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Comparative physiologists have two goals. The first is to explain mechanism, the study of how organisms are built functionally, "how animals work" (113). Studies of this type are undertaken at a variety of organizational levels: organismal, organ system, organ, tissue, cell, or molecular and biochemical. Ideally, a study will span several of these, integrating information from lower to higher levels and illuminating mechanisms from higher to lower. The second goal is to explain the diversity of functional systems found among organisms. In contrast to "how" questions, this goal attempts to answer "why" questions: Why is an organism built the way it is and why does it function in a particular way and not in another? How and why do physiological properties change through time?

The first goal entails equilibrium (in Lauder's sense [74]) studies—that is, those which examine the present-day associations among characters and do not undertake causal (in the sense of historical) explanations. In contrast, the second goal requires transformational or evolutionary, explanations and hence must deal, at least implicitly, with the ecology, genetics, and history of the organism and its population. For both goals, a primary reference point is the function of an organism in its natural environment. These goals are not unique to comparative physiology; they are broadly shared among the biological sciences (for example, behavioral science [124]).

In general, comparative physiologists have been much more successful in, and have devoted much more energy to, pursuing the former rather than the latter goal (37). Most of this Handbook is devoted to an examination of mechanism—how various physiological systems function in various animals. Such comparative studies are usually interpreted within a specific evolutionary context, that of adaptation. That is, organisms are asserted to be designed in the ways they are and to function in the ways they do because of natural selection which results in evolutionary change. The principal textbooks in the field (for example, refs. 33, 52, 102, 115) make explicit reference in their titles to the importance of adaptation to comparative physiology, as did the last comparative section of this Handbook (32). Adaptive evolutionary explanations are woven throughout the fabric of the science.

However, the assumption that adaptation is the sole explanation for natural diversity in living systems has been called into question (54, 80, 81, 132, 133) and has become a point of contention among many organismal and evolutionary biologists. The rote application of adaptive explanations for all physiological phenomena has effectively uncoupled comparative (ecological) physiology from evolutionary biology. Evolutionary biologists generally ignore and are ignorant of comparative physiology. Their courses do not discuss the evolution of physiological characters, and major textbooks in evolution (for example, ref. 43) contain virtually no reference to physiological phenomena. Comparative physiology should be a field central to evolutionary biology, given the importance of the functional characters that it studies and its avowed evolutionary orientation. If comparative physiology is to make a serious contribution to evolutionary biology, it must rethink its historical commitment to adaptationism.

An increasing number of studies have tested alternatives to adaptive explanations and examined the evolution of functional and structural characters. A diversity
of approaches is now available for the study of the evolution of physiological traits and mechanisms. Comparative physiologists are now almost uniquely positioned to utilize these approaches to understand how important functional characters have changed and may change through time. The types of organismal character that interest comparative (ecological) physiologists (for example, energy exchange, thermoregulation, and locomotor performance) are precisely those that everyone expects will be of importance to fitness and evolutionary success. Such studies must test (and not assume) the role of adaptation in shaping those characters.

The purpose of this chapter is to examine what we know about the evolution of physiological systems and the role of adaptation in shaping them. First, the several meanings of the term "adaptation" for physiologists are explored and their relevance to character evolution discussed. Then, alternatives to, or constraints on, adaptation are examined. Methods for the study of the evolution of physiological characters in both the field and the laboratory are then discussed, as well as their importance to future directions in comparative physiology.

THE MANY MEANINGS OF "ADAPTATION"

Physiologists and other organismal biologists employ the term "adaptation" to describe an especially wide variety of different biological responses, only some of which actually refer to evolutionary phenomena. Many of these uses are not widely shared with, or understood by, other biologists, a situation that easily may lead to confusion and misunderstanding. This section clarifies the multitude of uses and definitions of this word.

