

1. Adaptation and the evolution of physiological characters

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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, 82, 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 activate release of adrenal hormones with widespread physiological effects, including raising blood pressure, stimulating glucose mobilization, increasing heart rate, etc. If prolonged, the response may result in adverse physiological conditions and death. (3) *Adaptation can*

be the state of having become familiar with surroundings. 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 acclimatization), 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 sense 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 5) 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 and 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-

duction or mitochondria as adaptations for aerobic respiration.

Given this diversity of definitions, physiologists should be particularly careful to clarify the sense in which they are using the word “adaptation.” It is important to recognize that all of these are correct definitions in the sense that these are ways in which the word is actually used. We might regard some of these uses, particularly definitions 3 and 8, as unfortunate but not incorrect. An evolutionary biologist might insist that only definitions 5 and 6 are appropriate (and these are the senses in which it is generally used in this chapter). Such insistence, however, will not change common usage.

This set of definitions divides into two distinct categories, phenotypic and evolutionary adaptation: the first four refer to phenotypic alterations in individual organisms and the second four to multigenerational changes in populations of organisms. (This is the distinction made previously by Bock and von Wahlert [17] between *physiological* and *evolutionary* adaptation.) The first set encompasses changes in organisms ranging from very rapid to long-term developmental events that occupy the entire lifetime of an individual. In view of the very different nature of organismal and population processes, it is quite possible that phenotypic adaptation may have little in common with evolutionary changes and processes, even in response to similar environmental perturbations. For instance, mechanisms of temperature adaptation—in definitions 4 and 6—are usually quite distinct, the former involving alterations in protein concentration and the latter protein composition (61). Evolutionary adaptation clearly involves a genetic process. Phenotypic adaptation may also have a genetic basis (20, 109, 120), for example, differential gene activation (4, 71).

Is there a unifying theme in this diversity of definitions? The only common feature I see is *change through time* or *biological change in response to environmental change*. Temporal variation is the element that distinguishes adaptive from more static biological processes. One might be tempted to add “beneficial” to these common definitions, implying that these changes improve the functioning and/or fitness of the organisms in a changing environment. That is probably often true for definitions 1–4 and is certainly true for definitions 5–8. However, the mere fact of phenotypic alteration in a new environment cannot be taken as evidence of benefit. The fact that a lobster turns bright red in boiling water does not imply that becoming red is a positive reaction to minimize heat stress. Such effects may be direct responses to the environmental change and may be neutral or even detrimental in their impact on competitive fitness (62, 67, 79, 95). Phenotypic

adaptation may not necessarily predispose organisms to greater competitive fitness. For example, bacteria acclimated to a lower temperature (32°C) outcompete the same bacterial acclimated to a higher temperature (42°C) in both 32° and 42°C environments (79). The assumption that phenotypic adaptations are necessarily beneficial to the individuals in which they occur (29, 63, 106) is unwarranted and susceptible to the same criticisms brought against the assumption of evolutionary adaptation (79; see later under CRITICISMS OF ADAPTIVE INTERPRETATIONS).

A problem with the plethora of ways in which “adaptation” is used by physiologists and other organismal biologists is the blurring of distinctions between them and the assumption that all are related or equivalent. 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 response 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 means through which natural selection might have operated to produce the evolution of the character, generally by contending that the character benefitted survivorship 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 adaptation 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 program." 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 biological characters.

Particular criticism (54, 83) was directed to the assertion of optimality. Optimality approaches have played a prominent role in comparative biology—for example, in ecological energetics (125) and respiratory physiology (*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 designed 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 adaptationism 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 comparative biologists? Other criticisms of organismal biology, such as the irrelevance of integrative or systemic 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 commonly practiced and evolution. Most comparative biologists 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 applications of articles of faith undercuts the idea of a supportive linkage between comparative and evolutionary biology. If any character could unfailingly 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 perfection of organismal design as evidence of an omnipotent creator. The suggestion that organismal biologists simply exchanged one religious faith for another undermines their evolutionary credentials, or at least suggests that their approach and thinking have not been fundamentally altered by evolutionary thought. To be unconnected to evolution is to be unconnected 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 lampoons, 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 biologists. Some have simply ignored the controversy and continue to operate within an adaptationist framework. Others (for example, 19, 38, 59) have accepted the essentials of the criticism and have attempted more direct analyses of the adaptive process. These investigations examine adaptation as a hypothesis rather than as an assertion (37) and undertake comparative and experimental studies on the microevolution of functional characters. The approaches of some of these studies in evolutionary physiology are presented below, but before that, alternative hypotheses to adaptive explanations are discussed.

