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INTEGRATING HISTORICAL AND MECHANISTIC BIOLOGY ENHANCES THE STUDY OF ADAPTATION

KELLAR AUTUMN

Department of Biology, Lewis and Clark College

Portland, Oregon 97219–7899 USA

E-MAIL: AUTUMN@LCLARK.EDU

MICHAEL J. RYAN

Section of Integrative Biology C0930, University of Texas

Austin, Texas 78712 USA

E-MAIL: MRYAN@MAIL.UTEXAS.EDU

DAVID B. WAKE

Museum of Vertebrate Zoology and Department of Integrative Biology, University of California

Berkeley, California 94720–3160 USA

E-MAIL: WAKELAB@UCLINK.BERKELEY.EDU

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ABSTRACT

Adding a causal, mechanistic dimension to the study of character evolution will increase the strength of inferences regarding the evolutionary history of characters and their adaptive consequences. This approach has the advantage of illuminating mechanism and testing evolutionary hypotheses rigorously. We consider the advantages of combining mechanistic and historical biology in the study of behavior, physiology, and development. We present six examples to illustrate the advantages: (1) preexisting biases in sound perception in frogs; (2) preexisting biases in visual cues in swordtail

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fishes; (3) exploitation of prey location behavior for attraction of mates in water mites; (4) heterospecific mating in asexual molly fishes; (5) developmental foundation of morphological diversification in amphibian digits; and (6) locomotor performance at low temperature and the evolution of nocturnality in geckos. In each of these examples, the dominant role of history, combined with organismal integration, makes ignoring history a risky proposition.

UNDERSTANDING HOW LIFE works is the fundamental goal of biology. As modern biologists, we strive for a mechanistic explanation of phenomena that range from molecules to ecosystems. Mechanistic understanding involves distinguishing reproducible and testable causal patterns from noncausal or nontestable associations (Brandon 1996). With the advent of objective methods for generating and testing hypotheses of phylogenetic relationships (Hennig 1966), researchers actively began to incorporate phylogenetic perspectives into many areas of biological research focused on mechanistic understanding, including (as general examples) behavior, life history, functional and developmental morphology, and comparative physiology (Lauder 1982, 1990, 1991; Stearns 1992; Arnold 1993, 1994b; Niewiarowski 2001). This has meant that these and other fields, long isolated from systematic biology, gained historical perspective that has enriched interpretations, sometimes opening new pathways for understanding how history and biological mechanisms interact (e.g., Galis 1996). On the one hand, there has been enthusiastic integration of historical perspectives with mechanistic biology, as attested by a large literature that includes numerous symposia volumes (e.g., Harvey et al. 1996), collections of essays (e.g., Martins 1996), book chapters (e.g., Lauder 1991), and monographs (e.g., Brooks and McLennan 1991; Harvey and Pagel 1991; Garland and Carter 1994; Feder et al. 2000), in addition to a large number of research papers. On the other hand, authors have argued that the new methods are fundamentally a distraction, and that they obscure the central issues in fields such as behavioral evolution (Reeve and Sherman 1993, 2001) and comparative physiology (Mangum and Hochachka 1998). Our objective in this paper is to illuminate how intricately history and mechanism are integrated, and to show the value of a phylogenetic perspective in understanding patterns of evolution in behavior, morphology, and physiology. We present case studies that show how historical perspectives enable better understanding of mechanisms. We also argue that deep understanding of history and mechanism is invaluable for interpretation of results that otherwise could be seen as the workings of some ongoing dynamic (Ghiselin 1999). In particular we wish to counter the view that adequate understanding of complex patterns of evolution can be obtained by using an approach that relies solely on surrogates or proxies for fitness, such as performance or appearance, instead of the underlying and we believe more significant biological features of organisms.

In the following sections, we begin first by defining mechanism and history. Second, we present a logical treatment of the conflict that exists between integrative (historical, mechanistic) and purely adaptive approaches. Testability is a major advantage of the integrative approach we advocate. Next, we outline a protocol for testing integrative hypotheses and show how purely adaptive explanations are not scientifically valid unless the integrative explanation is first shown to be false. We use six examples from behavior, development, and physiology to illustrate the power and necessity of an integrative approach in the study of complex integrated systems.

MECHANISM AND HISTORY

Since mechanistic and historical biology are central concepts in our argument, we define what we mean by mechanism and history, and show how they can be integrated in answering evolutionary questions. Mechanism and history can be thought of as two dimensions or axes (Autumn et al. 1999) that each contain important information, but by themselves may be insufficient to address evolutionary questions involving complex integrated systems.

We use "mechanism" broadly to mean experimentally established, testable, causal

relationships among parts (Lauder 1991; Brandon 1996). The appropriate level or levels in the biological hierarchy for a mechanistic explanation will depend on the question (Jacob 1977). Since causal interactions may occur in both upward (small to large) and downward (large to small) directions (Bock 1989; Autumn 1995; Brandon 1996), it is not always possible to reduce the function of organisms to simple molecular causes (Savageau 1991). For this reason, mechanisms describe interactions that range from molecules to ecosystems.

Figure 1A shows a simple mechanism consisting of a lever arm, gears, and a belt. Each part of the mechanism is related causally to the production of torque, the measure of performance in this example. Variation in torque is a mechanistic consequence of variation in the moment arm and variation in the gear ratio. Let us imagine that there are two versions of this mechanism: *A* and *B*. *A* and *B* are similar in all respects except that one of the gears in *B* is larger than in *A*. Is the larger gear in *B* an adaptation for increasing torque? If the parts of *A* and *B* were laid out randomly on a table, there would be no way to predict whether a larger gear would increase torque because the order of gears in the mechanism will determine if increasing gear size increases or decreases torque. By adding information on how the mechanism is put together and how it works, one can predict the effects of a larger gear with a great deal of certainty. The strength of the prediction will depend on the strength of experimental and theoretical support for the mechanism in question. In this example, the causal effect of variation in the form of each part can be predicted by the equation:

$$Torque = Moment\ Arm \cdot \frac{Size\ of\ Gear\ 2}{Size\ of\ Gear\ 1} \quad (1)$$

or, where

$$\frac{Size\ of\ Gear\ 1}{Size\ of\ Gear\ 2} = Gear\ Ratio \quad (2)$$

and

$$Torque = \frac{Moment\ Arm}{Gear\ Ratio} \quad (3)$$

Accordingly, only by knowing how a mechanism is assembled, and by understanding the principles that underlie its operation, can one predict how changing one of the parts will change performance. If the only question is, "how does the mechanism work," one could use this dimension only. Evolutionary questions involve time and directional change, however. The mechanistic axis does not represent how the parts change, nor what actual patterns exist in nature. Even if we can say with certainty that changing the size of Gear 2 increases torque, we cannot say whether the direction of change was from large to small or from small to large. The mechanistic dimension lacks history.

We represent history as a second dimension that is complementary to the causal dimension. The historical axis shows how the parts have changed over evolutionary time. In Figure 1B, evolution has resulted in smaller gears, a larger moment arm, and an increase in torque. Any attempt to attribute the increase in torque to the inferred evolutionary changes in the parts would be purely correlational. Such inferences would not be testable without adding information about how the mechanism is assembled. In fact, if the gear ratio remained constant (as shown in Figure 1B), change in gear size would be independent from change in torque, and only changes in moment arm would have any effect on torque. Thus, by integrating the mechanistic and historical axes, the evolution of the system can be fully understood, and unfounded inferences can be avoided.

TESTING THE MECHANISTIC AND HISTORICAL EXPLANATION

The general form of most controversy that surrounds explanations based on history and mechanism versus those based on current utility alone has been as follows: one study (e.g., Wake 1991) argues that characters *A* (e.g., rules of development in salamanders) and *B* (e.g., small body size) mechanistically constrain character *C* (e.g., number of digits),