A physiologist or organismal biologist might well use adaptation in any of the following senses: (1) Adaptation can be the alteration of a sensory or nervous response under constant stimulus. The rate of firing of sensory cells or neurons usually changes acutely with the onset or offset of a stimulus. If that stimulus is maintained, the response may either decrease, as in pressure sensation, or increase over time, as in dark accommodation. Both are termed adaptation. (2) Adaptation can be a syndrome of physiological responses to environmental stress. Specifically, this usage refers to the General Adaptation Syndrome (116) in vertebrates, in which a variety of stressors may change the state of having become familiar with surrounding. This usage is colloquial, as in permitting an animal to adapt to experimental conditions. It usually refers to placing an organism in an apparatus or environment prior to beginning experimental measurements to minimize fright or activity. (4) Adaptation can be a functional change in an organism after exposure to new conditions or a new environment. Organisms frequently reorganize their physiological systems, particularly their rate processes, in response to changed conditions. The most familiar of these responses is temperature adaptation (acclimation or aclimatisation), but the term is also used to describe alteration of muscle structure and function to altered stress patterns, changes in osmotic and ionic regulation in different environmental media, etc. The term applies to such changes in both laboratory and natural conditions. The documentation and physiological basis of such plasticity is a particularly popular area of physiological study (61, 100). (5) Adaptation can be the process of improvement of fitness in a population of organisms in response to natural selection. This usage refers to the Darwinian evolutionary process, and it is in this sense that most evolutionary biologists use the term. It assumes that individuals with characteristics favored in their particular environment will reproduce more and that the favored characters will increase in frequency over generations. The literature in evolutionary biology dealing with the intricacies of this concept is large (readers are referred to the following books and articles for a more comprehensive discussion of evolutionary adaptation: 7, 16, 18, 81, 87, 89, 94, 104, 122, 132). (6) Adaptation can involve a character that originated due to natural selection. This usage refers to a structure or function that is the product of the preceding process. In this sense, a nasal salt gland may be an adaptation resulting from the process of adaptation (definition 2) to a saline environment. (7) Adaptation can involve a preexisting character that is beneficial to an organism in a particular environment or circumstance. The distinction between this and the previous definition is whether the character has been a target for selection in the current environment or is a feature that is adventitiously useful. Preadaptation, protoadaptation (44), and exaptation (55) connote ancestral features previously acquired that find a new utility in new circumstances. (8) Adaptation can involve a generally phylogenetically widespread structure or function necessary for, or promoting, survival, a feature of many living systems, sometimes crucial for existence but not evolved in reference to particular environmental circumstances in extant populations. For example, the use of DNA as a genetic material may be considered an adaptation for repro-
Adaptation of a sensory cell (definition 1) or of an organ system (definition 4) may or may not be evolutionary adaptations according to definition 6 or 7. The distinctions among these categories should be kept clearly in mind, and one should not be assumed to imply the other. Investigations of phenotypic adaptation (definitions 1–4) should study both their occurrence and their mechanistic basis (the most usual type of study, for example, refs. 61, 100) and their provision of benefit to the organism (less commonly examined [67]). Interpretations of evolutionary adaptation of physiological and morphological characters and their study are the topic of the remainder of this chapter.

CRITICISMS OF ADAPTIVE INTERPRETATIONS

Adaptation as the explanation for a biological phenomenon is easier to invoke than to demonstrate. For many characters, a satisfactory investigation into evolutionary development may be difficult or impossible. The respondent to this difficulty has often been to claim, rather than to investigate, adaptation. A mechanistic study of a morphological or physiological character frequently is concluded with the assertion of a plausible mechanism through which natural selection might have operated to produce the evolution of the character, generally by contending that the character benefited survival and reproduction. Indeed, the American Journal of Physiology: Regulatory, Integrative and Comparative Physiology specifically encourages such speculation (30). The problem with such assertions is that they often amount to dogmatic appeals to a particular evolutionary mechanism rather than a testable scientific hypothesis. Any such interpretation is only one of a multitude of different possible adaptive scenarios, let alone nonadaptive explanations, for the possession of a particular trait by a particular organism (80). This tendency to claim adaptation as the universal
explanation for biological diversity has evoked critical attack, not from opponents of evolutionary theory but from evolutionary biologists themselves. George Williams (132) maintained that ‘evolutionary adapta-
tion is a special and onerous concept that should not be used unnecessarily’ and discussed several alternative explanations for the possession of biological traits. Lewontin (81) and Gould and Lewontin (54) explicitly challenged the uncritical application of adaptation as a dogmatic assertion. In the latter article, they attacked what they characterized as the ‘adaptationist pro-
gram.’ They elevated the latter to a philosophical approach to biology and an agenda for its study and proceeded to criticize it as failing to consider alternative explanations for the evolutionary development of bio-
lógical characters.

Particular criticism (54, 83) was directed to the asser-
tion of optimality. Optimality approaches have played a prominent role in comparative biology—for example, in ecological energetics (125) and respiratory physiol-
ogy (symmorphosis; 123, 131). Gould and Lewontin (54) maintained that the adaptationist program seeks to demonstrate an optimal design of each organismal character. Any failure to find an optimal design in a particular trait is explained as a necessary compromise with other traits, resulting in an organism that is de-
signed as well as possible. Again, the failure to test these assertions was criticized. The entire approach was characterized (or caricatured) as ‘panglossian,’ a reference from Candide to Dr. Pangloss, who believed that everything happened for the best in this best of all possible worlds. ‘(Quoting Voltaire to lampoon adapta-
tionism is not original to Gould and Lewontin, but dates back to the turn of the century [6]).’

These critiques were widely influential and caused considerable consternation and controversy within the comparative and organismal communities. Why was this issue considered so important and troubling to biologist, such as the irrelevance of integrative or sys-
temic studies in comparison to molecular studies, have not perturbed the community in similar ways. There are several reasons for this concern.

First, these critiques centered on the philosophical and interpretive bases of the field, not on its choice of subject matter. Such fundamental criticisms cannot be dismissed as mere subjective prejudice.

