

ADAPTATION

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To Della and Susan

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Phylogenetic Systematics of Adaptation

"Hypotheses of ADAPTATION are basically comparative. To propose that a trait is adaptive implies that it promotes survival or reproductive success relative to other organisms lacking that trait."

ALLAN LARSON

JONATHAN B. LOSOS

Darwin's theory of natural selection was proposed to explain adaptation, the phenomenon that many organismal characteristics appear to have been designed to perform particular biological functions. Because natural selection is the only process within Darwinian evolutionary theory by which a character might be molded specifically to enhance organismal functions and survival, the evolutionary definition of adaptation now specifies that an organismal character constitutes an adaptation if it performs a function that is of utility to the organisms possessing it and if the character evolved by natural selection for that particular function (Gould and Vrba, 1982; Baum and Larson, 1991). The hypothesis that a character constitutes an adaptation makes specific predictions regarding the utility of the character, its phylogenetic origin, and how it may confer to its possessors an advantage for survival or reproductive success not provided by alternative characters. Testing these predictions requires multidisciplinary approaches that incorporate functional morphology, behavior, ecology, phylogenetic systematics, genetics, and natural history.

Hypotheses of adaptation and their tests are fundamentally comparative. To propose that a particular character is adaptive implies that the character confers an advantage which promotes the survival or reproductive success of its carriers relative to organisms lacking the trait. The character is to be compared specifically to phylogenetically antecedent conditions that occur as alternative variants within populations or in related evolutionary lineages. Adaptation is a meaningful hypothesis only if alternative possible explanations exist for the evolutionary origin and maintenance of organismal characters and their variation. We emphasize here the use of deductive methodology (see Mayr, 1982) and the importance of explicit alternative hypotheses because nonrigorous use of adaptive explanations has hindered evolutionary research (Lewontin, 1977). Several kinds of observations potentially can falsify the hypothesis of adaptation. For example, the hypothetically adaptive character may not enhance performance or survival

comparative approach

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* hypothesis that a character is an adaptation includes predictions on:

- (1) The utility of the character
- (2) The phylogenetic origin of it
- (3) How it may confer survival or reproductive success

relative to alternative variants that are its phylogenetic antecedents, or the character may originate in an ancestral lineage for which its current biological role would have been irrelevant (Greene, 1986a).

Studies of adaptation have followed two complementary evolutionary traditions, the first using systematic methodology to address macroevolutionary questions and the second using population genetic methodology to address microevolutionary ones. The macroevolutionary tradition uses phylogenetic analysis to test historical hypotheses of adaptation as explanations for the origins of the characters of species and higher taxa. The microevolutionary, or ecological genetic, tradition examines the action of natural selection on populational variation and is considered elsewhere in this book. The macroevolutionary aspects of adaptation constitute a major issue to which Darwin's theory of natural selection was addressed and it is the primary focus of this chapter.

The importance of using a phylogenetic perspective for macroevolutionary studies of adaptation is evident from the criticisms of Lewontin (1977) and Gould and Lewontin (1979). These authors discredit adaptationist studies conducted before such studies incorporated the rigorous concepts and methods of phylogenetic systematics. Their criticisms led directly to the reformulation of adaptationist studies in an explicitly phylogenetic perspective. We present the phylogenetic study of adaptation in the context of these criticisms. We then summarize in the form of a general protocol the procedures needed for testing macroevolutionary hypotheses of adaptation and discriminating adaptation from alternative explanations of character evolution. Statistical approaches useful for this procedure are reviewed. The procedures are illustrated using examples from the adaptive radiation of lizards of the genus *Anolis* on Caribbean islands.

I. Previous Criticism and Phylogenetic Revision of the Adaptationist Program

Lewontin's (1977) critique of adaptationist studies makes three principal criticisms: (1) Adaptationist studies often partition the organism into traits and the environment into problems that have no well-founded biological basis. Lewontin (1977) illustrates the arbitrariness of many such decisions using examples from sociobiological explanations of human behavior in which traits such as "indoctrinability" and "blind faith" have been considered adaptive biological characters. (2) Characters are studied in isolation from each other with the questionable assumption that there are no significant effects of interaction among characters in determining their utility to the organism. (3) All characters are assumed to be adaptive, with the main goal of adaptationist studies being to discover how they are adaptive rather than potentially to reject the adaptive explanation in favor of alternatives.

These criticisms reveal the importance of the initial stages of an adaptationist study, in which the investigator chooses the characters to be studied and generates hypotheses regarding their contributions to organismal survival or reproduction. Phylogenetic solutions to these three problems are now considered sequentially.

macro x microevol approaches

Lewontin's critiques of adaptationist studies.

A. Homology and Hypotheses of Adaptation → Phylogenetic solution to studies that partition organisms into traits @ environment into problems.

The principles of homology and phylogenetic analysis are indispensable for answering Lewontin's (1977) first criticism that adaptationist studies arbitrarily atomize the organism into traits and the environment into problems that the traits are designed to solve. The study of homology addresses this criticism by providing objective criteria for identifying characters as nonarbitrary components of organismal phenotype. The concept of homology used here combines elements of several published concepts (Patterson, 1982; Wagner, 1989; Hall, 1994). The characters to be studied as potential adaptations should exhibit three fundamental properties of homology: conservation, individualization, and uniqueness (Wagner, 1989). Conservation is the evolutionary persistence or stability of attributes of a character among lineages. Individualization denotes the separate developmental pathway of the character relative to the remainder of the organism. Uniqueness specifies that the distribution of the character defines a monophyletic group. We regard characters that satisfy these criteria as historically individualized components of the phenotype appropriate for study as hypothetical adaptations.

Patterson (1982) describes three empirical tests (similarity, congruence, and conjunction) by which one can judge whether a hypothetically adaptive character meets these basic criteria of homology. Although Patterson's (1982) tests were formulated within the conceptual framework of pattern cladism, we apply them using the principles of phylogenetic systematics (see de Queiroz, 1985).

Patterson's (1982) test of similarity examines shared attributes of a character among lineages. The form of a character, its developmental origin, and its position within the organism may reveal conserved patterns of similarity indicative of homology. These criteria may reveal that shared attributes among lineages are only superficial and not truly indicative of homology. For example, elongation of the trunk in the bolitoglossine salamanders *Lineatriton* and *Oedipina* fails the similarity test of homology because elongation occurs by lengthening individual vertebrae in *Lineatriton* and by increasing the number of vertebrae in *Oedipina* (Wake, 1966). Kaplan (1984) shows how intermediate forms observed in ontogeny, paleontological series, or interspecific comparisons among extant species may reveal similarities of morphological characters. Characters that meet rigorous criteria of similarity are then to be subjected to the tests of congruence and conjunction.

The test of congruence uses comparisons to additional taxonomic characters to ask whether the character being examined constitutes a synapomorphy of a monophyletic group. To the phylogenetic systematist, this test is equivalent to testing the hypothesis that the character had a single evolutionary origin. The test of congruence is failed if monophyly of the species sharing the character being examined is contradicted by the variation of other taxonomic characters (Patterson, 1982). The vertebrate "wing" (present only in bats and birds) fails the congruence test of homology because other synapomorphic characters (including presence of feathers, hair, and mammary

Paradigm = congruence = conjunction
* If fails congruence = Paradigm
* If fails similarity @ congruence = conjunction

3 properties of Homologies: (1) conservation, (2) Individualism, (3) Uniqueness. 3 empirical tests for Homology: (1) similarity, (2) congruence, (3) conjunction.

glands) identify monophyletic groups (birds, mammals) that contradict a group containing only the winged vertebrates (bats + birds). The test of congruence is most effective when many congruent taxonomic characters are available for the taxon being studied and the phylogeny of the taxon, therefore, is well resolved.

If alternative characters occur as a stable polymorphism within lineages that undergo successive events of evolutionary branching, the congruence test of homology may be failed by a character that is nonetheless homologous in the sense of having had a single evolutionary origin. Technical problems that result from this phenomenon, called lineage sorting, are considered in detail

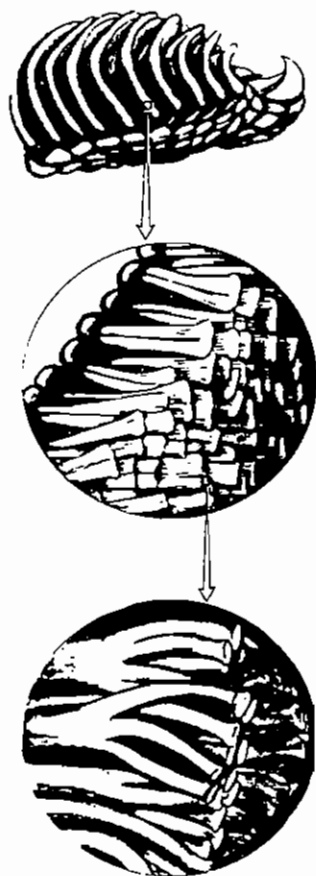


Figure 1 Toepads are composed of expanded scales that are termed lamellae. Lamellae are covered by microscopic setae; those of a geckonid lizard are illustrated (from Hildebrand, 1988; Reprinted by permission of John Wiley & Sons, Inc.).

by Roth (1991), who notes that the problem potentially occurs with characters that are emergent at the molecular or organismal levels (including morphological and behavioral characters).

The test of conjunction guards against inappropriate taxonomic comparisons of a character to a serial homologue that was produced by evolutionary duplication and divergence of characters within a lineage. The test is failed if two hypothetically homologous characters are observed together in the same organism (Patterson, 1982). For example, the hypothesis that the halteres of flies are homologous to hindwings of other insects would be falsified if both halteres and hindwings were observed in the same individuals.

