

Function and the Evolution of Phenotypic Stability: Connecting Pattern to Process¹

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SYNOPSIS. Phenotypes manifest a balance between the inherited tendency to remain the same (phenotypic stability) and the tendency to change in response to current environmental conditions (adaptation). This paper explores the role of functional integration and functional trade-offs in generating phenotypic stability by limiting the responses of individual characters to environmental selection. Evolutionarily stable configurations (ESCs) are systems of functionally interacting characters within which characters are “judged” by their contribution to system-level functionality. This “internal” component of selection differs from traditional “external” selection in that it travels with the organism wherever it goes and is maintained across a wide range of environments. External selection, in contrast, is by definition environment-dependent. The temporal and geographic constancy of internal selection therefore acts to maintain phenotypic stability even as environments change. Functional trade-offs occur when one character participates in more than one function, but can only be optimized for one. Participation of certain (“keystone”) characters in a trade-off potentially causes stabilization of an entire system owing to a cascade of functional dependencies on that character. Phylogenetic character analysis is an essential part of elucidating these processes, but patterns cannot be used as *prima facie* evidence of particular processes.

INTRODUCTION

Owing to genealogical descent, all organisms tend to resemble their recent ancestors, irrespective of the environments they inhabit. At the same time, each evolving lineage is subjected to a changing set of novel environmental demands to which organisms adapt. Phenotypes therefore manifest a balance between the inherited tendency to remain the same (phenotypic stability) and the tendency to change in response to current environmental conditions (adaptation). The nature of this balance is at the crux of our ignorance about how phenotypes evolve.

To understand the balance between evolutionary forces of change and conservation requires the integration of two mechanisms

into a coherent theory of phenotypic evolution: directional natural selection and constraints. Directional natural selection is by far the best understood mechanisms of the two. There is a mathematical theory, for example, explaining the population dynamic basis of genetic adaptation (Nagylaki, 1992) and there is ample empirical evidence of the action of natural selection in the wild (Endler, 1986). Far less understood are the mechanisms that lead to phenotypic stability in evolution. Among the processes that might lead to phenotypic stability are genetic, developmental and functional constraints. Developmental constraints are thought to be caused by the intrinsic nature of the developmental processes that limit the range of phenotypes available to natural selection (Maynard Smith *et al.*, 1985; Schwenk, 1995a). A good example is constraint on the pattern of digit reduction in amphibians, which has been shown to be caused by the developmental pattern of digit development (Alberch and Gale, 1983, 1985). Pleiotropic effects have also been

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proposed as a cause of character conservation. For instance the limitation of digit number to a maximum of five among recent amniotes cannot be explained by a developmental constraint *per se* since polydactyl limbs appear quite frequently. Wright (1968) and Lande (1978) attributed the lack of phylogenetic increase of digit number to deleterious pleiotropic effects of the mutations that cause polydactyly (a genetic constraint). Recently evidence was presented that the same mechanisms may explain the conservation of neck vertebrae number in eutherian mammals (Galis, 1999) as well as the conservation of the phylotypic stage in vertebrate evolution (Sander, 1983; Raff, 1996; Galis and Metz, 2001). Less commonly, function has also been suggested to play a role in constraint. Although seldom explicit, most notions of “functional constraint” relate to limitations on character evolution imposed by a character’s placement in a chain or web of functional dependency among a suite of characters (*e.g.*, Dullemeijer, 1980; Osse, 1983; Futuyma, 1998; Hall, 1998; Wagner and Schwenk, 2000).

In this paper we consider specifically the possible role of function in generating phenotypic stability. Most of the literature on stability or stasis has dealt either with individuated characters (*i.e.*, homology) or whole organisms (*i.e.*, *Baupläne*), probably because stability is most apparent at these phenotypic levels (*e.g.*, Wagner, 1989, 1999; Roth, 1991; Hall, 1994, for homology; Simpson, 1953; Wake *et al.*, 1983; Raff, 1996; Arthur, 1997; Hall, 1998, for *Baupläne*). However, function implies an intermediate hierarchical level reflecting dynamic relationships among a *set* of characters, as captured in the term “system” (Riedl, 1977; Wagner, 1986). Systems are suites or complexes of characters that produce a particular “output.” When they evince some level of coherence they are often described by morphologists as “functional units” or “character complexes” (*e.g.*, Bock and von Wahlert, 1965; Schwenk, 2001*b*). Since the processes leading to phenotypic stability are likely to vary among hierarchical levels (character, system, *Bauplan*) owing to the principle of emergence, each requires its