Second, the critiques exposed the rather tenuous connections between comparative biology as com-
monly practiced and evolution. Most comparative biol-
gists believe in the importance of evolution as the grand unifying concept in biology and think that their work both is in accord with it and provides support for it as a science. Gould and Lewontin’s (54) imputa-
tion that adaptive explanations are only rote applica-
tions of articles of faith undercuts the idea of a support-
ive linkage between comparative and evolutionary biology. If any character could unlawfully be explained by adaptation, it could equally well be explained by
some other cause, such as special creation. This is
uncomfortably close to the pre-Darwinian situation, in
which biologists regarded the uniqueness and perfec-
tion of organismal design as evidence of an omnipotent creator. The suggestion that organismal biologists sim-
ply exchanged one religious faith for another under-
mines their evolutionary credentials, or at least suggests that their approach and thinking have not been funda-
mentally altered by evolutionary thought. To be uncon-
ected to evolution is to be disconnected to the central
biological principle.

Third, a particularly disturbing aspect of the critique
was that such assertions of adaptation are not only unjustified but also unscientific. In other words, if
adaptive statements are not presented as hypotheses susceptible to falsification, the enterprise is not science.

What could be a more damning charge to a scientist?

Little wonder then that these critiques, challenging
fundamental values and interpretations of comparative biology, were taken so seriously.

Finally, the criticisms were delivered partly as lam-
poons, characterizing the approach as no more than a
series of ‘just so’ stories that might have been invented by Rudyard Kipling. Mockery perturbs beyond mere
criticism.

The response of the community to this criticism has
been mixed. Some (for example refs. 1, 23, 88, 102,
103, 121, 133), with varying levels of success, continue
to grapple with the concept of adaptation to make it
more palatable to organismal and comparative biolo-
gists. Some have simply ignored the controversy and
continue to operate within an adaptationist frame-
work. Others (for example, 19, 38, 59) have accepted
the essentials of the criticism and have attempted more
direct analyses of the adaptive process. These investiga-
tions examine adaptation as a hypothesis rather than
as an assertion (37) and undertake comparative and
experimental studies on the microevolution of func-
tional characters. The approaches of some of these
studies in evolutionary physiology are presented below, but before that, alternative hypotheses to adaptive ex-
planations are discussed.

ALTERNATIVES TO ADAPTIVE EXPLANATIONS

There are many possible explanations for the posses-
sion of a particular trait or character by an organism.
Adaptation in the sense of definition 5 above is only
one of them. Several categories of alternative explana-
tion, many of them cited by Gould and Lewontin (54),
are discussed below. All involve explanations for the
correlation of phenotypic characters within individuals
or between characters and environmental factors. Inter-
pretation of correlation in comparative studies is al-
ways difficult (52), particularly in regard to unravelling
the basis of the evolution of physiological characters.
Some of these explanations are subject to experimental
testing and thus permit an investigation of alternative
hypotheses to adaptation. Rejection, or at least consid-
eration, of such alternative hypotheses is an important
step in the investigation of the evolution of a character
and its adaptive basis (37).

An initial difficulty, however, lies in the stipulation
of exactly what constitutes a "character" (e.g. "trait"
or "feature") to be investigated. Any organism can
potentially be atomized into a series of thousands of
different traits, each requiring analysis and explana-
tion. In, for instance, the shape of every individual scale
on a fish an individual character of importance is and
it shaped separately by selection or another process?
Or is there a more broadly encompassing character of
interest, of which the shape of a set of scales, or of all
scales, on the fish is only a part? Because allometric,
phenotypic, and genetic correlation (see later under
Genetic Correlation and under Genetic Corre-
lation) are so widespread among different features of
an organism, the latter explanation is more likely.
However, identification of these broader characters is
difficult (101). Even the characteristic nature of single
traits may be in the process of being eliminated, as is presum-
able the case for vestigial organs (for example, non-
functional eyes in cave animals or pelvic girdles in
cetaceans). The trait may be neutral. The trait may even
have a new utility and benefit not present ancestrally,
in which case it is referred to as a preadaptation or
exaptation (55; see ref. 12 for an example).

Analysis of a character within a phylogenetic frame-
work can aid in the determination of its historical and
evolutionary development (19, 21, 22, 49, 59, 64, 75).
For example, the pattern of concordance between the
occupation of a specific environment and the appear-
ance of the character in a group of related taxa can
determine whether the character might be an
evolutionary response to the current environment of
the organism. By way of example, a phylogenetic study
of thermoregulation in Australian scincid lizards (66)
found that the evolution of heat tolerance was coinci-
dent with the occupation of desert environments. This
coincidence argues against high thermotolerance being
a preexisting (ancestral) condition in this group and
supports, but does not confirm, its adaptive nature.

To consider another example, desert-dwelling ring-
tails (Bassariscus astutus, a small carnivore in the rac-
coon family Procyonidae) have a low basal metabolic
rate and a high heat tolerance in comparison to most
other mammals (26). Low basal metabolic rates are
frequently found among desert-dwelling species of
mammal and bird and are generally thought to be
adaptations to environments of high temperature and
low productivity (5). Is this condition in ringtails a
specific adaptation that permitted this population to
occupy a desert environment? A phylogenetically based
examination (26, 48) found that the divergence in
metabolic rates between desert and montane popula-
tions of ringtail is statistically much greater than that
elsewhere in the procyonid lineage. This divergence is
consistent with an adaptive explanation that lower
metabolic rate was evolved in concert with the occupa-
tion of a hot and arid environment.

