicated experimental manipulation (104). A different kind of drawback associated with phylogenetic approaches has been their unfortunate tendency to impede, if not to stifle, comparative studies that employ traditional, nonphylogenetic approaches ["phylogenetic correctness" (73, p. 279)]. We advocate both greater

tolerance when investigators eschew phylogenetic approaches with good reason, and explicit discussion of the rationale for “phylogenetic incorrectness” when it is warranted.

Despite difficulties of implementation, the incorporation of phylogenetic approaches appears positive for evolutionary physiology. Such approaches have improved the choice of species, even when evolutionary considerations are not of primary concern. Moreover, phylogenetic considerations enhance the reliability of statistical inference, the kinds of evolutionary questions asked of physiological data, and the evolutionary relevance and robustness of comparative physiological studies.

Although comparative methods often infer ancestral physiological states from those of extant organisms, paleobiological studies may infer the physiological states of long-dead organisms from fossil anatomy (176, 177). Sometimes the essential features of such analyses (e.g. shared characters and parsimony) are implicit rather than explicit, and a phylogeny with appropriate character mapping may or may not be available. The form of the argument, however, is essentially parsimonious and phylogenetic (19). An excellent example of this approach is Hillenius’ study of endothermy in the mammalian lineage (97), in which evidence of nasal turbinates in fossil skulls suggests endothermy in theriocephalian therapsids. This result indicates that endothermy probably evolved before the emergence of mammals as a group and was therefore an inheritance rather than a novel evolutionary development in Mammalia.

Dormant stages of organisms can sometimes be resuscitated so that physiological states of recent “ancestors” can be determined directly. Many organisms in nature have diapausing or dormant eggs (87, 88) or seeds (130, 146) that persist in the environment for long periods (90). When resurrected, such time travelers can be compared with contemporary individuals in a common garden. Thus, ancestral and derived stocks from nature are compared directly, much the same way as with laboratory stocks with certain species [e.g. *Escherichia coli*, *Caenorhabditis elegans*, and *Drosophila melanogaster* (18)]. A fascinating example comes from a time-series analysis of *Daphnia* spp. from a lake that experienced eutrophication (and associated increases in cyanobacteria) in the 1960s and 1970s. After hatching dormant eggs of *Daphnia* spp. from sequential time periods, Hairston et al (89) found that *Daphnia* spp. rapidly evolved increased resistance to cyanobacteria in their diets during eutrophication. Future physiological studies can potentially explore the evolved mechanisms underlying such increased resistance.

Standing Genetic and Phenotypic Variability in Populations Evolutionary biology has established that the modes, rates, and outcomes of physiological evolution will depend critically on the pre-existing variation within the evolving population (or higher taxon), its heritability, and its relation to fitness. Because comparative studies are not sufficient to address these issues (129), evolutionary physiologists have increasingly attempted to characterize variation, heritability, and fitness consequences directly. These studies have used two types of approach. In

the physiology-to-genetics approach (2), evolutionary physiologists assume that physiological traits of interest have a genetic basis and seek to establish the genetic properties of this basis without ascertaining the identities and natures of the actual underlying genes. At the simplest level, an evolutionary physiologist might ask whether sprint speed varies or is invariant within a lizard population, with the outcome having implications for the evolutionary malleability of sprint speed in the population. This approach differs from that of typical physiology, in which an investigator might regard exceptionally fast or slow individuals as atypical and therefore exclude them from analysis (14). At a more complex level, evolutionary physiologists have applied the techniques and insights of quantitative genetics (see 49, 137) to estimate the heritability of diverse functional characters (both organismal and suborganismal), characterize phenotypic vs genotypic variation in traits, establish the relationship between traits and fitness, and quantify evolution. Locomotor performance (e.g. 42, 197, 203) and its mechanistic basis (e.g. 70, 71) have received particular attention.

A second approach, that of gene to physiology (2), examines the performance and fitness implications of discrete genes (or the products they encode) on organismal characters (114, 145, 212, 213): for example, lactate dehydrogenase on locomotor performance (164–166, 183) and temperature adaptation (63, 101, 196); hemoglobin on oxygen transport capacity (194); aminopeptidase on osmoregulatory ability (113, 114); alcohol dehydrogenase on ethanol tolerance (64–66); and phosphoglucose isomerase (31, 215–218), glycerol-3-phosphate dehydrogenase (6, 37, 126, 127), juvenile hormone esterase (86, 222–226), and troponin expression (139) on flight capacity.