We illustrate the tests of homology using the toepads present in almost all anoline lizards (Peterson, 1983; Fig. 1). These structures are composed of expanded subdigital scales, termed lamellae. The lamellae are covered with millions of microscopic setae. Electrons on the surface of the setae form bonds with electrons on the substrate, and the force of these intermolecular bonds allows adhesion to smooth surfaces (Cartmill, 1985). Electron microscopy establishes detailed similarity of structure among toepads of different anoles

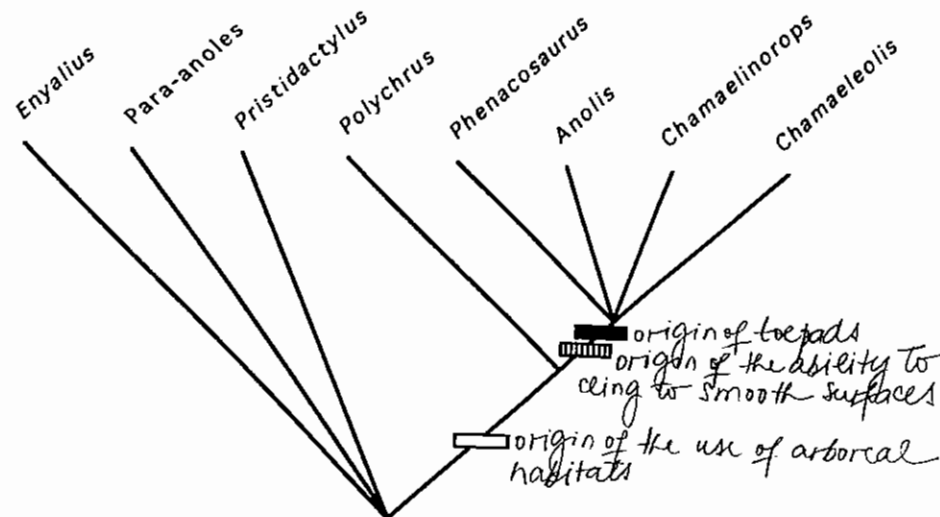


Figure 2 Evolution of toepads in anoline lizards. The black bar indicates origin of toepads, the hatched bar indicates origin of the ability to cling to smooth surfaces, and the open bar indicates origin of the use of arboreal habitats. The phylogeny is from Frost and Etheridge (1989). Relationships among the anoline genera are uncertain: *Chamaeleolis*, *Chamaelinorops*, and *Phenacosaurus* all possibly arose within *Anolis* (Hass et al., 1993; Jackman et al., unpublished). More detailed information on the natural history and/or phylogeny of *Enyalius*, *Pristidactylus*, and the para-anoles may produce different conclusions about when arboreality evolved, but will not alter the fundamental conclusion that it preceded the evolution of expanded toepads in the anoles.

and reveals no profound differences that would falsify similarity (Ruibal and Ernst, 1965; Peterson, 1983). Monophyly of the anoline lizards is established by several synapomorphies (Frost and Etheridge, 1989). The distribution of toepads is congruent with these characters (Fig. 2), except for secondary absence in several taxa (Peterson and Williams, 1981). Toepads are present on all digits and therefore constitute serially repeated structures within the organism. The presence of toepads on all digits, rather than specific attributes of the toepads of individual digits, constitutes the character being studied as a hypothetical adaptation. The character conceived in this way passes the conjunction test of homology because only a single set of toepads is present in any animal. Thus, toepads of anoline lizards meet the criteria of homology and constitute individualized components of the phenotype appropriate for testing as hypothetical adaptations.

The terms nonhomology and homoplasy describe characters that fail one or more of the three tests described above. Patterson (1982) categorizes the different kinds of nonhomology according to which of the three tests are passed or failed. We discuss below the use of homoplastic patterns of evolution to test the predictions of what we call general hypotheses of adaptation. Two categories of nonhomology are important in this context, parallelism and convergence. A character exhibits parallelism if it fails the congruence test, but passes similarity and conjunction. Parallelism involves more than one evolutionary origin of a characteristic that arises by similar developmental processes from similar ancestral conditions. Parallelism has been used to test hypotheses of developmental constraints on evolutionary change, as well as hypotheses of adaptation (Wake, 1991). A character exhibits convergence if it passes the test of conjunction, but fails both similarity and congruence. Convergence denotes the independent evolutionary origin of superficially similar characters and also has been invoked as a test of hypotheses of adaptation (Harvey and Pagel, 1991; Wake, 1991).

Toepads superficially similar to those of anoline lizards occur in two other groups of lizards, a monophyletic group within skinks (genera *Prasinohaema* and *Lipinia*) and the Gekkonidae. When compared to the toepads of anoles, toepads of these taxa fail the similarity test because they have conspicuous differences in the setae (Williams and Peterson, 1982). The congruence test is failed because of conflict with numerous characters used to examine the family-level relationships of lizards (Estes et al., 1988). The conjunction test is passed because only a single set of toepads is found in any animal. The toepads of gekkonids and skinks therefore are convergent with those of anoles and do not collectively constitute homologous characters.

Phylogenetic analysis also answers Lewontin's (1977) criticism of the arbitrary partitioning of the environment into problems that the characters must solve. Lewontin (1977) notes that organisms determine to some degree their effective environments through their active choice and utilization of resources. Physical and biological components of environments utilized by related species can be examined comparatively, and phylogenetic analysis can be used to identify the evolutionary succession of environmental factors that characterize the history of an evolving lineage. Utilization of environmental factors by populations can be examined analytically using the same principles and tests described above.

B. Hypotheses of Adaptation and Interactions Among Characters ⇒

solution to the problem of characters being studied in isolation from each other.
 Principles of homology are important also for answering Lewontin's (1977) second criticism, that adaptive studies of individual characters must not disregard their interactions with other characters in their contributions to organismal survival. Phylogenetic trees enable one to examine the historical associations of characters to identify cases of evolutionary nonindependence. Two different characters may characterize exactly the same taxa (Fig. 3a), in which case their phylogenetic histories coincide and tests of the adaptive status *must take interactions into account*.

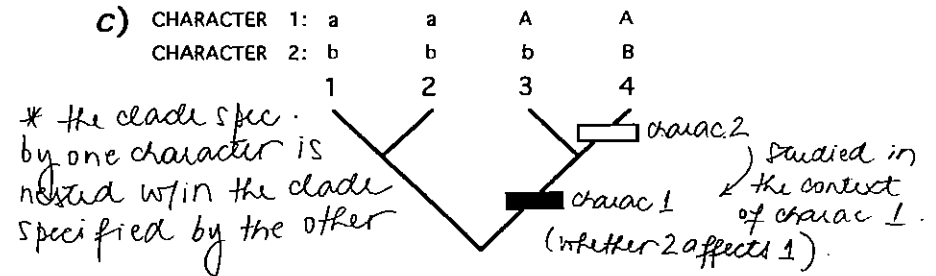
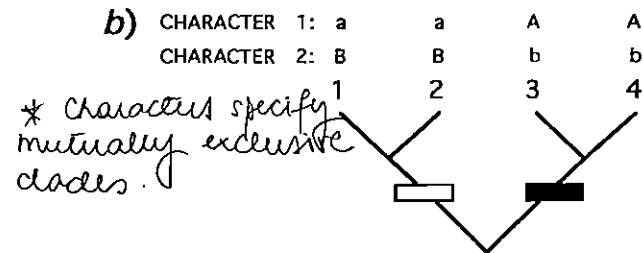
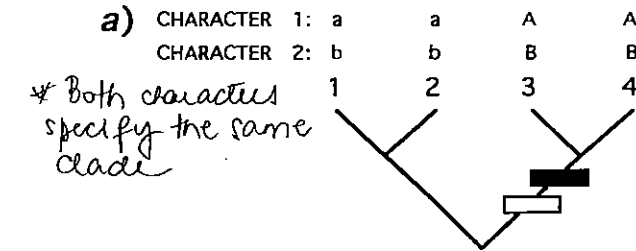


Figure 3 Possible relationships between two phylogenetically congruent characters: (a) the characters specify the same clade; (b) the characters specify mutually exclusive clades; or (c) the clade specified by one character is nested within the clade specified by the other.

of either character are done entirely in the context of the other one. Alternatively, the characters may originate on different lineages. The characters may occur on mutually exclusive lineages (Fig. 3b), in which case evolutionary associations among them are absent. If one character (1) arose on a lineage ancestral to the one on which a second character (2) arose (Fig. 3c), character 2 is studied in the context of character 1. To investigate the origins of character 1, however, it could be misleading to examine its utility only in taxa that also possess character 2, because character 1 arose in the absence of character 2. The use of appropriate phylogenetic comparisons can reveal whether the presence or absence of character 2 affects the biological role of character 1.

An example comes from communication in anoles. Anoles have a large extensible fan on the throat, termed a dewlap, that is used in social contexts by males and, in some species, by females (Fig. 4). Anoles also usually use stereotyped patterns of head-bobbing in such displays. Widespread occurrence of head-bobbing in iguanian lizards indicates that head-bobbing arose much earlier than the anoline dewlap in the evolutionary history of lizards (see Jenssen, 1977; Carpenter, 1986). The hypothesis that head-bobbing evolved initially as a biomechanical necessity for unfolding the dewlap or as an adaptation for displaying the dewlap thus would be inappropriate. A more appropriate hypothesis is that the dewlap evolved as an adaptation for communication and that head-bobbing may have been a prerequisite for the evolution of the dewlap.

The phylogenetic context will identify situations in which a hypothetical character is simply a structural consequence of organismal architecture, development, and allometry (Gould and Lewontin, 1979); such characters will

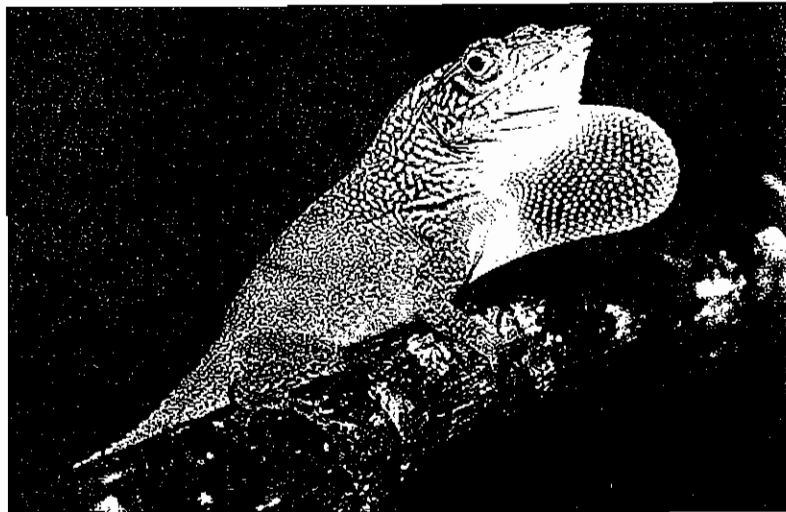


Figure 4 *Anolis grahami* using its dewlap during a display.