Analysis of character evolution within a phylogenetic
context may also permit determination of the ancestral
state and the directionality of evolution within any
descendent group. In addition, rates of evolution of
physiological characters may be estimated if phyloge-
netic divergence times are known. (The following refer-
cences are suggested for a discussion of the theory
and methods of such analyses: 7, 19, 39, 40, 46, 50, 59, 64,
75, 86; potential interpretive problems of historically
based adaptive studies are discussed in refs. 42, 76,
and 80.)

2. Developmental Pattern and Constraint

Change, and hence evolution, typically occurs in bio-
logical systems by modification of preexisting struc-
tures. Novelties, including adaptations, must therefore
arise sometime during ontogenetic development as an

I. Historical Inheritance

An organism may possess a character simply because
it was present in its ancestors. It may have no relation
to evolution in the current environment of the popula-
tion. The trait may even be detrimental, if only in
requiring energy for its formation and maintenance. It
may be in the process of being eliminated, as is presum-
ably the case for vestigial organs (for example, non-
functional eyes in cave animals or pelvic girdles in
cetaceans). The trait may be neutral. The trait may even
alteration of some developmental sequence. (An im-
portant exception to this generalization is the endosymbiotic incorporation of exogenous biological material.) Such familiar evolutionary patterns as neoteny and pedomorphosis appear to result from differential rates of maturation of different developmental fields or structures (53). (The role of development in initiating and constraining evolutionary novelty is exquisitely discussed in refs. 53, 59, 127, 132.) The necessity of operating on pre-existing structures and functions acts as a brake on potential evolutionary change. Improved function might be theoretically obtainable but would require such fundamental reworking of the developmental scheme as to be practically impossible.

Ontogenetic development is a complicated series of interactions among genes, gene products, and morphological structures. Timing of the interactions among these elements can have a crucial influence on ultimate shape and function. The problem for evolutionary change is to perturb the development program enough to produce a specific improvement but not so much as to interfere with other orderly interactions necessary to produce a functioning organism. Thus, adaptations frequently involve alterations rather late in the developmental scheme, such as rates of matura-
tion of specific organs or tissues. Alterations early in development may so fundamentally affect diverse structures that development is terminated or the result-
ing organism is not viable or competitive. Thus, early development often remains relatively conservative in comparison to later development.

Characters arising early in the developmental plan may thus be retained in adult organisms without apparent present utility. Some of these, such as the navel (or at least the umbilical cord associated with it), may have had considerable utility during development. Others, such as nipples in male mammals, may not. Other structural features may be rationalized only in terms of the operation of a developmental program, even at the expense of functional capacity in adults. Consider, for instance, the orientation of rods and cones in the vertebrate retina. These light-sensitive cells are pointed toward the pigmented layer and sclera of the eye and inverted with respect to incident illumination coming through the pupil and lens. Consequently, light must pass through several layers of neurons before reaching the photoreceptors, which must then send information back through those neurons, which then pass it back through the photoreceptor layer in the optic nerve. No engineer would have designed such a photosensitive circuit, and its explanation is not to be found in any adaptive function.

Other aspects of the structure, no matter how striking they may appear, may be completely incidental. Con-
sider, for instance, vertebrate blood. Its most apparent feature is its red color and its change in color with oxygenation state, but these are undoubtedly incidental properties arising from the use of iron in the oxygen-binding structure of hemoglobin. It is doubtful that the changing color of blood, convenient as it is for physiologists, was an important or selected factor in the early evolution of vertebrate hemoglobin as an oxygen carrier. Other reasons for blood color need not be invoked, nor is it even a separate character that must be explained. From this point of view, no further explanation, adaptive or otherwise, is required to account for the presence of red blood in vertebrates and blue blood in molluscs. Nor is it of adaptive importance that bones are white or that bile is green. It is not an adaptive feature that bones break when put under too much stress. These are correlated properties that require no further justification or explanation.

4. Phenotypic Size Correlation

Various traits, be they structural or functional, may be extensively correlated within an organism. For in-
stance, individuals having the largest heart size may also have the largest wings. This correlation may be a reflection of common genetic influence on these features (see later under Genetic Correlation, below) and/or a phenotypic association between characters (101). If the characters are phenotypically correlated, selec-
tion on one will affect the other and a priori it may be impossible to tell which is an adaptation and which is incidental to its design. Those that are crucial to its function will be taken into account in its construction, but unimportant factors will not be subjected to a designer's scrutiny. Properties such as density, thermal coefficient of expansion, or melting point may be im-
portant design criteria in some circumstances and irre-
relevant in others. Nevertheless, any structural material will possess a discrete value for the entire suite of physical properties.