ALTERNATIVES TO ADAPTIVE EXPLANATIONS

There are many possible explanations for the possession 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 explanation, 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. Interpretation of correlation in comparative studies is always 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 consideration, 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" (or "trait" or "feature") to be investigated. Any organism can potentially be atomized into a series of thousands of different traits, each requiring analysis and explanation. Is, for instance, the shape of every individual scale on a fish an individual character of importance and is 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 Phenotypic Size Correlation and under Genetic Correlation) 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 and obvious morphological structures may be debatable. Gould and Lewontin (54), for instance, asked whether the human chin can legitimately be considered a character which would require an explanation (adaptive or not). They maintained that it is simply the product of differential evolutionary development of two facial growth fields, requiring no further justification or explanation.

Possible explanations, in addition to adaptation, for the possession of a character by an organism include the following six.

1. 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 population. 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 presumably 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 framework 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 appearance of the character in a group of related taxa can help 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 coincident 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 ringtails (*Bassariscus astutus*, a small carnivore in the raccoon 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 populations 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 occupation 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 phylogenetic divergence times are known. (The following references 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 biological systems by modification of preexisting structures. Novelties, including adaptations, must therefore arise sometime during ontogenetic development as an

alternation of some developmental sequence. (An important 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 extensively discussed in refs. 53, 58, 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 developmental 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 alternations rather late in the developmental scheme, such as rates of maturation of specific organs or tissues. Alterations early in development may so fundamentally affect diverse structures that development is terminated or the resulting 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 design. Rather, its orientation is best understood in reference to the pattern of ontogenetic development of the eye arising from the brain. Thus, a structural fea-

ture of an organism may be best explained in terms of its development and not in terms of adult or current utility or design.

3. *Physical and Biomechanical Correlation*

Any structure will have an array of attendant properties 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 important design criteria in some circumstances and irrelevant 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. Consider, 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 instance, 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, selection on one will affect the other and a priori it may be impossible to tell which is an adaptation and which is merely being carried along in the course of evolution. If the characters are functionally coupled, as might be

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 may 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 (24, 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, an 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 analysis 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.

As an example, consider the extensive and well-controlled experiments of Rose, Service, and their co-workers (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, is therefore another potential source of variation in natural populations. Experimental evolutionary studies attempt to eliminate drift effects by maintaining large population sizes and replicated experimental and control lines (28, 78, 108).

Potential explanations for the presence of a trait in a population of organisms are many. Adaptation is but one of these. How then does one sort through these possibilities and determine the evolutionary source and adaptive importance of a particular feature of interest?

STUDYING THE EVOLUTION OF PHYSIOLOGICAL CHARACTERS

As Bock (16) has pointed out, studying the evolution of functional traits is not a simple undertaking. It is, however, one that has considerable reward because it 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 per-

spectives. 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 explanation 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 phenotypic traits would assist in evaluation of this alternative. The investigation of genetic correlation, explanation 5, and chance fixation, explanation 6, while highly desirable, are usually impractical or impossible in organisms most 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, 65). 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 presumptive adaptation (definition 6) to desert environments, 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 or groups (outgroups, sister taxa) to provide an indication of directionality and ancestral 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 phylogenetic 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 different degrees, all related to each other (39, 59). (Excellent and extensive discussions of the philosophy and methodology 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 scombroid 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 3, 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 association (50, 86), may then be sought directly with such techniques as principal component analysis. Failure to find correlated traits weakens the phenotypic correlation 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 unequal 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 manipulated 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, examination and rejection of these alternative hypotheses permits one to have more confidence that adaptation might be the real explanation for the character. Consideration and rejection of such alternatives is very much in the spirit of Williams' (132) assertion that adaptation "should be used only as a last resort. It should not be invoked when less onerous principles . . . are sufficient for a complete explanation." Since such comparative studies are the only means we have to examine the evolutionary history of natural living systems (manipulative 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 section, microevolutionary studies concentrate on analyzing the current processes of evolution. They concentrate 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 proceed. (The approach described here for microevolutionary analyses of physiological characters is taken from refs. 2, 10, 15, 16, 18, 73, which provide more detailed 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 bases: offspring must resemble their parents in regard to the