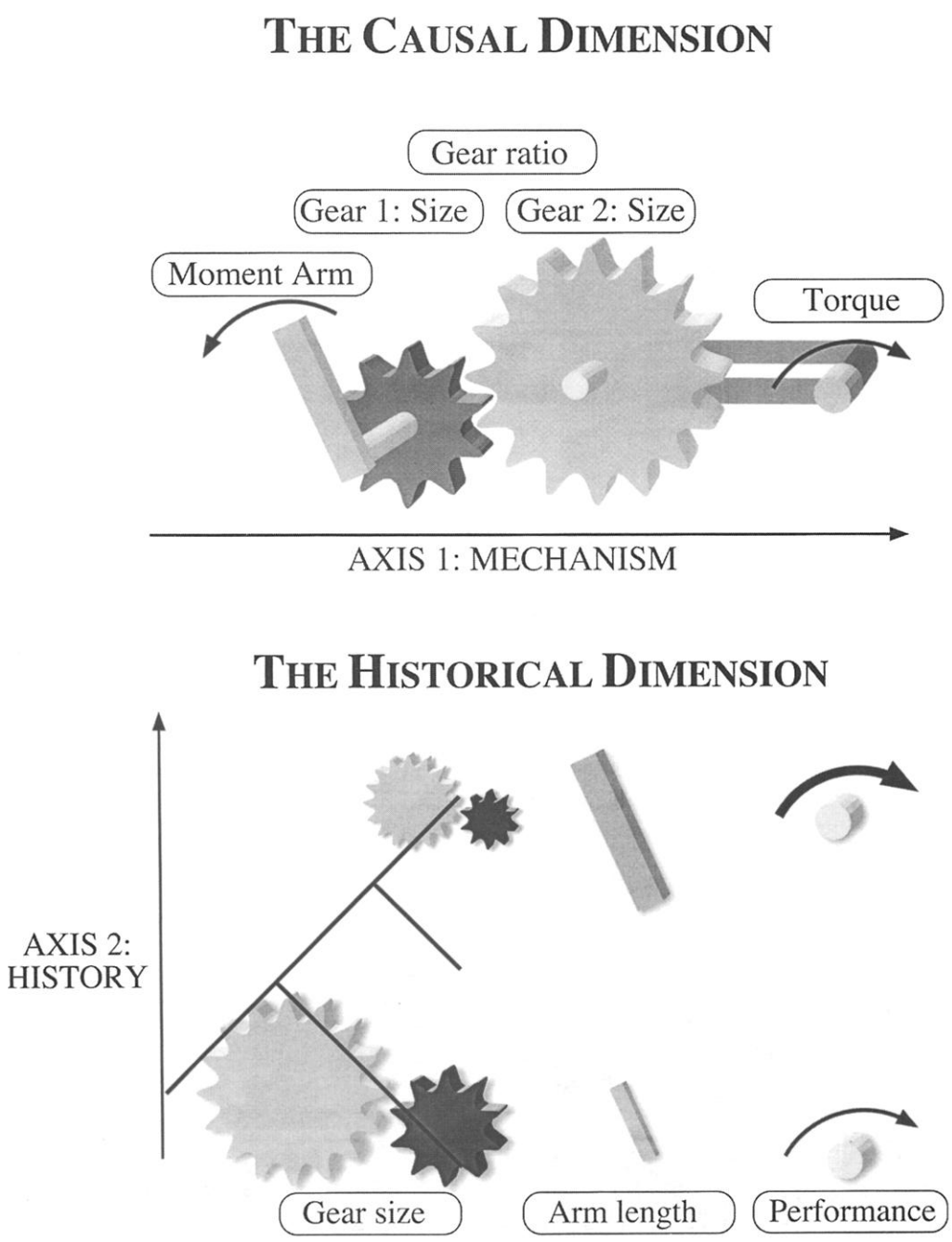


FIGURE 1. MECHANISTIC AND HISTORICAL AXES

A. Mechanistic axis. This represents the causal interactions among the parts. Here the lever and the gears work together to produce a torque. Variation in torque is a mechanistic consequence of variation in the moment arm and variation in the gear ratio. This axis does not tell us how these variables change and what actual patterns exist in nature. This dimension lacks history. **B.** Historical axis. This represents patterns of change in isolated parts over evolutionary time. By itself, this axis cannot provide strong inferences about the mechanistic causes of change. In this example, ancestral state is large gears, small moment arm, and low torque performance. In the derived condition, the gears have become smaller, the moment arm has become larger, and there is an increase in torque. Why did torque increase? One could explain this by correlated change in any of the variables. Possibly the smaller gears increase torque, or possibly the larger arm increases torque. It is not possible to tell without adding the mechanistic dimension. In this example the gear ratio remains the same, so change in gear sizes have no effect on torque.

and that because of the historical patterns of change in those characters, the ultimate cause of the current character state (e.g., digital reduction) was historical and acted mechanistically through *A* and *B* (see Arnold 1994a). An alternative approach (e.g., Reeve and Sherman 1993, 2001) argues that current fitness differences are both the proximate and ultimate cause of the current character states. How can the controversy be resolved? We suggest that the controversy cannot be resolved by theorizing about current fitness, proxies for fitness, or performance differences in populations—not even by actually measuring fitness. Fitness differences are a red herring in challenging mechanistic and historical hypotheses because mechanism and history determine the range of character variation that is possible. The challenge to the mechanistic hypothesis is to show that the hypothesized mechanism is incorrect. Logically, this should be done in an experimental context since one must demonstrate that characters *A* and *B* do not mechanistically constrain character *C*. Similarly, the appropriate way to challenge an historical hypothesis is to reject it on phylogenetic grounds. The challenge is to show that another historical hypothesis is more likely.

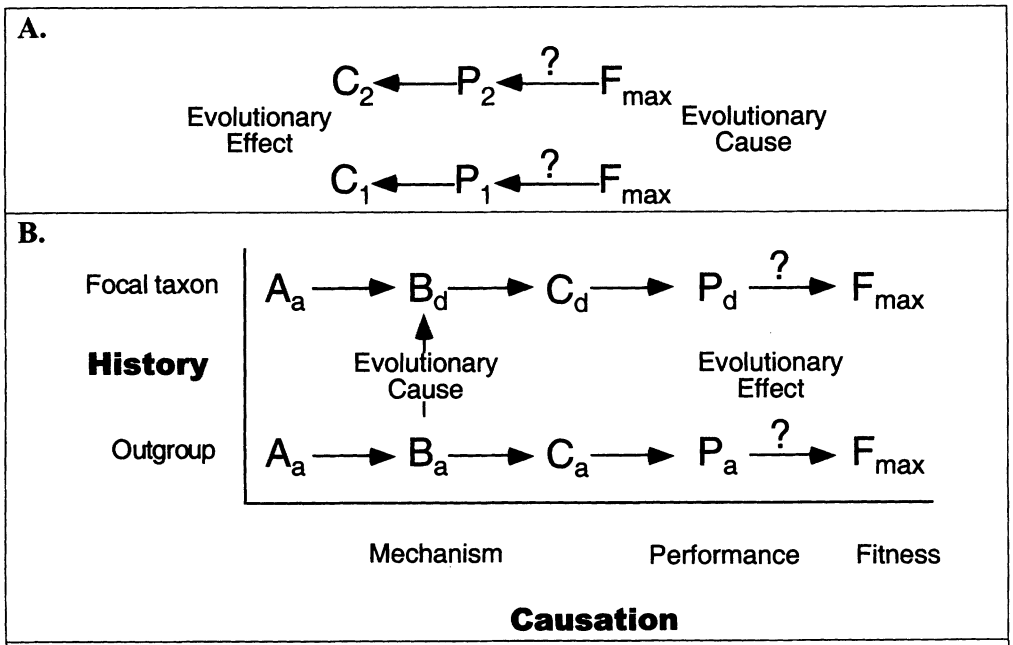
Since the goal of this paper is to highlight the importance of integrating mechanistic and historical biology to address evolutionary questions, it is reasonable to ask if we would have reached our conclusions without integrating mechanism and history. In each of the six case studies we present, a false conclusion would have been reached without an integration of mechanism and history. In the following sections, we present a summary of the six case studies, and identify the appropriate tests of the conclusions they reach. In each case, we present an evolutionary conclusion based on an integration of mechanistic and historical hypotheses, and an alternate hypothesis based only on maximizing fitness in current populations. We show that in each case, the alternate fitness-based hypothesis cannot be true unless the mechanistic or historical hypotheses are shown to be false (Figure 1).

The mechanistic and historical dimensions of the six examples show how and why mechanism and history must be integrated in order to reach a robust and meaningful conclusion. Figure 3 places each example in the context of the model shown in Figure 2. A common theme in these examples is that the range of phenotypes available for selection is constrained by the mechanisms that relate the characters in question, and by the phylogenetic history of the taxa in question.

FROG BEHAVIOR

Communication systems provide fertile ground for integrative studies. Evolutionary biologists want to know how mate recognition can reproductively isolate taxa, and Lorenz's use of display behavior in a phylogenetic analysis of the anatine ducks presaged the current practice of mapping behavioral characteristics onto phylogenies (Lorenz 1941). Animal communication is one of the social behaviors of great interest to behaviorists for over two millennia (Aristotle, translated by D'A W Thompson 1918; Darwin 1873). Neuroethology, the study of neural mechanisms of behavior, often focuses on communication; examples include the function and evolution of the song control nucleus in songbirds (Konishi 1994), and the mechanisms that underlie signal decoding in a variety of insects and frogs (Gerhardt 1994).

Communication systems are not only amenable to an integrative study but actually require such an approach for reliable interpretation of their evolution. The simplest communication system is a dyadic interaction of signal and receiver. Signal production, however, is not isolated from the rest of the animals' biology. In some insects such as *Drosophila,* song patterns produced by wing beating are inextricably linked to a morphology and physiology involved in and perhaps initially derived for self-powered flight (Ewing and Miyan 1986). The vocalizations of tetrapods are intertwined with the respiratory system (Gans 1973), and many visual stimuli, such as the long feathers of peacocks and widowbirds, are elaborations of morphologies involved in critical aspects of the animals' survival strategy, such as flight (Balmford et al. 1993). The sensory modality involved in signal reception is often used for other func-



C. Testing mechanistic and historical hypotheses.

1. Mechanistic hypotheses (horizontal axis)

- Alternate causal hypothesis: Fitness differences maintain state of C.
- Test must be mechanistic. First determine if change in A causes change in B, which in turn causes change in C. Next determine if change in C causes change in performance (P).
- Holding A constant will have mechanistic consequences that limit performance despite any theoretical fitness disadvantages.
- Changes in B will change performance despite any changes in fitness.

2. Historical hypotheses (vertical axis)

- Alternate historical hypothesis: Fitness differences caused evolution of C.
- Test must be historical. First, test the phylogenetic hypothesis that A_a, B_a, C_a are the ancestral character states at the focal taxonomic level. Next, test the phylogenetic hypothesis that A remained static, and that $B_a \rightarrow B_d$ and $C_a \rightarrow C_d$ occurred concordantly.
- If A remained static, and $B_a \rightarrow B_d$ and $C_a \rightarrow C_d$ occurred concordantly, and the causal hypotheses are true, then the alternate historical hypothesis must be false.

FIGURE 2. DIAGRAM ILLUSTRATING HOW INTEGRATING HISTORY AND MECHANISM CAN DISTINGUISH BETWEEN EVOLUTIONARY CAUSES AND EVOLUTIONARY SIDE EFFECTS

A. Alternate hypothesis based on maximizing fitness in current populations. Taxa 1 and 2 differ in a feature **C** that is associated with a difference in performance. Without mechanistic or historical knowledge, the inference is that the evolution and maintenance of the features in their respective taxa are driven by selection maximizing fitness within populations. **B.** With additional information about the mechanistic basis of variation in character **C**, the effects caused by stasis and change in other characters can be assessed. With additional information about the phylogenetic history of character **C** and its mechanistic basis, character polarity (ancestral and derived states) can be determined. By integrating mechanism and history, what was initially (see part **A**) thought to be an evolutionary cause can now be seen to be an evolutionary effect. **C.** The appropriate tests of the hypotheses outlined in part **B**, and the appropriate way to distinguish between the scenarios of parts **A** and **B**. The alternate (fitness based) hypotheses suggested by part **A** can only be valid if the mechanism or history outlined in part **B** is incorrect. Arguments based on current fitness in populations do not have any bearing on the hypotheses outlined in part **B**.

tions as well. Some moths, for example, have the ability to detect ultrasonic sounds, which could function in conspecific communication as well as detection of predatory bats, the function for which it might have been initially derived (Conner 1987). Thus one cannot understand the evolution of the signals used to communicate and the mechanisms used to decode the signals solely in the context of the communication system.