Biological structures are no different. The designing agent may have been selection, but selection will have operated only on features of functional importance. Other aspects of the structure, no matter how striking they may appear, may be completely incidental. Con-
sider, for instance, vertebrate blood. Its most apparent feature is its red color and its change in color with oxygenation state, but these are undoubtedly incidental properties arising from the use of iron in the oxygen-binding structure of hemoglobin. It is doubtful that the changing color of blood, convenient as it is for physiologists, was an important or selected factor in the early evolution of vertebrate hemoglobin as an oxygen carrier. Other reasons for blood color need not be invoked, nor is it even a separate character that must be explained. From this point of view, no further explanation, adaptive or otherwise, is required to account for the presence of red blood in vertebrates and blue blood in molluscs. Nor is it of adaptive importance that bones are white or that bile is green. It is not an adaptive feature that bones break when put under too much stress. These are correlated properties that require no further justification or explanation.
the case for heart size and large wings if selection favored endurance, both might be adaptive characters. However, it is also possible that only one might be of importance and the covariation of the second might falsely be assumed to be evidence of a functional association between the traits.

The most common source of phenotypic correlation is covariation of characters with size. Nearly all structural and functional characters vary with body size (24, 114). Bigger organisms generally have bigger organs so that a correlation between heart and wing size might reflect nothing more than body size heterogeneity within the population. Further, differential reproduction by individuals with large hearts and/or wings might also be misinterpreted as being due to either of these factors. In fact, selection may have been on body size itself or on performance related to body size independent of, or even in spite of, heart and wing size. Clearly size-related phenotypic correlation may greatly complicate any analysis of the functional or evolutionary importance of a single character. Therefore, attempts are commonly made to eliminate size effects. Frequently, however, the relationship between a character and body size is not straightforward. Departure from geometric similarity, in which linear elements scale as the cube root of volume, is termed allometry and is common in biological systems (34, 96, 105, 114). The structural and functional bases of the allometric scaling of most organismal characters, such as the scaling of basal metabolic rate to the three-quarter power of mass, are poorly understood and sometimes contentiously debated (60). Attempts to eliminate the influence of size by dividing by mass (for example, mass-specific metabolic rate), or any other allometric exponent assumed a priori, may fail to remove the effect of size completely. This failure may lead to incorrect functional or evolutionary conclusions because of remaining, though weaker, size correlations. One method for reducing the influence of size and other size-related characters is analysis of size-corrected residuals (8, 45, 59). A residual analysis may help eliminate body size as an explanation for the possession, or significance, of a character but does not eliminate the more general problem of disentangling other phenotypic correlations from evolutionary analyses.

5. Genetic Correlation
Two dissimilar phenotypic characters may share a common genetic basis. This genetic correlation can be generated by two different mechanisms. The first and most common is pleiotropy, in which a single gene affects more than one trait. For instance, a allele that increases testosterone secretion might be expected to influence numerous physiological, morphological, and behavioral characters. Second, a genetic association among traits may result from linkage disequilibrium, in which alleles at two loci are statistically associated and do not recombine randomly. The values of traits resulting from each locus thus tend to be associated with each other. Genetic correlations produced by either of these mechanisms have similar consequences. If selection favors one of the correlated characters, the second may also increase in the population, even if it is neutral or disadvantageous. In the latter case, the balance of selection coefficients will determine the directionality and rapidity of evolution. (For discussions of the effect of genetic correlation on the evolution of physiological traits, see refs. 3, 101).

The problem with genetic correlation as a confounding factor in evolutionary analysis is that breeding experiments are necessary to know whether traits are genetically correlated. Comparative phenotypic analyses alone will not permit determination of whether a trait stands by itself or is genetically correlated with other factors (2, 40, 69, 101). For many organisms of interest to physiologists, breeding experiments are very difficult or even impossible. Consequently, much of what we know about genetic correlations comes from experiments on laboratory-maintained organisms, such as mice or fruit flies. The results of these studies should give physiologists cause for serious concern about the genetic independence of the characters they study.

6. Physiological Correlation
As an example, consider the extensive and well-controlled experiments of Rose, Service, and their coworkers (reviewed in refs. 57, 107) on life history selection in Drosophila melanogaster. In the laboratory, selection for both early- and late-life reproduction was imposed on replicated populations. As anticipated, the pattern of reproduction evolved in response to this selection, as did longevity, later-reproducing flies living longer. In addition, however, a suite of diverse physiological characters also evolved in these experiments, including flight ability, desiccation tolerance, tolerance to ethanol vapor, and starvation resistance. Breeding experiments demonstrated that some of these physiological traits are genetically correlated with age-specific reproduction, the factor on which selection was imposed. In another set of experiments (63), selection on physiological characters in Drosophila led to associated changes in life-history characters.

Given these underlying genetic associations, it would not be correct to abstract one of these traits in a natural population and conclude that selection had operated adaptively in its formation. If, for example, one detected a low rate, or great tolerance, of water loss in a desert population of Drosophila, the temptation might be irresistible to conclude that selection had operated...
on that character. In fact, however, a reproductive or other life-history character may have been the target of selection and desiccation resistance may have been only a genetically correlated response.