Perhaps the initial motivation of these studies was from the discovery of unexpectedly large amounts of genetic variation in natural populations and attempts to test subsequent theory that most such variation was selectively neutral (114, 212, 213). This motivation continues as a debate on how allelic variation in genes of large effect can persist in populations without selection eliminating them (212, 213) and has its counterpart in the functional genomics of human disease. A repeated finding of gene-to-physiology studies, that the genes under investigation are often nonneutral, continues to provoke much interest. An additional goal, coincident with the emergence of evolutionary physiology, has been to rigorously explore the recursive relationships of genes to traits to organismal performance to fitness to genes against the background that these relationships can be nonstraightforward and nonobvious (61).

From its inception, a criticism of the gene-to-physiology approach has been that it is not sufficient to explain variation in complex physiological traits, which must be the product of numerous interacting genes. One response has been the exploitation of metabolic network theory to explain how single-gene variation can affect complex metabolic pathways and entire physiologies (212). Another is that advances in developmental biology, cell biology, and molecular physiology of model systems are revealing precisely how single gene changes can be manifested in whole-organism variation and how such variations have evolved (204).

The physiology-to-gene and gene-to-physiology approaches obviously converge with one another and with scientific progress in general. Already, the increasing technical sophistication of DNA arrays permits simultaneous screening of all genes of an organism or tissue for changes in expression in response to physiological change (33, 142, 198). Another development is increasing ease of precise genetic mapping, facilitating the description of quantitative trait loci. Quantitative trait loci, in turn, can establish or reject the polygeny of traits, permit the formal genetic analysis of quantitative traits, and facilitate the direct sequencing of the genes at the quantitative trait loci. On a more theoretical level, the two approaches bear on global genetic issues concerning organismal performance, such as pleiotropic effects on the evolution of physiological characters (41, 83, 185), the role of overall heterozygosity in influencing performance and fitness (112, 150), the effects of genetic correlation on rates of evolutionary change (2), and the relationship between numbers of deleterious mutations and overall viability and fitness (46, 154).

Past and Ongoing Natural Selection in the Wild Evolutionary biologists have developed a variety of methods to study the presence, intensity, and directionality of natural selection on character traits in wild populations (reviewed in 47, 151). Such studies of selection on physiological traits would be enormously valuable to our understanding of their evolution and current ecological importance. To date, however, relatively few such studies have been attempted (47), partly because of the difficulty of measuring physiological variables on very large numbers of animals. Available studies have concentrated on locomotor capacities (e.g. 16, 106, 148, 149, 209) and have sometimes demonstrated, through differential survival, that traits such as maximal speed and endurance contribute to fitness in natural populations. Such work forms an important bridge between the many laboratory studies on activity capacity and its mechanistic bases and its actual ecological and evolutionary significance. Future field selection studies may involve manipulation of such putative selective factors as predator density to test evolutionary hypotheses experimentally.

Although the above studies document patterns of selection on traits, they must be repeated over time to document actual evolutionary responses to selection. An alternative way to document short-term evolutionary responses involves monitoring shifts in species recently introduced into novel environments (159). An example is *Drosophila subobscura*, which was introduced from the Old World into North and South America in the late 1970s and spread rapidly on both continents (5). In only one to two decades, the North American populations have evolved a latitudinal wing-length cline, which parallels that in native Old World populations (105).

Experimental Approaches: Manipulating Variation and Diversity to Test Hypotheses Although natural experiments most clearly reflect the actual past and ongoing processes of physiological evolution as they occur in the wild, they