own theory of causality (Wagner and Schwenk, 2000; Schwenk, 2001*b*). Here we consider the mechanisms whereby system-level function might lead to constraints on phenotypic evolution. We use the examples of lizard feeding and vomeronasal systems to illustrate our models and show that dynamic interactions among characters could promote very long-term phenotypic stability in functional systems. As they depend on functional analysis for their elucidation, these interactions may be cryptic and not apparent during standard character analyses. We show that phylogenetic patterns, although essential for identifying and characterizing the evolutionary processes leading to stability, cannot provide *prima facie* evidence for particular processes or mechanisms, hence our ability to use pattern to predict process is severely limited. There are at least two ways that functional interactions among characters can lead to phenotypic stability: *functional integration* and *functional trade-off*. These are discussed separately in the following sections.

FUNCTIONAL INTEGRATION AND PHENOTYPIC STABILITY

Evolutionarily stable configurations

Wagner and Schwenk (2000) developed a model of functional integration among characters leading to the evolution of phenotypic stability. They called such phenotypically stabilized systems *evolutionarily stable configurations*, or ESCs. Here we provide an overview of the ESC concept and illustrate it with the example of lingual prey capture in lizards.

ESCs are suites of characters (from two to many) that functionally interact to produce a particular output. The set of interacting characters constitutes a functional “system,” such as an organ system or the feeding system, and the functional output is its “proper function” (after Millikan, 1984). The proper function is essentially the system’s *raison d’être*, the over-arching function it is adapted to perform (*e.g.*, the prehension of food with the tongue; see Wagner and Schwenk [2000] for further discussion). An ESC is evident as a stable part of the phenotype that has persisted

through long periods of time, through cladogenesis and across a wide range of environments. This evolutionary stability arises from the functional interactions among characters within the system and is therefore an emergent property of system-level functioning. Its stability does not, therefore, depend on the presence of developmental constraints or constancy of the environment in which the organisms live.

Phenotypic stability arises because the set of anatomical and functional relationships within an ESC severely limits the universe of advantageous phenotypic variation possible in its constituent characters. The strength and nature of the limitation is determined by the impact of character variation on system-level functionality. As such, given a random mutation leading to phenotypic change in one character, the mutation can persist if the functional interactions of that character with others are unaffected or enhanced in terms of the system's proper function (*i.e.*, whole-system function). If, however, the change impairs or disrupts system functionality (*e.g.*, feeding performance is reduced), the individual manifesting the mutation will have reduced fitness relative to others in the population and the mutation will be selected against. Given the precisely coordinated nature of character interactions in many systems, the majority of phenotypic changes are likely to be deleterious. As such, the predominant form of selection on most characters will be stabilizing. The strength of the stabilizing selection is proportional to the "tightness" of the "fit" required between two characters when they interact. A tight fit implies low tolerances (in an engineering sense) and even a small change in character phenotype will affect system function. A looser fit implies higher tolerances and therefore a greater threshold for potential phenotypic change. Since performance of the proper function requires the coordination of several or many characters, a deleterious change in one character's functional interaction(s) has a cascading effect throughout the system, causing reduced functionality or even failure of the system, as a whole. Thus, any phenotypic change in an ESC component character is "tested" by system-

level functionality. Changes that impair performance of the proper function sufficiently to have fitness consequences for the organism will be selected against.

The significance of this scenario is that the stabilizing selection acting within an ESC is "internal," *i.e.*, it springs from the demands of system functionality, an intrinsic attribute of the organism. *Internal selection* therefore travels with the organism wherever it goes and exerts its pressures more-or-less constantly across a wide range of environments. This is distinct from the typical, Darwinian notion of selection deriving from pressures exerted by the biotic and abiotic environment inhabited by the organism. Such *external selection* is, by definition, environment-dependent (Whyte, 1965; Arthur, 1997; Wagner and Schwenk, 2000). Wagner and Schwenk (2000) defined internal selection as rank-invariance of fitness among morphs across a range of environments and rank-dependence of fitness on the particular combination of character states in the system. In other words, the rank-order in fitness of individuals varying in some character remains constant across a range of environments (absolute fitness values might vary) and the fitness effects of character variation depend on the particular set of characters that interact (the characters are functionally integrated).