trait. 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 do 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 scalation pattern in lizards [41]), or a major gene product (for example, lactic dehydrogenase in fish [98, 99], phosphoglucose 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-

tion, see ref. 117) of the effects of such factors as size and other size-correlated variables on the trait is advisable.

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 natal population and recaptured 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 (126).

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 will either by itself.

INCORPORATING 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 evolutionary change. In addition to trying to understand how organisms are built and work today, comparative biologists must attempt to understand how today's organisms became the way they are by studying how organismal properties and physiological mechanisms have changed through time.

Adaptation as the sole explanation for all aspects of

organismal function and design has too long dominated physiological thinking. It was an easy afterthought to justify results in terms that seemed possible in view of the current environmental circumstances of an organism. Its axiomatic application disinclined physiologists to the investigation of evolutionary questions. Testable alternatives to adaptation exist, and their consideration greatly enriches our appreciation of the evolutionary process.

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 equilibrium 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. Critiques 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 undertake such studies. The field has many traditional strengths, including the detailed understanding of functional mechanisms, an excellent technological base, a strong experimental ethic, and a long-standing emphasis on the importance of the integration of characters at the organismal level. Evolutionary biology has much 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.

I thank the following individuals for their very perceptive and helpful comments on the manuscript: T. Bradley, A. Cullum, T. Garland, R. Huey, R. Lenski, A. Leroi, J. McLister, S. O'Steen, M. Rose, and T. Watkins. Support was provided by National Science Foundation grants IBN-9118346, -9208662, -9420155, and -9507416.

REFERENCES

- Alexander, R. McN. Apparent adaptation and actual performance. In: *Evolutionary Biology*, edited by M. K. Hecht, B. Wallace, and R. J. MacIntyre. New York: Plenum, 1991, vol. 25, p. 357-373.
- Arnold, S. J. Morphology, performance and fitness. *Am. Zool.* 23: 347-361, 1983.