have limitations. They can be poorly controlled, their sampling of organisms and environmental factors can be biased, they are often nonreplicated and unrepeatable, and the signal-to-noise ratio of the trait under investigation can be insubstantial. Most importantly, genetic, epigenetic, and phenotypic linkages among traits mean that seldom will a gene, trait, or suite of genes/traits of interest vary in isolation without covariation of interacting elements, thereby confounding the interpretation of natural experiments. Therefore, as in most other areas of the life sciences, evolutionary physiology includes a strong component of intentional manipulation or engineering of genes or traits of interest, but with outcomes typically characterized in relation to performance and/or fitness. A long heritage of experimental physiology has provided a wealth of surgical and pharmacological techniques to manipulate the traits themselves as well as diverse means of manipulating specific internal or external environmental variables. Joining these are laboratory and field techniques from experimental ecology for manipulating the number and variety of interacting organisms and their environments, experimental evolution approaches, and genetic engineering of allelic series, knock-outs, knock-ins, complementation, rescue, etc. Most of these techniques themselves have the drawback of manifesting processes that seldom, if ever, occur in nature and thus, by themselves, can reveal little about the likelihood or potential for physiological evolution in nature. For this reason, the complementary analysis of natural and deliberate experiments may yield the greatest insights (59). Thus, for example, whereas the analysis of *Ldh-B* genotype in natural populations of the fish *Fundulus* can implicate *Ldh-B* genotype as a likely component of fitness, it can neither establish that *Ldh-B* genotype is sufficient for variation in fitness nor exclude the influence of linked genes (165). Such demonstrations require manipulations such as the replacement of a genotype's allozyme with an alternative allozyme via microinjection (165). Whether evolution is likely to achieve comparable manipulations, however, can emerge only from study of the natural *Fundulus* populations.

Phenotypic Engineering A powerful approach to studying the mechanistic and adaptive significance of phenotypic characters is to manipulate them directly and subsequently study the performance and/or fitness consequences in the laboratory or the field (190). Such approaches have been termed “allometric engineering” when involving manipulations of body size (192) or as “phenotypic engineering” for more general manipulations (107, 108). An example of the latter involves manipulating butterfly wing color (by altering developmental temperature or by painting) and then monitoring field-released individuals for heat balance, mating success, and survival (e.g. 109–111). Other investigators have engineered intestinal transport capacity (27, 92), milk production capacity (93), hormone status (107, 108), and total body size (187, 191, 221). Performance consequences of such changes can be examined either in staged encounters in the laboratory or in animals released into natural populations. These latter studies then become a type of natural selection study, as discussed below.

Phenotypic engineering permits direct experimental tests of the significance of a character and is thus a valuable tool to expand upon comparative studies. It can not only expand the magnitude of variation in traits beyond that occurring in nature (188, 193), but also verify causal, mechanistic links between traits [e.g. between body size and performance (192)].

A complementary approach involves manipulating the environment rather than the phenotype. The classical methods involve reciprocal transplants (34, 157) and common gardens (189). The latter are widely used to factor out environmental effects in studies of geographic or interspecies variation (68, 69).

Genetic Engineering An evolutionary physiologist may choose to manipulate the gene(s) encoding a trait rather than manipulating the trait directly (51, 169). Such manipulations have long been possible, if not simple, in genetically tractable organisms such as yeast, *Drosophila*, and many bacteria. A few examples for bacteria concern the consequences of excess tryptophan synthesis for growth (44), the effects of lactose permease and beta-galactosidase expression on metabolic flux (45), and interactions between structural and regulatory genes that control expression of an efflux protein and their effects on growth in the presence and absence of an antibiotic (128, 156). Advances in molecular biology already (or will soon) make such manipulations feasible at any level down to the individual nucleotide, and in an expanding diversity of experimental species.

One example concerns the heat-shock genes, whose expression was correlated with inducible stress tolerance and thus were implicated as a mechanism of stress tolerance (52, 57). In yeast, this implication was first confirmed for the single-copy gene *HSP104* when deleting the gene abolished a significant component of inducible thermotolerance, and reintroducing the gene restored inducible thermotolerance to control levels (179). Moreover, site-directed mutagenesis of a single nucleotide in a critical region of this gene was sufficient to abolish inducible thermotolerance, whereas control mutagenesis had no effect (158). For other heat-shock genes and in more complex multicellular eukaryotes (where multiple copies of some heat-shock genes have evolved), more complex techniques are necessary (e.g. 220). Increasing the haploid copy number of the *hsp70* gene from 5 to 11 in *Drosophila*, for example, dramatically increases the resistance of whole larvae and pupae to natural thermal stress (56, 171, 172), and gut-specific expression of the *hsp70* gene off a heterologous promoter protects the gut against heat-induced damage during feeding (60). Many additional transgenic manipulations of heat-shock genes are now available and confirm the suspected consequences of these genes for fitness (52, 57). Similar genetic manipulations will undoubtedly be one of the most exciting and productive areas in future studies of evolutionary physiology.