The two central questions in sexual selection are: (1) how does signal variation in males influence their attractiveness to females; and (2) why do females find some signals more attractive than others. In the túngara frog, *Physalaemus pustulosus,* males can add a component, a chuck, to the basic whine component of the advertisement call. Females prefer whines with chucks to whines without chucks. Bigger males produce lower frequency chucks, and females prefer lower frequency chucks. This results in larger males having greater mating success. But females benefit as well; by mating with larger males, females have more of their eggs fertilized (Ryan 1985). An interpretation based solely on the current function of this system is that male túngara frogs evolved chucks to signal large size as females coevolved a preference for such calls. A closer examination of the sensory mechanisms involved in signal processing, together with knowledge of the phylogenetic relationships and signal variation of close relatives, yields a different interpretation, however.

Frogs have two peripheral auditory organs whose frequency tuning contributes importantly to decoding acoustic signals. The amphibian papilla (AP) is more sensitive to lower frequencies, usually below 1500 Hz, while the basilar papilla (BP) is more sensitive to higher frequencies. In the túngara frog, the AP's most sensitive frequency is ca. 700 Hz while that of the BP is 2100 Hz (Ryan et al. 1990). The whine has most of its energy below 1000 Hz, while the chuck has most of its energy above 1500 Hz. Phonotaxis experiments combined with the neurophysiological data show that the AP is critical in the initial decoding of the whine, while the BP is critical in the initial decoding of the chuck (Wilczynski et al. 1995).

Although the tuning of the AP and BP tends to match the dominant frequencies of the whine and chuck, respectively, the BP is tuned on average slightly below the dominant frequency of the average chuck in the population: 2100 Hz for the BP and 2500 Hz for the call. This suggests that the female's behavioral preferences for lower frequency chucks might result from the better match of the calls to the sensitivity of the BP. Computer models integrating an average tuning curve and digitized calls drawn at random from a populations of túngara frogs support this contention (Ryan et al. 1990), as do a number of phonotaxis experiments that use synthetic stimuli (Wilczynski et al. 1995). Thus the combination of studies of function and mechanism shows that females gain a reproductive advantage by preferring the lower frequency chucks of bigger males, and this preference is mediated by the tuning of the BP.

Chucks are derived within one of the two clades (the *P. pustulosus-P. petersi* clade) of the *Physalaemus pustulosus* species group (Ryan and Drewes 1990; Cannatella et al. 1998). Of the ca. 40 species in the genus, only some populations of the túngara frog's sister species, *P. petersi* (which might include more than one species; cf. Cannatella et al. 1998), are known to add a call suffix. Results of behavioral experiments were combined with phylogenetic information to determine if female preference for chucks evolved in concert with chucks (the coevolution hypothesis) or if females had a preexisting preference for chucks that was exploited by males (sensory exploitation hypothesis; both hypotheses reviewed in Kirkpatrick and Ryan 1991; Shaw 1995; Ryan 1998). These hypotheses can be discriminated by reconstructing the phylogenetic history of the species group and inferring the historical sequence by which chucks and preferences for chucks evolved.

P. coloradorum is a member of the *Physalaemus pustulosus* species group. It is a member of a clade of species west of the Andes that diverged from the *P. pustulosus-P. petersi* clade after the evolution of the chuck (Ryan and Drewes 1990; Cannatella et al. 1998). Phonotaxis experiments show that *P. coloradorum* females prefer their own calls with *P. pustulosus* chucks added over the normal chuckless

calls of their conspecific males (Ryan and Rand 1993). Both *P. pustulosus* and *P. coloradorum* prefer calls with chucks. Parsimony suggests that this preference was inherited from a common ancestor, although it is possible that females of *P. coloradorum* coincidentally evolved the same preference for traits not existing in their own males.

Studies of the population biology of túngara frogs show that females gain a reproductive advantage by preferring whines with lower frequency chucks. The phylogenetic analysis, however, rejects the hypothesis that female túngara frogs evolved this preference. Instead, both the preference for chucks and the neural tuning that biases females toward lower frequency chucks are present in a close relative, *P. coloradorum*, and appear to have arisen prior to the divergence of these species. This preexisting bias was responsible for the selection that favored chucks when they arose. At this point, it is not clear why there is a preexisting preference for chucks. The answer might be adaptive; chucks might increase the ability of females to detect the call in background noise or locate it in space. It might not be obviously adaptive; the BP is a frequency channel that is not used in communication by most species in the *Physalaemus pustulosus* species group. Once species recognition is released by reception of the whine, further acoustic stimulation may merely enhance the physiological and behavioral response of the receiver.

Knowledge of the mechanisms involved in call preference can prevent misguided speculation about other adaptive scenarios. Reeve and Sherman (1993:25) suggested that *P. coloradorum* as well as *P. pustulosus* should be selected to prefer low frequency calls since these calls should be produced by larger males. They suggest that this shared preference could explain the presence of similarly tuned auditory responses. As noted above, however, frogs have two auditory papillae sensitive to airborne sound. The amphibian papilla is tuned to lower sounds, and in both *P. pustulosus* and *P. coloradorum* it is maximally sensitive to the species' whinelike advertisement call. The basilar papilla is tuned to high frequency sounds, and is critical in sensing the *P. pustulosus* chuck. The *P. coloradorum* call does not contain frequencies in the maximum sensitivity range of its own BP. Thus a shared preference for low frequency whines in both species, which involves the AP, could not explain why *P. coloradorum* and *P. pustulosus* have BP organs with similar tuning. Nevertheless, Reeve and Sherman's comment, however misguided, is in the spirit of the approach we recommend—knowledge of the animal's entire biology might be necessary to understand specific functions; it is important, however, to get the biology right.

Why do female túngara frogs prefer calls with chucks to calls without chucks and prefer lower frequency chucks to higher frequency chucks?

Mechanism: The tuning of the basilar papilla (BP) is sensitive to the dominant frequency of the chuck, and is critical in its initial neural processing. The BP of the female is tuned to respond to the frequency range of the chuck, and is more responsive to slightly lower than average frequency chucks in the population. Since large males make lower frequency chucks, females prefer larger males. As a consequence, females gain a reproductive advantage.

History: The tuning of the BP is similar among species in the *Physalaemus* species group, and thus represents an ancestral character state. The túngara frog is well nested within *Physalaemus*, and the chuck represents a derived character state. The tuning of the BP and therefore the preference for chucks existed prior to the evolution of the chucks themselves.

Integration of mechanism and history: Females gain a reproductive advantage by preferring whines with lower frequency chucks, but selection for this female preference is not the evolutionary cause. Instead, the reproductive advantage gained by preference for whines with lower frequency chucks is an evolutionary effect due to the way frog brains work and how they have evolved.

Testing the conclusion: Since females do have greater reproductive success when they mate with larger males, one could have concluded

that this was a selective advantage over those that did not prefer larger males, that this was the cause of preference for chucks, and that the evolution of AP and BP tuning was driven by this advantage. This alternate hypothesis based on current fitness maximization is contradicted by the mechanistic and phylogenetic hypotheses. Choice of larger males and reproductive advantage are the evolutionary side effects, not the evolutionary cause (Figure 3). In fact there is no advantage because there is no alternative to preference for larger males. Selection for choosing larger males is not possible unless the mechanistic or the historical hypotheses can be rejected. Challenges to this conclusion are likely to come from advances in the neurobiology of frogs, or from a systematic revision of *Physalaemus*.

Swordtail Behavior

Behaviors associated with the "sword" of fishes of genus *Xiphophorus* (which also includes swordless platyfish) are important in mate recognition. Basolo (1990a) showed that females of *X. helleri* preferred males with longer tails. Rosenthal and Evans (1998) used video playbacks to show that this female preference was based on the sword itself and not other correlated characters. Basolo (1990b, 1995) also appended swords to two species of platyfish, *X. maculatus* and *X. variatus*. In both of the species, females preferred the sworded conspecifics to normal swordless ones. A phylogenetic analysis of swordtails by Rosen (1979) and Rauchenberger et al. (1990) argued for monophyly of swordtails. Since swords are present in this group and absent in platyfish and other fishes in the family Poeciliidae, Basolo argued that there was a preexisting bias for swords, and male swordtails exploited this female bias when evolving the caudal extension. Meyer et al. (1994) presented an alternative interpretation of the phylogenetic relationships within *Xiphophorus* that questioned the preexisting bias interpretation of Basolo, although recent analyses (Borowsky et al. 1995; Marcus and McCune 1999) tend to be more supportive of the previous phylogenetic hypotheses (Rosen 1979; Rauchenberger et al. 1990). Regardless of the relationships within the genus, Basolo's interpretation was supported by experiments that show female preference of males with artificially added swords in the closely related genus *Priapella*.

Females of many species prefer larger males (Ryan and Keddy-Hector 1992). The sword is only one of several phenotypes that could enhance male attractiveness; perhaps large body size is what many females appear to find so attractive. In a study using video playbacks, Rosenthal and Evans (1998) manipulated the male phenotype in otherwise identical sequences of a courting male. In addition to the obvious control experiments, they removed the sword from the male but increased the male's size (maintaining natural proportions) such that it was the same length as the male with the sword. Females showed no difference in preference between the sworded and swordless male that were the same body length. This supports the contention that evolution of the sword might exploit a preexisting bias for large size that might be widespread through the poeciliids.

Why do female swordtails prefer males with longer swords?

Mechanism: Female platyfish prefer large males. Females measure male size by estimating total body length, and the presence of a sword increases total body length. Thus, female swordtails prefer sworded males over sworded or swordless males of shorter body length.

History: Preference for large males predates the evolution of swordtails. Swordtails are monophyletic and share a common sworded ancestor. Thus, preference for sword predates evolution of the sword.

Integration of mechanism and history: Females prefer males with longer swords because the sword exploits a preexisting bias toward preference for large males.