6. Chance Fixation
In considering evolution of a physiological character, we generally think of selection on standing genetic variability in a large, outbreeding population. Under such conditions, it is highly unlikely that the frequency of deleterious characters will increase or become fixed in the population. Natural mating populations, however, may be neither large nor randomly breeding. If effective population size is small (for example, under 100), an allele may increase and even become fixed in the population by chance alone. This problem is exacerbated by fluctuations in these already low population sizes, which may occasionally drop to very few breeding individuals (a bottleneck). Probability of random fixation of an allele and its attendant traits increases greatly in such situations. Further, any propensity within such small populations toward nonrandom mating or an unequal sex ratio of breeding individuals within the population further reduces the effective population size and increases the likelihood of genetic drift. In general, we do not know effective population size in natural demes of different organisms or the importance of drift in influencing the distribution of alleles and traits within them. Drift, as opposed to adaptation, fulfills the second goal of organismal and comparative biology—to explain how organisms came to be the way they are, not just how they work in the here and now. Examining physiological processes from an evolutionary viewpoint can give us valuable new perspectives. For example, physiological studies have traditionally emphasized central tendency (for example, averages or regression values) and to look upon individual variation as an unfortunate and suspect complicating factor (8). From an evolutionary perspective, however, individual variation is a prime precondition for the operation of selection and possible evolution of a trait within a population. Individual variation from this viewpoint is something of interest in its own right. New questions then become apparent in regard to this variability: What are its correlated properties among individuals? Is the trait inherited? Is it an important trait? For instance, in regard to maximal locomotor speed, what, physiologically and morphologically, makes a fast individual fast? Is being fast an inherited trait? Is it important to be fast? Similar questions could be asked about urine-concentrating ability, digestive efficiency, maximal oxygen consumption, etc. These questions arise directly from an interest in studying the evolution of organismal characters.

Here I consider two different sorts of studies evolutionary physiology. The first concerns conclusions about comparisons of characters among different populations, species, genera, or higher taxa, examining the endpoints of different phylogenetic lineages. This is the more familiar kind of comparative study. The second type investigates the process of evolution, the origin and operation of selection and other processes on characters, within a single population. The former may be considered macroevolutionary and the latter, microevolutionary. The former concentrates more on pattern analysis, the latter on analysis of adaptive process. The former, usually of necessity, involves relatively few individuals (for example, a dozen) in each taxon; the latter requires observations on hundreds of individuals or more. (For discussions of interrelationships between intra- and interpopulation or -species studies, see refs. 34, 47, 49, 111.)