Selection Studies Selection experiments on populations of organisms in the laboratory, long a mainstay of geneticists and evolutionary biologists, have also been incorporated into evolutionary physiology (18, 77). These permit the direct observation of evolutionary change resulting from an alteration in the selective environment

and allow physiologists to experiment on physiological evolution itself. Laboratory experimentation facilitates control of the environment and selective factors, maintenance of nonselected populations, and replication of experimental groups, permitting a rigorous statistical evaluation of the evolutionary response.

Experimental selection studies follow three designs: natural selection in the laboratory, artificial truncation selection, and laboratory culling (173). The first manipulates an environmental variable (e.g. temperature or water or nutrient availability) and monitors the consequent changes in replicated experimental populations for many generations. In laboratory natural selection, the experimenter does not directly choose which organisms possessing which characters will be permitted to breed: those that are fittest in the new experimental environment will produce more offspring, irrespective of which characters result in higher fitness. In contrast, artificial truncation selection permits only organisms possessing certain traits to breed. This type of selection is familiar from plant and animal breeding. It requires the a priori establishment of the traits to be selected and the screening of individual organisms prior to reproduction. Laboratory culling creates a selective environment that permits only a small portion of each population to survive to reproduce. Choice of the type of selection experiment depends on the principal experimental goal. Testing of hypotheses concerning environmental adaptation would probably employ either natural selection in the laboratory or laboratory culling, while the production of organisms with certain combinations of physiological traits might be done through artificial truncation selection.

Perhaps the greatest utility of selection experiments in evolutionary physiology is their ability to test general predictions concerning physiological evolution, specifically in regard to patterns and consequences of evolutionary adaptation (12, 13, 18). Many formal and informal models of environmental adaptation begin with assumptions concerning evolutionary constraints and patterns. For example, in regard to adaptation to the thermal environment, most models (e.g. 78, 131) assume that adaptation to one thermal environment requires loss of fitness in other environments (trade-off) and consequent changes in the range of temperatures tolerated (niche shift). The ability to do direct experiments changes these assumptions into testable hypotheses, subject to falsification. In regard to these particular assumptions, experimental studies on evolutionary adaptation of bacterial populations to different temperatures in general fail to support them (17, 152). While the expected pattern of fitness trade-off and niche shift occurred in one thermal environment, it was completely absent in four others. Some studies of *Drosophila*, however, are consistent with these assumptions (32, 160). Such results question facile assertions and assumptions concerning the course that evolution will or must take and require revision of evolutionary models that incorporate such assumptions.

Conclusion: On Finding the Right Organism for Study Although we present the foregoing analytical approaches individually, they clearly have the greatest power when used in complementary fashion (59). Each approach is best suited to

reveal certain aspects of physiological evolution but may ignore or obscure others. To return to the example of heat-shock proteins in *Drosophila*, genetic engineering can unambiguously establish the phenotypic and fitness consequences of variation in Hsp70 protein expression (56, 58, 60, 117, 119–121, 171–172), but it can establish neither the likelihood that such variation will evolve, persist, or even occur in natural populations nor the ecological relevance of such variation (59). That goal requires direct studies of variation (20, 118, 122) and its ecology (54, 171, 172) in natural populations, but these, in turn, cannot unambiguously establish the physiological phenotypes of the genes under study. Similarly, comparative studies of past physiological evolution (e.g. the evolution of endothermy or diving bradycardia; see above), descriptive studies of contemporary physiological or morphological evolution (e.g. 105, 134), and experimental or laboratory evolution (see above) each provides special insights. The latter suite of approaches, however, may be most powerful when each approach is applied to a common species or population of interest and/or when new techniques are deployed. For the moment, the joint requirements of these approaches and techniques may be so restrictive as to exclude problems (e.g. the evolution of endothermy or diving bradycardia) and species (e.g. endothermic tuna) of traditional interest to physiologists. Thus, an increasingly common practice in evolutionary physiology has been to study nontraditional (at least for physiologists) models such as *Drosophila*, *E. coli*, and *C. elegans* either alongside or in place of the more traditional exotic subjects of ecological and comparative physiologists. “Choosing the right organism” for study is a hallowed tradition in physiology (116, 123, 208) and is yielding truly surprising outcomes. For example, genomic screens of *E. coli* undergoing experimental evolution at high temperatures suggest that the same genes are evolving in independent evolutionary trials (170). In pursuing such models, approaches, and techniques and in searching for insights from allied fields, evolutionary physiologists are continuing a long tradition of multidisciplinary physiology but in new directions.