Testing the conclusion: An alternate hypothesis based on current fitness maximization is that females are being selected to choose "superior" males, which possess large and/or larger swords. The mechanistic and phylogenetic analyses contradict the alternative

Example	Ancestral characters	Derived characters	Integration of A & B	Performance	Hypothetical fitness advantage
1. Frog behavior	Auditory tuning	Addition of a call suffix	Female prefers more complex calls	Mate choice	Mating success
2. Swordtail behavior	Female preference for large male	Sword	Female prefers swords	Mate choice	Mating success
3. Water mite behavior	Response to water surface vibration	Male production of surface vibration	Female attracted to water surface vibration	Mate Choice	Mating success
4. Molly behavior	Female preference for larger conspecific male	Parthenogenesis	Female prefers larger heterospecific male	Mate Choice	None
5. Salamander limb development	Bifurcation rule	Large genome; small body	Digital reduction	Reduced function	Burrowing
6. Gecko physiology	Q_{10} and T_o of $\dot{V}_{O_2 max}$	Low C_{min}; low T_b	MAS	Increased but submaximal endurance capacity	Locomotion; Tolerance for high daytime temp.

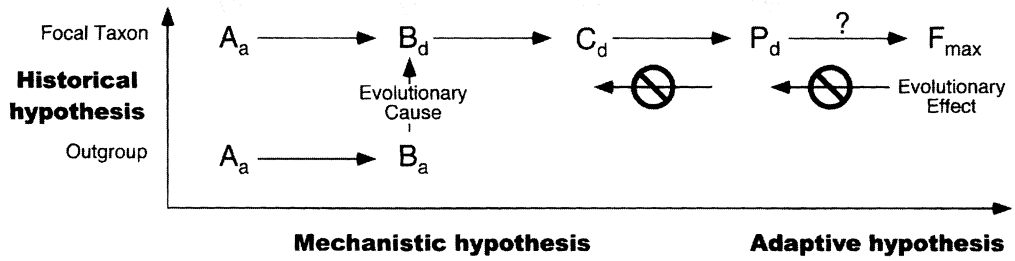


FIGURE 3. KEY TO FIGURE 2 FOR THE SIX EXAMPLES PRESENTED IN THE TEXT

In each example, the mechanistic and historical hypotheses contradict the alternate adaptive hypothesis (Figure 2A) based on current selective processes. Ancestral characters (A_a) and derived characters (B_d) determine mechanistically the state of character C, which in turn determines performance (P). Thus, derived performance (P_d) is the evolutionary effect, and the derived characters (B_d) are the evolutionary cause. Therefore, it is not possible for selection to directly affect the state of character C (denoted by Ø), and the adaptive hypothesis based only on current fitness maximization must be false unless the historical or mechanistic hypotheses can be rejected.

hypothesis that female swordtails evolved this preference as a result of selection for male quality. Selection for choosing "superior" males identified by larger swords is not possible unless the mechanism or the history is false. Challenges to this conclusion are likely to come from advances in the study of platyfish behavior, or from a systematic revision of *Xiphophorus* and related taxa.

WATER MITE BEHAVIOR

Preexisting bias for male courtship signals in water mites may result from selection in a context unrelated to mate choice (Proctor 1991, 1993). Male water mites (*Neumania papillator*) produce water surface vibrations that attract females. Females approach these vibrations, and upon reaching their source they are courted and mated by the male. These mites hunt at the water surface for copepods swimming below. Male and female mites find their prey by localizing the source of the water vibrations generated by the locomotion of the copepods. The water surface vibrations produced by the courting males and the swimming prey are similar in form.

Foraging and sex are linked: hungry females are more likely to mate since they are more likely to be attracted to the water surface vibrations (Proctor 1991, 1993). Responses to sensory input have multiple functions, but knowing this does not establish whether a particular response arose simultaneously in both functions (foraging and sex) or evolved initially in one. A phylogenetic analysis showed that the use of water surface vibrations in foraging preceded the use of similar vibrations in male courtship. Thus, the most parsimonious hypothesis is that response to water surface vibrations evolved initially in foraging and was then exploited for male courtship.

Why are female water mites attracted to water surface vibrations created by males?

Mechanism: Water mites locate copepod prey by the water surface vibrations they create in the water when they move. Male water mites create water surface vibrations that match the frequency of those created by the copepod prey. Males obtain matings when female mites are attracted to the water surface vibrations caused by males.

History: The use of water surface vibrations in foraging preceded the use of similar vibrations in the attraction of females by males.

Integration of mechanism and history: Males gain a fitness advantage by exploiting a preexisting sensory bias in females.

Testing the conclusion: The mechanistic and phylogenetic analyses contradict the alternative adaptive hypothesis that female water mites evolved their preference as a result of selection for locating males. Any reproductive advantage for the female gained by male production of water surface vibrations is an evolutionary side effect, not the evolutionary cause. Challenges to this conclusion are likely to come from advances in the study of behavior, and/or a systematic revision, of water mites.

MOLLY BEHAVIOR

Historical analysis can reveal the existence of currently functionless traits. It often is assumed that behavioral traits must be adaptive. An alternative is that the presence of these traits might be due to evolutionary persistence rather than current maintenance by selection. The Amazon molly (*Poecilia formosa*), a small poeciliid fish, is an all-female species that reproduces by gynogenesis. It is thought to have evolved from hybridization between a male *P. latipinna* and a female *P. mexicana* (Turner 1982). Female Amazons mate with males of either one of these species to obtain the sperm necessary for embryogenesis, but this sperm is not incorporated into the genome of the offspring. Female Amazon mollies, like other poeciliid fishes and many other organisms, show a preference for large males that is statistically indistinguishable from that of its two parental species, *P. latipinna* and *P. mexicana*. The evolutionary maintenance of these preferences might be explained by direct selection if mating with larger males has an immediate influence on female reproductive success. Thus females should mate with larger males if these males provide more or better resources such as food or nesting sites, are better fathers because of increased protection of the young or mate, or if larger males fertilize more eggs. Alternatively, indirect selection can result in large male mating preferences if there is genetic variation for the preference for large size, and if there is linkage disequilibrium between genes associated with these preferences and those associated with large male size. Under this scenario of Fisher's runaway sexual selection theory, the preference increases in frequency as a correlated response to direct evolution of the trait (Andersson 1994).

The peculiar mating system of Amazon mollies provides an opportunity to test the indirect and direct selection hypotheses for the maintenance of this female preference. The indirect selection hypothesis is immediately rejected. Since there are no males in the species there can be no correlated evolution of trait and preference. Some of the direct selection hypotheses can also be rejected since the males mated by Amazon mollies offer no parental care or resources to even their conspecific mates, let alone heterospecifics. Large male size does not influence female reproductive success (Marler and Ryan 1997). There is no relationship between body size of male *P. latipinna* and the fecund-

ity of females they mated. This eliminates the obvious explanations for the evolutionary maintenance of mating preference for large males, and supports an alternative hypothesis that this preference was inherited from the parental species and has persisted in Amazon mollies without being maintained by selection.

Why do asexual all-female mollies prefer larger males?

Mechanism: Females of both sexual and asexual species of molly prefer to mate with large males. Mating with heterospecific males is required for embryogenesis in asexual Amazon mollies, but sperm is not incorporated into the genome of the offspring of Amazon mollies.

History: Preference for large males is an ancestral character state of the species that gave rise to the Amazon molly. Asexual reproduction evolved in the Amazon molly.

Integration of mechanism and history: Female Amazon mollies prefer large males because preference for large males was inherited from their sexual ancestors, and not because selection currently maintains this preference.

Testing the conclusion: The mechanistic and phylogenetic analyses contradict the alternative adaptive hypothesis that Amazon mollies evolved a preference for large males as a result of selection for male quality. The conclusion based on integrating mechanism and history is strong because there is no fitness advantage of choosing larger males. Selection for choosing large males is not possible unless the mechanism and the history are false. Challenges to this conclusion are not likely, unless it can be shown that sperm is incorporated into the genome of the Amazon molly, and that the Amazon molly did not evolve from species that already exhibit a preference for large males.

AMPHIBIAN LIMB DEVELOPMENT

The pentadactyl limb is highly conserved in the evolution of terrestrial vertebrates. The earliest amphibians had more than five digits (Coates 1994), but very early in the history of tetrapods, evolution reduced the numbers of digits down to four or five in the forelimb and five in the hind limb. Those numbers characterize most generalized tetrapods that have lived, with exceptions involving further reductions. Both genetic and developmental mechanical constraints have been proposed as reasons for this conservation, and as explanations as to why polydactyly is so uncommon and unstable (Shubin et al. 1997). Reductions in digital number frequently are adaptive in nature (e.g., the mechanically specialized skeleton of the bird wing is reduced to three highly modified digits; Burke and Feduccia 1997). There are also alternative explanations for digital reduction, however.

Digits are numbered from I to V, counting from preaxial to postaxial. When digital reductions occur they typically involve losses of the outer digits, I or V, or occasionally I and II. Only one instance of the loss of both digits IV and V is well documented, in the hand of theropod dinosaurs (Sereno and Novas 1992). Developmental constraints may cause the outermost digits to be lost first. Proximal portions of the limb are formed first during morphogenesis, then more distal portions condense and finally digits appear. A distinct developmental axis is apparent in frogs and amniotes that extends through the postaxial limb, specifically through digit IV. A digital arch then extends preaxially, and digits III, II, and I are formed in temporal sequence, with digit V usually being added relatively early and in an opposite direction (Shubin and Alberch 1986). Amphibians differ from amniotes in that no living species has more than four digits on the forelimb. Salamanders differ further both from frogs and all amniotes that possess digits in that the axis of the developing limb extends through digits I and II rather than digit IV. The digital arch of salamanders starts at the primordium of an element unique to amphibians, the basale commune, and grows postaxially so that the sequence of digit formation is I-II (near simultaneous)-III-IV-V (Shubin and Alberch 1986).

Digital reduction has proceeded beyond the four digits of the forelimb in both frogs and salamanders. In frogs, miniaturized species (and only such species) have only four

toes, and in salamanders reduction to four or fewer digits has occurred repeatedly in phylogenetically distinct lineages (Duellman and Trueb 1986). Digital reduction is always preaxial in frogs, whereas in salamanders it is invariably postaxial (Shubin and Wake 1996).

Digital reduction has been studied extensively, and explanations fall into two general and somewhat competing categories: adaptive (e.g., Lande 1978) and incidental to other phenomena (e.g., Alberch and Gale 1985; Wake 1991). We propose a resolution to this controversy that illustrates the synergism between history, mechanism, and adaptation that we advocate.