In summary, an ESC is a set of characters united by common participation in a series of coordinated interactions serving a single proper function, the "purpose" for which the system has evolved. Since a tightly integrated web of functional interactions can tolerate little phenotypic deviation in constituent characters before system functionality is adversely affected, internal selection will constrain phenotypic evolution and the system eventually "gels" into an evolutionarily stable configuration. Internal selection arises as an emergent property of system function and is therefore an intrinsic, organismal attribute.

ESCs, internal selection and phenotypic stability

It is important to recognize that phenotypic stability in the context of ESCs does not imply immutability. In other words,

evolution of the ESC phenotype can occur as long as character evolution remains within the circumscribed sphere of the ESC. As such, the ESC is a closed set of phenotypes under the operation of adaptation to the proper function so that phenotypic changes always remain within the ESC (just as the set of even integers is a closed set under the operation of addition). Therefore, the ESC potentially encompasses a *set* of phenotypes that are variable among lineages, but variation is expressed only within a restricted orbit. Phenotypic stability in this sense is relative; ESC phenotypes in different lineages are recognizable as the “same” thing, but they are not necessarily identical. In the lizard feeding example described below, for example, lizard families differ in anatomical details, such as lingual papillary form, but the mechanism and kinematic sequence of prey capture are nonetheless highly conserved. The ESC is thus evident as an historical entity and it is this historical persistence that requires explanation.

The qualities of an ESC imbuing it with evolutionary stability are touched on above. Essential is the fact that the internal rules of functionality inherent to internal selection apply in a potentially diverse set of environments. Consequently, internal stabilizing selection acting on system phenotype transcends environmental boundaries, both spatially and temporally. This implies that as environments (and therefore external selection regimes) change through geologic time, the strictures of internal selection remain, acting to maintain the phenotypic *status quo*. A critical point is that *the action of internal selection on an individual character can be in direct opposition to the action of external selection*. In other words, environmental selection tending to modify a character in some adaptive way will be ineffective if the fitness loss associated with reduced ESC functionality is greater than the fitness gain associated with modifying the character (an analogy is the relationship between “natural [or viability] selection” and “sexual selection”). Thus, any single character is subject to a balance of internal and external selection pressures. As long as the internal component of selection is stronger than the external component, the char-

acter will be unresponsive to environmental pressure to change. Since tightly integrated systems are likely to serve critical survival functions (such as feeding, locomotion or escape), the resistance to pointillistic, adaptive modification of individual characters might be very great, indeed. ESCs, as a whole, therefore tend to resist environmental perturbation as long as the environments encountered do not exceed the range tolerated by ESC system function. This range of environments, though potentially broad, is nevertheless finite. A critical change in the environment might modify the internal optimality rules governing ESC function, eliminating internal selection on its characters and setting the stage for its disintegration. Thus, a key change in the environment is one way an ESC can be disrupted and its characters freed for alternative evolutionary pathways. Wagner and Schwenk (2000) identified other possibilities. ESCs can therefore manifest very long-term phenotypic stability, but they are not evolutionary dead-ends.

Example of an ESC: the evolution of lizard feeding systems

Feeding obviously plays a critical role in survival and therefore the contribution of the feeding system to fitness is likely to be substantial. In vertebrates, feeding is often a complex affair divisible into as many as five functionally distinct stages: subjugation, ingestion, processing, intra-oral transport, and swallowing (Schwenk, 2000a). Each stage requires the coordinated activity of many parts whose anatomical and dynamic relationships must be tightly integrated to produce the required outcome. Thus internal selection on components of the feeding system is likely to be strong, providing a fertile ground for the evolution of ESCs.

The ability of many lizards to apprehend small food items with the tongue (lingual ingestion) exemplifies the tightly integrated nature of the feeding system (Schwenk, 2000b) and serves as a putative ESC (with proper function, “the prehension of small food items with the tongue”). This example was developed in detail by Wagner and Schwenk (2000). We review it here and

compare it to data on the lizard vomeronasal, chemosensory system in the following section to develop the notion of “functional trade-offs.” These examples are then used to consider the relationship between phylogenetic pattern and evolutionary process.

“Lizards” are non-snake, non-amphisbaenian squamate reptiles. Squamates and their sister taxon, *Sphenodon* (tuatara) constitute the group Lepidosauria. Squamata is further divided into two basal clades, Iguania and Scleroglossa. Iguania includes three families, Iguanidae, Agamidae and Chamaeleonidae. Scleroglossa is a diverse and speciose group including all remaining lizard families, plus snakes and amphisbaenians (Fig. 1).