3. Arnold, S. J. Genetic correlation and the evolution of physiology. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 189–215.
4. Baldwin, J., and P. W. Hochachka. Functional significance of isoenzymes in thermal acclimatization: acetylcholinesterase from trout brain. *Biochem. J.* 116: 883–887, 1970.
5. Bartholomew, G. A. Energy metabolism. In: *Animal Physiology: Principles and Adaptations*, edited by M. S. Gordon, G. A. Bartholomew, A. D. Grinnell, C. B. Jorgensen, and F. N. White. New York: Macmillan, 1982, p. 46–93.
6. Bateson, W. Heredity and variation in modern lights. In: *Darwin in Modern Science*, edited by A. C. Seward. Cambridge: Cambridge Univ. Press, 1909, p. 85–101.
7. Baum, D. A., and A. Larson. Adaptation reviewed: a phylogenetic methodology for studying character macroevolution. *Syst. Zool.* 40: 1–18, 1991.
8. Bennett, A. F. Inter-individual variability: an underutilized resource. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 147–169.
9. Bennett, A. F. The accomplishments of ecological physiology. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 1–8.
10. Bennett, A. F., and R. B. Huey. Studying the evolution of physiological performance. *Oxf. Surv. Evol. Biol.* 7: 251–284, 1990.
11. Bennett, A. F., and R. E. Lenski. Evolutionary adaptation to temperature. II. Thermal niches of experimental lines of *Escherichia coli*. *Evolution* 47: 1–12, 1993.
12. Bennett, A. F., and R. E. Lenski. Evolutionary adaptation to temperature. V. Adaptive mechanisms and correlated responses in experimental lines of *Escherichia coli*. *Evolution* 50: 493–503, 1996.
13. Bennett, A. F., R. E. Lenski, and J. E. Mittler. Evolutionary adaptation to temperature. I. Fitness responses of *Escherichia coli* to changes in its thermal environment. *Evolution* 46: 16–30, 1992.
14. Block, B. A., J. R. Finnerty, A. F. R. Stewart, and J. Kidd. Evolution of endothermy in fish: mapping physiological traits on a molecular phylogeny. *Science* 260: 210–214, 1993.
15. Boake, C. R. B., ed. *Quantitative Genetic Studies of Behavioral Evolution*. Chicago: Univ. of Chicago Press, 1994.
16. Bock, W. J. The definition and recognition of biological adaptation. *Am. Zool.* 20: 217–227, 1980.
17. Bock, W. J., and G. von Wahlert. Adaptation and the form-function complex. *Evolution* 19: 269–299, 1965.
18. Brandon, R. N. *Adaptation and Environment*. Princeton: Princeton Univ. Press, 1990.
19. Brooks, D. R., and D. A. McLennan. *Phylogeny, Ecology, and Behavior*. Chicago: Chicago Univ. of Chicago Press, 1991.
20. Bull, J. J. Evolution of phenotypic variance. *Evolution* 41: 303–315, 1987.
21. Burggren, W. W. Does comparative respiratory physiology have a role in evolutionary biology (and vice versa)? In: *Physiological Strategies for Gas Exchange and Metabolism*, edited by A. J. Woakes, M. K. Grieshaber, and C. R. Bridges. Cambridge: Cambridge Univ. Press, 1991, p. 1–13.
22. Burggren, W. W., and W. E. Bemis. Studying physiological evolution: paradigms and pitfalls. In: *Evolutionary Innovations*, edited by M. H. Nitecki. Chicago: Univ. of Chicago Press, 1990, p. 191–228.
23. Cain, A. J. The perfection of animals. *Biol. J. Linn. Soc.* 36: 3–29, 1989.