Figure 5 *Anolis lutrogularis*, one of the largest anoles, illustrating a large and rugose head. Compare it to the smaller, presumably more ancestral, head structure illustrated by *Anolis grahami* (Fig. 4).

originate as part of a complex of related features of organismal design that are phylogenetically inseparable from each other. Concordant evolution of two characters suggests that purely structural or developmental explanations for their coupling be investigated prior to testing hypotheses of adaptation. For example, large and rugose heads have evolved independently several times within *Anolis* (Fig. 5). In each case, the evolution of these features occurred simultaneously with an increase in body size (see Cannatella and de Queiroz, 1989). The hypothesis that large heads are simply an allometric consequence of an increase in body size, and not an independent character, should be tested and rejected before postulating adaptive explanations specifically for the large heads. Even if these characters are not necessarily coupled developmentally, adaptive explanations for large heads must be formulated within the context of large body size because of the phylogenetic association of these characters; large heads may be adaptive only in animals having large body size. The phylogenetic context therefore restricts nonarbitrarily the combinations of characters that should be examined to test biologically meaningful hypotheses of interaction among characters.

C. Alternatives to Adaptation

Lewontin's (1977) third major criticism of adaptationist studies is that all characters are assumed adaptive, causing the investigator to search only for the means by which a character is adaptive rather than subjecting the hypothesis of

→ solution to the fact that most characters are frequently assumed to be adaptive.

adaptation to potential falsification. The revised definition of adaptation given by Gould and Vrba (1982), in which the concepts of adaptation, exaptation, and nonadaptation are logically separated, provided the first step in answering this criticism. The hypothesis of adaptation makes several predictions, one of which is that a character confers an advantage relative to its phylogenetic antecedents. If the character cannot be distinguished from its phylogenetic antecedents with respect to conferring an advantage to the organisms possessing it, it is termed a nonadaptation (Fig. 6; Baum and Larson, 1991).

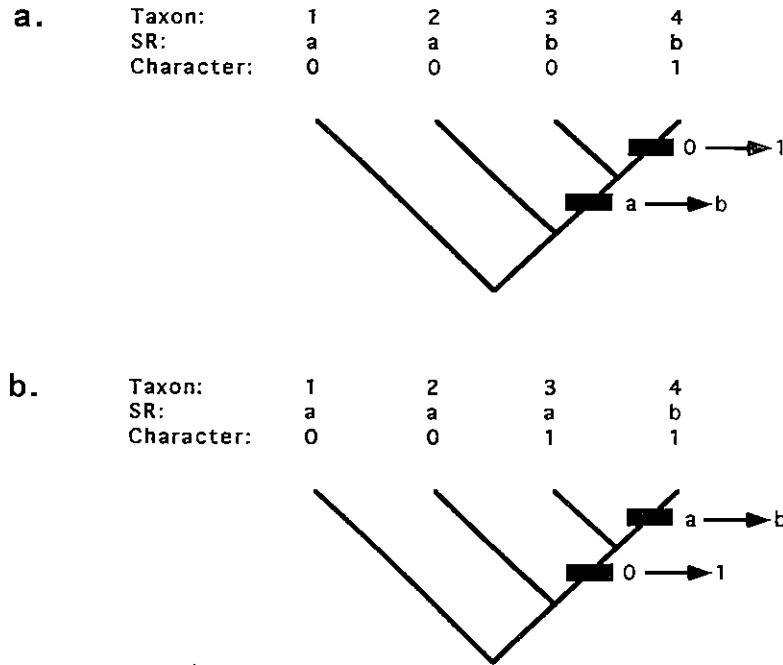


Figure 6 Illustration of the phylogenetic predictions of hypotheses of adaptation, exaptation, nonadaptation, and disadaptation using the selective regime (SR; after Baum and Larson, 1991). Hypotheses are illustrated with reference to taxon 4. (a): A clade having a derived character (1) is phylogenetically nested within a clade having a derived selective regime (b). Adaptation predicts that utility or performance of the derived character (1) will exceed that of the ancestral condition (0) in selective regime b. Primary nonadaptation predicts that characters 0 and 1 have equivalent utility under selective regime b. Primary disadaptation predicts that character 0 has greater utility than character 1 in selective regime b. (b) A clade having a derived selective regime (b) is nested within a clade having a derived character (1). Exaptation predicts that the utility of the derived character (1) exceeds that of the ancestral character (0) in selective regime b. Secondary nonadaptation predicts that characters 0 and 1 are equivalent in utility within selective regime b. Secondary disadaptation predicts that character 0 has greater utility than character 1 in selective regime b.

terms:

- adaptation
- primary nonadaptation
- primary disadaptation
- exaptation

alternative hypothesis of character evolution.

If the character is found to confer an advantage, the hypothesis of adaptation requires further that the character's evolutionary origin occurred by natural selection for its currently observed biological role. This criterion makes several predictions potentially falsifiable by phylogenetic analysis. The biological role currently observed for the character must have been present at its origin. This prediction is tested by examining the character and its utility in different species in a phylogenetic context (Fig. 6). Thorough testing requires comparative studies of functional morphology and selective factors affecting the character (Baum and Larson, 1991) and detailed studies of the natural history of the species being studied (Greene, 1986b, 1994). The hypothesis that a character evolved as an adaptation for a biological role is falsified if that role is associated only with a subset of taxa nested within the clade possessing the character; in this case, the current utility of the character evolved after the character itself (Fig. 6). Using the terminology of Gould and Vrba (1982), such a character is termed an exaptation for the biological role being examined. The history of character evolution in a group of species often may comprise a nested hierarchy of exaptations and adaptations. A character may acquire biological roles other than ones for which it arose by natural selection, and its newly acquired role may lead to selectively driven modification of that character and others. These modifications represent adaptations arranged phylogenetically in a nested hierarchy. The broader goal of studies of adaptation, therefore, can be seen as that of examining the interaction of adaptive, exaptive, and nonaptive influences on evolutionary diversification (see Arnold, 1994).

The nested hierarchy of adaptations and exaptations is illustrated by the evolution of the dewlap in anoles. The dewlap is expanded using the second ceratobranchial bone, a component of the hyoid apparatus. The hyoid apparatus is homologous to the gill arches of aquatic vertebrates; the second ceratobranchial of tetrapods is a homology nested within the more inclusive homology that includes the corresponding gill arches of aquatic vertebrates. The gill arch to which the second ceratobranchial is homologous presumably arose by selection for a respiratory function and constitutes an adaptation for respiration. The gill arches were coopted for feeding in tetrapods, making gill arches an exaptation for feeding, but the derived characteristics of the hyoid apparatus that arose in the context of feeding, including the second ceratobranchial bone, constitute adaptations for feeding. The second ceratobranchial bone then was coopted in anoles for expanding the dewlap and is an exaptation for communication. Derived characteristics of the anoline second ceratobranchial bone evolved for expanding the dewlap constitute adaptations for communication.

One should consider the alternative hypotheses of character evolution to be investigated, especially when hypotheses of adaptation are falsified. Vrba and Gould (1986) present a taxonomy of the deterministic and stochastic processes that may underlie the evolution of characters. They emphasize that evolutionary studies in the past often have emphasized adaptive interpretations because alternative hypotheses involving nonrandom processes were largely absent from traditional Darwinian theory, which invoked random drift as the main alternative to natural selection. They emphasize the importance of a hierarchical expansion of traditional Darwinism to include deterministic

processes acting at the species level (species selection) and the genomic level (selfish DNA) whose consequences often include evolutionary sorting of the organismal-level characters that are usually the focus of adaptationist studies. Organismal characters generated through the indirect action of species selection or selfish DNA are not necessarily expected to have a biological role conferring advantages at the organismal level. The evolutionary study of the effects of selfish DNA and species selection on evolution of organismal characters is a new field. We anticipate that as phylogenetic approaches are used more extensively to test hypotheses of adaptation, rejection of adaptive explanations will lead to greater investigation of these alternative processes as factors affecting the evolution of organismal characters. Vrba and Gould (1986) offer a more detailed discussion of the predictions of hypotheses of species selection and selfish DNA.

So far we have emphasized single processes (natural selection for a particular biological role, exaptation, species selection, selfish DNA, stochastic processes) as the primary explanations of character evolution. The actual evolutionary history of a group undoubtedly features numerous processes acting simultaneously or in sequence to influence the evolution of organismal characters. Different factors may act synergistically or in opposition to each other. Empirical discrimination of alternative causal factors will be most effective when they act in opposition to each other. As the concept of exaptation implies, different biological roles may be performed by a single character and the different roles may exert conflicting selective influences on the differential survival and reproduction of alternative characters. Likewise, species selection and natural selection may have opposite effects on the evolutionary sorting of character variation.

This chapter emphasizes the initial stages of testing hypotheses of adaptation and should be viewed as the beginning of an iterative, deductive process. Hypotheses of evolutionary process often make specific predictions regarding evolutionary pattern that permit rigorous testing and potential falsification. Complex interactions among factors influencing organismal evolution, such as those discussed by Vrba and Gould (1986), may obscure the role of natural selection. Initial results therefore will reveal whether selective factors prevail over any conflicting influences on the evolution of organismal characters. If predictions of natural selection are falsified, alternative causal hypotheses of character evolution should be investigated (Baum and Larson, 1991). If the predictions of natural selection are upheld, the hypothesis of adaptation is subjected to a more refined and detailed investigation (Baum and Larson, 1991; Coddington, 1994).

Because the study of adaptation requires the use of many evolutionary concepts whose contexts and meanings have varied, and because precise definitions are needed for employing phylogenetic methodology, we provide specific definitions and explanations of key concepts in Table 1.

II. A General Protocol for Testing Hypotheses of Adaptation

The hypothesis that a character evolved as an adaptation makes two predictions: (1) that the character evolved in the context of a particular

Prediction of an adaptation:

- (1) the character evolved in the context of a particular selective regime,
- (2) the character is more advantageous than phylogenetic antecedents in that regime.