Lingual prehension of small food items is used universally by *Sphenodon* and all iguanians. In contrast, identical food items are grasped with the teeth and jaws in virtually all scleroglossan lizards (reviewed by Schwenk, 2000b). Lingual prehension involves protrusion of the tongue beyond the jaws as the food item is approached with a lunge. The tongue is protracted as it is pushed forward by the hyobranchial apparatus (tongue and throat skeleton) and cantilevered beyond the mandible on the lingual process extending within it. The tongue tip is tethered to the end of the mandible by muscles so that as it protrudes it is forced to curl ventrally, presenting the tongue’s dorsal, papillose surface anteriorly toward the prey (Fig. 2). Papillae are long and densely glandular. The tongue contacts the food item forcefully, pushing it down into the papillae, maximizing the area of contact. A combination of interlocking and wet adhesion creates a remarkably effective adhesive mechanism so that during rapid tongue retraction inertial forces do not dislodge the prey item. The jaws fly open to accommodate the tongue and adherent prey during retraction, then snap shut, trapping or crushing the prey. The tongue is then slowly retracted the rest of the way within the mouth and the system is “reset” for the next feeding stage. The entire sequence takes as a little as 20 milliseconds and represents the finely coordinated action of

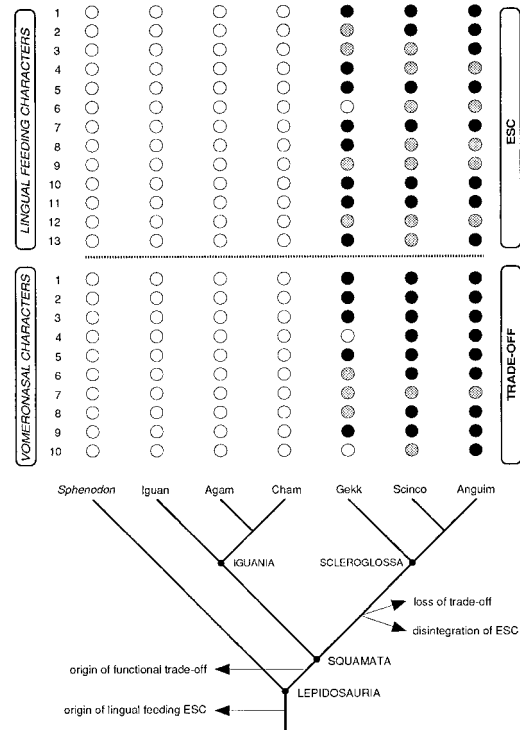


FIG. 1. Phylogeny of squamate reptiles and the evolution of feeding and vomeronasal systems. Circles represent characters listed in Tables 2 and 3 and discussed in the text. Open circles indicate the plesiomorphic state, black circles the derived state, stippled circles variable states. Terminal iguanian taxa are the families Iguanidae (Iguan), Agamidae (Agam) and Chamaeleonidae (Cham). Terminal scleroglossan taxa are suprafamilial groups, Gekkota (Gekk), Scincomorpha (Scinco) and Anguimorpha (Anguim). Phylogeny based on Estes *et al.* (1988) as discussed in Schwenk (2000b).

tongue, jaws, hyobranchial apparatus, head and whole-body.

A fundamentally similar system characterizes ingestion in all iguanian taxa, regardless of taxonomic affiliation, environment, diet or other ecological variable (Schwenk, 2000b; Wagner and Schwenk, 2000). Since a virtually identical system is found in the outgroup, *Sphenodon*, lingual prey prehension is inferred to represent the plesiomorphic condition (Schwenk and Throckmorton, 1989). Chameleons represent the only non-trivial departure from the ancestral phenotype within Iguania, with a ballistic projection phase inserted into the sequence just before prehension, but the