Alberch and Gale (1985) argued that digital reduction in both frogs and salamanders is a result of developmental truncation, in which the digit formed last is that which is lost as a simple consequence of the failure of the digit to undergo morphogenesis. Limb development has been portrayed as a series of segmentation and bifurcation events during which cell masses must reach a certain size (in terms of numbers of cells) before proceeding through either event (Oster et al. 1988). Experimental reductions in the rate of cell division resulted in the absence of the most preaxial digit in the frog studied, and of the most postaxial digit in the salamander studied, in accordance with expectations from the Shubin-Alberch model of limb morphogenesis. A cause of digital loss is thus reasonably well known. Reduction in the numbers of cells in a primordium can be accomplished as an incidental outcome of reduction in overall organismal size, which results in fewer cells in cell aggregations and organ primordia, providing cell size remains constant. If cell size increases and body size remains constant, there also will be fewer cells in primordia. Salamanders have enormous genomes. The smallest salamander genome is larger than that of all other tetrapods (except a few Australian myobatrachid frogs), and there is a well-documented positive relationship between cell size and genome size (Roth et al. 1994). A small reduction in the size of an organism that has a large genome, or a small increase in the size of the genome in an organism of constant body size, would have the side effect of reducing the number of cells in a primordium; this effect would accentuate through development, having its greatest impact near the end of limb morphogenesis when the last digits are forming. A miniaturized species with a large genome size (e.g., the four-toed plethodontid genus *Batrachoseps;* Sessions and Larson 1987) would, in effect, have a double dose and be a likely candidate for digital reduction if this formulation is correct. These observations show that there is a well-understood mechanism in amphibians for explaining digital reduction.

History provides a means of testing whether this proximal understanding is a sufficient explanation for the ultimate causes of digital reduction in amphibians. For example, one can predict that miniaturized frogs belonging to different phylogenetic lineages might lose the first toe but never the fifth. This is, in fact, the case, because miniaturized terminal taxa in phylogenetically distant lineages (such as species of *Psyllophryne* of the family Brachycephalidae, and *Mertensophryne* of the family Bufonidae; Alberch and Gale 1985) have undergone digital loss. In frog genera that belong to different families, phalangeal reduction in the first digit occurs. Phylogenetic analysis shows that miniaturization has occurred independently, and that digital reduction has evolved independently. Whereas only miniaturized species of frogs have lost a digit, there are many miniaturized frogs that retain the ancestral number of digits. Thus, while miniaturization increases the likelihood of digital reduction, it does not dictate it.

Using similar logic, one can predict that miniaturized salamanders, especially those with large genomes, belonging to different phylogenetic lineages might lose the fifth toe but never the first. Such is the case, as demonstrated by Wake (1991), who showed that miniaturized terminal taxa in three distinct lineages of the family Plethodontidae had lost the fifth toe. Furthermore Wake showed that rare variant animals in other miniaturized lineages are sometimes found that have only four toes, and occasionally they are asymmetrical in a single organism. Miniaturized species in other families (e.g., *Salamandrina terdigitata* of the family Salamandridae) also have only four toes, and invariably it is the fifth that is absent. But not all miniaturized

plethodontids have four toes. And a further complication is that there are some relatively large species that have lost digits (members of the families Amphiumidae and Proteidae) and even limbs (members of the family Sirenidae). These species are not only large, however, they are also paedomorphic—all retain either larval morphology as adults, or some larval traits such as open gill slits as adults—and all have very large genomes (*Necturus* of the Proteidae has the largest tetrapod genome). Thus, using the arguments of Hanken and Wake (1993), they can be said to be biologically miniaturized, if not physically miniaturized. *Necturus* has only four toes (V is absent), and *Proteus* (also Proteidae) has only two, with digits III, IV, and V being absent, as predicted by the Shubin-Alberch model. Dipnoans are basal outgroups of tetrapods that have fins with a central axis, but their fins are remarkably reduced in relation to ancestral fishes that had fins that resembled limbs far more than do those of modern dipnoans (Shubin 1995). Again, there is an important correlation. Living dipnoans have by far the largest genomes of any vertebrates, and they are paedomorphic relative to more basal extinct taxa (Bemis 1984).

Miniaturization must often be adaptive in amphibians (Wake 1991), and we postulate that the advantages of miniaturization with respect to habitat use, predation avoidance, early age at first reproduction, or other factors are more than sufficient to offset any disadvantage that might arise from digital loss. Reeve and Sherman (1993) urge that one adopt a strict adaptationist perspective in which one seeks an immediate adaptive advantage to digital reduction, but evolutionary reduction has always posed difficulties for strictly adaptationist explanations. They speculate that alternative phenotypes might have reduced fitness because of possible disruptions of the original developmental program. In essence, they want to change the question from constraint on the production of form to constraint on adaptation (Amundson 2001). Such a perspective might be appropriate for lineages such as lizards, where stages in limb and digital reduction can be found in living taxa that appear to be elongating and adapting slowly to snakelike locomotor behavior. Lande (1978) argued that it might take millions of years for distal to proximal limb reduction, but in cases where such reduction was geologically rapid, only weak selection pressures were necessary. The situation in frogs and salamanders appears to be quite different, however. In salamanders, digits come and go as complete organs, as witness the cases of asymmetry found in single individuals (Wake 1991) and extreme variation found within and among populations of a single species (e.g., the hynobiid salamander *Hynobius lichenatus;* Hasumi and Iwasawa 1993). From our perspective it is far more satisfying to seek an explanation that takes full account of mechanism, history, and adaptation, rather than automatically give precedence to direct adaptation alone, which risks the loss of useful information that other perspectives can provide.

Why do some small salamanders have only four toes?

Mechanism: Development of the limbs of vertebrates entails several cell-level morphogenetic processes. Cells destined to give rise to mesopodial skeletal elements cluster together to form condensations. As the condensations grow in size by accretion (but most importantly by cell division), they either segment or bifurcate; they segment as they elongate, and as they round out they bifurcate. There is a bias in the direction of cell proliferation that is determined by positional information in the limb itself. The combined effects of segmentation and bifurcation are the proximal mechanisms that underlie sequential formation of phalanges and digits as well as the mesopodial elements. Salamanders have much larger genomes than other tetrapods, and within the Plethodontidae, members of the tribe Bolitoglossini—a deeply nested clade to which *Batrachoseps* belongs—have the largest genomes of terrestrial salamanders. Furthermore, there is an empirical relationship between genome size and cell volume, with volume increasing in direct proportion to haploid genome size. Small adult size combined with large cell size means that condensations will have few cells, and in some parts of the developing limb perhaps an insuffi-

cient number to undergo segmentation or bifurcation.

History: A synapomorphy for the Order Caudata (salamanders) is a switch from postaxial to preaxial dominance in limb development (Shubin 1995; Shubin and Wake 1996). In frogs and amniotes the first digit to appear is number four. A digital arch forms and grows in a preaxial direction, bifurcating and segmenting to give rise to digits three, two, and one, in that order. Before this sequence is completed, digit five forms in a postaxial direction. In salamanders there is precocial development of digits one and two, and from them the digital arch grows posteriorly, bifurcating and segmenting to give rise sequentially to digits three, four, and five. Thus digit one is the last formed in frogs and amniotes, but digit five is the last formed in salamanders.

Salamanders also have experienced a dramatic increase in genome size relative to all other tetrapods, and this has major implications for limb development (Sessions and Larson 1987). Because salamanders are relatively small as juvenile (and adult) organisms, the number of cells is dramatically reduced relative to numbers in other tetrapods of comparable size (Hanken and Wake 1993; Roth et al. 1994). Condensations of cells do not form unless a certain minimal number are present, and when condensations do form they do not bifurcate or segment until a certain indeterminate number of cells are present.

Integration of mechanism and history: The combination of an historical shift in positional dominance in limb development and an historical increase in genome and cell size, in conjunction with particular morphogenetic mechanisms and small overall size, leads to the repeated evolution of four-toed salamanders in which the missing toe is the last formed, or number five. Digital reduction also occurs in very small frogs, even in absence of large genomes and cells, and it is invariably the first digit that is lost. These patterns are predictable from a combined understanding of history and mechanism. Small frogs and small salamanders do not invariably lose a digit; doubtless there is a role for stabilizing selection in maintaining the general design principles of the limbs. But in the absence of sustained selection digits are lost as a result of a default process related to the combination of history and mechanism.

Testing the Conclusion: Without the integration of mechanism and history, one could conclude that digitally reduced salamanders have a fitness advantage over individuals with 5 toes, and that this fitness advantage underlies the pattern of digital reduction. Instead, an understanding of the rules of development and the phylogenetic history of salamanders and frogs strongly suggests that any differences in performance due to digital reduction are evolutionary side effects. Selection cannot act directly on toe number because variation in toe number is limited by the number of cells in the developing limb bud, which is in turn a mechanistic consequence of cell number and cell size. Challenges to this conclusion are likely to come from advances in developmental biology, and from systematic revisions of the Amphibia.

GECKO LOCOMOTION

THE NOCTURNALITY PARADOX

In ectotherms, the rate of physiological processes decreases exponentially as body temperature drops below the thermal optimum. Diurnal lizards use behavioral thermoregulation to maintain body temperatures near their thermal optima, typically 35–45°C (Cowles and Bogert 1944; Avery 1982; Huey 1982). As one would predict from an adaptive perspective, the thermal optima for a variety of physiological and performance variables are typically close to the temperatures diurnal lizards experience in nature (Huey 1982). The evolution of nocturnality is interesting because nocturnal lizards actively forage at temperatures 10–35°C below the body temperatures (and thermal optima) of typical heliothermic, diurnal lizards. Nocturnal activity thus represents a significant challenge to the evolutionist. For example, given typical rate-temperature effects (Bennett 1982), a relatively moderate nocturnal body temperature of 25°C would result in a 50–75% decrease in performance in a diurnal lizard. From an adaptive perspective, one would expect lizards to evolve thermal optima that are similar to

body temperatures during activity (Huey et al. 1989). Therefore nocturnal lizards should have thermal optima that are similar to the body temperatures they experience at night. In other words, both nocturnal and diurnal lizards should function best at their respective activity temperatures. If the thermal optima of nocturnal lizards have decreased, it follows that nocturnal lizards should have evolved greater performance than diurnal lizards at low temperature, and to have done this at the cost of reducing performance at high temperature.