TABLE I
Phylogenetic Definitions

<i>Adaptation</i>	An organismal character produced by natural selection for a particular biological role (modified from Gould and Vrba, 1982; Baum and Larson, 1991).
<i>Aptation</i>	An organismal character that confers utility to the organism regardless of whether its phylogenetic origin featured natural selection (from Gould and Vrba, 1982).
<i>Biological role</i>	An action or use of a character by the organism during the course of its life history (from Bock and von Wahlert, 1965; Bock, 1979).
<i>Character</i>	A component of organismal phenotype subject to cladistic analysis and to Patterson's (1982) tests of homology. A character that passes Patterson's (1982) tests may be termed a homology or homologous character.
<i>Disaptation</i>	An organismal character whose utility to the organism is demonstrably inferior to that of a phylogenetically antecedent character (from Baum and Larson, 1991).
<i>Effect</i>	The biological role of an exaptation, a use for which the character was coopted, but which was not a factor in the character's evolutionary origin (from Gould and Vrba, 1982).
<i>Exaptation</i>	An organismal character that has been coopted for a use unrelated to its origin. An exaptive character originally may have been an adaptation for a different use or a nonaptation (from Gould and Vrba, 1982).
<i>Function</i>	The biological role of an adaptation, the use for which the character evolved by natural selection (from Gould and Vrba, 1982).
<i>Homology</i>	A component of the organismal phenotype exhibiting the evolutionary properties of conservation, individualization, and uniqueness (Wagner, 1989; see text); empirically identifiable using Patterson's (1982) tests of similarity, congruence, and conjunction.
<i>Homoplasy</i>	Possession by two or more species of a shared attribute that was not derived from their most recent common ancestor; embraces convergence, parallelism, and evolutionary reversal (modified from Futuyma, 1986).
<i>Natural selection</i>	Those interactions between heritable organismal character variation and the environment that cause differences in rates of birth or death among varying organisms in a population (from Vrba and Gould, 1986).
<i>Nonaptation</i>	An organismal character that confers no utility for organismal survival or reproduction relative to phylogenetically antecedent characters. A primary nonaptation is one whose origin cannot be ascribed to the direct action of natural selection. Nonaptation also may arise secondarily by loss of the utility of a character (modified from Gould and Vrba, 1982; Baum and Larson, 1991).
<i>Parsimony</i>	Resolution of homoplasy among phylogenetic characters by choosing as the best working hypothesis the phylogenetic topology that minimizes homoplasy.
<i>Performance advantage</i>	A result of comparative functional analysis showing that a character has enhanced utility not associated with an alternative character (from Greene, 1986a).
<i>Selective regime</i>	Critical aspects of organismal/environmental interaction that are postulated by a hypothesis of adaptation to be significant factors influencing natural selection of the characters being studied. It provides predictions of how natural selection would direct evolution of a character under study as contrasted with other potential influences including stochastic factors, genetic or developmental correlations with other selected characters, or incidental effects of species selection or selfish DNA (from Baum and Larson, 1991).
<i>Sorting</i>	Differential rates of birth or death among varying organisms in populations resulting from any deterministic or stochastic causes (from Vrba and Gould, 1986).

* Would there ever be selection for disaptation?
How does it arise?

* Why do they use the term utility and NOT fitness?

selective regime; and (2) that the character is more advantageous than phylogenetic antecedents in that context. Generally applicable protocols for testing these predictions have been formulated by Greene (1986a), Coddington (1988, 1990), and Baum and Larson (1991). These protocols are compared and contrasted by Baum and Larson (1991) and, although their details differ, they share common goals. A synopsis of the protocol described by Baum and Larson (1991), which incorporates many features of the earlier protocols and adds some new procedures, is given below. Some minor modifications have been incorporated. We present this protocol as a series of six steps to be applied iteratively in testing hypotheses of adaptation.

Step 1: Formulation of Hypotheses of Adaptation - A hypothesis of adaptation must be formulated by making specific statements regarding (1) the character hypothesized to be adaptive, (2) the taxa in which the character is observed and to which the hypothesis of adaptation pertains, and (3) the postulated biological role of the character. The phylogenetic methodology is potentially applicable to taxa of any rank, but we agree with Cracraft (1990) that the most effective resolution of the evolutionary history of a character occurs when comparisons involve closely related species (Cracraft, 1989). The characters studied usually will be fixed or nearly fixed in the species studied except for the effects of recurring mutation. Some cases of intraspecific polymorphism may be appropriate for study if outgroup comparison provides an unambiguous inference of evolutionary polarity of the character states.

Step 2: Reconstruction of Phylogeny - Any methodology that produces a rooted tree relating the taxa containing the character being studied and their most closely related outgroups can be used for this study (see Swofford et al., 1996). Phylogenetic inferences should be most robust when based upon a large number of characters drawn from diverse morphological, molecular, and behavioral systems, and examined for any systematic conflicts among characters (see Larson, 1994). We agree with de Queiroz (1989) that it is not necessarily circular to consider the same characters as sources of phylogenetic information and subject to analysis as potential adaptations. When a phylogenetic hypothesis is based on a large number of characters, however, the phylogenetic hypothesis generally will not depend heavily on the effects of the particular characters being investigated as potential adaptations.

Step 3: Scoring and Phylogenetic Partitioning of Characters - As noted above, every effort must be made to establish homology relations among the characters studied as potential examples of adaptation. The procedures noted here for assessing homology of the characters being studied as potential adaptations are the same procedures applied to all morphological or behavioral characters used to generate the phylogeny in the previous step. Patterson's (1982) tests of similarity, conjunction, and congruence are applied, with congruence assessed using the phylogenetic analysis described in the previous step. A detailed discussion of criteria used for testing similarity of characters is given by Kaplan (1984). Developmental information can be particularly important for determining whether similarity among characters is indicative of homology or only a superficial similarity produced by convergence. Although the importance of developmental information is uncontroversial, the ways in which the information is used to assess homology are highly controversial (Patterson, 1982; de Queiroz, 1985; de Pinna, 1991). A particularly controversial issue is

CHARACTER CONFLICT!

on

Assessing
homology
- incongruence is
resolved
using
Maximum
parsimony
Testing
Hypothesis of ADAPTATION:

Formulate Adap. Hyp \Rightarrow Reconstruct Phylogeny \Rightarrow Assess Homology of character \Rightarrow Assess "Homology" of the selective regime \Rightarrow Infer selective regime in which a character arise + test the biological role of a character \Rightarrow classify the trait

whether a character should be conceived and measured at only a single stage (usually the adult) in the organism's life cycle or whether the character should be conceived as an ontogenetic transformation transcending different stages of the organism's life history (de Queiroz, 1985; de Pinna, 1991).

Incongruence among characters is usually resolved using the criterion of maximum parsimony, although other methods have been proposed. In many cases, more than one equally parsimonious optimization of character changes on the phylogenetic tree will be possible, precluding unambiguous assessment of homology. The implications of the alternative optimizations for testing hypotheses of adaptation must be examined (Maddison and Maddison, 1992; Losos and Miles, 1994).

Step 4: Scoring and Phylogenetic Partitioning of Selective Regimes - The critical factors comprising the selective regime depend on the specific hypothesis of adaptation being tested. The selective regime can incorporate abiotic climatic factors, biotic environmental factors, organismal features, or any combination of these factors. A detailed study of the natural history of the species being studied is generally required for the realistic characterization of selective regimes (Greene, 1986b). Because selective regimes comprise organismal/environmental interactions, they are subject to the same criteria of homology and phylogenetic analysis that apply to morphological characters as discussed in the previous step. Comparative studies of selection acting within populations of related extant species provide potentially the most effective test of the macroevolutionary stability of selective regimes.

Step 5: Assessing the Biological Role or Utility of Characters - Results of the previous two steps are combined to infer selective regimes for the lineages on which a character arises. Of particular interest are the selective regimes under which a character evolved and the selective regimes of extant species that possess the character. The selective regime forms the framework for testing hypotheses of the biological role or utility of the hypothetically adaptive character. The hypothesis of adaptation postulates that the character performs a specific function and that this performance enhances the organism's survival or reproductive success. Predictions of the hypothesis of selective origin of a character therefore can be tested in two ways: (1) functional morphological studies to test the prediction that possession of the character enhances performance, and (2) studies to test the prediction that possession of the character increases rates of survival or reproductive success. Both approaches encompass numerous specific tests and provide valid tests of the selective hypothesis. The particular approaches chosen will depend on the characteristics of the organisms being studied and the ease by which appropriate manipulations and observations can be made.

The most direct approach for evaluating the utility of a character is to study its use in a task, such as the ability to escape predators, that is important for survival or reproductive success. Performance is measured to test the hypothesis that a character provides greater utility than the antecedent character in the relevant selective regime. Several different kinds of comparative and experimental approaches can be used to obtain the relevant measurements. Whenever possible, naturally occurring variation should be used to obtain the alternative characters whose performance is to be compared. When this option is not possible, experimental manipulation can be used to simulate the

alternative characters (e.g., Carothers, 1986; Emerson and Koehl, 1990). Physical models also may be useful for examining how alternative characters affect performance (Rudwick, 1964; Fisher, 1985). Phylogenetic methods are used to infer the utilities of characters as they occurred in ancestral lineages. Detailed coverage of the testing of utility of characters is provided by Fisher (1985).

Measurement of performance is usually the most useful way to test predictions of hypotheses of adaptation because the predictions made are very specific. The question that the character's performance actually enhances survival or reproductive components of fitness (Lauder et al., 1993) can be examined by measuring within populations the rates of survival or reproduction of individuals possessing the hypothetically adaptive character versus its evolutionary antecedent. As with the measurement of performance, use of naturally occurring polymorphism is desirable, although appropriate character variation could be induced using genetic or phenotypic manipulations. Studies of this sort frequently look at the effect of character manipulation on survival (e.g., Kettlewell, 1973; Schluter, 1994), but less frequently examine reproductive aspects of fitness.

Step 6: *Classifying Traits into Categories of Utility/Historical Genesis* - The data gathered above permit characters to be categorized as adaptations, exaptations, nonaptations, or disaptations (Baum and Larson, 1991; Arnold, 1994). A character qualifies as an aptation if it demonstrates significantly greater utility than its phylogenetic antecedent for performing a task that promotes organismal survival or reproductive fitness in a particular selective regime. If the selective regime for which the character is advantageous characterizes the lineage on which it arose, the character is termed an adaptation. The character retains the status of adaptation in all lineages that retain the selective regime present at its origin. A subsequent evolutionary change of selective regime may cause the character to be exaptive, nonaptive, or even disaptive on more recent lineages. A character is termed an exaptation where it is advantageous for a selective regime that arose subsequent to its evolutionary origin. Prior to the change of selective regime, the character may have occupied any of the alternative categories.