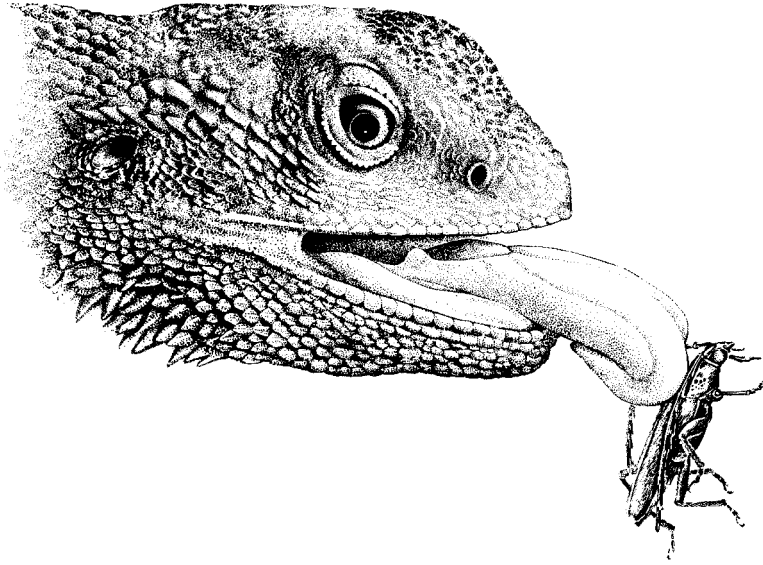


FIG. 2. The lingual feeding ESC phenotype: prey capture in an iguanian lizard (*Pogona barbata*, Agamidae). See text for discussion. From Schwenk (2000b), reproduced with permission of Academic Press, San Diego.

system remains fundamentally the same (Wagner and Schwenk, 2000). Scleroglossans, in contrast, evolved a novel, jaw-based prehension mechanism. We suggest that the lingual prehension system has been phenotypically stabilized in Iguania by the action of internal selection acting across a wide range of environments to maintain the integrated nature of the prehension mechanism.

The inference of strong, stabilizing internal selection is strengthened by circumstantial evidence for concomitant external selection acting on the tongue in opposition to its role in feeding function (Table 1). In all squamates, including iguanians, the tongue is also used for vomeronasal chemoreception. In a behavior called “tongue-flicking,” the tongue is rapidly protruded tip-first to sample environmental chemicals, then returned to the mouth where the chemicals are introduced into paired vomeronasal organs through small openings in the palate (Halpern, 1992; Schwenk, 1995b). Although both lingual ingestion and tongue-flicking require tongue protrusion, the mechanism for each is fundamentally different: in feeding it is whole-tongue movement coupled to hyobranchial protraction, tongue-tip curled; in flicking it is hy-

drostatic elongation of the anterior-most part of the foretongue independent of hyobranchial movement, tongue-tip pointed (Schwenk, 2000b). Modification of the foretongue to optimize hydrostatic elongation and tongue-flicking would interfere with the foretongue’s functional role in prey prehension (Table 1). Thus putative external selection favoring the evolution of enhanced tongue-flicking and chemoreception is opposed by internal selection acting on the tongue to maintain functionality of the prey capture system. In other words, the lingual prehension ESC has remained phenotypically stable despite environmental selection tending to change it.

Stabilization of the prehension system in iguanians implies retention of plesiomorphic character states related to ingestion. This prediction is confirmed by phylogenetic character analysis (Table 2 and Fig. 1): iguanians retain ancestral states in relevant characters of the tongue, hyobranchium and skull. In contrast, scleroglossans show variable, mostly derived states in the same characters. In other words, the pattern is consistent with the idea that internal selection within the ESC has limited phenotypic evolution in the characters related to lingual ingestion, but once a novel prehension sys-

TABLE 1. *Functional and morphological trade-offs in foretongue optimization for prehension (feeding) versus tongue-flicking (chemoreception).**