HOW EVOLUTIONARY PHYSIOLOGY CAN INFORM EVOLUTIONARY BIOLOGY, EVOLUTIONARY AND POPULATION GENETICS, AND SYSTEMATICS

Much current research in evolutionary biology proceeds without explicit or detailed reference to the mechanisms encoded by the genes. Amechanistic (214) evolutionary biology has several bases, some of which are pragmatic: Statistical analyses of the genotype alone can yield considerable insights into evolution, as can the amechanistic scoring of traits to phylogenetic relationships. Also, incorporating functional analyses into evolutionary studies can be both logistically and intellectually challenging. But other bases of amechanistic evolutionary biology are ideological, stemming from a belief that detailed understanding of the phenotype is irrelevant or insufficient for understanding of evolution, or it can contribute

little. Thus Mayr (143, p. 115) has written: "...the mechanistic approach, although quite indispensable in the study of proximate causations, is usually quite meaningless in the analysis of evolutionary causations," and Gould (80, p. 101) has written "...the flowering of [functional studies of evolution] has yielded a panoply of elegant individual examples and few principles beyond the unenlightening conclusion that animals work well." Watt (214) has analyzed these ideological bases in detail. Clearly, a mechanistic approaches will continue to reward evolutionary biologists for years to come. We disagree, however, with the premise that functional and/or integrative studies have little to offer evolutionary biology. Our purpose here (as also stated in 72, 104, 138, 214) is to emphasize the value that mechanistic approaches can add to the research programs of evolutionary biologists.

The inclusion of mechanistic perspectives is becoming increasingly important and, indeed, necessary in the following areas of evolutionary biology:

1. Understanding the implications of genetic diversity. As evolutionary biologists increasingly examine the actual nucleotide sequences of genes under study and their variation, their principal challenge will be to explain the origin and consequences of such variation. Foreseeably, demonstrating that a given nucleotide is/isn't under selection or comparing a sequence to a null model may no longer represent an acceptable level of proof. Instead, meeting this challenge may require tests of hypotheses of the functional significance of variants, which in turn will require detailed understanding of the function these genes' products perform in intact organisms in natural environments (i.e. an evolutionary and ecological functional genomics).
2. Practical implications of evolutionary theory. While evolutionary biology has historically been the most curiosity-driven of the biological sciences, its bearing is increasing on applied issues of great significance to the national research agenda (147). These include the origin and spread of disease, conservation of biodiversity, global climate change, impact of genetically modified and exotic organisms, and evolutionary paradigms in engineering of drugs, biomaterials, and organisms. The devising of meaningful solutions to such problems will require detailed understanding of mechanisms underlying organismal function (e.g. 22).
3. Environmental influences on evolutionary diversification. Much evolutionary research and interpretation occur in an environmental context. Environmental stress, for example, is a recurrent motif in evolutionary studies (21, 99, 100). Theoretical models and laboratory studies of the role of the environment in evolution, however, are now far in advance of rigorous characterizations of natural environments and their impact on organisms. These disparate aspects must be brought into register.

Clearly, interaction with evolutionary (and nonevolutionary) physiologists could provide the mechanistic expertise for which the foregoing three examples call and could therefore substantially enhance the research programs of evolutionary

biologists. In the past, the different goals and research foci of evolutionary biologists and physiologists have tended to isolate these two communities. The growth of evolutionary physiology, in which these communities focus on common problems and speak a common language, therefore represents a novel opportunity for evolutionary biologists to partner with mechanistic biologists and for physiologists to reciprocate to further the influx of evolutionary thought into their discipline.

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