If a character is found not to differ significantly in utility from its phylogenetic antecedent, nonaptation constitutes the working hypothesis for further investigation of its evolution. A subsequent study of the natural history of the species possessing the character may reveal undiscovered components of the selective regime or biological roles that may lead ultimately to rejection of the hypothesis of nonaptation; until nonaptation is rejected, however, the character cannot be considered an adaptation or exaptation. Primary nonaptation denotes a character that arose by means other than natural selection, whereas secondary nonaptation denotes a formerly aptive character whose utility was lost by an evolutionary change in the selective regime (Baum and Larson, 1991).

Disaptation describes characters whose utility in a particular selective regime is significantly less than that of their phylogenetic antecedents. Formerly aptive or nonaptive characters can become disaptations through an evolutionary change of selective regime. Primary disaptation implies that the character replaced one of superior utility at its origin, which runs counter to

the theory of natural selection. Empirical results suggesting primary disaptation probably indicate an erroneous assessment of selective regime or homology of characters, particularly lack of individualization from other features subject to selection (see Baum and Larson, 1991). Note that primary nonaptations and primary disaptations are the only categories for which origin of a character by natural selection is explicitly rejected.

As this discussion illustrates, the adaptive status of a character is not necessarily stable during its evolutionary history and may be categorized differently at different points in its evolutionary history. Characters that qualify as adaptations must arise as such; characters whose evolutionary origin is nonadaptive do not become adaptations secondarily by subsequent evolutionary change. Characters never originate as exaptations, but may become exaptive only through evolutionary change in the selective regime and utility of the character (Fig. 6). Characters may be nonaptive or disaptive either by their evolutionary origin or secondarily by evolutionary changes in selective regime.

Rigorous discrimination of the categories described above occurs only when phylogenetic resolution is precise. An ambiguous situation occurs, for example, when evolutionary changes in both the character and selective regime occur on the same branch of the phylogenetic tree. In such cases, one cannot determine whether the character arose before or after the new selective regime, precluding discrimination of adaptation and exaptation. If appropriate variation occurs, one would expect response to a new selective regime to occur rapidly; entry into the new selective regime and character change then would not be separated by phylogenetic branching and would be reconstructed on the same branch of the evolutionary tree. Similarly, exaptation could occur rapidly if a new character leads to modification of the selective regime which would be expected, for example, for characters termed key innovations (Baum and Larson, 1991). However, in many cases of exaptation, the new selective regime will not be immediately available for a variety of reasons (e.g., the new regime occurs in other biogeographic areas or results from subsequent evolution of other taxa). In these cases, changes in the character and selective regime are more likely to be separated by a branching of lineages. For this reason, Arnold (1994) argues that if one observes many instances of simultaneous evolution of a character and a selective regime, adaptation is the preferred working hypothesis.

The evolution of toepads in anoles, mentioned previously, can be used to illustrate this protocol for testing hypotheses of adaptation. Toepads are widely considered adaptive for arboreality, but this hypothesis has not been tested rigorously. The manner in which this hypothesis can be tested is straightforward. The hypothesis states that the evolution of toepads conferred increased clinging capability and occurred in the context of a selective regime favoring enhanced arboreality. Cartunill (1985) identifies two ways in which arboreality may be achieved, by grasping and by adhesion. The presence of claws in lizards provides some grasping ability, but toepads potentially provide both improved grasping ability and adhesion. The phylogenetically antecedent condition of anoline digits is to possess claws, but lack toepads; the presence of toepads in addition to claws is the derived condition. Arboreality in anoles, therefore, encompasses at least two selective regimes potentially affecting the

evolution of toepads for clinging. Toepads and their setae may enhance the grasping of narrow supports and provide adhesion on smooth surfaces. The phylogenetic hypothesis necessary to examine this question is presented in Fig. 2. We already have discussed the evidence that toepads pass empirical tests of homology. Almost all anoles are arboreal; the few that are not have become terrestrial secondarily (Peterson and Williams, 1981). Phylogenetic reconstruction indicates that arboreality evolved before the evolution of toepads. Functional studies demonstrate that all 17 species of *Anolis* examined can cling to a smooth surface (Fig. 7; Losos, 1990a; Irschick et al., in press); lizard species without toepads are unable to cling to such surfaces (Losos, unpublished; *Polychrus*, however, has not been examined). Functional studies are not yet available to examine the potential utility of toepads for grasping narrow branches. Thus, we can reject the null hypothesis of functional equivalence for comparing digits that contain toepads versus the phylogenetically antecedent condition in which they are absent. The evolution of toepads is associated functionally and phylogenetically with increased clinging ability, consistent with the hypothesis that toepads constitute an adaptation to adhesion on smooth arboreal surfaces. Further tests of this hypothesis might include experimental removal of the setae to examine their effect on clinging in the lab or survival in the field, detailed behavioral studies (Greene, 1986b, 1994) to refine understanding of the utility of the pad, and measurements of grasping ability on rough or narrow surfaces.

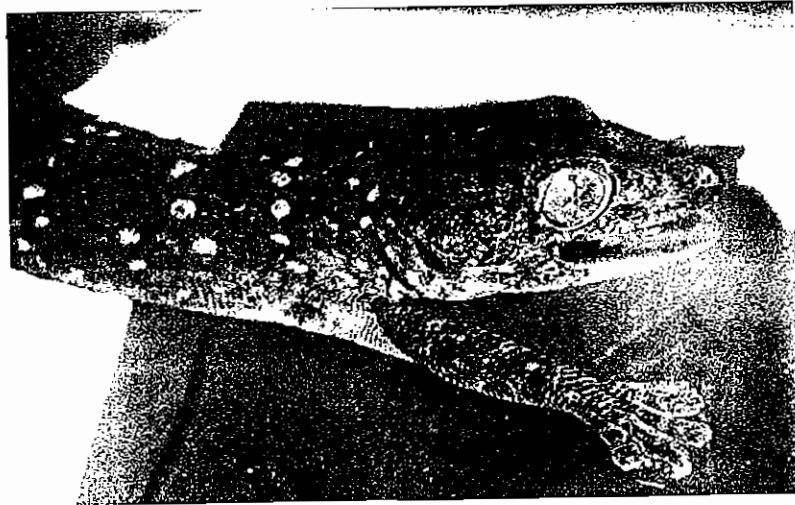


Figure 7 Measurement of clinging ability in a tokay gecko (*Gekko gecko*). Lizards are placed on a force plate and pulled downward, permitting measurement of the adhesive force generated (see Irschick et al., in press). Photograph by D. J. Irschick.

III. Using Homoplasy to Test General Hypotheses of Adaptation

Parallelism and convergence produce common attributes in nonhomologous characters of different taxa. Common attributes produced by parallelism may constitute genuine similarity whereas convergence produces only superficial resemblances among characters. The different lineages in which parallel or convergent evolution are observed may share a selective regime through common ancestry or may have evolved comparable selective regimes via parallel or convergent evolution. Parallelism permits a nearly exact replication of tests of adaptive hypotheses, and convergence permits repeated testing of adaptive hypotheses for the common attributes of convergently evolved characters. Each of the characters related by parallel or convergent origin should individually pass the tests of homology and be subjected to the phylogenetic tests of adaptation described in the protocol outlined above.

Parallelism and convergence also permit testing what we call general hypotheses of adaptation. If similar attributes have evolved multiple times in different lineages, one can test the general hypothesis that evolution occurred in each case under similar conditions of selective regime. This comparative approach, which has been used in evolutionary biology since the field's inception, has been reviewed by Pagel (1994a). Common attributes of different characters are hypothesized to be functionally analogous (McLennan and Brooks, 1993; Wenzel and Carpenter, 1994); this hypothesis should be tested by incorporating analyses of performance when investigating a correlation between the evolution of common attributes and selective regime (e.g., Losos, 1990a; Arnold, 1994; Coddington, 1994).

A general hypothesis of adaptation is strongest if one observes many independent evolutionary origins of similar attributes occurring in the contexts of comparable selective environments. Statistical methodology can be employed to test the null hypothesis that an observed association between shared attributes of characters, functional performance, and selective regime occurred by chance. The wide variety of statistical methods that has been proposed in the past few years is too great to review here; interested readers may consult reviews by Maddison and Maddison (1992) and Losos and Miles (1994).

One commonly used statistical approach is the concentrated changes test (Maddison, 1990; Maddison and Maddison, 1992; Sillén-Tullberg, 1993) [see Pagel (1994b) for a recent method that relies on maximum likelihood rather than parsimony]. Given two attributes (one of which could be a selective regime), #1 and #2, each of which has two states, *A* and *a* and *B* and *b*, this test asks whether the evolution of *a* → *A* in character #1 occurs more often when the state of character #2 is *B* than would be expected by chance. For studies of adaptation, this translates to asking whether a particular character evolved in taxa occupying a specific selective regime more often than expected by chance. This test is necessary because if most members of a lineage occupy a particular selective regime, then one would expect most instances of character evolution to occur in taxa occupying that selective regime by chance alone. For small numbers of taxa and instances of character evolution, exact calculations of probability are possible, whereas for larger numbers, simulation is necessary. Either type of analysis can be performed using MacClade (Maddison and

Charac. #1 @ #2 selective Reg
 Aa // Bb
 a → A ⇒ test statistically whether this change
 occurs more often than expected
 by chance.

Maddison, 1992).

The evolution of lizard toepads is useful for illustrating this approach. As mentioned earlier, toepads evolved convergently in three lineages of lizards. We therefore can test the general hypothesis that lizard toepads represent an adaptation to arboreality. Within geckos, homology of the toepads is clear (Ruibal and Ernst, 1965), but within skinks, microstructure of the toepad varies (Williams and Peterson, 1982), which suggests that toepads of different species might not be homologous. For this analysis, assumption of homology within skinks is statistically conservative because it minimizes the number of times that toepads have evolved in arboreal selective regimes. Measurements of performance are scant for geckos, toepad-bearing skinks, and their outgroups, but existing data suggest that all toepad-bearing lizards have enhanced clinging ability (Irschick et al., in press).

Consequently, the first hypothesis to test is that the evolution of toepads and clinging ability is phylogenetically associated in lizards. Testing this hypothesis requires a fully resolved phylogeny for lizards (including snakes that are phylogenetically part of the lizard clade), which is currently unavailable. For heuristic purposes, we present a phylogeny (Fig. 8) that follows Estes et al. (1988) for interfamilial relationships among limb-bearing lizards (i.e., excluding pygopodids, snakes, and other legless, and thus padless, squamates).