Trait optimization for lingual prehension	Trait optimization for tongue-flicking
Foretongue shape	
a. high diameter: blunt, rounded, wide, deep—maximize surface area for prey contact; muscular support for prey weight and inertia	a. small diameter: narrow, tapered and/or spatulate—optimization of hydrostatic elongation mechanism
Tongue-tip form	
a. unclleft—tip is slightly notched in iguanians (unknown functional significance), but a deeper bifurcation would interfere with prehension	a. pointed, bifurcated or forked—sharp tips promote turbulence during flick, max. chemical uptake; forking enhances trail-following
Tongue protrusion mechanism	
Whole-tongue protrusion coupled to hyobranchial retraction:	Hydrostatic foretongue elongation uncoupled to hyobranchial protraction:
a. long, robust lingual process to support and guide tongue	a. short lingual process does not penetrate foretongue; won't interfere with protrusion
b. anatomical coupling between most of tongue and hyobranchium	b. tongue-hyobranchial anatomical coupling restricted to hindtongue
c. genioglossus muscles insert anteriorly with some fibers running anteriorly to tip to tether foretongue to mandible	c. genioglossus muscles insert posteriorly so as not to interfere with foretongue elongation; few or no fibers running to tip
d. weakly developed circular fibers restricted to anteriormost part of foretongue	d. well developed circular fibers for hydrostatic elongation
e. partitioned hyoglossus bundles with large intrinsic component tied to transverse septum	e. discrete hyoglossus bundles for hydrostatic elongation
Surface features	
Adapted to adhesive mechanism for prehension of food:	Adapted to friction-reduction and biomechanics of chemical collection and delivery:
a. high-profile (long) papillae—absorb impact energy; for frictional interlocking; translation of shear into tensile stress; form surface to prey, increasing 3-D surface area of contact	a. low-profile papillae or none—minimize friction during rapid tongue-flicking; smooth surface for fluid-spreading related to mechanics of chemical sampling
b. dense mucous epithelium—sticky mucus for adhesion	b. no mucous glands; fluid on surface mostly serous or sero-mucous from sublingual glands
c. papillary apices narrow, often rugose—interdigitating/interlocking with prey surfaces	c. papillary apices broad, smooth and keratinized—promote fluid-spreading
Bending and oscillation	
Bending and oscillating detrimental to prehension function; tongue protruded straight with tip curled ventrally, rigid, with hyobranchial support:	Minimally dorsoventral bending during flick in some cases with rapid, multiple oscillations; often lateral bending to direct flick toward some target; dynamic, with no hyobranchial support:
a. weakly developed dorsal longitudinal fibers	a. well developed dorsal longitudinal fibers
b. little or no oblique fiber system	b. well developed oblique fiber system

* Trait optimization for lingual prehension is restricted to *Sphenodon* and iguanian lizards (some minor differences for chamaeleonids). Although iguanians are capable of modest tongue-flicking, trait optimization for this function only occurs within Scleroglossa. Modified from Tables 2 and 3 in Wagner and Schwenk (2000).

tem evolved in ancestral scleroglossans (jaw prehension; discussed by Wagner and Schwenk, 2000), these characters were “released” from the this constraint and freed to respond to other (external) selection pressures—for example, optimization of the tongue for tongue-flicking (see next). Thus, phylogenetic character analysis is consistent with the ESC hypothesis.

FUNCTIONAL TRADE-OFFS AND PHENOTYPIC STABILITY

Phenotypic stability of an ESC is explained as the result of functional integration among a set of characters related through their performance of a single (proper) function. Integration is manifested as internal selection. An alternative functional mechanism that might lead to phenotypic

TABLE 2. *Lingual feeding ESC characters.**

1.	foretongue papillae high profile
2.	end of tongue blunt, rounded and deep
3.	tongue tip unclenched or slightly notched
4.	foretongue highly glandular
5.	foretongue epithelium unkeratinized
6.	lingual process (of hyobranchium) robust
7.	lingual process at least 50% of resting tongue length
8.	lingual process penetrates corpus of tongue, surrounded by verticalis musculature
9.	gape kinematics during ingestion (prey prehension) include distinct slow-open I, slow-open II, fast-open, fast-close, and (usually) slow-close phases
10.	tongue protruded extra-orally during ingestion with max. protrusion at end of slow-open II (at tongue-prey contact)
11.	tongue tip curled ventrally during protrusion with dorsal surface arched, papillary surface curled around apex and tongue tip tucked ventrally, anchored near tip of lower jaw
12.	tongue movements coupled to hyobranchial movements during ingestion, transport and swallowing
13.	mesokinetic skull movement absent during prehension and all phases of feeding

* See Figure 2. Character descriptions are of the plesiomorphic (ESC) character state. Based on characters 1–13 in Wagner and Schwenk (2000), Table 1.

stability is found in functional trade-offs. Trade-offs arise when a character participates in more than one function. Whereas in an ESC, stability is, in a sense, the direct result of internal selection, in a trade-off, stability is an *indirect* effect of the conflict inherent to the optimization of one character for two (or more) different functions. If selection acting on a character for one function is strong relative to selection acting on that character for a second function, selection for the second function will be ineffective. The character will fail to evolve in a way that would optimize its role in the second function. In short, an ESC involves integration of multiple characters in performance of a single function, whereas trade-offs occur when a single character performs multiple functions.

Functional trade-offs are likely to be ubiquitous because few characters participate in only one function. Most character phenotypes are a “compromise” reflecting the balance of selection forces stemming from multiple functions. However, functional trade-offs may have far-reaching consequences. This might occur when trade-offs involve certain critical characters and when there is a steep imbalance in the conflicting sources of selection pressure. The functional trade-off between optimization of the foretongue in lizards for prehensile function *versus* chemosensory function, alluded to above, exemplifies this condition.