24. Calder, W. A. *Size, Function, and Life History*. Cambridge, MA: Harvard Univ. Press, 1984.
25. Cavicchi, S., D. Guerra, V. Latorre, and R. B. Huey. Chromosomal analysis of heat-shock tolerance in *Drosophila melanogaster* evolving at different temperatures in the laboratory. *Evolution* 49: 676–684, 1995.
26. Chevalier, C. D. Aspects of Thermoregulation and Energetics in the Procyonidae (Mammalia: Carnivora). Irvine: Univ. of California, 1991 Ph.D. thesis.
27. Cohan, F. M., and A. A. Hoffmann. Genetic divergence under uniform selection. II. Different responses to selection for knock-down resistance to ethanol among *Drosophila melanogaster* populations and their replicate lines. *Genetics* 144: 145–163, 1986.
28. Cohan, F. M., and A. A. Hoffmann. Uniform selection as a diversifying force in evolution: evidence from *Drosophila*. *Am. Nat.* 134: 613–637, 1989.
29. Cossins, A. R., and K. Bowler. *Temperature Biology of Animals*. London: Chapman and Hall, 1987.
30. Dantzler, W. H. Perspectives. *Am. J. Physiol.* 263 (Regulatory Integrative Comp. Physiol. 34): R1, 1992.
31. Dial, B. E., and L. L. Grismer. A phylogenetic analysis of physiological–ecological character evolution in the lizard genus *Coleonyx* and its implications for historical biogeographic reconstruction. *Syst. Biol.* 41: 178–195, 1992.
32. Dill, D. B., E. F. Adolph, and C. G. Wilber, eds. *Adaptation to the Environment*. Washington, DC: Am. Physiol. Soc., 1964.
33. Eckert, R., D. Randall, and G. Augustine. *Animal Physiology: Mechanisms and Adaptations* (3rd ed.), New York: Freeman, 1988.
34. Emerson, S. B., and S. J. Arnold. Intra- and interspecific relations between morphology, performance, and fitness. In: *Complex Organismal Functions: Integration and Evolution in Vertebrates*, edited by D. B. Wake and G. Roth. Chichester, UK: Wiley, 1989, p. 295–314.
35. Endler, J. A. *Natural Selection in the Wild*. Princeton: Princeton Univ. Press, 1986.
36. Falconer, D. S. *Introduction to Quantitative Genetics* (3rd ed), London: Longman, 1989.
37. Feder, M. E. The analysis of physiological diversity: the prospects for pattern documentation and general questions in ecological physiology. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 38–75.
38. Feder, M. E., A. F. Bennett, W. W. Burggren, and R. B. Huey, eds. *New Directions in Ecological Physiology*. Cambridge: Cambridge Univ. Press, 1987.
39. Felsenstein, J. Phylogenies and the comparative method. *Am. Nat.* 125: 1–15, 1985.
40. Felsenstein, J. Phylogenies and quantitative characters. *Annu. Rev. Ecol. Syst.* 19: 445–471, 1988.
41. Fox, S. F. Natural selection on morphological phenotypes of the lizard *Uta stansburiana*. *Evolution* 29: 95–107, 1975.
42. Frumhoff, P. C., and H. K. Reeve. Using phylogenies to test hypotheses of adaptation: a critique of some current proposals. *Evolution* 48: 172–180, 1994.
43. Futuyma, D. J. *Evolutionary Biology* (2nd ed.), Sunderland, MA: Sinauer, 1986.
44. Gans, C. *Biomechanics: An Approach to Vertebrate Biology*. Philadelphia: Lippincott, 1974.
45. Garland, T., Jr. Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* 247 (Regulatory Integrative Comp. Physiol. 18): R806–R815, 1984.
46. Garland, T., Jr. Rate tests for phenotypic evolution using phylo-