Given the phylogeny in Fig. 8, expanded toepads and enhanced clinging capability are inferred to have evolved simultaneously three times and no times separately. Using MacClade, we calculate that the probability of three events of toepad evolution occurring simultaneously with the evolution of clinging ability is $P = 0.000008$. Each instance of evolution of toepad morphology and enhanced clinging ability occurs on an arboreal lineage. The probability of this occurring by chance is $P = 0.028$. Thus, these analyses uphold the hypothesis that expanded toepads in lizards have evolved as an adaptation to increase the clinging ability in arboreal situations.

The relationship between general hypotheses of adaptation and hypotheses specific for a particular character in a particular taxon is complex. A statistically significant association between the shared attributes of characters and selective regime has been interpreted as strengthening the hypothesis that each character is individually adaptive as proposed. However, a particular character may depart from the general trend and fail to constitute adaptation even if other characters sharing attributes with it are adaptive. Furthermore, a particular character may be adaptive as proposed even if the general hypothesis of adaptation is not upheld.

IV. Adaptation and Quantitative Characters

The protocol just outlined emphasizes discrete (or categorical) characters. Essentially the same conceptual approaches can be used to study adaptive evolution in quantitative (or continuous) characters, although details of the phylogenetic analysis will differ. A number of methods based on parsimony can be used to reconstruct the evolution of continuous characters; one of these is mathematically essentially identical to the one used for categorical characters (Swofford and Maddison, 1987). For continuous characters, most extant taxa

will differ in their character states as a result of sampling, genetic drift, and other processes. Consequently, reconstructions often will reveal evolutionary change occurring on many branches of the tree. Thus, rather than testing the hypothesis that the character evolved within a particular selective regime, as one would do with a categorical character, one might test the hypothesis that character evolution has been greater in lineages experiencing the selective regime than in lineages not exposed to it. Furthermore, because populations often contain substantial genetic variation for continuous characters (Falconer, 1981), one might expect characters to evolve quickly after the lineage enters a new selective regime. Using this assumption, one can test the more restrictive hypothesis that evolutionary change of a character is greater on branches experiencing a change of selective regime.

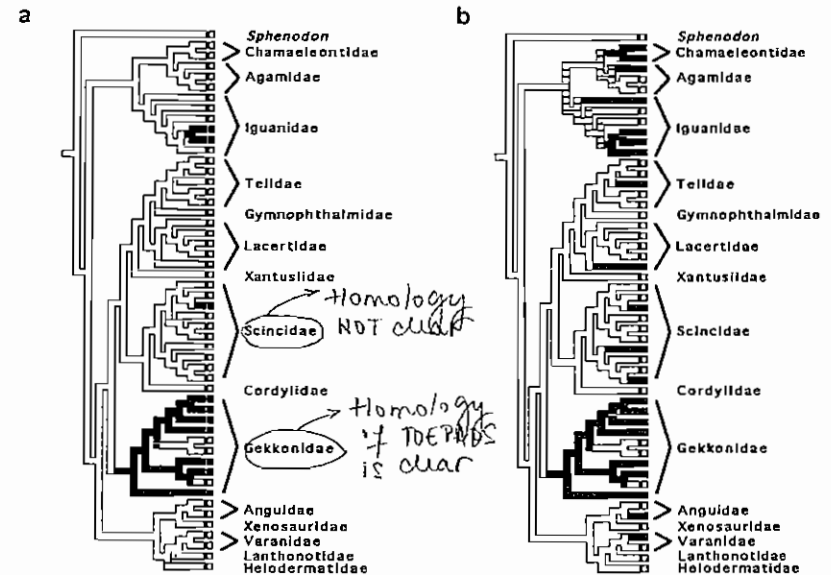


Figure 8 (a) Toepads and clinging ability are distributed identically in lizards and are shown together. Black bars represent lineages characterized by expanded pads and clinging ability. Interfamilial relationships follow Estes et al. (1988). The number of lineages illustrated within each family is roughly proportional to the number of species within each family, but is not meant to depict specific relationships; rather, the tree is presented for heuristic purposes. Figure prepared using MacClade (Maddison and Maddison, 1992). (b) Evolution of use of arboreal habitats. Hatched lineages indicate ambiguous reconstruction of ancestral habitats. In this case, arboreality could have arisen once in the Acrodonta (=Chamaeleontidae, Agamidae, and Iguanidae) followed by three separate losses of arboreality for a total of four evolutionary changes. However, an equally parsimonious reconstruction would postulate four independent origins of arboreality with no reversals; other equally parsimonious resolutions also exist.

This approach can be applied to cases in which comparable selective regimes have arisen multiple times. For example, evolution of body size in Lesser Antillean anoles is hypothesized to be greater on the lineages that moved from a one-species to a two-species island than on the remaining branches showing no change in the number of species. In this case, the selective regime refers to size of prey; in sympatry, anoles diverge in body size to utilize different sizes of prey (Schoener, 1970). The hypothesis was upheld for anoles of the northern Lesser Antilles, but not for those in the southern Lesser Antilles (Losos, 1990b).

In some cases, the focal character, performance, and selective regime all will be continuous variables. Several examples of continuous selective regimes are temperature, dimensions of structural habitat, and size of prey. For studies in which all three parameters are continuous variables, the hypothesis of adaptation would predict that all three variables are correlated in their evolution. Numerous methods have been advanced to investigate the correlated evolution of continuous characters. They are reviewed elsewhere (e.g., Harvey and Pagel, 1991; Gittleman and Luh, 1992, 1994; Maddison and Maddison, 1992; Miles and Dunham, 1993; Losos and Miles, 1994; Purvis et al., 1994; Westneat, 1995) and the field is changing rapidly with new developments. Broadly speaking, however, three explicitly phylogenetic approaches exist:

1. Evolutionary reconstructional methods ("Directional Methods" of Harvey and Pagel, 1991) use one of several algorithms based on parsimony, or other criteria (e.g., Harvey and Purvis, 1991; Lynch, 1991), to reconstruct evolutionary change of each variable. Then, the amounts of change occurring on all lineages are calculated for all variables and examined for correlated evolution.

2. Autocorrelational methods (Cheverud et al., 1985; Gittleman and Kot, 1990; see also Lynch, 1991) were developed to examine the extent to which character values among extant taxa reflect their phylogenetic relationships; for example, is the similarity between pairs of taxa a function of their relatedness? This approach uses a phylogeny to generate a matrix of phylogenetic relatedness among all taxa. This matrix is then used in a maximum likelihood-based regression in which species' values are estimated using the weighted average of related species, the weights being specified by the matrix of phylogenetic relationships. If, in fact, no relationship exists between observed and estimated values for each species, then the autocorrelation coefficient will not differ significantly from zero and phylogenetic information may not be informative in studying character evolution (see Martins, 1996). Presumably, this result occurs when characters evolve so rapidly that closely related species are not necessarily similar phenotypically. These methods can be used also to calculate how different each species' phenotypic value is from that predicted based on its phylogenetic relationships. This method has been used to examine whether a correlation exists between such "specific values" calculated for several variables (reviewed in Purvis et al., 1994; Martins, 1996), although the underlying evolutionary basis for this approach is not clear (Losos and Miles, 1994).

3. The independent contrasts method (Felsenstein, 1985) is the most widely used method for studying correlated evolution. The rationale underlying

reconstruct
the evol
change of
each var. =>
Examine
correlated
evolution

9.9

this method is that each ancestral node in a phylogenetic tree gives rise to two descendants (either extant species or other ancestral nodes). The amount of difference in value of a continuous character between these two descendants, termed a contrast, represents the amount of evolutionary change that has accrued since the descendants diverged from their common ancestor; however, contrary to directional methods, independent contrasts do not specify on which of the two branches the evolutionary change occurred. For a fully resolved phylogenetic tree with n taxa, $n - 1$ contrasts exist. Statistical methods are used to calculate contrasts using a model of character evolution and the lengths of branches in units of expected evolutionary change (proportional to time in a model of gradual evolution). Contrasts calculated for several variables are then examined for correlation (see Garland et al., 1992 for details). In the absence of a fully resolved phylogeny, one can calculate contrasts for phylogenetically nonoverlapping pairs of extant species (Felsenstein, 1985; Burt, 1989).

We illustrate the study of adaptation in quantitative characters using independent contrasts (data are presented in Table 2). Distantly related species of *Anolis* that occupy similar structural habitats often are morphologically similar (Williams, 1972, 1983). One character that often differs among species occupying different habitats is relative length of the hindlimb (Moermond, 1979; Williams, 1983; Pounds, 1988; Losos, 1990a), which is related to running ability (Losos, 1990c; Losos and Irschick, 1996). Analysis of principal components of several variables of the structural habitat indicates that the second axis reflects a trade-off between low, broad supports (high values on PC II) versus high, narrow supports (low values on PC II). One might hypothesize that this variation in habitat use is associated with variation in length of limbs; lizards having high values on PC II might have evolved long legs to run quickly on the ground in pursuit of prey and to confront conspecifics, whereas lizards having low scores on PC II have shorter limbs to enhance locomotion on narrower supports. The following hypothesis of adaptation is suggested: species of *Anolis* have adapted to changes in structural habitat by modifying the relative length of the hindlimb to maximize capability for effective locomotion.

Figure 9 presents the phylogeny used in this analysis and indicates 13 independent contrasts. Given the lack of knowledge concerning modes of character evolution, the most conservative approach is to try several maximally distinctive models to examine whether different assumptions qualitatively alter the results of the analysis. Consequently, each analysis was run twice, once assuming a model of gradual evolution using the branch lengths indicated in the figure, which were derived from molecular systematic studies, and once assuming that change occurred only at speciation events; hence lengths of all branches were set equal [see Garland et al. (1992) for further details on use of independent contrasts].