Although this model makes sense as a thought experiment, the data strongly contradict it. Nocturnal lizards are capable of greater locomotor performance at low temperatures than are comparable diurnal lizards (Autumn et al. 1994; Autumn et al. 1997; Autumn et al. 1999), but low temperatures remain suboptimal for many physiological functions, including locomotion (Huey et al. 1989; Autumn and Full 1994; Autumn et al. 1994; Autumn and Denardo 1995; Autumn et al. 1997; Autumn et al. 1999). The evolution of nocturnality seems to represent a paradox: geckos have evolved greater performance at low temperatures yet their performance is greater at high temperatures they never experience during activity. How can this paradox be resolved? Is more information needed about the behavioral ecology of geckos to understand the selective forces involved? In this case, behavioral ecology cannot answer the question.

A traditional phylogenetic analysis cannot resolve the paradox either. As in the gear ratio example (Figure 1), simply knowing the order of events on a cladogram is not sufficient information to infer the causal basis of an evolutionary change (Lauder 1991). Instead, the resolution of the nocturnality paradox requires the integration of the physiological mechanisms that underlie sustained locomotion with the evolutionary history of the gecko clade.

MECHANISTIC PARAMETERS UNDERLYING VARIATION IN SUSTAINED LOCOMOTOR PERFORMANCE

The capacity for an animal to sustain locomotion is known as endurance capacity. The physiology underlying variation in endurance capacity is similar in humans (Brooks and Fahey 1985), lizards (Bennett 1982), amphibians (Gatten et al. 1992), and invertebrates (Full 1997). Locomotion for periods longer than approximately 10 minutes is sustained by aerobic metabolism. The maximum rate of aerobic metabolism, or $\dot{V}_{O_2max}$, plays a large part in determining the endurance capacity of an animal. The speed of locomotion at which $\dot{V}_{O_2max}$ is reached is termed the maximum aerobic speed (MAS), which sets the upper limit for sustainable locomotion and is largely responsible for determining endurance capacity. $\dot{V}_{O_2max}$ is only one of two variables that set the MAS, however. The minimum cost of locomotion (C_{min}) is equivalent to the inverse of fuel economy. Animals with greater fuel economy (lower C_{min}) have greater MAS because they cover more ground per unit of fuel. The relationship between these variables can be represented by the equation,

$$MAS = \frac{\dot{V}_{O_2\,max} - y_0}{C_{min}}, \qquad (4)$$

where the idling cost (y_0) is approximately 1 to 1.5× the resting metabolic rate.

There is a cascade of effects (Figure 4) from the large decrease in body temperature associated with the evolution of nocturnality. In order to quantify what effect this had on performance, it is necessary to account for the other related variables in the system. Since aerobic capacity is strongly temperature dependent, colder lizards will have lower endurance, all else being equal. Both fuel economy and aerobic capacity are strongly dependent on body mass, so one must factor out body mass in comparisons of MAS among lizards that differ in size. This is not as simple as it might seem. In order to explain a change in performance (MAS), it is necessary to account for the separate effects of change in the three physiological variables that determine MAS: y_0, $\dot{V}_{O_2max}$, and C_{min}.

Estimates of Mechanistic Parameters are Sensitive to Phylogenetic Bias

To make matters more complex, the relationships between mass, aerobic capacity, and

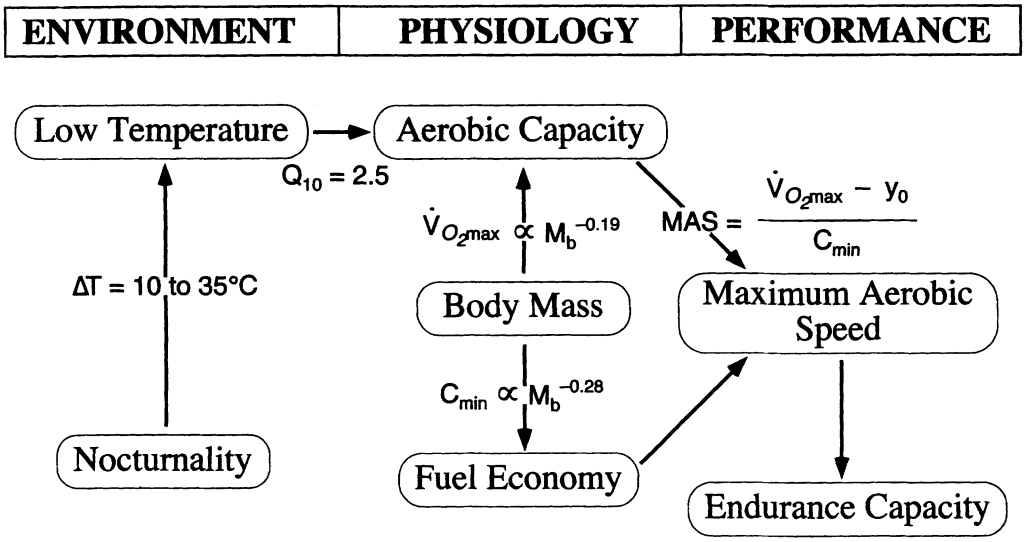


FIGURE 4. OUTLINE OF THE INTEGRATION OF THE ENVIRONMENTAL AND PHYSIOLOGICAL VARIABLES UNDERLYING ENDURANCE CAPACITY IN NOCTURNAL LIZARDS
For a detailed explanation, see Autumn et al. (1999).

C_{min} are allometric, and are based on measurements of a phylogenetically biased sample of species. Measurement of MAS, y_0, $\dot{V}_{O_2max}$, and C_{min} in lizards is extremely time consuming, and members of some lizard taxa are not sufficiently cooperative to run steadily on a treadmill. Not surprisingly, the sample of species for which all of the variables have been measured remains relatively small and phylogenetically biased. In particular, varanoid species thought to have unusually high $\dot{V}_{O_2max}$ dominate the sample of diurnal lizard data. As Felsenstein (1985) and many others (e.g., Harvey and Pagel 1991; Garland 1992;) have emphasized, species cannot be treated as independent data points. In order to compare species that differ in body mass, it is necessary to filter out the phylogenetic bias caused by sampling species that are differentially related by common ancestry. The estimates of the allometric coefficients may be confounded with phylogenetic effects (Garland and Ives 2000), yet precise values of the allometric coefficients are necessary if animals that differ in body mass are to be compared. Accordingly, an integration of mechanism (allometric effects) and history (phylogenetic effects) is necessary to resolve the paradox of how nocturnal lizards could have evolved increased endurance at low temperature and yet remain suboptimal.

In order to use a phylogenetically biased sample of lizard taxa to tease apart the causal effects of variation in temperature, body mass, y_0, $\dot{V}_{O_2max}$, C_{min}, and MAS, an explicit integration of mechanism and history is critical (Autumn et al. 1999). This involves a phylogenetic analysis of mechanism, in contrast to a traditional phylogenetic analysis of isolated characters.

RESOLVING THE NOCTURNALITY PARADOX WITH AN INTEGRATION OF MECHANISM AND HISTORY

Lizards are ancestrally diurnal, are physiologically optimized for diurnal temperatures, and are therefore capable of maximal performance in the environment they experience in nature. Consider the effect of an ecological shift to a nocturnal environment on the ancestral lizard physiology: low temperatures reduce y_0 and $\dot{V}_{O_2max}$, but C_{min} is thermally insensitive (John-Alder and Bennett 1981; John-Alder et al. 1983; Bennett and John-

Alder 1984; Lighton and Feener 1989; Full and Tullis 1990; Autumn et al. 1994; but see Weinstein and Full 1994 for crabs). The effect of a reduced y_0 and $\dot{V}_{O_2max}$ is a reduced MAS. This explains why diurnal lizards have poor endurance at low temperature. Two questions remain: (1) Why do geckos have relatively poor endurance at low temperature? In other words, why are geckos suboptimal at the temperatures they experience during activity? (2) Why do geckos have increased endurance and MAS at low temperature relative to comparable diurnal lizards? What is the physiological basis for the increased performance capacity and can it explain why geckos remain suboptimal at low temperatures? Answering these questions requires a comparison of y_0, $\dot{V}_{O_2max}$, C_{min}, and MAS in lizards that differ greatly in body mass and phylogenetic history.

Evolution of the Maximal Rate of Oxygen Consumption

Evolution of an increased $\dot{V}_{O_2max}$ at low temperature would increase MAS and endurance, and would explain question (2) above. Optimality theory predicts that the thermal optimum for $\dot{V}_{O_2max}$ should coincide with activity temperatures. $\dot{V}_{O_2max}$ is strongly temperature sensitive in diurnal lizards; a 10°C decrease in body temperatures causes a 50–75% decrease in $\dot{V}_{O_2max}$ (Bennett 1982). $\dot{V}_{O_2max}$ is also strongly mass dependent. Allometric analysis has played a central role in the comparative physiology of metabolism (e.g., Kleiber 1961), and would seem to be the proper tool for the job. A comparison accounting for body mass and temperature should reveal if geckos evolved an increased $\dot{V}_{O_2max}$ (Figure 5A). Recent studies, however, that focused deeply on particular lizard clades (varanids: Thompson and Withers 1997; geckos: Autumn et al. 1999) have inadvertently created a phylogenetic bias in the $\dot{V}_{O_2max}$ data set. Varanids are thought to have unusually high $\dot{V}_{O_2max}$, and the inclusion of six relatively closely related varanid species (Thompson and Withers 1997) may amount to "phylogenetic pseudoreplication" of a single evolutionary event rather than six independent values (Garland and Adolph 1994). Fortunately, advances in phylogenetic comparative methods (Felsenstein 1985; Garland et al. 1992; Garland and Ives 2000) provide a simple solution. Instead of treating species as independent samples, the method of phylogenetically independent contrasts compares pairs of sister taxa (Garland et al. 1993; Figure 5B). The method reduces the influence of sister taxa that have similar values by common ancestry. For example, the contrasts between varanid sister taxa (each with high $\dot{V}_{O_2max}$) might be numerically small, while the single contrast between the ancestor of varanids and its sister taxon might be numerically large. A phylogenetically correct analysis reveals, surprisingly, that when geckos evolved nocturnality, they did not evolve an increased $\dot{V}_{O_2max}$ at low temperature. In fact, there is no evidence that the thermal optimum for $\dot{V}_{O_2max}$ decreased at all (Autumn and Full 1994). $\dot{V}_{O_2max}$ at 25°C is nearly identical in both nocturnal and diurnal lizards, once the effects of body mass and phylogeny are accounted for. This answers question (1) of how geckos are suboptimal for endurance at low temperature: low temperatures cause a decrease in $\dot{V}_{O_2max}$ in lizards, and geckos are no exception. The answer to question (2) of how geckos have increased performance at low temperature requires a measurement of the other variables that affect MAS.