- genetically independent contrasts. *Am. Nat.* 140: 509–519, 1992.
47. Garland, T., Jr., and S. C. Adolph. Physiological differentiation of vertebrate populations. *Annu. Rev. Ecol. Syst.* 22: 193–228, 1991.
 48. Garland, T., Jr., and S. C. Adolph. Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.* 67: 797–828, 1994.
 49. Garland, T., Jr., and P. A. Carter. Evolutionary physiology. *Annu. Rev. Physiol.* 56: 579–621, 1994.
 50. Garland, T., Jr., P. H. Harvey, and A. R. Ives. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41: 18–32, 1992.
 51. Garland, T., Jr., R. B. Huey, and A. F. Bennett. Phylogeny and thermal physiology in lizards: a reanalysis. *Evolution* 45: 1969–1975, 1991.
 52. Gordon, M. S., G. A. Bartholomew, A. D. Grinnell, C. B. Jorgensen, and F. N. White. *Animal Physiology: Principles and Adaptations*. New York: Macmillan, 1982.
 53. Gould, S. J. *Ontogeny and Phylogeny*. Cambridge, MA: Belknap, 1977.
 54. Gould, S. J., and R. C. Lewontin. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc. R. Soc. Lond. B Biol. Sci.* 205: 581–598, 1979.
 55. Gould, S. J., and E. Vrba. Exaptation—a missing term in the science of form. *Paleobiology* 8: 4–15, 1982.
 56. Grant, P. R., B. R. Grant, J. N. M. Smith, I. J. Abbott, and L. K. Abbott. Darwin's finches: population variation and natural selection. *Proc. Natl. Acad. Sci. U.S.A.* 73: 257–261, 1976.
 57. Graves, J. L., E. C. Toolson, C. Geong, L. N. Vu, and M. R. Rose. Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. *Physiol. Zool.* 65: 268–286, 1992.
 58. Hall, B. K. *Evolutionary Developmental Biology*. London: Chapman and Hall, 1992.
 59. Harvey, P. H., and M. D. Pagel. *The Comparative Method in Evolutionary Biology*. Oxford: Oxford Univ. Press, 1991.
 60. Heusner, A. A. Energy metabolism and body size. I. Is the 0.75 mass exponent of Kleiber's equation a statistical artifact? *Respir. Physiol.* 48: 1–12, 1982.
 61. Hochachka, P. W., and G. N. Somero. *Biochemical Adaptation*. Princeton: Princeton Univ. Press, 1984.
 62. Hoffmann, A. A. Acclimation: increasing survival at a cost. *Trends Ecol. Evol.* 10: 1–2, 1995.
 63. Hoffmann, A. A., and P. A. Parsons. *Evolutionary Genetics and Environmental Stress*. New York: Oxford Univ. Press, 1991.
 64. Huey, R. B. Phylogeny, history, and the comparative method. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 76–98.
 65. Huey, R. B., and A. F. Bennett. A comparative approach to field and laboratory studies in evolutionary ecology. In: *Predator–Prey Relationships*, edited by M. E. Feder and G. Lauder. Chicago: Univ. of Chicago Press, 1986, p. 82–98.
 66. Huey, R. B., and A. F. Bennett. Phylogenetic studies of coadaptation: preferred temperatures versus optimal performance temperatures of lizards. *Evolution* 41: 1098–1115, 1987.
 67. Huey, R. B., and D. Berrigan. Testing evolutionary hypotheses of acclimation. In: *Animals and Temperature: Phenotypic and Evolutionary Adaptation*, edited by I. A. Johnston and A. F. Bennett. Soc. Exp. Biol. Sem. Ser. 59, 1996, p. 205–237.
 68. Huey, R. B., A. E. Dunham, K. L. Overall, and R. A. Newman. Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiol. Zool.* 63: 845–872, 1990.