Evolution of the vomeronasal chemosensory system in squamate reptiles

Circumstantial evidence for selection acting on the tongue for the improvement of tongue-flicking (and therefore vomeronasal chemoreception) was adduced above to bolster the case for internal selection acting to maintain the tongue’s adhesive, prehensile phenotype. However, we did not consider the ramifications of this situation for the vomeronasal system. Owing to its participation in two separate functions, feeding and chemoreception, the tongue is the locus of a clear functional trade-off. The tongue is, itself, an integrated, subsidiary functional unit (Schwenk, 2001a) comprising a subset of the lingual feeding ESC characters. Biomechanical considerations indicate that in almost every feature, optimization of the tongue for chemosensory tongue-flicking is in direct opposition to its optimization for feeding (Table 1; see also Wagner and Schwenk, 2000). Since the tongue is a critical component of the lingual feeding ESC, internal selection maintaining feeding function is exceptionally strong. As such, the negative fitness consequences of modifying the tongue to enhance chemosensory function would be severe. Thus, as long as the lingual prehension ESC persists, as it does in *Iguania*, the tongue is unresponsive to selection acting to this end.

The significance of this trade-off goes

TABLE 3. *Vomeranasa chemosensory system characters (see Fig. 2).**

1. intrinsic circular fiber system of tongue well developed (3)
2. taste buds reduced or absent on foretongue (4)
3. vomeronasal organs (esp. sensory epithelium) well developed (5)
4. vomeronasal fenestrae independent of choanal groove (8)
5. nucleus sphericus (amygdaloid formation) well developed (11)
6. tongue-flicks commonly directed to air without substrate contact (14)
7. tongue-flicks sometimes consist of multiple oscillations (15)
8. chemosensory location of food without visual cues (18)
9. chemosensory identification of predators (21)
10. ability to follow scent trails

* In contrast to Table 2, descriptions are of derived character states. For characters 1–9, numbers in parentheses indicate the corresponding character in Schwenk (1993). Character 10 is based on Schwenk (1994).

beyond tongue phenotype, however. It has a limiting effect on evolution of the entire vomeronasal chemosensory system. This is evident from the pattern of vomeronasal specialization following the release of the tongue from the ESC in the scleroglossan lineage: the foretongue is rapidly modified to enhance hydrostatic elongation and tongue-flicking. Importantly, optimization of the tongue for tongue-flicking further permits ancillary specialization of the vomeronasal organs, their central nervous system projections and the behaviors dependent on the system. Thus, in ancestral scleroglossans, release of the tongue from its functional constraint in feeding initiated a cascade of phenotypic changes related to the vomeronasal system. A phylogenetic character analysis is consistent with this hypothesis (Table 3, Fig. 1). Vomeronasal system characters remain in their plesiomorphic state within the Iguania, but in Scleroglossa they show variable, mostly derived states. In this sense the tongue represents a “keystone character” (or set of characters) because of its central role in chemical sampling and delivery. Specialization of virtually all other parts of the vomeronasal system was contingent upon initial specialization of this critical component. Thus, retention of the vomeronasal system in an unimproved state throughout the iguanian radiation (as evident by comparison to the Scleroglossa) is the indirect result of functional trade-offs involving the tongue.

Functional trade-offs as a consequence of ESCs

Functional trade-offs leading to phenotypic stability in system-level phenotypes

may be promoted by the presence of ESCs. This is true only because ESCs tend to subject potentially large sets of characters to strong internal selection. Virtually all characters serve multiple functions and are therefore subject to conflicting demands, but participation of a character in an ESC would shift the balance of selection forces strongly in one direction, greatly increasing the likelihood of a trade-off in its service to other functions or systems. Whether or not the phenotypic consequences of a trade-off extend beyond the individual character to other, functionally contingent characters depends on the particular role played by the character in its secondary system. When a so-called keystone character is involved, phenotypic stability may extend downstream to a suite of related characters owing to a web of functional dependencies.