Macroevolutionary Studies
Comparative macroevolutionary studies attempt to explain the differential distribution of characters among different biological groups. The first, and probably most valuable, step in the evolutionary analysis of such data is the abandonment of the preconception that any differences that may be observed are necessarily adaptive. Adaptation should be considered as only one of a series of competing hypotheses to be investigated (37, 80). The alternative hypotheses most easily investigated are historical inheritance (explanation 1; see earlier under ALTERNATIVES TO ADAPTIVE EXPLANATIONS) and phenotypic correlation (explanation 4). Developmental constraint, explanation 2, is a special case
of exploitation 1, and rejection of the latter would also
reject the former. Biomechanical correlation, explanation
3, is largely a problem of too narrow a definition of
a character; a proper search for correlated pheno-
typic traits would assist in evaluation of this alterna-
tive. The investigation of genetic correlation, explanation
5, and chance fixation, explanation 6, while highly desir-
able, are usually impractical or impossible in or-
ganisms more commonly the subject of such studies.
Crucial to the evaluation of the historical hypothesis
is a knowledge of the phylogenetic relationships of the
groups of interest (21, 49, 51, 59, 64, 67). These
relationships will determine which groups should be
part of the study, and thus this information must
precede data collection. For example, to study a pre-
surmountable adaptation (definition 6) to desert environ-
ments, different subgroups (for example, populations or
species), some of which live in deserts and some of
which do not, must be included. Further, the character
must be measured in a closely related group of groups
(outgroups, sister taxa) to provide an indication of
directionality and basal state of the character. Thus,
a minimum of three groups, and preferably many more,
is required in any such analysis (48). Assistance from
a systematist familiar with the group in question is
usually highly valuable in determining these phyloge-
netic relationships.
An important complicating factor in the analysis of
such data is the lack of statistical independence of
different subgroups in a study, since they are, to differ-
ent degrees, all related to each other (39, 59). (Excellent
and extensive discussions of the philosophy and meth-
odology of this approach can be found in the references
cited earlier under Historical Inheritance section.) Some
interesting and instructive examples of its application:
are studies of thermoregulation in scorpion fishes
(14), locomotor performance in anoline lizards (84, 85),
and rates of metabolism and water loss in geckos
(31). If a character can be shown by this analysis not
to have been present in the ancestor, the historical
inheritance alternative can be rejected.
Analysis of phenotypic correlation, explanation 4,
requires an examination of the statistical associations
of the character with other traits. Body size is the most
likely confounding correlate, so the best starting place
is to determine whether body size varies among the
groups compared. If body size does not differ among
groups, size and other factors correlated with it are
unlikely to complicate the analysis. Correlations with
other traits, after correction for phylogenetic associa-
tion (50, 86), may then be sought directly with such
techniques as principal component analysis. Failure to
find correlated traits weakens the phenotypic correla-
tion hypothesis. If correlated traits are found, any or
all of them may have to be considered as being an
alternative target of adaptation. If body sizes are un-
equal among the groups compared, an analysis of
phylogenetically independent contrasts of the variable
of interest on body size should be undertaken (see
methods in refs. 43, 72). A promising experimental
approach for directly testing the importance of body
size and its influence on numerous different traits is
allometric engineering (117, 118). Body size is manipu-
lated by such techniques as yolk removal or hormonal
supplementation, permitting the direct measurement of
the effect of body size itself, uncomplicated by diverse
correlations inherent in intergroup comparative studies.
In comparative macroevolutionary studies of this
sort, adaptation as a hypothesis for the existence of a
character cannot be tested directly. However, examina-
tion and rejection of these alternative hypotheses per-
mits one to have more confidence that adaptation
might be the real explanation for the character. Consid-
eration and rejection of such alternatives is very much
in the spirit of Williams' (132) assertion that adapta-
tion "should be used only as a last resort. It should
never be invoked when less onerous principles . . . are
sufficient for a complete explanation." Since such com-
parative studies are the only means we have to examine
the evolutionary history of natural living systems (ma-
nipulative experiments being impossible), it is well
worth being as careful as possible in their analysis and
interpretation.
Microevolutionary Studies
In contrast to the analysis of the historical patterns
resulting from evolution described in the previous sec-
tion, microevolutionary studies concentrate on analyz-
ing the current processes of evolution. They concen-
trate on a single population or series of populations
and analyze the possibility and the actual operation of
selection on biological characters. They show what is
possible in the evolution of physiological characters—
for example, which traits will respond to selection,
which traits are correlated with each other, and how
rapidly and extensively adaptive evolution may pro-
cceed. (The approach described here for microevolution-
ary analyses of physiological characters is taken from
refs. 2, 10, 15, 16, 18, 73, which provide more de-
tailed discussions.)
For a biological character to be able to respond to
selection, it must be both variable and heritable (35).
That is, within a population, individuals must differ
from each other with respect to the character, and
some of this variation must have a genetic basis: off-
spring must resemble their parents in regard to the
Most physiological characters are quantitative (having many character states or being continuously distributed). These traits may be under the influence of many different genes and are consequently amenable to analysis through the techniques of quantitative genetics (36). One of the first steps of a microevolutionary study might be the demonstration of variability and heritability in the character of interest. The character cannot respond evolutionarily to selection if it is not both variable and heritable. However, most physiological traits that have been examined apparently possess these properties (8, 49, 93). For many physiological traits, such as rate processes, interindividual variability is quite high, with coefficients of variation of around 20%–30%. Given this relatively great variability, physiologists have also sought, usually successfully, to demonstrate that these traits are measurably repeatable through time for an individual (8, 68). In addition, these traits have moderate, but nonetheless highly significant, levels of heritability (8, 49, 93, 119).

Such investigations frequently require that the organisms be brought into the laboratory to breed, or at least to deliver offspring, so that patterns of inheritance can be analyzed and determined. Measurements of the character in large numbers of individuals are required. If the potential for response to selection can be demonstrated, the effect of the character on survival or reproduction within the natural population can be examined (see ref. 35 for a discussion of methods of demonstrating natural selection). The character in question may be an organismal trait, such as locomotor performance (for example, burst speed in lower vertebrates [10, 70, 90, 128]), a particular physiological or morphological character (for example, bill shape in birds [56] or scalar pattern in lizards [41]), or a major gene product (for example, lactic dehydrogenase in fish [98, 99], phosphoglucone isomerase in butterflies [130], or leucine aminopeptidase in molluscs [72]). A group of individuals of measured character state within the population is followed through time to determine whether those with low, intermediate, or high values of the character are more or less likely to survive and reproduce in the natural environment (see refs. 35, 73, 110 for a discussion of methods). Care must be taken to include all ages as selection might be particularly intense on newborns or some other group and might go undetected if some ages were excluded from examination (112). This approach is basically correlative and subject to the problems pointed out earlier under Phenotypic Size Correlation. If differential survival in organisms with high values of a particular trait can be demonstrated, it is still possible that selection may be operating on a correlated trait and not on the one in question (91). Hence, analysis (and possibly manipula-

After demonstration of the correlation of the trait with differential survival (and perhaps identification of the portion of the life cycle affected), the use of the trait under natural conditions—that is, its ecological importance—should be examined. Why is it that having a particular condition of the character is beneficial? Arnold (2) advocated dividing such studies into two more manageable portions, termed the performance and fitness gradients. In the former, the effect of a character on a performance trait (for example, escape ability) is examined in the laboratory; in the latter, the performance trait is analyzed in its natural context.