 69. Huey, R. B., L. Partridge, and K. Fowler. Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution* 45: 751–756, 1991.
 70. Jayne, B. C., and A. F. Bennett. Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* 44: 1204–1229, 1990.
 71. Johnson, T. P., and A. F. Bennett. The thermal acclimation of burst escape performance in fish: an integrated study of molecular and cellular physiology and organismal performance. *J. Exp. Biol.* 198: 2165–2175, 1995.
 72. Koehn, R. K., R. I. E. Newell, and F. W. Immermann. Maintenance of an aminopeptidase allele frequency cline by natural selection. *Proc. Natl. Acad. Sci. U.S.A.* 77: 5385–5389, 1980.
 73. Lande, R., and S. J. Arnold. The measurement of selection on correlated characters. *Evolution* 37: 1210–1226, 1983.
 74. Lauder, G. V. Form and function: structural analysis in evolutionary morphology. *Paleobiology* 7: 430–442, 1981.
 75. Lauder, G. V., R. B. Huey, R. K. Monson, and R. J. Jensen. Systematics and the study of organismal form and function—advances in systematics are defining new directions for functional morphology and comparative physiology. *Bioscience* 45: 696–704, 1995.
 76. Lauder, G. V., A. M. Leroi, and M. R. Rose. Adaptations and history. *Trends Ecol. Evol.* 8: 294–297, 1993.
 77. Lenski, R. E., and A. F. Bennett. Evolutionary response of *Escherichia coli* to thermal stress. *Am. Nat.* 142: S47–S64, 1993.
 78. Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. *Am. Nat.* 138: 1315–1341, 1991.
 79. Leroi, A. M., A. F. Bennett, and R. E. Lenski. Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proc. Natl. Acad. Sci. U.S.A.* 91: 1917–1921, 1994.
 80. Leroi, A. M., M. R. Rose, and G. V. Lauder. What does the comparative method reveal about adaptation? *Am. Nat.* 143: 381–402, 1994.
 81. Lewontin, R. C. Adaptation. *Sci. Am.* 239: 156–169, 1978.
 82. Lewontin, R. C. Sociobiology as an adaptationist program. *Behav. Sci.* 24: 5–14, 1979.
 83. Lewontin, R. C. The shape of optimality. In: *The Latest on the Best: Essays on Evolution and Optimality*, edited by J. Dupre. Cambridge, MA: MIT Press, 1987, p. 151–159.
 84. Losos, J. B. Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* 60: 369–388, 1990.
 85. Losos, J. B. The evolution of form and function: morphology and locomotor performance ability in West Indian *Anolis* lizards. *Evolution* 44: 1189–1203, 1990.
 86. Martins, E. P., and T. Garland, Jr. Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. *Evolution* 45: 534–557, 1991.
 87. Mayr, E. Adaptation and selection. *Biol. Zentralbl.* 101: 161–174, 1982.
 88. Mayr, E. How to carry out the adaptationist program? *Am. Nat.* 121: 324–334, 1983.
 89. Michod, R. E. On adaptedness and fitness and their role in evolutionary explanation. *J. Hist. Biol.* 19: 289–302, 1985.
 90. Miles, D. B. Habitat related differences in locomotion and morphology in two populations of *Urosaurus ornatus*. *Am. Zool.* 27: 44A, 1987.
 91. Mitchell-Olds, T., and R. G. Shaw. Regression analysis of natural selection: statistical and biological interpretation. *Evolution* 41: 1149–1161, 1987.