PHYLOGENETIC PATTERN AND EVOLUTIONARY PROCESS

Phylogenetic character analysis is an essential starting point for identifying phenotypic stability. By definition, stability represents the failure to evolve or the retention of ancestral character states. Thus, within a focal clade we expect phenotypic stability to be manifested as symplesiomorphy in a set of characters. This is exactly the pattern evident in the two examples presented here (Fig. 1). Obviously, in any phylogenetic analysis a subset of the characters will retain the ancestral state. However, the significant pattern here is that the symplesiomorphic characters are all related through participation in a common functional system, suggesting a causal connec-

tion. Otherwise we would expect a randomized pattern of plesiomorphic and derived states within the system (or a randomized distribution of primitive character states across a variety of systems). Since an ESC has a single origin and is disintegrated relatively rarely, we expect it to be characteristic of a higher clade or a significant part of it (Wagner and Schwenk, 2000). In other words, it should be represented monophyletically or, because it can be lost, paraphyletically. A polyphyletic distribution of an ESC, however, is not likely (these would represent different historical entities). The same pattern should hold for system phenotypes stabilized indirectly by functional trade-offs in keystone characters.

Recognition of stabilized systems is greatly facilitated in cases where the mechanistic basis of the stability is lost in a sister or descendant clade, as in the example here. Without such a comparison, support for the mechanistic hypothesis (ESC or functional trade-off) is weakened. Thus, the hypothesis of phenotypic stability driven by functional integration (an ESC) or functional trade-off is minimally dependent on a phylogenetic character analysis demonstrating the appropriate pattern of character state distributions across the phylogeny and a functional analysis that plausibly links the implicated characters. Does the phylogenetic pattern, alone, implicate a particular process? Figure 1 shows clearly that it does not. Identical patterns of symplesiomorphy *vs.* variable, derived states are evident for both lizard examples, stabilization through functional integration in the feeding system (top) and stabilization through functional trade-off in the vomeronasal system (bottom). Thus, a pattern of symplesiomorphy in functionally-related characters does not point to the specific evolutionary process involved.

Can the pattern, at least, reject the two remaining explanations for stability: external (environmental) stabilizing selection and developmental constraint? Not entirely. In particular, the neo-Darwinian null model for stasis, a stable environment (Simpson, 1953), is especially difficult to eliminate. Although environments might appear to vary among stabilized taxa at a gross scale,

it is conceivable that in those parameters directly relevant to the focal system it is, in fact, stable. Thus one must consider not only general habitat, but the specific attributes of the environment likely to affect a particular system before we can reject Simpson's (1953) notion of "a long eventless course of relatively unchanging conditions" as the basis for phenotypic stability.

Although the possibility of developmental constraint operating across a diverse suite of characters associated through function seems remote, it may not be. Characters subjected to long-term stabilizing selection tend to become canalized (*e.g.*, Wagner *et al.*, 1997), hence the internal selection characteristic of ESCs might ultimately lead to a set of developmentally constrained characters. However, the rapid modification of constituent characters following dissolution of the ESC suggests that most characters are not strongly canalized. A more likely possibility is that functionally-related characters are constrained by pleiotropic effects of the genes controlling their expression. As such, the genetic architecture underlying a functionally related character set evolves so that the set behaves, evolutionarily, as a single character (Cheverud, 2001). Functional interactions among independent characters probably drive the evolution of the genetic architecture initially, however, and the architecture can, itself, evolve so that the character complex can ultimately disintegrate.

In sum, phylogenetic analysis is an essential part of elucidating the evolutionary processes responsible for phenotypic stability. A monophyletic or paraphyletic distribution of symplesiomorphy in functionally-related characters across higher clades is the expected phylogenetic pattern of stability caused by functional integration or functional trade-off. Although consistent with a model of functional constraint, however, such patterns cannot entirely reject the alternative hypotheses of external stabilizing selection and genetic/developmental constraint. Additional data on relevant aspects of the associated environments and patterns of character evolution in related clades can lend additional credibility to the hypothesis of functional constraint, but ultimately phy-

logenetic pattern cannot serve as direct evidence for any evolutionary process.

CONCLUSIONS

The partitioning of organisms into tightly integrated systems implies that, even in the absence of other constraining factors, individual characters may not be free to evolve according to the dictates of the external environment. Characters are often shaped by the systems in which they serve, largely buffered from the effects of external selection pressures. Integrated suites of genetically and developmentally autonomous characters potentially behave as evolutionary units. However, the composite nature of such units becomes apparent in comparative analyses that include parts of the clade in which the functional coherence of the system has been disrupted. It is in the comparison of functionally constrained and unconstrained sister lineages that the effects of integration and trade-off are most evident. Perhaps the key point of this analysis is to suggest that as organisms evolve, they set the context for their own future evolution.

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