Many physiological characters (for example, rate processes) may already be regarded as complex performance traits in this context. Demonstration of the ecological effect of the performance trait—for example, exactly how it aids in predator escape, food acquisition, mate attraction, starvation resistance, etc.—may be quite difficult to accomplish. Focal observations on individual organisms in nature [97] can establish patterns of behavior or organismal interaction (for example, what predators are present and how organisms avoid becoming prey). These may be used to provide a credible rationale for the ecological operation of the character. However, the stochastic nature of selection may require similar observations on hundreds of individuals to demonstrate the effect of a known character state (10). In addition, the presence of the observer will have an unknown, and perhaps unwelcome, effect on the results of the study.

Such microevolutionary studies are large and complex but not impossible. Their feasibility depends greatly on the choice of character and study organism. Ideally, the organism should live in a discrete, easily observed population of manageable size. It should produce groups of offspring of known parentage, which can be compared to each other. Organisms should be readily collected, measured, marked, and returned without damage to their natural population and recollected again later. The character should have temporal stability and a connection to a performance trait of presumptive importance in nature. These desirable features restrict the field of potential study organisms but do not preclude all interesting ones.

It should be borne in mind that only very rarely is any study complete, including mechanistic analyses done in the laboratory. Some steps are missing in even the best and most complete investigations. Getting partial answers may be better than waiting forever to discover the perfect system (37, 129).

In the search for experimental systems for the study
of evolutionary physiology, some investigators have turned to a variety of laboratory-cultured organisms, such as fruit flies and bacteria. These organisms offer several advantages. They can be maintained in discrete populations of large size, minimizing chance fixation effects while allowing the continuous generation of new genetic variation by mutation and recombination. Small organisms with very rapid generation times may be used, permitting observation of the time course as well as trajectory of evolutionary change. In some organisms, direct measurement of competitive (Darwinian) fitness is possible so that magnitude and rate of adaptation can be determined. Further, the replicated nature of the experiment permits an assessment of the repeatability, or inevitability, of any evolutionary pattern and a statistical analysis of its significance.

This approach has been termed "natural selection in the laboratory" (108) and involves the imposition of a novel environment on replicated experimental populations, while maintaining control populations for comparison. It is distinct from "artificial selection" (as in animal or plant breeding) in which the experimenter chooses particular characters and permits breeding on their basis alone. In the former approach, only the environment is stipulated by the investigator and selection works on any characters it can. Examples include experimental physiological studies on the evolution of temperature adaptation in fruit flies (25, 63, 69) and bacteria (11, 13, 77, 92) and ethanol resistance in fruit flies (27). These laboratory-based studies of microevolutionary physiology are meant to supplement, not supplant, research on natural populations. The two approaches are mutually supportive and informative.

Organisms did not acquire their present forms and functional capacities by special creation. These traits will either have evolved from characters preexisting in their ancestors or have arisen by natural selection. This approach has been termed "natural selection in the laboratory" (108) and involves the imposition of a novel environment on replicated experimental populations, while maintaining control populations for comparison. It is distinct from "artificial selection" (as in animal or plant breeding) in which the experimenter chooses particular characters and permits breeding on their basis alone. In the former approach, only the environment is stipulated by the investigator and selection works on any characters it can. Examples include experimental physiological studies on the evolution of temperature adaptation in fruit flies (25, 63, 69) and bacteria (11, 13, 77, 92) and ethanol resistance in fruit flies (27). These laboratory-based studies of microevolutionary physiology are meant to supplement, not supplant, research on natural populations. The two approaches are mutually supportive and informative and will lead to more rapid progress in our understanding of the evolution of physiological characters than would either by itself.

INTEGRATING AN EVOLUTIONARY PERSPECTIVE INTO PHYSIOLOGICAL STUDIES

Organisms did not acquire their present forms and functional capacities by special creation. These traits evolved from characters preexisting in their ancestors. They were shaped sometimes by adaptation, sometimes by other forces; sometimes they were constrained from changing completely by other forces; sometimes they were constrained from changing partially by other forces. These varied forces, while maintaining control populations for comparison, produced organisms with specific traits by natural selection in the laboratory (108). The overall pattern of evolution of physiological characters reflects the interaction of adaptation and other forces, while maintaining control populations for comparison.

Over the past half-century, comparative studies have emphasized the equilibrium (74) interaction of organism and environment. Comparative (ecological) physiology rose to prominence as an independent field, with many attendant successes (see ref. 9), operating largely within an equilibrial frame of reference. However, the understanding of the evolution of physiological systems did not keep pace with the expanding knowledge of their mechanistic bases and ecological correlates. The dominance of adaptation often precluded critical evolutionary investigations. Critics of this adaptationist view, although unsettling, freed comparative biology to begin investigations of alternative explanations for the evolution of organismal characters. It is now both possible and highly desirable to incorporate an evolutionary component into comparative physiological studies (10, 21, 38).

Comparative (ecological) physiology (along with its companion field, functional morphology) is in a very strong position to gain from work on the evolution of important organismal and physiological characters and their mechanistic bases. Evolutionary physiology is emerging as an important new discipline, to the mutual benefit of both comparative physiology and evolutionary biology.

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