Evolution of the Minimum Cost of Locomotion

One of the most compelling questions in the fields of physiology and biomechanics is: why does fuel economy differ among animals? This question can be divided into two classes of mechanism: mass dependent and mass independent. This is an important distinction because variation that is mass independent is likely to be the result of a different mechanism than variation that is mass dependent. Figure 6A shows the energetic cost of locomotion—or the inverse of fuel economy—versus body mass in mammals and lizards. Note that the metabolic cost scales strongly with body mass. Lower values on the *y*-axis mean better fuel economy. Larger animals have better fuel economy. The lizards have a slope that is 33% lower than the mam-

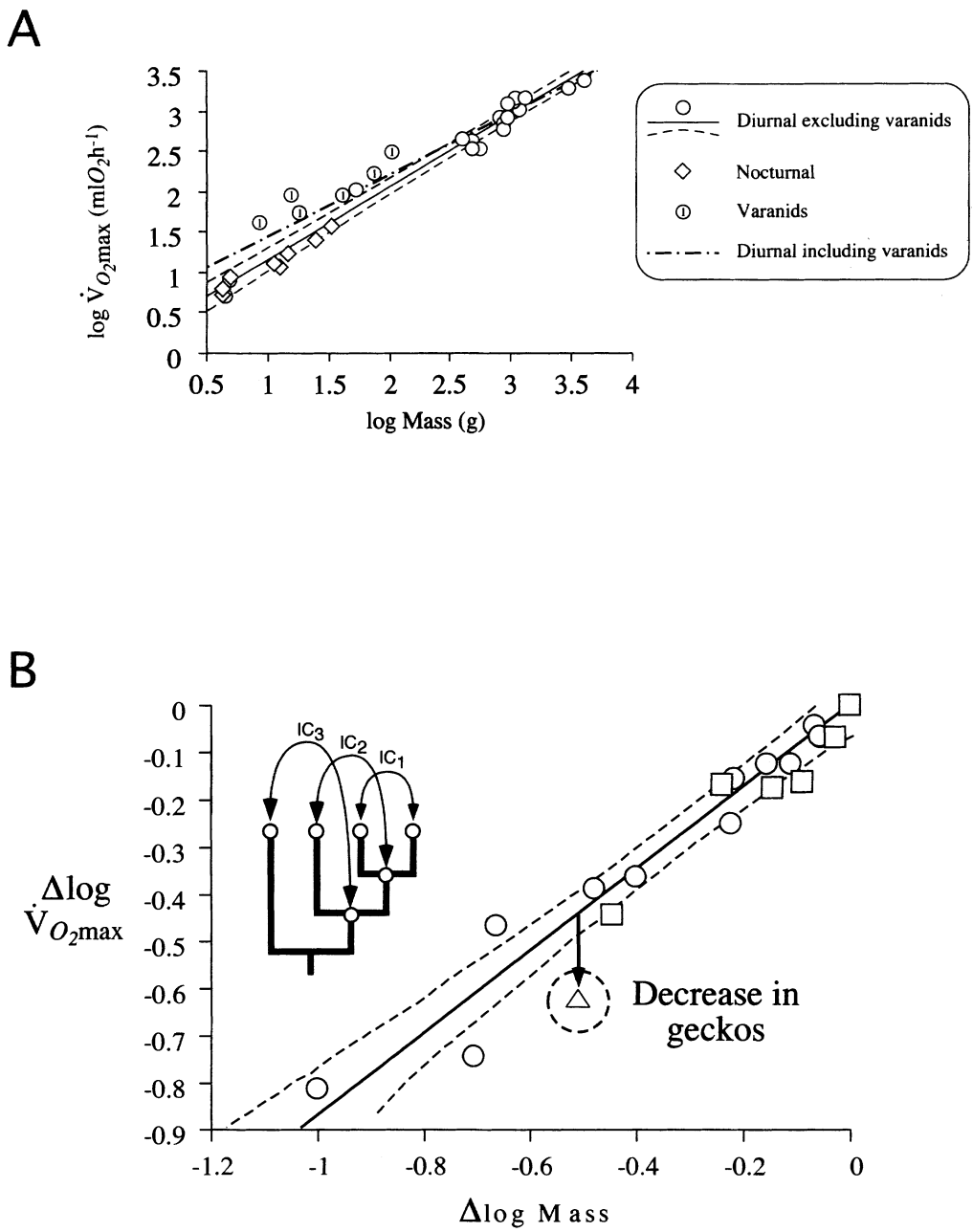


FIGURE 5. SCALING OF MAXIMUM RATE OF AEROBIC METABOLISM

A. Allometric analysis of body mass and $\dot{V}_{O_2max}$ (Q_{10} scaled to 35°C) in diurnal and nocturnal lizards. Phylogenetic pseudoreplication of varanid species (numeral 1) makes interpretation of differences between geckos (diamonds) and diurnal lizards (circles) difficult. **B.** Phylogenetically independent contrasts of body mass and $\dot{V}_{O_2max}$ (at activity temperatures). Solid line represents contrasts between diurnal lizard sister taxa (squares). Contrasts between gecko sister taxa fall within the 95% confidence limits of the diurnal lizard regression, indicating that body mass has a similar effect on $\dot{V}_{O_2max}$ in all lizards. Once the confounding effect of a phylogenetically biased sample (see **A**) is removed, it is evident that $\dot{V}_{O_2max}$ (at activity temperatures) decreased substantially in the gecko clade (triangle), and that this decrease is fully acounted for by the decrease in body temperature associated with the evolution of nocturnality. (Modified from Autumn et al. 1999.)

mals. This means that fuel economy in lizards may have a fundamentally different relationship to body mass than mammals. This would strongly challenge Kram and Taylor's (1989) mass-dependent hypothesis that ground contact time determines energetic cost of locomotion. The difference in slope implies that the mass-dependent mechanism that relates body mass to C_{min} is different in mammals and lizards. The next step would be to study energetics, muscle function, and biomechanics to find out what is the cause of difference in mass dependence in lizards. This could take a lifetime and a substantial amount of grant support—especially since each data point on Figure 6A can take 3 months to collect. When we separate the data on nocturnal geckos (Figure 6B) from the data on diurnal lizards, however, it becomes clear that the difference in slope between lizards and mammals (Figure 6A) was a phylogenetic artifact. This is due to the fact that geckos and diurnal lizards differ greatly in fuel economy. Body mass has similar effects on fuel economy in both geckos and diurnal lizards, however. A phylogenetically correct independent contrasts analysis (Figure 6C) adds statistical support to this conclusion. Only by considering history can we reveal that the mass dependence is the same within the geckos and diurnal lizards, and that mass-independent mechanisms may be important in explaining the difference in C_{min} between geckos and diurnal lizards. Without considering phylogeny one could have invested a huge amount of time and resources looking for a mass-dependent mechanism that does not exist. In this example, ignoring history leads one down an unproductive research direction while embracing history elucidates mechanism.

Thus, C_{min} in lizards does not represent an unusual mass-dependent scaling relationship; rather, geckos have a low C_{min}. A low C_{min} is a shared, derived character of the gecko clade (Autumn et al. 1999). This answers question (2) of how geckos have increased performance at low temperature: since C_{min} is temperature independent, it has the effect of increasing MAS (and endurance capacity) at all temperatures (Figure 7).

Without an understanding of the mechanisms that underlie endurance, the combination of suboptimality and increased performance at low temperature in geckos seemed paradoxical. An integration of mechanism and history reveals that there is no paradox after all. Low temperatures are suboptimal for geckos because $\dot{V}_{O_2max}$ is thermally sensitive, and the thermal optimum for $\dot{V}_{O_2max}$ is similar in nocturnal and diurnal lizards. Geckos have greater MAS at low temperature because they evolved a low C_{min}. The increase in performance is substantial: nocturnal geckos at low temperature are operating at about 2–3 times the MAS of diurnal lizards at low temperature (Autumn et al. 1999). MAS in a gecko at low temperature is only about 50% of the MAS of a diurnal lizard at high temperature, however.

Why are geckos suboptimal at the temperatures they experience during activity, and yet have increased endurance and MAS at low temperature relative to comparable diurnal lizards?

Mechanism: The maximal rate of aerobic metabolism ($\dot{V}_{O_2max}$) is strongly thermally sensitive in ectotherms. The thermal optimum for $\dot{V}_{O_2max}$ is high (30–45°C) in lizards, and geckos are no exception. The minimum cost of locomotion (C_{min}) is thermally insensitive in all terrestrial vertebrates. Endurance capacity is determined largely by the maximum aerobic speed (MAS), which in turn is dependent on C_{min} and $\dot{V}_{O_2max}$ by the equation, $MAS = \frac{\dot{V}_{O_2max} - y_0}{C_{min}}$. The C_{min} and $\dot{V}_{O_2max}$, and thus MAS, scale allometrically with body mass.

History: The thermal sensitivity and the thermal optimum for $\dot{V}_{O_2max}$ remained largely evolutionarily static in lizards in general, and geckos in particular. Accounting for a phylogenetically biased sample and for differences in body mass, geckos and other lizards have similar $\dot{V}_{O_2max}$ at low nocturnal temperatures. Accounting for a phylogenetically biased sample and for differences in body mass, geckos evolved C_{min} 1/2 to 1/3 that of other lizards. Geckos evolved a reduced MAS when they evolved to be active at low nocturnal temperatures, but not as low a value of MAS as predicted for diurnal lizards at the same low temperatures.