We first tested the hypothesis that structural habitat is an important selective regime affecting locomotion. If this hypothesis is correct, then locomotor behavior should have evolved concordantly with structural habitat. In support of this hypothesis, evolutionary increases in the use of low habitats were associated with evolutionary increases in the frequency of running and decreases in the frequency of walking (i.e., contrasts that were large and positive for PC II were correlated with large positive contrasts for change in running frequency and large negative contrasts for change in walking frequency;

e.g. - Distantly related spp. of *Anolis* => occupy similar structural habitats => morphologically similar.
- HYPOTHESIS: spp. have adapted to changes in structural habitat by modifying the length to ↑ locomotion
see next page (fig. 9)

TABLE 2
Data for Anolis Species in Independent Contrasts Example¹

	Snout-vent length (mm)	Hindlimb length (mm)	Sprint speed (m/s)	Run frequency (% all moves)	Walk frequency (% all moves)	PC II score
<i>A. cristatellus</i>	4.156	3.985	-2.154	0.869	0.548	0.783
<i>A. cuvieri</i>	4.844	4.559	-2.172	---	---	-1.676
<i>A. evermanni</i>	4.132	3.869	-1.988	0.600	0.821	-0.118
<i>A. gundlachi</i>	4.176	4.054	-2.154	0.714	0.621	1.124
<i>A. krugi</i>	3.884	3.660	-1.966	0.533	0.781	0.456
<i>A. poncensis</i>	3.781	3.440	-1.952	0.660	0.720	0.471
<i>A. pulchellus</i>	3.774	3.482	-1.917	0.452	0.896	-0.170
<i>A. stratulus</i>	3.794	3.489	-1.784	0.838	0.631	-1.517
<i>A. garmani</i>	4.681	4.367	-2.293	0.739	0.736	-0.381
<i>A. grahmi</i>	4.112	3.816	-2.087	0.778	0.596	-0.707
<i>A. lineatopus</i>	4.035	3.830	-2.079	0.861	0.402	0.730
<i>A. opalinus</i>	3.867	3.528	-1.981	0.987	0.461	0.424
<i>A. sagrei</i>	3.892	3.589	-1.988	0.844	0.445	1.707
<i>A. valencienni</i>	4.286	3.656	-1.897	0.420	1.012	-1.125

¹ Data from Losos (1990a); no locomotor behavioral data are available for *cuvieri*.

Morphological and sprint data are ln transformed; locomotor behavioral data are arcsine square-root transformed.

Fig. 10a; Table 3). Hence, the assumption that this aspect of structural habitat may be an important selective regime for locomotion appears reasonable.

We then examined whether the evolutionary changes of morphology, locomotor capabilities, and habitat are associated. We removed the effect of body size on hindlimb length and sprinting capability, but not structural habitat (see Losos, 1990a), by calculating residuals of each variable regressed upon snout-vent length, a common proxy for body size. These residuals were calculated using the contrasts for each variable. With the effect of size removed, evolution in limb length is positively associated with evolutionary change in sprinting capabilities (Fig. 10b; Table 3). In turn, an evolutionary change in structural habitat is associated with a change in sprinting capability (Fig. 10c; Table 3). Hence, we suggest that relative hindlimb length in these lizards has evolved as an adaptation to changes in structural habitat. Further tests of this hypothesis would include a more detailed examination of anoline behavior and use of habitats, and the effect of limb length on survival and reproduction in natural populations.

V. Criticisms of Phylogenetic Approaches to the Study of Adaptation

Adaptation and phylogenetic analyses are inherently controversial subjects; inevitably, criticisms will be made regarding the phylogenetic methodology for studying adaptation advocated here. Two critiques of these methods have

TABLE 3
Results of Independent Contrasts Analyses¹

	Gradual model		Speciational model	
	r ²	P	r ²	P
Run frequency vs. habitat use (PC II)	0.28	0.062	0.27	0.070
Walk frequency vs. habitat use	0.43	0.016	0.45	0.013
Relative sprint speed vs. relative hindlimb length	0.66	0.001	0.68	0.001
Habitat use vs. relative hindlimb length	0.50	0.005	0.56	0.002

¹All analyses are regressions through the origin. Successful standardization of contrasts was verified prior to analyses. See Garland et al. (1992) for details.

appeared (Leroi et al., 1994; Frumhoff and Reeve, 1994). Here we discuss these critiques and conclude that their arguments do not provide a compelling reason to abandon phylogenetic methods for the study of adaptation.

Leroi et al. (1994) acknowledge that the phylogenetic methodology that we describe will identify adaptations accurately in some instances, such as the repeated evolution of "crown of thorns" bristles in flea lineages whose hosts live in hazardous habitats (Traub, 1980) or fringes on the toes of phylogenetically diverse lizards that utilize sandy substrates (Luke, 1986). Nonetheless, Leroi et

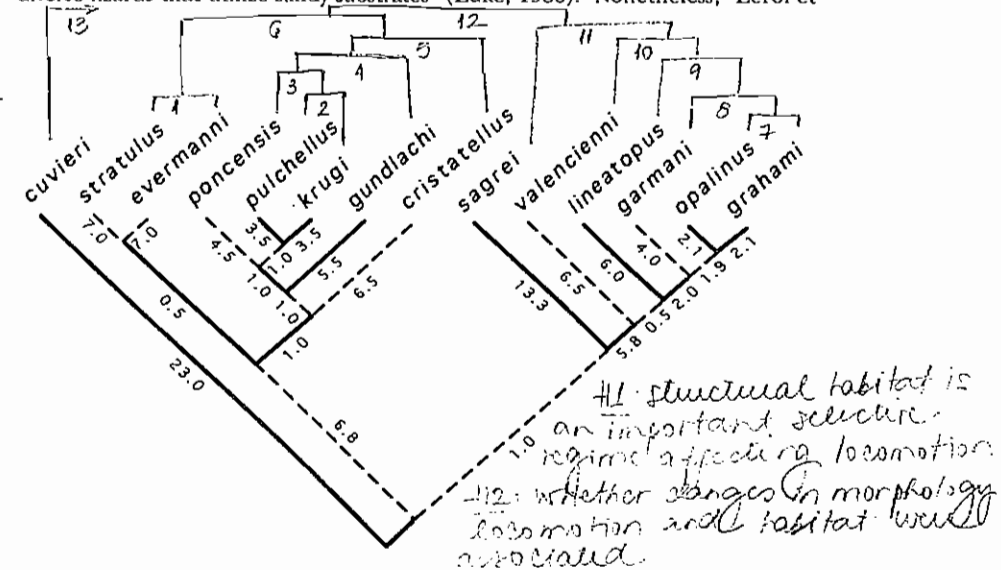
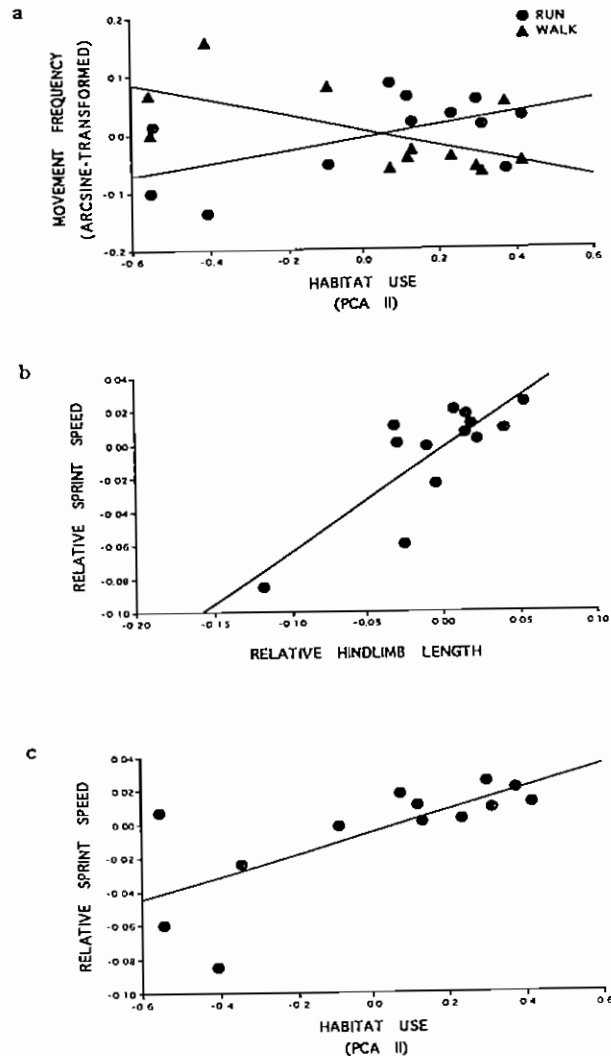


Figure 9 Phylogeny of *Anolis* lizards used in the example of independent contrasts. Thirteen contrasts are indicated by the black and hatched lines. Each contrast represents the two branches diverging from an ancestral node. Numbers represent the lengths of branches, which are not drawn to scale. Sources of phylogenetic information are discussed in Losos (1990a, 1992).



al. (1994) consider such cases exceptions, arguing (p. 383) that "phylogenetic patterns will often suggest that a trait is an adaptation when in fact it is not and suggest that it is not, when in fact it is." This conclusion stems from three major sources: consideration of the genetics of character evolution, a critique of the concept of the selective regime, and a reliance on inductive methods of scientific inquiry. We discuss these criticisms together with criticisms concerning the use of parsimony (Frumhoff and Recve, 1994).

A. Genetics of Character Evolution: Microevolutionary and Macroevolutionary Perspectives

The critique of Leroi et al. (1994) contains an extended argument that genetic information is critical for evaluating the role of adaptive explanations in character evolution. In essence, Leroi et al. (1994) view the phylogenetic study of adaptation as if its goal were to identify the genetic details of the response to natural selection occurring in a single population in the evolutionary past. This is a misrepresentation of the goals of macroevolutionary studies of adaptation. Although we agree that genetic information is important for understanding the responses to selection occurring within a population on a microevolutionary time scale, we do not agree that lack of such information compromises phylogenetic tests of the role of adaptation occurring on a macroevolutionary time scale. In a microevolutionary study, the evolutionary response of a population to selection on character variation depends critically on the specific genetic system or background underlying this variation. In contrast, for a macroevolutionary study, we begin with the observation that a character has evolved and ask whether natural selection can explain its origin.

Leroi et al. (1994) note that a character may evolve for reasons other than natural selection, such as pleiotropy or genetic correlation. The purpose of the methodology outlined in this chapter is to identify and test predictions that are specific to a hypothesis of character evolution by natural selection, but would not be made by alternative hypotheses. In their criticisms of phylogenetic approaches, Leroi et al. (1994) overlook the emphasis on functional studies (e.g., Greene, 1986a; Coddington, 1990; Baum and Larson, 1991) which provide a means of testing predictions specific to the hypothesis of natural selection. Other potential explanations, including developmental or genetic correlations of the character being studied with other selected characters, do not predict an increase in utility or a specific biological role for the character being studied.