92. Mongold, J. A., A. F. Bennett, and R. E. Lenski. Evolutionary adaptation to temperature. IV. Adaptation of *Escherichia coli* at a niche boundary. *Evolution* 50: 35–43, 1996.
93. Mousseau, T. A., and D. A. Roff. Natural selection and the heritability of fitness components. *Heredity* 59: 181–197, 1987.
94. Munson, R. Biological adaptation. *Phil. Sci.* 38: 200–215, 1971.
95. Padilla, D. K., and S. C. Adolph. Plastic inducible morphologies are not always adaptive: the importance of time delays in a stochastic environment. *Evol. Ecol.* 10: 105–117, 1996.
96. Peters, R. H. *The Ecological Implications of Body Size*. Cambridge: Cambridge Univ. Press, 1983.
97. Pough, F. H. Organismal performance and Darwinian fitness: approaches and interpretations. *Physiol. Zool.* 62: 199–236, 1989.
98. Powers, D. A. A multidisciplinary approach to the study of genetic variation within species. In: *New Directions in Ecological Physiology*, edited by M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey. Cambridge: Cambridge Univ. Press, 1987, p. 102–130.
99. Powers, D. A., T. Lauerma, D. Crawford, and L. DiMichele. Genetic mechanisms for adapting to a changing environment. *Annu. Rev. Genet.* 25: 629–659, 1991.
100. Precht, H., J. Christofersen, H. Hensel, and W. Larcher. *Temperature and Life*. Berlin: Springer-Verlag, 1973.
101. Price, T., and T. Langen. Evolution of correlated characters. *Trends Ecol. Evol.* 7: 307–310, 1992.
102. Prosser, C. L. *Adaptational Biology: Molecules to organisms*. New York: Wiley, 1986.
103. Quellar, D. C. The spaniels of St. Marx and the Panglossian paradox: a critique of a rhetorical programme. *Q. Rev. Biol.* 70: 485–489, 1995.
104. Reeve, H. K., and P. W. Sherman. Adaptation and the goals of evolutionary research. *Q. Rev. Biol.* 68: 1–32, 1993.
105. Reiss, M. J. *The Allometry of Growth and Reproduction*. Cambridge: Cambridge Univ. Press, 1989.
106. Rome, L. C., E. D. Stevens, and H. B. John-Alder. The influence of temperature and thermal acclimation on physiological function. In: *Environmental Physiology of the Amphibians*, edited by M. E. Feder, and W. W. Burggren. Chicago: Univ. of Chicago Press, 1992, p. 183–205.
107. Rose, M. R. Genetics of increased lifespan in *Drosophila*. *Bioessays* 11: 132–135, 1989.
108. Rose, M. R., J. L. Graves, and E. W. Hutchison. The use of selection to probe patterns of pleiotropy in fitness characters. In: *Insect Life Cycles: Genetics, Evolution and Co-Ordination*, edited by F. Gilbert. New York: Springer-Verlag, 1990, p. 29–42.
109. Scheiner, S. M., and R. F. Lyman. The genetics of phenotypic plasticity. II. Response to selection. *J. Evol. Biol.* 4: 23–50, 1991.
110. Schuller, D. Estimating the form of natural selection on a quantitative trait. *Evolution* 42: 849–861, 1988.
111. Schluter, D. Bringing population and phylogenetic approaches to the evolution of complex traits. In: *Complex Organismal Functions: Integration and Evolution in Vertebrates*, edited by D. B. Wake and G. Roth. Chichester, England: Wiley, 1989, p. 79–95.
112. Schluter, D., T. D. Price, and L. Rowe. Conflicting selection pressures and life history trade-offs. *Proc. R. Soc. Lond. B Biol. Sci.* 246: 11–17, 1991.
113. Schmidt-Nielsen, K. *How Animals Work*. Cambridge: Cambridge Univ. Press, 1972.
114. Schmidt-Nielsen, K. *Scaling: Why is Animal Size so Important?* Cambridge: Cambridge Univ. Press, 1984.
115. Schmidt-Nielsen, K. *Animal Physiology: Adaptation and Environment* (4th ed.). Cambridge: Cambridge Univ. Press, 1990.
116. Selye, H. *The Story of the Adaptation Syndrome*. Montreal: Acta, 1952.
117. Sinervo, B., P. Doughty, R. B. Huey, and K. Zamudio. Allometric engineering: a causal analysis of natural selection on offspring size. *Science* 258: 1927–1930, 1992.
118. Sinervo, B., and R. B. Huey. Allometric engineering: an experimental test of the causes of interpopulational differences in performance. *Science* 248: 1106–1109, 1990.
119. Sorci, G., J. G. Swallow, T. Garland, Jr., and J. Clobert. Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. *Physiol. Zool.* 68: 698–720, 1995.
120. Stearns, S. C. The evolutionary significance of phenotypic plasticity. *Bioscience* 39: 436–445, 1989.
121. Stephens, D. W., and J. R. Krebs. *Foraging Theory*. Princeton: Princeton Univ. Press, 1986, p. 206–215.
122. Stern, J. T. The meaning of “adaptation” and its relation to the phenomenon of natural selection. *Evol. Biol.* 4: 39–66, 1970.
123. Taylor, C. R., and E. R. Weibel. Design of the mammalian respiratory system. I. Problem and strategy. *Respir. Physiol.* 44: 1–10, 1981.
124. Tinbergen, N. On aims and methods in ethology. *Z. Tierpsychol.* 20: 410–433, 1963.
125. Townsend, C. R., and P. Calow. *Physiological Ecology: An Evolutionary Approach to Resource Use*. Sunderland, MA: Sinauer, 1981.
126. Travisano, M., J. A. Mongold, A. F. Bennett, and R. E. Lenski. Experimental tests of the roles of adaptation, chance, and history in evolution. *Science* 267: 87–90, 1995.
127. Waddington, C. H. *New Patterns in Genetics and Development*. New York: Columbia Univ. Press, 1962.
128. Watkins, T. B. Predator-mediated selection on burst swimming performance in tadpoles of the Pacific tree frog, *Pseudacris regilla*. *Physiol. Zool.* 69: 154–167, 1996.
129. Watt, W. B. Biochemistry, physiological ecology, and population genetics: the mechanistic tools of evolutionary biology. *Funct. Ecol.* 5: 145–154, 1991.
130. Watt, W. B., R. C. Cassin, and M. S. Swan. Adaptation at specific loci. III. Field behavior and survivorship differences among *Colias* PGI genotypes are predictable from *in vitro* biochemistry. *Genetics* 103: 725–739, 1983.
131. Weibel, E. R., C. R. Taylor, and H. Hoppeler. The concept of symmorphosis: a testable hypothesis of structure function relationship. *Proc. Natl. Acad. Sci. U.S.A.* 88: 10357–10361, 1991.
132. Williams, G. C. *Adaptation and Natural Selection*. Princeton: Princeton Univ. Press, 1966.
133. Williams, G. C. A defense of reductionism in evolutionary biology. *Oxf. Surv. Evol. Biol.* 2: 1–27, 1985.