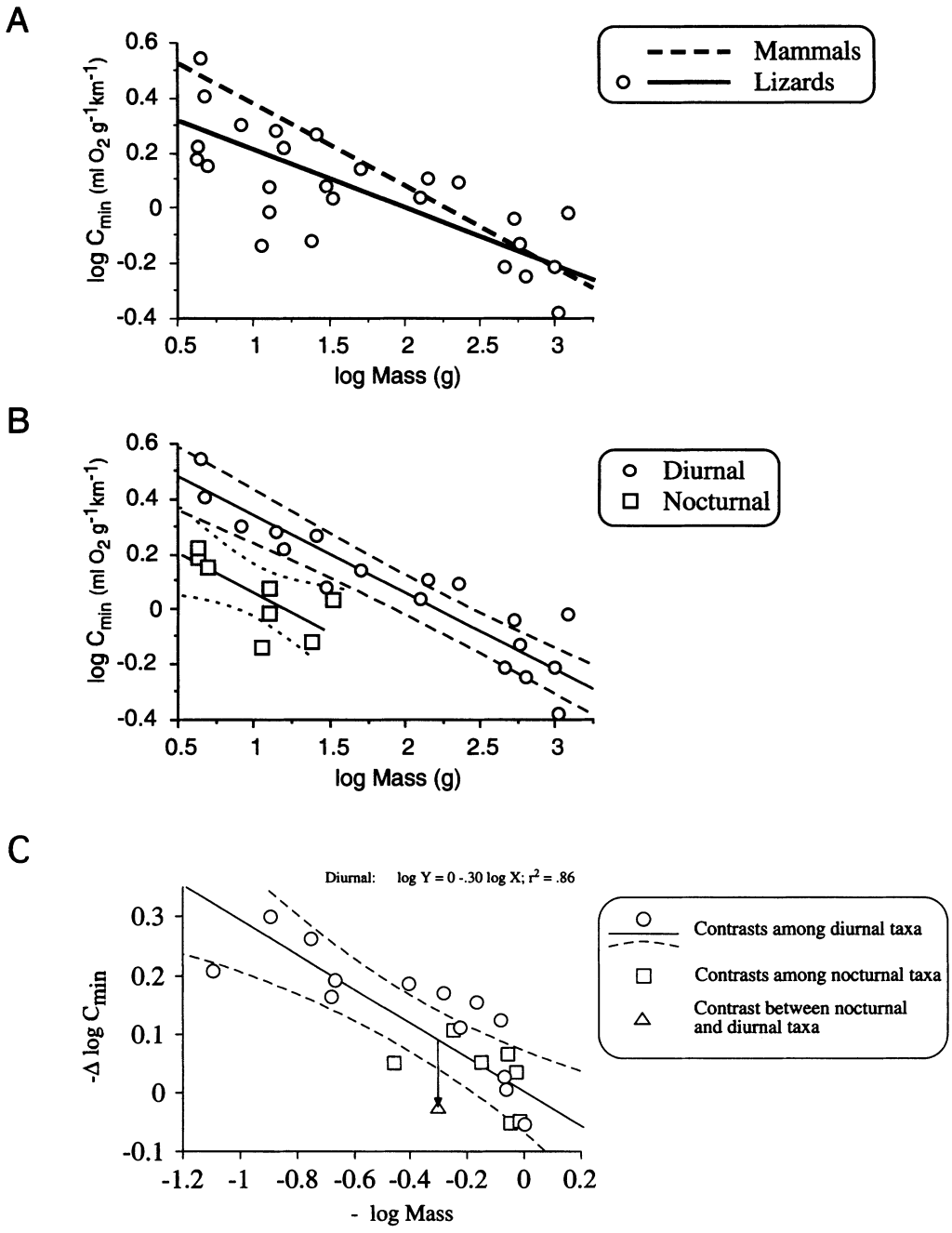


FIGURE 6. SCALING OF MINIMUM COST OF LOCOMOTION

A. Allometric relationship between body mass and minimum cost of locomotion (C_{min}). Circles represent data for lizards (Autumn et al. 1999). The allometric slope in lizards (-0.21; solid line) is 33% lower than the slope measured in mammals (-0.31; Taylor et al. 1970). **B.** Allometric relationship between body mass and C_{min}, within the gecko clade (squares), and within nongeckos (circles). The allometric slope in geckos and nongeckos is similar, but geckos have a lower C_{min} than nongeckos of a given body mass. **C.** Phylogenetically independent contrasts in log C_{min} and log body mass. The solid line represents a regression through contrasts among non-gecko sister taxa (circles). Contrasts among gecko sister taxa fall within the 95% confidence limits of the regression, indicating that evolution in body mass is associated with a similar change in C_{min} in geckos and nongeckos. The contrast between geckos and nongeckos fell outside the confidence limits of the regression, indicating that a low C_{min} is a shared derived character of geckos. (Modified from Autumn 1999 and Autumn et al. 1999.)

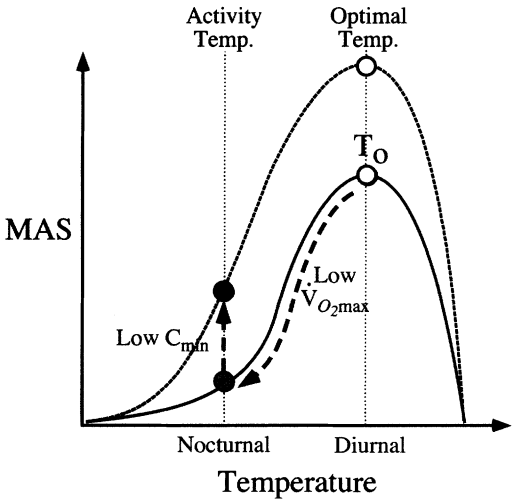


FIGURE 7. THEORETICAL ILLUSTRATION OF THE RESOLUTION OF THE NOCTURNALITY PARADOX

The solid curve represents the diurnal ancestor of geckos, and the dotted curve represents nocturnal geckos. The dashed arrows show how performance decreased because of the thermal sensitivity of $\dot{V}_{O_2max}$, and also increased due to the adaptive effect of decreasing C_{min}. A decreased C_{min} increases performance at all temperatures, but since the geckos are active at low temperature, performance is still submaximal.

Integration of mechanism and history: Geckos gained a performance advantage at low temperature when they evolved a low C_{min}, but remain suboptimal for sustained locomotion at the temperatures they experience during activity. Suboptimality is a historical legacy of high thermal sensitivity and thermal optimum of $\dot{V}_{O_2max}$ inherited by the diurnal ancestor of geckos that lived approximately 160 million years ago.

Testing the conclusion: An alternate hypothesis based on current fitness maximization is that current selection is responsible for submaximal performance at low temperature in geckos. Without the integration of mechanism and history, one could have concluded that balancing selection or phylogenetic inertia (Huey et al. 1989) was responsible for the paradoxically increased but submaximal performance at low temperature in geckos. Instead, the mechanistic and historical evidence strongly suggests that suboptimality is an evolutionary side effect, not the evolutionary cause, because the major changes took place 160 million years ago (Autumn et al. 1999), and the most thermally sensitive variable ($\dot{V}_{O_2max}$) did not evolve. Challenges to this conclusion are likely to come from advances in physiology and biomechanics of terrestrial locomotion, and from systematic revisions of the Squamata.

CONCLUSION

The historical and mechanistic approaches we present enhance understanding of patterns and processes of organismal evolution. Understanding the historical and mechanistic foundations of phenotypes has the potential to increase the accuracy and efficiency of research. Perhaps most importantly, we present a protocol for testing historical and mechanistic explanations. In order for our fields to progress, we must move beyond rhetoric toward rigorous tests of our hypotheses.

Objections to the use of historical approaches have taken two fundamentally different directions. First, a great strength of decades of research in behavioral biology, functional morphology, and organismal physiology has been the strong focus on proximate causal mechanisms. Mapping of traits on a phylogenetic hypothesis has been viewed as a regressive encouragement of correlational approaches that threaten the genuine progress that has been associated with the focus on causal mechanisms (Mangum and Hochachka 1998).

Second, the incorporation of evolutionary perspectives in fields such as social behavior has led to a sharp focus on phenotypes and their contribution to fitness. This trend has contributed to ongoing debates over the meaning and definition of adaptation. We did not directly address the issue of whether adaptation should be defined historically or by current utility. In the context of the case studies we presented here, the definition of adaptation is less important than a robust understanding of why organismal traits take the form they do. We are especially concerned that exclusion of historical and mechanistic biology from adaptive explanations leads to the substitution of a superficial

and largely untestable narrative (see O'Hara 1988) for deep understanding of the trait itself by assigning the significance of variants in the trait to fitness a priori. Every aspect of the phenotype potentially contributes to fitness, so some workers (e.g., Reeve and Sherman 1993) interpret an overly historical perspective as fostering the idea that features of organisms persist that are nonadaptive. While discovery of nonadaptive traits is a possibility, it is by no means a foregone conclusion of an integrative approach.

In the case studies we presented, there was a synergy between mechanistic and historical biology that led to discoveries that would have been impossible without this approach. We believe that this integrative approach will advance the field of evolutionary biology more rapidly than an approach targeted solely at fitness and local adaptation because more information is used to reach conclusions, and because conclusions will be easily testable. Biologists should not be threatened by the opportunity to use new tools (e.g., new phylogenetic comparative methods; Schluter et al. 1997; Garland and Ives 2000) to answer mechanistic questions, while at the same time increasing the strength of their evolutionary conclusions. The most interesting questions are generally the ones that involve complex systems. Such systems, however, do not lend themselves to easy answers based on thought experiments. Conclusions made without knowledge of the causal linkage among the parts of a complex integrated system are untestable and are likely to be false. Even if researchers are mechanistically oriented so that evolutionary questions do not interest them, phylogenetic methods may be necessary to make valid comparisons among species. Even if researchers are interested in adaptation and not in mechanism, understanding of mechanism may be necessary to reach a robust and rigorous answer. Let us not slow the advancement of evolutionary biology by assuming that what is outside our field is either simple or irrelevant. Instead, let us progress more rapidly by integrating mechanistic and historical biology in the study of evolution.

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