Leroi et al. (1994) proposed an alternative method by which phylogenetic information could be used to study character evolution. They suggest (p. 397)

Figure 10 Analysis of locomotor adaptations in *Anolis*. (a) The relationship between habitat use and frequencies of walking and running. Habitat use is represented by the second axis in an analysis of principle components (Losos, unpublished). Each point represents one contrast in a phylogenetic analysis of independent contrasts using the gradual model from Table 3. Regression lines are for heuristic purposes; analyses using contrasts require regression through the origin (Garland et al., 1992). (b) The relationship between relative limb length and relative sprint speed. Variables are residuals calculated from regressions against snout-vent length. Residuals are calculated using values of contrasts. (c) Relationship between relative sprint speed and habitat use.

that "patterns of selection, genetic variation, covariation, and interaction might be measured for several species belonging to a single monophyletic group" and this information could then be optimized onto a phylogenetic tree to infer historical events. They illustrate this method as a means of testing whether a genetic correlation was responsible for correlated evolution of two traits. Certainly, there can be long-term patterns of correlation among characters and such correlations will be of interest in evaluating hypotheses of character evolution. Epigenetic or developmental factors producing correlation of characters can be stable on a macroevolutionary time scale and may be manifested in patterns of pleiotropy and epistasis observed within populations at any given period of evolutionary history. Rather than an alternative to our approach, this methodology is an appropriate extension of it because it tests phylogenetic predictions specific to other processes that could be responsible for character evolution. Hypotheses of developmental constraint, for example, can be tested phylogenetically using a protocol comparable to the one that we describe for studying adaptation; this approach, however, is in no manner a replacement for the study of natural selection.

B. Critique of the Use of Selective Regime

Leroi et al. (1994) criticized the concept of selective regime as being defined so broadly as to be useless. As with any methodology, the use of selective regimes can be abused. The concept is meaningful only if one can identify with precision important components of organismal/environmental interaction that may influence the action of selection on character variation. Oddly, Leroi et al. (1994) accept the selective regime postulated by Luke (1986), which they state to be "type of substrate," but are critical of the use of "scansorial/arboreal vs. terrestrial" as different selective regimes in plethodontid salamanders (Baum and Larson, 1991).

In both cases, as well as those discussed in this chapter, it is important that the selective regime be described in sufficient detail to predict whether selection would favor individuals with some capabilities over individuals with others. Certainly, the hypothesis that an attribute increases locomotor performance on sand, as fringes do (Carothers, 1986), would evolve in taxa living on sandy substrates is reasonable and worth testing, as is the hypothesis stated earlier that a feature that enhances the ability to hold onto a rough surface would evolve in arboreal and scansorial salamanders. Thus, the concept of the selective regime has utility when properly employed.

In this vein, the criticisms of Leroi et al. (1994) regarding the analysis of arboreal vs. terrestrial selective regimes in plethodontid salamanders represent a superficial reading and misrepresentation of Baum and Larson (1991). Contrary to the statements of Leroi et al. (1994), Baum and Larson provide an extensive discussion of the relationships among morphology, function, and arboreality in salamanders, as evidenced by the comparison of several different plethodontid lineages that occupy different arboreal selective regimes. Leroi et al. (1994) also misrepresent Baum and Larson (1991) by stating that the evolution of derived attributes studied as adaptations in *Aneides* did not occur in other salamanders, when, in fact, several similar instances of such evolution

were explicitly discussed (pp. 14 and 15), allowing a test of a general hypothesis of adaptation.

C. Use of Parsimony for Reconstructing the Evolution of Characters and Selective Regimes

Frumhoff and Reeve (1994) identify an important assumption of phylogenetic approaches to the study of adaptation, namely, that the evolution of a character can be reconstructed accurately. If reconstructions of character changes are incorrect, mistaken conclusions may follow. Frumhoff and Reeve (1994, p. 173) identify assumptions implicit in parsimony: "Character state optimization will accurately reveal the timing and direction of historical transitions between character states only if the rate of character change within lineages is low relative to the rate of ... cladogenesis. If, in particular, transitions between character states are sufficiently frequent within a species lineage, the phylogenetic 'memory' of these traits will decay such that their mapping onto a cladogram may bear little relation to the actual sequence of character state transitions between ancestral and derived taxa..." We also agree with Frumhoff and Reeve (1994, p. 174) that "parsimonious inferences of the ancestral states of a given character may often not be robust and should be interpreted with caution." Schultz et al. (1996) provide a thorough reanalysis of this argument, however, and show that the error rates for reconstruction of ancestral characters are considerably lower than the estimates of Frumhoff and Reeve (1994).

One must be aware of parsimony's underlying assumptions and take care to assess whether they are likely to be violated. For example, characters that differ greatly among even closely related species may experience rates of evolution too great to be studied using older phylogenetic divergences. As discussed earlier, phylogenetic autocorrelation may be an appropriate means of identifying such situations. Parallel evolution in closely related taxa will be more difficult to detect. However, if it occurs as a result of parallel evolution in selective regimes, as Frumhoff and Reeves speculate, then, in fact, the procedure will be conservative by identifying one instance of evolution of a trait in a particular selective regime when, in fact, multiple instances occurred.

Frumhoff and Reeve (1994) also doubt that the evolution of selective regimes will be conserved enough to permit accurate reconstruction via parsimony. As with character evolution, some selective regimes may evolve too rapidly for these methods to be useful. Nonetheless, the similarity in environmental circumstances that often characterize large, old clades (e.g., the arboreal habitats of *Anolis*, the aquatic lifestyle of whales) indicates that selective regimes often evolve slowly enough to be useful in phylogenetic studies of adaptation.

Any phylogenetic study of adaptation must examine characters and selective regimes at a phylogenetic level for which evolutionary changes are observed, but not extensively superimposed on single lineages. The problems associated with the uses of parsimony discussed above are diminished in importance if the most appropriate phylogenetic level is established; closely related lineages are used to investigate rapidly evolving characters and more

distantly related lineages are compared to study more slowly evolving characters. Undoubtedly, certain characters and taxa will be more amenable than others to studies of adaptation because of historical details regarding rates of evolution, branching of lineages, and extinction. Although these factors are beyond the control of the investigator, choice of taxa and characters amenable to thorough phylogenetic analysis can ensure that studies of adaptation can be productive. As illustrated by our examples from anoles, characters of interest to evolutionary ecologists often are amenable to phylogenetic analysis. If the criticisms of Frumhoff and Reeve (1994) are taken to their logical extreme, the concepts of homology and phylogeny would be meaningless and functional properties of characters would not be generalizable beyond the species level. This conclusion is equivalent to discrediting the entire field of phylogenetic systematics, as well as comparative anatomy, palaeontology, and, in fact, all of comparative biology.

D. Hypothetico-deductive vs Inductive Approaches to Scientific Inquiry

Criticisms of phylogenetic approaches to studying adaptation invariably emphasize the inability to demonstrate in a positive sense the occurrence of natural selection in the evolutionary past. Expectation of positive demonstration or proof reflects an inductive approach to science that we consider inappropriate, not only to the study of historical processes, but to studies of any natural phenomenon. Rather than attempting to show by induction that natural selection has acted in a particular population, we derive predictions from the hypothesis that natural selection has acted and seek empirical data that potentially would falsify that hypothesis. We argue above that the historical hypothesis of adaptive origin of a character makes specific, testable predictions that are not made by alternative hypotheses explaining the evolutionary origin of a character. These hypotheses, even for explaining unique evolutionary events, make numerous predictions whose testing requires studies of morphology, function, development, phylogeny, genetics, ecology, and behavior. Empirical results in all of these fields provide potential falsifiers of hypotheses of adaptation and sources for refining those hypotheses.

Associated with the inductive arguments used to criticize phylogenetic studies of adaptation is the statement that results of the proposed phylogenetic study of adaptation may be in error. There is no methodology in any aspect of evolutionary biology (or any science) that is guaranteed to produce a completely correct result in a single study. The usefulness of a general methodology, such as the one presented here, is to provide a basis for repeated empirical testing of hypotheses. The methodology, if applied in an iterative manner, should lead to correction of errors (Baum and Larson, 1991; Coddington, 1994). The diverse and specific predictions of hypotheses of adaptation ensure repeated testing of predictions and potential falsification of the hypotheses. Hypotheses of adaptation, or of any of the alternative explanations for the evolutionary origin of a character, are viewed from the deductive framework as working hypotheses whose successive testing leads to improvement of those hypotheses. By correcting errors, application of the

deductive method approximates the truth, although we do not reach a point in the investigation at which absence of error is assured.

The testing of historical hypotheses in evolutionary biology does not require a compromise of the principles of hypothetico-deductive science. [See Mayr (1982) for further discussion of this issue.] We reject in principle the inductive arguments used to discredit the testing of historical hypotheses of adaptation. Further criticism and improvement of the testing of historical hypotheses of adaptation should be made using an explicitly deductive framework.

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Laboratory Evolution: The Experimental Wonderland and the Cheshire Cat Syndrome

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I. Introduction: Victorian Schoolgirls Revisited

On an earlier occasion, we proposed that evolutionary theories "are like sheltered, upper-class, Victorian schoolgirls, dressed up entirely in white and severely scrubbed" (Rose et al., 1987, p. 95). This simile arose from our view that, while theories may be pretty to look at, they may not be that robust after all when confronted with the dangers of the real, empirical world. Indeed, we went further and proposed that the confrontation of theories with data could be likened to taking these schoolgirls on a trip through a jungle. There is a problem, however, with this situation. Many schoolgirls will die for reasons that are more accidental than a test of their true fitness. It will just happen that Prudence will be bitten by a venomous snake, while Cicely fortuitously escaped death by running ahead to talk with Abigail. To test the schoolgirls more appropriately, an environment combining danger with some type of order is preferable.

Our simile can be continued at this point by recalling *Alice's Adventures in Wonderland* by Lewis Carroll. In this charming tale, a classic Victorian schoolgirl has a fantastical adventure in which she is stringently tested, but also somehow free from mortal danger. For us, the experimental realm of laboratory evolution provides the comparable Wonderland to that of Alice. It is possible to test theories with greater fairness in the laboratory, in that the number of confounded,